Knotweed (*Polygonum cuspidatum*)

Resveratrol, Grape Skin, & Grape Seed

Within the whole grape are a group of polyphenols, constisting of antioxidant phytonutrients such as resveratrol, anthocyanins, catechins, and quercetin, are believed to help the body's cells resist damage by free radicals. The same disease-fighting properties are thought to be delivered by both fresh grapes, with the seeds, and red wine.

Within the **skin skin and leaves** is a particularly important polyphenolic compound, the phytoalexin **resveratrol,** which has recently demonstrated an array of health promoting actions. It also possesses chemopreventative properties and functions as a plant defense molecule.

Resveratrol is a natural product occurring in the skins of grapes and various other plants, including peanuts and ***Polygonum cuspidum*** (Japanese knotweed). It is an immune-enhancing cytokine that protects the plant from fungal attack.

The **grape seed,** contains other important polyphenolic compounds**,** including anthocyanidins, proanthocyanidins (GSPE) and oligomer proanthocyanidins. Grape seed extract has been extensively studied as an anti-inflammatory agent for the treatment of hemorrhoids, swollen joints, athletic injuries, post-surgical edema, and post-surgical lymphedema, edemaprotective,[4,9] cancer inhibition,[8] cardiovascular protective,[3] improves visual function,[6] possesses general anti-oxidative and age inhibiting ability.[7] A synergistic effect has been demonstrated when grape seed and skin extract are taken together. [38]

Active Constituents

Grape Skin

- Saponins

  beta-sitosterol-6'-linolenoyl-3-O-beta-D-glucopyranoside (1), beta-sitosterol (2), beta-sitosterol-3-O-beta-D-glucoside (3), oleanolic acid (4), oleanolic aldehyde (5), **resveratrol** (6), (+)-epsilon-viniferin (7), (-)-catechin (8), and 1-triacontanol (9). (10) ellagic acid, ellagic acid glycosides

Polyphenolic Phytoalexin - a class of plant antibiotic

- Resveratrol (Trans-3, 4', 5-trihydroxylstilbene)

  alpha-Viniferin - a trimer of Resveratrol

  Piceatannol & Pterostilbene - analogs of Resveratrol [111]
- oligomeric anthocyanins [136]

Grape seed

Polyphenol Flavonoids

- -catechins, epicatechin, procyanidin, and some dimers and trimers, specifically:
- -anthocyanidins,
- -proanthocyanidins (GSPE),
- -oligomer proanthocyanidins (OPCs, aka PCO's and pycnogenols) [112]

Note: Polyphenolics generally increase as fruit ripenes and the highest concentrations are located in the skins. Free ellagic acid, ellagic acid glycosides, and total ellagic acid ranged from 8 to 162, 7 to 115, and 587 to 1900 mg/kg, respectively, in the skin of ripe grapes. Hot-pressed juices contained considerably lower polyphenolic concentrations than were present in whole grapes. Five anthocyanidins were present in each cultivar in variable concentrations (delphinidin > petunidin > malvidin + peonidin > cyanidin). Antioxidant capacity was appreciably influenced by cultivar, maturity, and location in the fruit with good correlations to soluble phenolics found in both methanolic and ethyl acetate extracts (r = 0.83 and 0.92, respectively). [114]

Mechanism of Effects

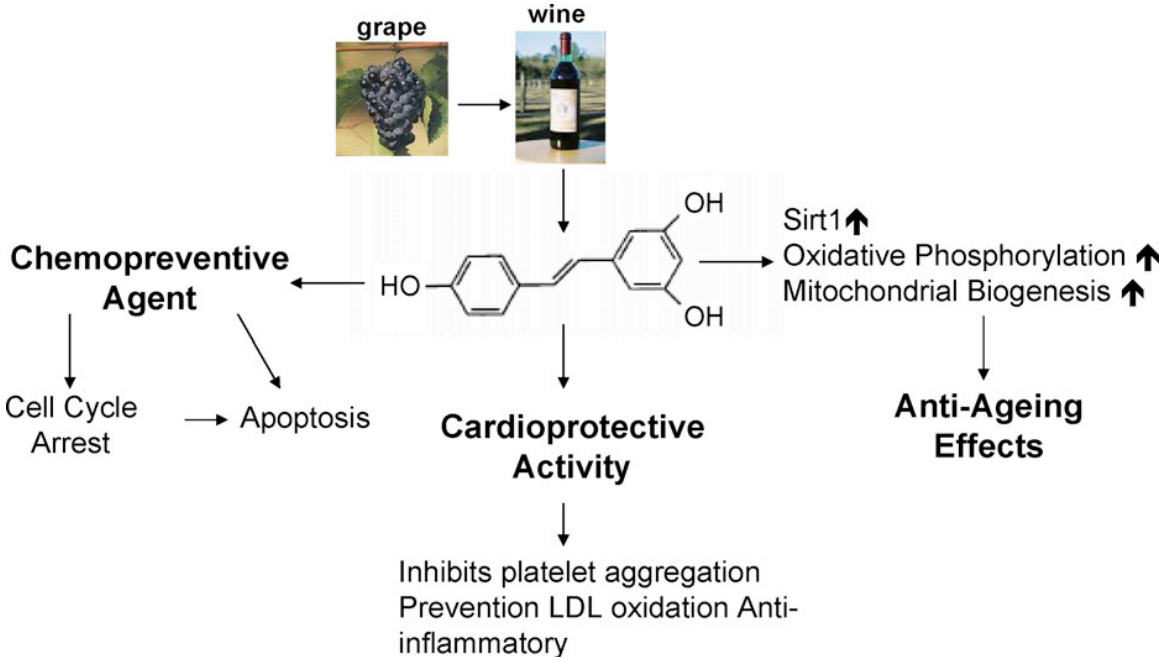
Resveratrol is found in a number of plants, including grape skins, raspberries, mulberries and peanuts. Its job in nature is to fight fungus during the rainy season, and it is especially prevalent in grapes used in making red wine.

The countries around the Mediterranean basin have different diets, religions and cultures. The diet of Crete represents the traditional diet of Greece prior to 1960. Analyses of the dietary pattern of the diet of Crete shows a number of protective substances, such as selenium, glutathione, a balanced ratio of n-6/n-3 essential fatty acids (EFA), high amounts of fibre, antioxidants (especially resveratrol from wine and polyphenols from olive oil), vitamins E and C, which have been shown to be associated with lower risk of cancer. [104]

Resveratrol phytoalexin, a naturally occurring plant cytokine, is found in grapes, wine, and other plant products. Its job in nature is to fight fungus during the rainy season, and it is especially prevalent in grapes used in making red wine. It has been shown to have anti-inflammatory, anti-oxidant, cell-repair, phyto-estrogen and anti-tumor activities. The discovery of resveratrol has important implications for increasing the effectiveness of cancer therapy, with some clinical trials using resveratrol already showing encouraging results. Resveratrol also helps to control atherosclerosis, heart disease, arthritis, and autoimmune disorders.

Grape seed flavonoids are responsible for giving many fruits, in particular berries, their dark purple and blue color. Their free radical scavenging effects are 20-50 times greater than vitamin C or E. They also reinforce the natural cross linking of collagen that forms the matrix of connective tissue, a very important function during any post-surgical healing, and they are anti-inflammatory in that they prevent the release and synthesis of compounds that promote inflammation such as histamines, serine proteases and prostaglandins. [5] OPCs are primarily known for their antioxidant activity and have been reported to demonstrate antibacterial, antiviral, anti-carcinogenic, anti-inflammatory,anti-allergic and vasodilatory actions. [45]

There is much gained through the synergy of these two contributors to grape beverages. Isolates of certain biologically active components of seeds and skins of grapes when separated from the whole, were found in pne study to be less effective compared to the whole grape extract, including the skin and seed. The best way to get that synergy is to use both the seed and skin proponents. There is a significant positive effects on platelet aggregation, endothelial function and LDL oxidation from consuming the whole grape. [38]

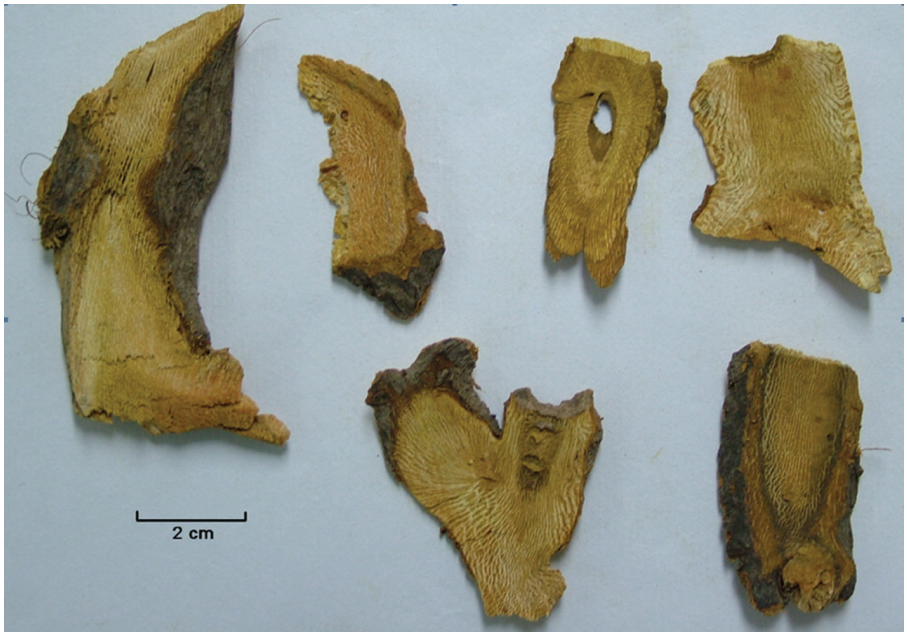


Japanese Knotweed (*Polygonum cuspidatum*), Hu Zhang

Polygonum cuspidatum is richest known source of resveratrol, has traditionally been used in traditional Chinese Medicine, (TCM) to treat cancer.

Polygonium c. has been widely used in China for thousands of years to treat and prevent diseases. TCM has been proven safe and effective, and it is being considered as one of the important types of complementary and alternative medicine and receives increasing attention worldwide. The dried root of Polygonum cuspidatum Sieb. et Zucc. (also known as "Hu Zhang" in Chinese) is one of the medicinal herbs listed in the Pharmacopoeia of the People's Republic of China. Hu Zhang is widely distributed in the world. It can be found in Asia and North America and is used as folk medicine in countries such as Japan and Korea. In China, Hu Zhang is usually used in combination with other TCM

herbs. The therapeutic uses of those Hu Zhang-containing TCM prescriptions or formulations are for treating cough, hepatitis, jaundice, amenorrhea, leucorrhea, arthralgia, burns and snake bites. Recent pharmacological and clinical studies have indicated that Hu Zhang has antiviral, antimicrobial, anti-inflammatory, neuroprotective, and cardioprotective functions. This review gives a summary of the reported therapeutic effects of the active compounds and the different extracts of Hu Zhang.[192]



In TCM cancer is generally believed to be caused by physiologically 3 factors: (1) phlegm (this is an accumulation of excess fluid which has congealed in a particular part of the body) and (2) blood stasis (this is a partial or complete obstruction of blood circulation in a particular part of the body). In addition to tumors or masses, cancer also involves (3) tissue destruction (necrosis), which is viewed in TCM as being caused by the presence of heat toxins in the body. So we have phlegm, blood stasis and heat toxins as the 3 factors, and in TCM, Hu Zhang is said to influence all 3 of those factors.
***Polygonum c.*,** according to TCM activates the blood circulation and removes blood stasis, and its secondary functions include draining heat out and transforming (eliminating) phlegm, and clearing heat and eliminating toxins. Modern research has discovered potential applications of this herb for cancer -- several compounds have shown possible usefulness, including **resveratrol, emodin**, and **chrysophanol**. These compounds have shown antitumor, antimetastatic, chemopreventive, chemical carcinogenesis-inhibitive, oncogene signal transduction-inhibitive and immune-modulating properties. Resveratrol is a new anticancer composition which higher efficiency in *Polygonum c*, [192] that regulates mRNA expression of several genes involved in cell cycle control, apoptosis, metastasis, cell-cell adhesion, and ER and AR signaling pathway. [193]

Resveratrol: a multitargeted agent for age-associated chronic diseases.

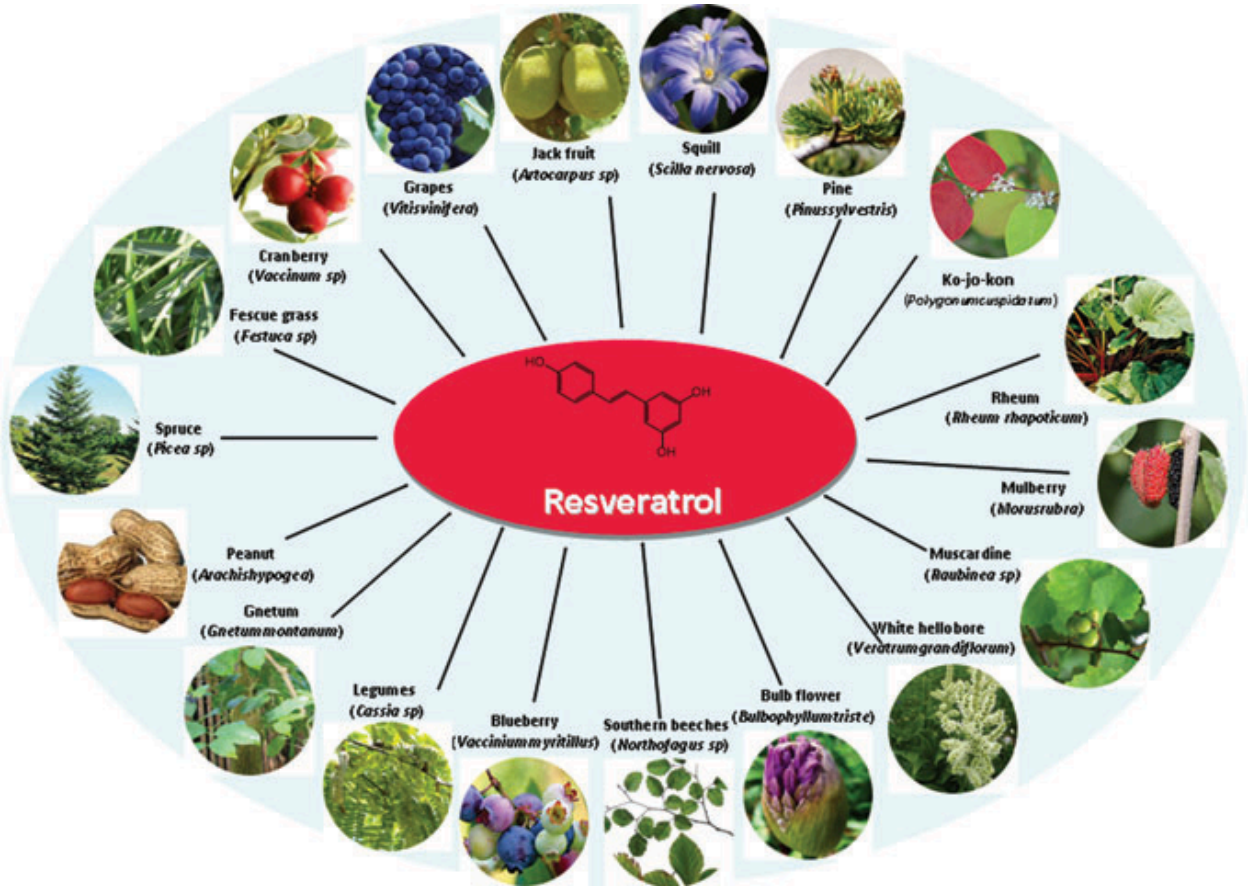
Extensive research within the last decade has revealed that most chronic illnesses such as cancer, cardiovascular and pulmonary diseases, neurological diseases, diabetes, and autoimmune diseases exhibit dysregulation of multiple cell signaling pathways that have been linked to inflammation. Thus mono-targeted therapies developed for the last two decades for these diseases have proven to be unsafe, ineffective and expensive. Although fruits and vegetables are regarded to have therapeutic potential against chronic illnesses, neither their active component nor the mechanism of action is well understood. Resveratrol (trans-3, 5, 4'-trihydroxystilbene), a component of grapes, berries, peanuts and other traditional medicines, is one such polyphenol that has been shown to mediate its effects through modulation of many different pathways. This stilbene has been shown to bind to numerous cell-signaling molecules such as multi drug resistance protein, topoisomerase II, aromatase, DNA polymerase, estrogen receptors, tubulin and F1-ATPase. Resveratrol has also been shown to activate various transcription factor (e.g; NFkappaB, STAT3, HIF-1alpha, beta-catenin and PPAR-gamma), suppress the expression of antiapoptotic gene products (e.g; Bcl-2, Bcl-X(L), XIAP and

survivin), inhibit protein kinases (e.g; src, PI3K, JNK, and AKT), induce antioxidant enzymes (e,g; catalase, superoxide dismutase and hemoxygenase-1), suppress the expression of inflammatory biomarkers (e.g., TNF, COX-2, iNOS, and CRP), inhibit the expression of angiogenic and metastatic gene products (e.g., MMPs, VEGF, cathepsin D, and ICAM-1), and modulate cell cycle regulatory genes (e.g., p53, Rb, PTEN, cyclins and CDKs). Numerous animal studies have demonstrated that this polyphenol holds promise against numerous age-associated diseases including cancer, diabetes, Alzheimer, cardiovascular and pulmonary diseases. In view of these studies, resveratrol's prospects for use in the clinics are rapidly accelerating. Efforts are also underway to improve its activity in vivo through structural modification and reformulation. Our review describes various targets of resveratrol and their therapeutic potential.[288]

Multiple molecular targets of resveratrol: Anti-carcinogenic mechanisms.
Extensive in vitro studies revealed multiple intracellular targets of resveratrol, which affect cell growth, inflammation, apoptosis, angiogenesis, and invasion and metastasis. These include tumor suppressors p53 and Rb; cell cycle regulators, cyclins, CDKs, p21WAF1, p27KIP and INK and the checkpoint kinases ATM/ATR; transcription factors NF-kappaB, AP-1, c-Jun, and c-Fos; angiogenic and metastatic factors, VEGF and matrix metalloprotease 2/9; cyclooxygenases for inflammation; and apoptotic and survival regulators, Bax, Bak, PUMA, Noxa, TRAIL, APAF, survivin, Akt, Bcl2 and Bcl-X(L). In addition to its well-documented anti-oxidant properties, there is increasing evidence that resveratrol exhibits pro-oxidant activity under certain experimental conditions, causing oxidative DNA damage that may lead to cell cycle arrest or apoptosis. [245]

Resveratrol

- Anti-Carcinogenic - inhibits proliferation, angiogenesis, tumor invasion, metastasis, enhances immune response, and induces apoptosis.
- Chemopreventive - anti-inflammatory, estrogenic/anti-estrogenic
- Potent inhibitor of NF kappa B, AP-1, COX-2, PG_2, TNF-alpha, IL-6; also inhibits inducible Nitric Oxide Synthase.
- Powerful Anti-Oxidant - inhibits lipid peroxidation, food preservative, free radical scavenger, glutathione sparing
- Immune Cell Function - modulates cytokine activity
- Cardioprotective - anti-atherosclerotic, improves venous insufficiency,
- Lung health: treatment for COPD
- Skin Protectant - antimicrobial, enhances diabetic wound care, inhibits MMP-8
- Gene normalizing



Resveratrol as a potent and diverse inhibitor of cancer

Resveratrol, besides cardioprotective effects, resveratrol exhibits anticancer properties, as suggested by its ability to suppress proliferation of a wide variety of tumor cells, including lymphoid and myeloid cancers; multiple myeloma; cancers of the breast, prostate, stomach, colon, pancreas, and thyroid; melanoma; head and neck squamous cell carcinoma; ovarian carcinoma; and cervical carcinoma. The growth-inhibitory effects of resveratrol are mediated through cell-cycle arrest; upregulation of p21Cip1/WAF1, p53 and Bax; down-regulation of survivin, cyclin D1, cyclin E, Bcl-2, Bcl-xL and clAPs; and activation of caspases. Resveratrol has been shown to suppress the activation of several transcription factors, including NF-kappaB, AP-1 and Egr-1; to inhibit protein kinases including IkappaBalpha kinase, JNK, MAPK, Akt, PKC, PKD and casein kinase II; and to down-regulate products of genes such as COX-2, 5-LOX, VEGF, IL-1, IL-6, IL-8, AR and PSA. These activities account for the suppression of angiogenesis by this stilbene. Resveratrol also has been shown to potentiate the apoptotic effects of cytokines (e.g., TRAIL), chemotherapeutic agents and gamma-radiation. Phamacokinetic studies revealed that the target organs of resveratrol are liver and kidney, where it is concentrated after absorption and is mainly converted to a sulfated form and a glucuronide conjugate. In vivo, resveratrol blocks the multistep process of carcinogenesis at various stages: it blocks carcinogen activation by inhibiting aryl hydrocarbon-induced CYP1A1 expression and activity, and suppresses tumor initiation, promotion and progression. Besides chemopreventive effects, resveratrol appears to exhibit therapeutic effects against cancer. Data in humans have revealed that resveratrol is pharmacologically quite safe. [130]

Pleiotropic mechanisms facilitated by resveratrol metabolites and resveratrol rather than resveratrol alone

Resveratrol has demonstrated cancer chemopreventive activity in animal models and some clinical trials are underway. In addition, resveratrol was shown to promote cell survival, increase lifespan and mimic caloric restriction, thereby improving health and survival of mice on high-calorie diet. All of these effects are potentially mediated by the pleiotropic interactions of resveratrol with different enzyme targets including COX-1 (cyclo-oxygenase-1) and COX-2, NAD+-dependent histone deacetylase SIRT1 (sirtuin 1) and QR2 (quinone reductase 2). Nonetheless, the health benefits elicited by resveratrol as a direct result of these interactions with molecular targets have been questioned, since it is rapidly and extensively metabolized to sulfate and glucuronide conjugates, resulting

in low plasma concentrations. To help resolve these issues, we tested the ability of resveratrol and its metabolites to modulate the function of some known targets in vitro. In the present study, we have shown that COX-1, COX-2 and QR2 are potently inhibited by resveratrol, and that COX-1 and COX-2 are also inhibited by the resveratrol 4'-O-sulfate metabolite. We determined the X-ray structure of resveratrol bound to COX-1 and demonstrate that it occupies the COX active site similar to other NSAIDs (non-steroidal anti-inflammatory drugs). Finally, we have observed that resveratrol 3- and 4'-O-sulfate metabolites activate SIRT1 equipotently to resveratrol, but that activation is probably a substrate-dependent phenomenon with little in vivo relevance. Overall, the results of this study suggest that in vivo an interplay between resveratrol and its metabolites with different molecular targets may be responsible for the overall beneficial health effects previously attributed only to resveratrol itself.[261]

Chemopreventive: In vivo research

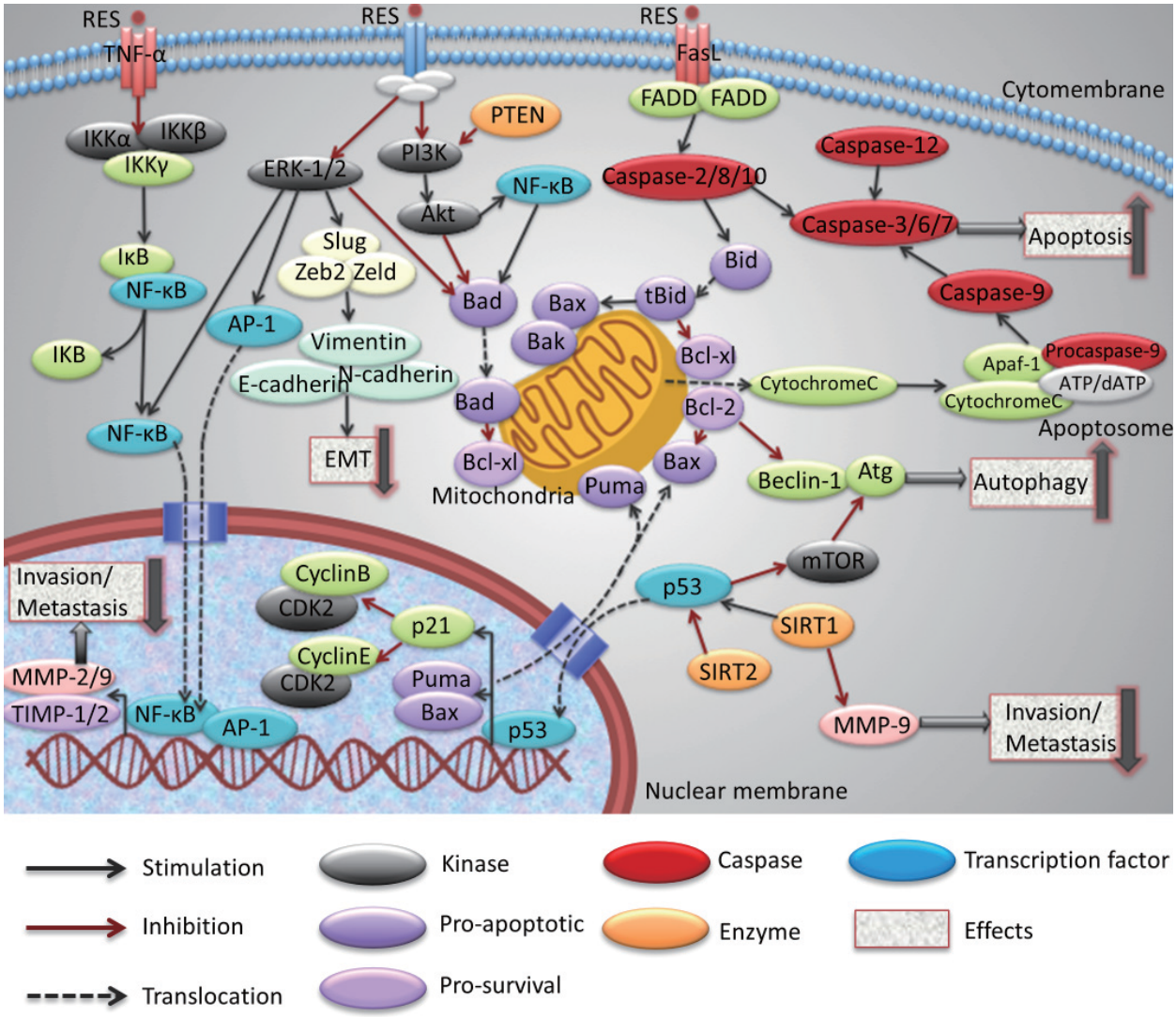
Despite scepticism concerning its bioavailability, a growing body of in vivo evidence indicates that resveratrol has protective effects in rodent models of stress and disease. Comprehensive and critical review of all the in vivo data on resveratrol suggest it is potential a therapeutic agent for humans. [161]

Resveratrol's anti-cancer effects against specific types of cancer

Cancer Type	Genes and Pathways Targeted	Effect(s)
Breast	p53, PTEN, p27, p21, p70S6K, pS6RP, Src-Stat3, Akt, Bcl2, NF-κB, calpain, MMP-9, cyclin D, Cdk4, ribonucleotide reductase, CYP1A1, telomerase; nitric oxide (NO) production, reactive oxygen species (ROS)	apoptosis, growth arrest, cell migration
Prostate	Caspases-3 and -9, p53, p21, p27, Bax, Bak, Bid, Bad, MKP5, PI3K, Akt, cyclins B/D1/E, Cdk1/4, Bcl2, Src-Stat3, ROS	apoptosis, cell viability, proliferation rate, cell-cycle arrest
Colon	AMPK, cathepsin D, caspase-2, cytochrome c, ATF3, Cdk7, p34Cdc2; ROS	apoptosis, cell growth
Pancreatic	MIC-1, cytochrome c, caspase-3, Src-Stat3, NF-κB	apoptosis, cell growth
Ovarian	Cdc2, ATM/ATR, chk1/2, Cdc25C, H2A.X, Akt, HIF-1a, VEGF	autophagocytic death, cell-cycle arrest
Thyroid	p53, c-fos, c-jun, p21	apoptosis
Multiple myeloma	c-fms, CD14, CD11a, $1,25(OH)_2D_3$ nuclear receptor (VDR), Bax, Apaf-1, Cathepsin K, RANK, NFATc1, NF-κB (nuclear translocation), Bcl2, Bcl-x(L), XIAP, Mcl-1, MMP-2, MMP-9	apoptosis
Leukemia	NO	apoptosis, cell growth
B-cell lymphoma	p27, p53, CD69, BCL6, Myc, Akt, p70S6K	apoptosis, cell-cycle arrest, glycolysis
Squamous cell carcinoma	p21, p27, Cyclins A/E/D1/D2 Cdk2/4/6, pRb, MEK1, pERK1/2, c-Jun, AP-1, HIF-1α, VEGF, Akt, E2F1-5, DP1/2	cell-cycle arrest

Athar, M, Back, JB, Kopelovich, L, Bickers, DR, and Kim, AL **2009**. Multiple molecular targets of resveratrol: anti-canrcinogenic mechanisms. **Arch. Biochem and Biophys.** 486:95-102.

Anti-tumor effects and molecular mechanisms of resveratrol.



Guohua Han1, Jufeng Xia1, Jianjun Gao1,2, Yoshinori Inagaki1, Wei Tang1,*, Norihiro Kokudo1, Anti-tumor effects and cellular mechanisms of resveratrol, Drug Discoveries & Therapeutics. 2015; 9(1):1-12.

Resveratrol in human cancer chemoprevention - Choosing the 'right' dose.

There is now robust preclinical evidence to suggest that resveratrol possesses cancer chemopreventive properties. A series of clinical pilot studies has provided insights into its pharmacokinetics, and data on its human antineoplastic pharmacodynamics start to emerge. It is likely that resveratrol will be developed further in the clinic as a putative cancer chemopreventive agent. The question that remains unresolved is: What is the most suitable dose of resveratrol for effective cancer preventive intervention? Mechanistic studies in cells in vitro have almost invariably used concentrations of resveratrol in the 10(-5) to 10(-4) M range, which is much higher than those which can be achieved in the human biophase after consumption of doses up to 1 g. Many of the preclinical efficacy studies in rodent models of carcinogenesis have employed doses, which are dramatically above those which can be ingested with the diet. New experimental paradigms need to be used to obtain information on pharmacological changes elicited by resveratrol when present at very low concentrations or when administered at dietary-relevant doses.[285]

Anti-cancer actions
INHIBITS PROLIFERATION

Upregulates p21 CIP1/WAF1

Breast Cancer

Dr. Anait S. Levenson and colleagues from Northwestern University in Chicago investigated the effects of resveratrol in breast cancer cells stably transfected with wild-type estrogen receptor (wtER) and mutant ER (mutER). Resveratrol showed dose-dependent growth inhibitory effects in breast cancer cells. ICI blocked the growth inhibitory action of estradiol but not of resveratrol, "suggesting that the antiproliferative effects of resveratrol also involve ER-independent pathways", the team writes.

In gene array studies, resveratrol upregulated more than 80 genes, most profoundly p21 CIPI/WAF1, which is associated with cancer cell growth arrest. [20]

Decreases Cell Proliferation

Cancers, such as breast, esophageal, stomach, lung, bladder, and prostate, depend on environmental factors and diet for growth and evolution. Dietary micronutriments have been proposed as effective inhibitory agents for cancer initiation, progression, and incidence. Among them, polyphenols, present in different foods and beverages, have retained attention in recent years. Red wine is a rich source of polyphenols, and their antioxidant and tumor arresting effects have been demonstrated in different in vitro and in vivo systems. In the present study, we have measured the anti-proliferative effect of red wine concentrate, its total polyphenolic pool, and purified catechin, epicatechin, quercetin, and resveratrol (all of which account for more than 70% of the total polyphenols in red wine) on the proliferation of hormone sensitive (MCF7, T47D) and resistant (MDA-MB-231) breast cancer cell lines. Our results indicate that polyphenols, at the picomolar or the nanomolar range, decrease cell proliferation in a dose- and a time- dependant manner. In hormone sensitive cell lines, a specific interaction of each polyphenol with steroid receptors was observed, with IC50s lower than previously described. Interaction of polyphenols with steroid receptors cannot fully explain their inhibitory effect on cell proliferation. In addition, discrete antioxidant action on each cell line was detected under the same concentrations, both by modifying the toxic effect of H2O2, and the production of reactive oxygen species (ROS), after phorbol ester stimulation. Our results suggest that low concentrations of polyphenols, and consecutively, consumption of wine, or other polyphenol-rich foods and beverages, could have a beneficial antiproliferative effect on breast cancer cell growth. [15]

In tissue culture: Resveratrol inhibited formation of estrogen-dependent and chemical carcinogen-induced lesions in mouse mammary organ culture in the absence of 17-beta-estradiol, mixed estrogen agonist/antagonist activities in mammary cancer cell lines; in the presence of 17-beta-estradiol, reservatrol functions as an anti-estrogen.

In mice: Resveratrol in the presence of 17-beta-estradiol, reduced mammary tumorigenesis carcinogen-induced preneoplastic lesions. Mammary tumors are inhibited reservatrol may have beneficial effects on tumor prevention in other types of cancer as well as breast.

Stops Breast Cancer Growth

A new research report appearing in the October 2011 issue of The *FASEB Journal* shows that resveratrol, the "healthy" ingredient in red wine, stops breast cancer cells from growing by blocking the growth effects of estrogen. This discovery, made by a team of American and Italian scientists, suggests for the first time that resveratrol is able to counteract the malignant progression since it inhibits the proliferation of hormone resistant breast cancer cells. This has important implications for the treatment of women with breast cancer whose tumors eventually develop resistance to hormonal therapy.

"Resveratrol is a potential pharmacological tool to be exploited when breast cancer become resistant to the hormonal therapy," said Sebastiano Andò, a researcher involved in the work from the Faculty of Pharmacy at the University of Calabria in Italy.

To make this discovery, Andò and colleagues used several breast cancer cell lines expressing the estrogen receptor to test the effects of resveratrol. Researchers then treated the different cells with resveratrol and compared their growth with cells left untreated. They found an important reduction in cell growth in cells treated by resveratrol, while no changes were seen in untreated cells. Additional experiments revealed that this effect was related to a drastic reduction of estrogen receptor levels caused by resveratrol itself.

"These findings are exciting, but in no way does it mean that should people go out and start using red wine or resveratrol supplements as a treatment for breast cancer," said Gerald Weissmann, M.D., Editor-in-Chief of The *FASEB Journal*. "What it does mean, however, is that scientists haven't finished distilling the secrets of good health that have been hidden in natural products such as red wine."[282]

Modulates MED28 (magicin/EG1) expression and inhibits epidermal growth factor (EGF)-induced migration in MDA-MB-231 human breast cancer cells.

Resveratrol and pterostilbene exhibit diverse biological activities. MED28, a subunit of the mammalian Mediator complex for transcription, was also identified as magicin, an actin cytoskeleton Grb2-associated protein, and as

endothelial-derived gene (EG-1). Several tumors exhibit aberrant MED28 expression, whereas the underlying mechanism is unclear. Triple-negative breast cancers, often expressing epidermal growth factor (EGF) receptor (EGFR), are associated with metastasis and poor survival. The objective of this study is to compare the effect of resveratrol and pterostilbene and to investigate the role of MED28 in EGFR-overexpressing MDA-MB-231 breast cancer cells. Pre-treatment of resveratrol, but not pterostlbene, suppressed EGF-mediated migration and expression of MED28 and matrix metalloproteinase (MMP)-9 in MDA-MB-231 cells. Moreover, overexpression of MED28 increased migration and the addition of EGF further enhanced migration. Our data indicate that resveratrol modulates the effect of MED28 on cellular migration, presumably through the EGFR/phosphatidylinositol 3-kinase (PI3K) signaling pathway, in breast cancer cells.[283]

Inhibits Progression through the S Phase of the Cell Cycle: Piceatannol as an Anti-Cancer Agent

We studied the effects of piceatannol on growth, proliferation, differentiation and cell cycle distribution profile of the human colon carcinoma cell line of Caco-2. Growth of Caco-2 and HCT-116 cells was analyzed by crystal violet assay, which demonstrated dose- and time-dependent decreases in cell numbers. Treatment of Caco-2 cells with piceatannol reduced proliferation rate. No effect on differentiation was observed. Determination of cell cycle distribution by flow cytometry revealed an accumulation of cells in the S phase. Immunoblotting demonstrated that cyclin-dependent kinases (cdk) 2 and 6, as well as cdc2 were expressed at steady-state levels, wheras cyclin D1, cyclin B1 and cdk 4 were down-regulated. The abundance of p72 was also reduced, wheras the protein level of cyclin E was enhanced. Cyclin Q levels were enhanced only at concentrations up to 100 µmol/L. These changes also were observed in studies with HCT-116 cells. On the basis of our findings, piceatannol can be considered to be a promising chemopreventive or anticancer agent. **[16]**

Antitumor activity: Decreases the Expression of CyclinB1 and p34cdc2 Protein

To study the antitumor activity of resveratrol and its effect on the expression of cell cycle proteins including cyclin D1, cyclin B1 and p34cdc2 in transplanted liver cancer of murine.

METHODS: Murine transplanted hepatoma H22 model was used to evaluate the in vivo antitumor activity of resveratrol. Following abdominal administration of resveratrol, the change in tumor size was recorded and the protein expression of cyclin D1, cyclin B1 and p34cdc2 in the tumor and adjacent non-cancerous liver tissues were measured by immunohistochemistry.

RESULTS: Following treatment of H22 tumor bearing mice with resveratrol at 10 or 15 mg/kg bodyweight for 10 days, the growth of murine transplantable liver cancer was inhibited by 36.3 % or 49.3 %, respectively. The inhibitory effect was significant compared to that in control group. The level of expression of cyclin B1 and p34cdc2 protein was decreased in the transplantable murine hepatoma 22 treated with resveratrol whereas the expression of cyclin D1 protein did not change.

CONCLUSION: Resveratrol exhibits anti-tumor activities on murine hepatoma H22. The underlying anti-tumor mechanism of resveratrol might involve the inhibition of the cell cycle progression by decreasing the expression of cyclinB1 and p34cdc2 protein.**[22]**

Blocks S and G2 phases of the Cell Cycle in Lymphoma cells

Resveratrol induces anti-proliferation and arrests the S phase in human histiocytic lymphoma U937 cells. Resveratrol induces arrest in the S phase at low concentrations (30-60 microM), but high concentrations do not induce S phase accumulation in U937 cells. Removal of resveratrol from the culture medium stimulates U937 cells to reenter the cell cycle synchronously, as judged by the expression patterns of cyclin E, A and by fluorescent activated cell sorting analysis. These data demonstrate that resveratrol causes S phase arrest and reversible cell cycle arrest. Thus, resveratrol provides an important new cell cycle blocker as well as a cancer chemopreventive agent. **[29]**

Lymphoma amd Multiple Myeloma: Inhibition and increases effectiness of Taxol

Resveratrol (trans-3,4,5-trihydroxystilbene) has received attention for its potential chemopreventive and antitumor effects in experimental systems. Recent evidence suggests that paclitaxel, alone or in combination with other drugs, can be effectively used in the treatment of non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM). This study investigated whether resveratrol can sensitize NHL and MM cell lines to paclitaxel-mediated apoptosis and to delineate the underlying molecular mechanism of sensitization. Both resveratrol and paclitaxel negatively modulated tumor cell growth by arresting the cells at the G(2)-M phase of the cell cycle. **Low concentrations of resveratrol exerted a sensitizing effect on drug-refractory NHL and MM cells to apoptosis induced by paclitaxel**. Resveratrol selectively down-**regulated the expression of antiapoptotic proteins Bcl-x(L) and myeloid cell differentiation factor-1 (Mcl-1) and up-regulated the expression of proapoptotic proteins Bax and apoptosis protease activating factor-1 (Apaf-1).** Paclitaxel down-regulated the expression of Bcl-x(L), Mcl-1, and cellular inhibitor of apoptosis protein-1 antiapoptotic proteins and up-regulated Bid and Apaf-1. Combination treatment resulted in apoptosis through the formation of tBid, mitochondrial membrane depolarization, cytosolic release of cytochrome c and Smac/DIABLO, activation of the caspase cascade, and cleavage of poly(adenosine diphosphate-

ribose) polymerase. Combination of resveratrol with paclitaxel had minimal cytotoxicity against quiescent and mitogenically stimulated human peripheral blood mononuclear cells. Inhibition of Bcl-x(L) expression by resveratrol was critical for chemosensitization and its functional impairment mimics resveratrol-mediated sensitization to paclitaxel-induced apoptosis. Inhibition of Bcl-x(L) expression by resveratrol was due to the inhibition of the extracellular signal-regulated kinase 1/2 (ERK1/2) pathway and diminished activator protein-1-dependent Bcl-x(L) expression. The findings by resveratrol were corroborated with inhibitors of the ERK1/2 pathway. This study demonstrates that in resistant NHL and MM cell lines resveratrol and paclitaxel selectively modify the expression of regulatory proteins in the apoptotic signaling pathway and the combination, via functional complementation, results in synergistic apoptotic activity. [160]

Exhibits Cytostatic and Anti-estrogenic Activities, Inhibiting Endometrial Carcinoma

Trans-3,4',5-trihydroxystilbene was reported recently to serve as an estrogen agonist with cultured MCF-7 cells transfected with estrogen response element-luciferase reporter plasmids. As currently shown, treatment of cultured human endometrial adenocarcinoma (Ishikawa) cells with resveratrol (concentrations as high as 10 [mu]M) did not significantly increase the levels of an estrogen-inducible marker enzyme, alkaline phosphatase. To the contrary, when alkaline phosphatase was induced by treatment with 1 nM of 17[beta]-estradiol (E2), resveratrol exhibited a dose-dependent decrease in activity (IC50 = 2.3 [mu]M). Furthermore, when Ishikawa cells were treated with resveratrol as a single agent, estrogen-inducible progesterone receptor (PR) was not enhanced, and PR expression induced by treatment with E2 was inhibited by resveratrol in a dose-dependent fashion at both the mRNA and protein levels. In addition, resveratrol mediated suppression of a functional activity of PR as demonstrated by down-regulation of [alpha]1-integrin expression induced by E2 plus progesterone. With transient transfection experiments conducted with Ishikawa cells, antiestrogenic effects were confirmed by dose-dependent inhibition of E2-induced estrogen response element-luciferase transcriptional activity. Because resveratrol antagonized estrogenic effects in Ishikawa cells, competitive binding analyses were performed to examine the potential of displacing [3H] E2 from human estrogen receptor (ER). Resveratrol showed no discernable activity with ER-[alpha], but with ER-[beta], E2 was displaced with an IC50 of 125 [mu]M. However, mRNA and protein expression of ER-[alpha] but not ER-[beta] were suppressed by resveratrol in Ishikawa cells, in the concentration range of 5-15 [mu]M. In addition, in the presence or absence of E2, resveratrol inhibited Ishikawa cell proliferation in a time-dependent manner with cells accumulating in the S phase of the cycle <=48 h. This effect was reversible. Analysis of some critical cell cycle proteins revealed a specific increase in expression of cyclins A and E but a decrease in cyclin-dependent kinase 2. These data suggest resveratrol exerts an anti-proliferative effect in Ishikawa cells, and the effect may be mediated by both estrogen-dependent and -independent mechanisms. [41]

Inhibits Cell Division: Cervical Cancer

Resveratrol has been shown to significantly alter the cellular physiology of tumor cells, as well as block the process of initiation and progression. At least one mechanism for the intracellular actions of resveratrol involves the suppression of prostaglandin (PG) biosynthesis. The involvement of PGs and other eicosanoids in the development of human cancer is well established. PGs are synthesized from arachidonic acid via the cyclooxygenase pathway and have multiple physiological and pathological functions. In addition, evidence has arisen suggesting that PGs may be implicated in the cytotoxic and/or cytoprotective response of tumor cells to ionizing radiation (IR). As such, we hypothesized that tumor cells may exhibit changes in the cellular response to IR following exposure to resveratrol, a naturally occurring compound that inhibits cyclooxygenase-1 (COX-1) activity. Thus, clonogenic cell survival assays were performed using irradiated HeLa and SiHa cells pretreated with resveratrol prior to IR exposure, and resulted in enhanced tumor cell killing by IR in a dose-dependent manner. Further analysis of COX-1 inhibition indicated that resveratrol pretreatment: (1), inhibited cell division as assayed by growth curves; and (**2**), induced an early S phase cell cycle checkpoint arrest, as demonstrated by fluorescence-activated cell sorting, as well as bromodeoxyuridine pulse-chase analysis. These results suggest that resveratrol alters both cell cycle progression and the cytotoxic response to IR in two cervical tumor cell lines. [3]

Cervical cancer: Suppresses hypoxia-inducible factor 1alpha and VEGF

Human papillomavirus (HPV)-16 oncoproteins, E6 and E7, are associated with enhanced tumor angiogenesis in human cervical cancers. The purpose of this study was (a) to investigate whether expression of HPV-16 E6 and E7 oncoproteins induces hypoxia-inducible factor 1alpha (HIF-1alpha) and vascular endothelial growth factor expression in cervical cancer cells; and (b) to assess the effect of resveratrol on 16 E6- and E7-induced HIF-1alpha and VEGF gene expression. HPV-16 E6- and E7-transfected cervical cancer cells express increased HIF-1alpha protein and VEGF expression. These stimulatory effects were abrogated by cotransfection with either HIF-1alpha siRNA or treatment with resveratrol. Blocking extracellular signal-regulated kinase 1/2 (ERK 1/2) and phosphoinositide-3-kinase by PD98059 and LY294002, respectively, abolished 16 E6- and E7-induced HIF-1alpha and VEGF expression. Functionally, we showed that HPV-16 E6- and E7-transfected cervical cancer cells

stimulated in vitro capillary or tubule formation, and these angiogenic effects could be abolished either by cotransfection with HIF-1alpha siRNA or by treatment with resveratrol. HPV-16 oncoproteins contribute to enhanced angiogenesis in cervical cancer cells via HIF-1alpha-dependent VEGF expression. Resveratrol suppresses 16 E6- and E7-induced HIF-1alpha-mediated angiogenic activity and, thus, is a promising chemotherapeutic agent for human cervical cancer. [195]

Anti-Proliferative Effects

Resveratrol is a constituent of the human diet that has been shown to inhibit cellular processes associated with tumor initiation, promotion and progression. In this study, we examined the effect of synthetic resveratrol on the proliferative capacity of immortal and neoplastic human breast epithelial cells in culture. MCF-7, an estrogen receptor-positive breast cancer cell line, MCF-10F, an immortal estrogen receptor-negative breast epithelial cell line, and MDA-MB-231, a malignant estrogen receptor-negative breast epithelial cell line, were treated with 5, 10, 20 or 40 microg/ml resveratrol, and their proliferative activities were determined with the WST-1 colorimetric assay after periods of time ranging from 24 to 144 h of treatment. Our results showed that this phytoalexin inhibited the proliferation of human breast epithelial cells in a dose- and time-dependent manner. Treatment of cells with resveratrol reduced the number of viable cells and prevented the exponential growth of the three cell lines examined. These observations indicate that resveratrol has a direct anti-proliferative effect on human breast epithelial cells that is independent of the estrogen receptor status of the cells. Thus, this dietary compound is a potential chemopreventive agent for both hormone responsive and non-responsive breast cancers. **[28]**

Inhibits Prostate Cancer-cell Proliferation, Promotes Cell Differentiation, Phytoestrogenic

Resveratrol has remarkable efficacy against prostate cancer cells, with molecular targets ranging from cell cycle regulation to induction of apoptosis. [195]

Resveratrol is a natural phytoestrogen. It has been reported to promote differentiation of murine MC3T3-E1 osteoblasts and to inhibit proliferation of prostate cancer cell lines. In the present study we tested the effects of resveratrol on the increased proliferation of human AHTO-7 osteoblastic cell line induced by conditioned media (CM) from a panel of carcinoma cell lines. Resveraatrol was found to modulate AHTO-7 proliferation in a tamoxifen-sensitive mechanism at lower concentrations, but failed to induce the osteoblast differentiation marker alkaline phosphatase (ALP) in contrast to vitamin D3. The proliferative response of AHTO-7 cells to conditioned media from carcinoma cell lines was diminished (30-71.**4**% inhibition) upon pretreatment with 0.**5** microM resveratrol. **Highest inhibition was demonstrated for pancreas (BxPC3, Panc-1), breast (ZR75-1) and renal (ACHN) carcinoma cell line supernatants** whereas the effect on colon carcinoma (SW620, Colo320DM) cell CM and prostate cancer (PC3, DU145 and LNCaP) CM was less pronounced. Direct addition of resveratrol affected only supernatants of cell lines (<25% inhibition) exhibiting growth stimulatory activity for normal WI-38 lung fibroblasts. Resveratrol inhibited proliferation of DU145 and LNCaP cells in concentrations exceeding **5** microM, altered cell cycle distribution of all prostate cancer cell lines in concentrations as low as 0.**5** microM, but did not inhibit the production of osteoblastic factors by these lines. In conclusion, resveratrol failed to induce ALP activity as marker of osteoblast differentiation in human osteoblastic AHTO-7 cells, however, inhibited their response to osteoblastic carcinoma-derived growth factors in concentrations significantly lower than those to reduce growth of cancer cells, thus effectively modulating tumor - osteoblast interaction. **[2]**

Inhibits prostate cancer, in vivo, down-regulates Androgen receptor

A recent in in vivo experiment to explore its effect of resveratrol in the Transgenic Rat for Adenocarcinoma of Prostate (TRAP) model, featuring the rat probasin promoter/SV 40 T antigen was performed. Resveratrol suppressed prostate cancer growth and induction of apoptosis through androgen receptor (AR) down-regulation, without any sign of toxicity. Resveratrol not only downregulated androgen receptor (AR) expression but also suppressed the androgen responsive glandular kallikrein 11 (Gk11), known to be an ortholog of the human prostate specific antigen (PSA), at the mRNA level. The data provide a mechanistic basis for resveratrol chemopreventive efficacy against prostate cancer. [218]

Triggers Apoptosis: By Activating Caspases 9 and 3

More recently, since the first report on the apoptosis inducing activity of resveratrol in human cancer cells, the interest in this molecule as a potential chemotherapy agent has significantly intensified. Not only has its role as an anti-cancer agent been corroborated, but the precise mechanisms of the anti-cancer activity or resveratrol are being elucidated. Our group has been active in studying the cross talk between the caspase family of protease and mitochondria, in drug-induced apoptosis. In this regard, we have shown that the anti-cancer activity of resveratrol could be attributed to its ability to trigger apoptosis in **human leukemia and breast carcinoma cells.** The cytotoxicity of resveratrol is restricted against these transformed cell types due to its ability to selectively up-regulate CD95-CD95L interaction on the tumor cell surface, unlike normal peripheral blood cells. Despite the involvement of the CD95 signaling pathway, apoptosis induced by resveratrol is not accompanied by robust caspase

8 activation, but **involves mitochondrial release of cytochrome C and downstream activation of caspases 9 and 3.** We also extrapolate these in vitro findings in a murine model of carcinogensis, and demonstrate in vivo induction of apoptosis in mouse skin papillomas. [13]

Induces p53: Mediated Apoptosis

The anti-tumor activity of resveratrol occurs through p53-mediated apoptosis. In this study, we have elucidated the potential signaling components underlying resveratrol-induced p53 activation and induction of apoptosis. We found that in a mouse JB6 epidermal cell line, resveratrol activated extracellular-signal-regulated protein kinases (ERKs), c-Jun NH2-terminal kinases (JNKs), and p38 kinase and induced serine 15 phosphorylation of p53. Stable expression of a dominant negative mutant of ERK2 or p38 kinase or their respective inhibitor, PD98059 or SB202190, repressed the phosphorylation of p53 at serine 15. In contrast, over-expression of a dominant negative mutant of JNKI had no effect on the phosphorylation. Most importantly, ERKs and p38 kinase formed a complex with p53 after treatment with resveratrol. Strikingly, resveratrol-activated ERKs and p38 kinase, but not JNKs, phosphorylated p53 at serine 15 in vitro. Furthermore, pretreatment of the cells with PD98059 or SB202190 or stable expression of a dominant negative mutant of ERK2 or p38 kinase impaired resveratrol-induced p53-dependent transcriptional activity and apoptosis, whereas constitutively active MEK1 increased the transcriptional activity of p53. These data strongly suggest that both ERKs and p38 kinase mediate resveratrol-induced activation of p53 and apoptosis through phosphorylation of p53 at serine 15. [33]

Triggers Apoptosis by Interfering with Heat Shock Protein 70: Suppression of Prostate Cancer

70 KDa heat shock proteins (HSPs70), either as a constitutive or inducible form, are expressed at very high levels in malignant human tumors of various origin. In different cell types, they are known to play an anti-apoptotic role. Resveratrol has been shown to be active in inhibiting multistage carcinogenesis, inducing apoptotic cell death. With the present study, a possible relationship between HSP70 expression and cell death elicited by resveratrol in DU-145 cells, which mimic the late hormone-refractory stages of prostate carcinoma, was investigated. To this end, we treated DU-145 with different concentrations. (50, 100 and 200 microM) of resveratrol and cell viability, by tetrazolium salts assay (MTT) and membrane breakdown, by lactic dehydrogenase (LDH) release, were measured. The possible induction of oxidative stress was evidenced both by performing a fluorescent analysis of intracellular reactive oxygen species (ROS) production, or evaluating the amount of nitrite/nitrate (NO) in culture medium. In addition, the expression of HSP70 level, evaluated by immunoblotting, was examined and compared with caspase-3 activity and DNA damage, determined by Single Cell Gel Electrophoresis assay.

The data clearly indicate that the addition of resveratrol to DU-145 reduces cell viability and increases membrane breakdown, in a dose-dependent way, without interfering with ROS production or NO synthesis, unless 200 microM resveratrol was added. Furthermore, at low concentration (50-100 microM) resveratrol is able to raise HSP70 levels but, at high concentration (200 microM), the measured levels of protective HSP70 were unmodified with respect to that of the control values. These results confirm the ability of resveratrol to **suppress the proliferation of human prostate cancer cells with a typical apoptotic feature, interfering with the expression of HSP70**. [31]

Induces Apoptosis in Prostate Cancer: Caspase-Mediated

This study was conducted to determine the effects of resveratrol on prostate cancer cell viability through apoptosis induction and the significance of the three hydroxyl groups on resveratrol to the measured effect. Hormone-sensitive LNCaP cells and hormone-insensitive DU 145 cells were treated with resveratrol, tri-methoxy-resveratrol, or diethylstilbestrol (DES; the positive control for toxicity and apoptosis). Cell viability was determined by using an MTS assay. Apoptosis was determined by the appearance of apoptotic morphology, annexin V-FITC-positive intact cells, and caspase activation. Resveratrol and DES decreased viability in LNCaP cells, but only resveratrol-treated cells expressed apoptotic morphology, annexin V-FITC-positive cells, and caspase activation. Tri-methoxy-resveratrol had no effect on DU 145 cell-viability and was less toxic to LNCaP cells than resveratrol. **Resveratrol was toxic to cells regardless of whether the cells were hormone-responsive or -unresponsive.** This finding suggests that the cell's hormone responsive status is not an important determinant of the response to resveratrol. Furthermore, the hydroxyl-groups on resveratrol are required for cell toxicity. Finally resveratrol but not DES induced caspase-mediated apoptosis. [17]

Inhibits Prostate Cancer: Inhibits Bcl-2

A study was conducted to evaluate the chemopreventive/antiproliferative potential of resveratrol against prostate cancer and its mechanism of action. Treatment with resveratrol (0-50 micromol/L for 24 hours) resulted in a significant (a) decrease in cell viability, (b) decrease of clonogenic cell survival, (c) inhibition of androgen (R1881)-stimulated growth, and (d) induction of apoptosis in androgen-responsive human prostate carcinoma (LNCaP) cells. Interestingly, at similar concentrations, resveratrol treatment did not affect the viability or rate of apoptosis in normal human prostate epithelial cells. Furthermore, our data showed that resveratrol-treatment resulted in significant dose-dependent inhibition in the constitutive expression of phosphatidylinositol 3'-kinase and

phosphorylated (active) Akt in LNCaP cells. Resveratrol treatment for LNCaP cells was also found to result in a significant (a) loss of mitochondrial membrane potential, (b) inhibition in the protein level of antiapoptotic Bcl-2, and (c) increase in proapoptotic members of the Bcl-2 family, i.e., Bax, Bak, Bid, and Bad. Taken together, our data suggested that resveratrol causes an inhibition of phosphatidylinositol 3'-kinase/Akt activation that, in turn, results in modulations in Bcl-2 family proteins in such a way that the apoptosis of LNCaP cells is promoted. [155]

Prostate cancer: Inhibits EGF/TGFalpha

The development of androgen-independent prostate cancer (AI PrCa) involves constitutive Erk1/2 activation sustained by the epidermal growth factor/transforming growth factor-alpha/EGF receptor (EGF/TGFalpha/EGFR) axis and other trophic signaling mechanisms in neoplastic human prostate epithelial cells in vivo. In this report, we show that growth-inhibitory concentrations of the dietary phytochemical resveratrol suppress EGFR-dependent Erk1/2 activation pathways stimulated by EGF and phorbol ester (12- O -tetradecanoyl phorbol 13-acetate, TPA) in human AI PrCa PC-3 cells in vitro. Because protein kinase C (PKC) is the major cellular receptor for phorbol esters and taking into consideration that resveratrol is PKC-inhibitory, we investigated resveratrol effects on cellular PKC isozymes associated with the suppression of TPA-induced Erk1/2 activation. The PKC isozyme composition of PC-3 cells was defined by Western analysis of the cell lysate with a comprehensive set of isozyme-selective PKC Ab's. PC-3 cells expressed PKCalpha, epsilon, zeta, iota, and PKD (PKCmicro), as did another human AI PrCa cell line of distinct genetic origin, DU145. The effects of resveratrol on TPA-induced PKC isozyme activation were defined by monitoring PKC isozyme translocation and autophosphorylation. Under conditions where resveratrol suppressed TPA-induced Erk1/2 activation, the phytochemical produced isozyme-selective interference with TPA-induced translocation of cytosolic PKCalpha to the membrane/cytoskeleton and selectively diminished the amount of autophosphorylated PKCalpha in the membrane/cytoskeleton of the TPA-treated cells. These results demonstrate that resveratrol abrogation of a PKC-mediated Erk1/2 activation response in PC-3 cells correlates with isozyme-selective PKCalpha inhibition. The results provide evidence that resveratrol may have value as an adjuvant cancer therapeutic in advanced prostate cancer. [156]

Inhibit prostate cancer and increases effectiveness of chemotherapy (vinorelbine)

Resveratrol and propolis increased necrotic cell features after treatment, and apoptotic modifications. Also measured were cell cycle progression to study a correlation with p21 and p53, two well-known cell cycle checkpoints. Both Resveratrol and propolis modulate cell cycle distribution, increasing p53 levels, being able to rescue DU145 from death. The results presented suggest chemotherapy based on resveratrol and propolis, alone or in combination with vinorelbine, as a potential useful tool for prostate cancer therapy; the increase in cell cycle control and the modulation of HSPs expression reinforce this suggestion. [165]

In another study the effects of resveratrol (100 and 200 microM), and of the ethanolic extract of propolis (50 and 100 microg/ml), were tested in androgen-resistant prostate cancer cells (DU145), a cell line resembling the last stage of prostate carcinoma. A comparison between the activity of these micronutrients and vinorelbine bitartrate (Navelbine), a semi-synthetic drug normally used in the therapy of prostate cancer, was conducted. Several biochemical parameters were tested, such as cell viability (MTT assay), cell membrane integrity (lactate dehydrogenase release), cell redox status (nitric oxide formation, reactive oxygen species production, reduced glutathione levels), genomic DNA fragmentation (COMET assay) with special attention on the presence of apoptotic DNA damage (TUNEL test), and possible mitochondrial transmembrane potential alteration (deltapsi). Our results point out the anticancer activity of resveratrol and propolis extract in human prostate cancer, exerting their cytotoxicity through two different types of cell death: necrosis and apoptosis, respectively. The data obtained suggest the possible use of these micronutrients both in alternative to classic chemotherapy, and in combination with very low dosage of vinorelbine (5 microM). [166]

Prostate cancer inhibition:

Researchers at the University of Alabama at Birmingham (UAB) came to the conclusion after a study of male mice that were fed resveratrol. The findings were published Saturday in the online edition of Carcinogenesis. The nutrients in red wine have shown anti-oxidant and anti-cancer properties. In the study resveratrol-fed mice showed an 87 percent reduction in their risk of developing prostate tumors that contained the worst kind of cancer-staging diagnosis. The mice that proved to have the highest cancer-protection effect earned it after seven months of consuming resveratrol in a powdered formula mixed with their food. Other mice in the study, those fed resveratrol but still developed a less-serious form of prostate cancer, were 48 percent more likely to have their tumor growth halted or slowed when compared to mice who did not consume the compound, according to the study. [199]

Prostate cancer: Resveratrol and Grape (Muscadine) Skin Extract suppression – down-regulates Akt activity, ups PTEN and P21

Muscadine grapes contain unique phytochemical constituents compared with other grapes and are potentially a source for novel compounds with antitumor activities. This study compared the antitumor activities of muscadine

grape skin extract (MSKE), which we show contains no resveratrol, with that of resveratrol using primary cultures of normal prostate epithelial cells (PrEC) and the prostate cancer cell lines RWPE-1, WPE1-NA22, WPE1-NB14, and WPE1-NB26, representing different stages of prostate cancer progression. MSKE significantly inhibited tumor cell growth in all transformed prostate cancer cell lines but not PrEC cells. Prostate tumor cell lines, but not PrEC cells, exhibited high rates of apoptosis in response to MSKE through targeting of the phosphatidylinositol 3-kinase-Akt and mitogen-activated protein kinase survival pathways. The reduction in Akt activity by MSKE is mediated through a reduction in Akt transcription, enhanced proteosome degradation of Akt, and altered levels of DJ-1, a known regulator of PTEN. In contrast to MSKE, resveratrol did not induce apoptosis in this model but arrested cells at the G(1)-S phase transition of the cell cycle associated with increased expression of p21 and decreased expression of cyclin D1 and cyclin-dependent kinase 4 proteins. [200]

Curcumin and resveratrol (Liposome) in combination reduces prostate cancer incidence in PTEN knockout mice

Curcumin and resveratrol, as liposome encapsulated were tested in combination in male B6C3F1/J mice, and in prostate-specific PTEN knockout mice. In vitro assays using PTEN-CaP8 cancer cells were performed to investigate the combined effects curcumin with resveratrol on (i) cell growth, apoptosis and cell cycle (ii) impact on activated p-Akt, cyclin D1, m-TOR and androgen receptor (AR) proteins involved in tumor progression. HPLC analysis of serum and prostate tissues showed a significant increase in curcumin level when liposome encapsulated curcumin coadministered with liposomal resveratrol ($p < 0.001$). Combination of liposomal forms of curcumin and resveratrol significantly decreased prostatic adenocarcinoma in vivo ($p < 0.001$). In vitro studies revealed that curcumin plus resveratrol effectively inhibit cell growth and induced apoptosis. Molecular targets activated due to the loss of phosphatase and tensin homolog (PTEN) including p-Akt, cyclin D1, mammalian target of rapamycin and AR were downregulated by these agents in combination. Findings from this study for the first time provide evidence on phytochemicals in combination to enhance chemopreventive efficacy in prostate cancer. These findings clearly suggest that Curcumin and Resveratrol in combination may reduce prostate cancer incidence due to the loss of the tumor suppressor gene PTEN. [247]

Induces apoptosis in chemoresistant cancer cells via modulation of AMPK signaling & the induction of ROS

Resveratrol has been reported to possess therapeutic effects for various cancers including colon cancers. In this article, the molecular basis of resveratrol with emphasis on its ability to control intracellular signaling cascades of adenosine monophosphate (AMP)-activated protein kinase (AMPK) responsible for inducing apoptosis in drug-resistant cancer cells was investigated. Recently, the evolutionarily conserved serine/threonine kinase, AMPK, emerges as a possible target molecule of cancer control. We have investigated the effects of resveratrol on apoptosis in relation to AMPK in HT-29 cells shown chemoresistant to a cancer chemotherapeutic drug, etoposide. Resveratrol exhibited a variety of molecular events in etoposide-based combination therapy in HT-29 colon cancer cells including the AMPK activation, inhibition of cell growth, induction of apoptosis, and reactive oxygen species (ROS) generation. The involvement of AMPK signaling cascade in resveratrol-based cancer therapy was clearly shown by comparing the conditions of AMPK activated states and inactivated states. We have identified ROS as an upstream regulator of AMPK. Further investigation warrants to elucidate the mechanism by which resveratrol generates ROS and AMPK activation. [197]

Suppresses Pancreatic Cancer by Inhibiting Leukotriene A4 Hydrolase.

The anticancer effects of red wine have attracted considerable attention. Resveratrol (3,5,4'-trihydroxy-trans - stilbene) is a well-known polyphenolic compound of red wine with cancer chemopreventive activity. However, the basis for this activity is unclear. We studied leukotriene A(4) hydrolase (LTA(4)H) as a relevant target in pancreatic cancer. LTA(4)H knockdown limited the formation of leukotriene B(4) (LTB(4)), the enzymatic product of LTA(4)H, and suppressed anchorage-independent growth of pancreatic cancer cells. An in silico shape similarity algorithm predicted that LTA(4)H might be a potential target of resveratrol. In support of this idea, we found that resveratrol directly bound to LTA(4)H in vitro and in cells and suppressed proliferation and anchorage-independent growth of pancreatic cancer by inhibiting LTB(4) production and expression of the LTB(4) receptor 1 (BLT(1)). Notably, resveratrol exerted relatively stronger inhibitory effects than bestatin, an established inhibitor of LTA(4)H activity, and the inhibitory effects of resveratrol were reduced in cells where LTA(4)H was suppressed by shRNA-mediated knockdown. Importantly, resveratrol inhibited tumor formation in a xenograft mouse model of human pancreatic cancer by inhibiting LTA(4)H activity. Our findings identify LTA(4)H as a functionally important target for mediating the anticancer properties of resveratrol.[268]

Liver Fibrosis: Inhibits EGF

The grape-derived polyphenol resveratrol is anti-proliferative for human liver myofibroblasts, which may be beneficial for the treatment of liver fibrosis. However, its mechanism of action is ill understood. Here, we have studied how resveratrol interfered with signaling pathways used by epidermal or platelet-derived growth factors to

induce the proliferation of these cells. We found that resveratrol inhibited epidermal growth factor or platelet-derived growth factor-induced DNA synthesis. Resveratrol did not, however, decrease epidermal growth factor receptor autophosphorylation or activation of extracellular regulated kinases, but strongly inhibited the phosphorylation of Akt and of its substrate forkhead related transcription factor. **This suggested that resveratrol inhibited epidermal growth factor-induced mitogenic signaling** through inhibition of the phosphatidylinositol 3-kinase /Akt pathway. The phosphatidylinositol 3-kinase inhibitor LY 294002, also, inhibited epidermal growth factor-dependent DNA synthesis and Akt phosphorylation but did not decrease extracellular regulated kinases phosphorylation. In contrast, resveratrol inhibited platelet-derived growth factor-stimulated receptor autophosphorylation and every subsequent signaling step. Resveratrol did not directly inhibit phosphatidylinositol 3-kinase activity measured on immunoprecipitates from epidermal growth factor-stimulated myofibroblasts, but it strongly reduced the autophosphorylation of the phosphatidylinositol 3-kinase downstream target phospho-inositide-dependent kinase-1 that phosphorylates Akt. We, thus, show that resveratrol has growth factor-specific effects: it inhibits platelet-derived growth factor signaling via reduced receptor activation, whereas it reduces epidermal growth factor-dependent DNA synthesis via inhibition of the phosphatidylinositol 3-kinase/Akt pathway, possibly through inhibition of phospho-inositide-dependent kinase-1 activity. [164]

Inhibits Colon Cancer: Decreases Ornithne Decarboxylase Activity

Resveratrol inhibits colon cancer by causing a significant decrease of ornithine decarboxylase (ODC) activity, a key enzyme of polyamine biosynthesis, which is enhanced in cancer growth. ODC inhibition resulted in the reduction of the intracellular putrescine content, indicating that polyamines might represent one of several targets involved in the anti-proliferative effects of resveratrol. [14]

Suppresses Colitis and Colon Cancer Associated with Colitis.

Ulcerative colitis is an idiopathic, chronic inflammatory disease of the colon associated with a high colon cancer risk. Here, we used a dextran sulfate sodium (DSS) mouse model of colitis, which resembles human ulcerative colitis pathology. Resveratrol mixed in food ameliorates DSS-induced colitis in mice in a dose-dependent manner. Resveratrol significantly improves inflammation score, downregulates the percentage of neutrophils in the mesenteric lymph nodes and lamina propria, and modulates CD3(+) T cells that express tumor necrosis factor-alpha and IFN-gamma. Markers of inflammation and inflammatory stress (p53 and p53-phospho-Ser(15)) are also downregulated by resveratrol. Because chronic colitis drives colon cancer risk, we carried out experiments to determine the chemopreventive properties of resveratrol. Tumor incidence is reduced from 80% in mice treated with azoxymethane (AOM) + DSS to 20% in mice treated with AOM + DSS + resveratrol (300 ppm). Tumor multiplicity also decreased with resveratrol treatment. AOM + DSS-treated mice had 2.4 +/- 0.7 tumors per animal compared with AOM + DSS + 300 ppm resveratrol, which had 0.2 +/- 0.13 tumors per animal. The current study indicates that resveratrol is a useful, nontoxic complementary and alternative strategy to abate colitis and potentially colon cancer associated with colitis.[258]

Resveratrol inhibits colitis-induced colon cancer in animals

Resveratrol, a naturally occurring polyphenolic antioxidant, has been shown to exhibit chemoprophylactic effects on cancer development. Previously, we reported that 2,3',4,4',5'-pentamethoxy-trans-stilbene (PMS), a methoxylated resveratrol derivative, exerted a highly potent anti-proliferative effect on human colon cancer cells as compared with its parent compound. In the present study, the chemopreventive effect of PMS was evaluated in a mouse model of colitis-associated colon carcinogenesis. Experimental approach: Seven-week-old Balb/c mice were injected i.p. with 10 mg.kg(-1) azoxymethane (AOM). After 1 week, 3% dextran sodium sulphate (DSS) was administered in the drinking water for 7 days followed by 14 days of tap water for recovery, and this cycle was repeated twice. Key results: Intragastric administration of PMS (25, 50 mg.kg(-1) body weight) for 16 weeks significantly reduced the multiplicity of colonic neoplasms by 15% and 35% ($P < 0.01$) respectively. Moreover, PMS at 50 mg.kg(-1) inhibited colon cancer cell proliferation and promoted apoptosis. Such changes were accompanied by reduction of Akt (protein kinase B) phosphorylation, inactivation of beta-catenin and down-regulation of inducible nitric oxide synthase. In parallel, in vitro studies also demonstrated that PMS inhibited proliferation and induced apoptosis in the murine colon adenocarcinoma cell line Colon26 with concomitant inhibition of Akt phosphorylation and inactivation of beta-catenin. Conclusions and implications: PMS effectively suppressed colon carcinogenesis in an AOM/DSS animal model and may merit further clinical investigation as a chemoprophylactic agent against colitis-associated colon cancer in humans.[260]

Inhibits Protein Kinase C

Protein kinase D (PKD) is a member of the protein kinase C (PKC) superfamily with distinctive structural, enzymological and regulatory properties. Identification of the cellular function(s) of PKD has been hampered by the absence of a selective inhibitor. Recently, Stewart et al. showed that resveratrol inhibited PKD, but not various PKC isoforms, in vitro. Here we confirmed that the activity of PKD is indeed inhibited in vitro by resveratrol (IC(50)

approximately 200 microM). Additionally, we assessed the inhibition by resveratrol of PKD activity in intact cells, by Western blotting with a phosphospecific PKD antibody which recognizes the autophosphorylated enzyme. In this setting, very high concentrations of resveratrol were required to achieve inhibition of PKD autophosphorylation (IC(50) approximately 800 microM). Since resveratrol produces other pharmacological effects (e.g., cyclooxygenase inhibition) at lower concentrations than those required to inhibit PKD in intact cells, its value as a selective tool to investigate the cellular function(s) of PKD is questionable. [4]

Synergistic with 5-FU against colon cancer

Resveratrol (Res) can modulate multiple cellular pathways relevant for tumorigenesis but is less effective in colon cancer compared to breast cancer. To increase the chemopreventive potential of Res in combination with 5-fluorouracil (5-FU), a systematic study was carried out in colon cancer cells. HCT-116 cells were treated with Res and 5-FU and several cell-based assays, such as MTT, clonogenic, wound healing, DAPI, comet assay, and Western blot, were performed. A significant inhibition of cell proliferation, migration, and increased apoptosis were observed when moderate concentration of Res (15 microM) was associated with very low concentration of 5-FU (0.5 microM). This combination caused apoptosis by blocking the cells at S phase and enhanced the DNA damage. Expression levels of p-JNK and pp38 were increased without affecting pERK. 5-FU could be used as a therapeutic modality to improve efficacy of Resbased chemotherapy against colon cancer.[281]

Reverses Tumor-Promoter-Induced Inhibition of Gap-Junctional Intercellular Communication (GJIC)

Resveratrol is a promising agent for the prevention of cancer. We investigated the effect of resveratrol on gap-junctional intercellular communication (GJIC) in WB-F344 rat liver epithelial cells because inhibition of GJIC is an important mechanism of tumor promotion. Seventeen to 50 microM resveratrol increased GJIC significantly by a factor of 1.**3** compared with solvent vehicle controls, when the WB-F344 cells were exposed to resveratrol for 6 h. Most tumor promoters, including the phorbol ester TPA and the insecticide DDT, block GJIC. Resveratrol at 17-50 microM also significantly prevented down-regulation of GJIC by TPA and DDT, by a factor of **2**.7 and 1.8, respectively. This recovery of GJIC from TPA inhibition was partly correlated with hindered hyperphosphorylation of Cx43. In conclusion, resveratrol was found to enhance GJIC and counteract the effects of tumor promoters on GJIC, and this is likely a mechanism that contributes to the anti-promotional and anti-carcinogenic properties of resveratrol. [27]

Anti-cancer: Enhances Gap-Junctional Intercellular Communication (GJIC)

To investigate the effects of the resveratrol on proliferation and gap-junctional intercellular communication (GJIC) in human liver cancer cell line HepG2. METHODS: MTT assay was used to observe the effects of resveratrol on HepG2 cell growth, and the distribution of cell cycles was detected with flow cytometry (FCM). The effects of resveratrol on GJIC of HepG2 cells labeled with 5'-CFDA/AM was examined with fluorescence redistribution after photobleaching (FRAP) and confocal microscope. RESULTS: The results of MTT assay indicated that the proliferation of HepG2 cells was significantly inhibited by resveratrol in a time- and dose-dependent manner. Resveratrol could arrest HepG2 cell growth in S phase, inhibit DNA synthesis and induce cell apoptosis. Furthermore, the levels of GJIC increased sharply after resveratrol treatment of the cells. CONCLUSION: Resveratrol is capable of inhibiting HepG2 cell proliferation, causing cell growth arrest at S phase and inducing cell apoptosis. Increased GJIC level contributes to the effect of resveratrol in HepG2 cell proliferation inhibition and its cancer chemopreventive activity. [171]

Inhibits tumor cell growth and enhanced the apoptosis of tumor cells

Resveratrol (Res) inhibited the tumor cell growth and enhanced the apoptosis of tumor cells in time-concentration-dependent manners showing the phenomenon of obvious G(0)/G(1) blocking and apoptotic peak. The maximal tumor inhibition rate came up to 42.76%. Furthermore, Res improved function of T, B lymphocytes, killing activity of NK cells, release of antibodies, and the total complement activity in serum. It also increased contents of IL-2 and TGF-beta1, but reduced that of IL-8 and VEGF. CONCLUSION: Res can not only affect tumor cells directly but also exert anti-tumor efficiency through reinforcing cell-mediated, humoral immune response and accommodating lymphocytes to secrete cytokines. [147]

Phyoestrogenic: Agonist/Antagonist for Estrogen Receptors

Epidemiological evidence indicates that phytoestrogens inhibit cancer formation and growth, reduce cholesterol levels, and show benefits in treating osteoporosis. At least some of these activities are mediated through the interaction of phytoestrogens with estrogen receptors alpha and beta (ER-alpha and ER-beta). Resveratrol was shown to bind ER in cytosolic extracts from MCF-7 and rat uteri. However, the contribution of ER-alpha vs. ER-beta in this binding is unknown. Here we report that **resveratrol binds ER-beta and ER-alpha with comparable affinity, but with 7,000-fold lower affinity than estradiol (E2). Thus, resveratrol differs from other phytoestrogens that bind ER-beta with higher affinity than ER-alpha.** Resveratrol acts as an estrogen agonist and stimulates ERE-driven reporter gene activity in CHO-K1 cells expressing either ER-alpha or ER-beta. The

estrogen agonist activity of resveratrol depends on the ERE sequence and the type of ER. Resveratrol-liganded ER-beta has higher transcriptional activity than E2-liganded ER-beta at a single palindromic ERE. This indicates that those tissues that uniquely express ER-beta or that express higher levels of ER-beta than ER-alpha may be more sensitive to resveratrol's estrogen agonist activity. For the natural, imperfect EREs from the human c-fos, pS2, and progesterone receptor (PR) genes, resveratrol shows activity comparable to that induced by E2. We report that resveratrol exhibits E2 antagonist activity for ER-alpha with select EREs. In contrast, resveratrol shows no E2 antagonist activity with ER-beta. These data indicate that resveratrol differentially affects the transcriptional activity of ER-alpha and ER-beta in an ERE sequence-dependent manner. [30]

Suppresses Over-Active Breast Cancer P450 Enzyme

Introduction: Cytochrome P450 1B1 (CYP1B1) catalyzes the bioactivation of numerous procarcinogens and it is expressed in tumor cells, including human breast cancer cells. To study CYP1B1 gene expression, it is important to have an accurate, precise, reproducible, specific, and quantitative method.

MCF-7 human breast carcinoma cells were treated with beta-naphthoflavone (BNF; 50 microM), emodin (0.1-3 microM), trans-resveratrol (2.5-20 microM), or 0.1% dimethylsulfoxide (DMSO; vehicle control). Total cellular RNA was isolated and reverse transcribed. cDNA samples were quantified by a fluorescence assay and a constant amount (1 ng) was amplified in a real-time DNA thermal cycler (LightCycler). Results: Melting curve analysis and agarose gel electrophoresis of the amplicons resulted in a single peak and a single band, respectively. The identity of the amplicon was confirmed to be CYP1B1 by sequencing analysis. The standard curve for the real-time PCR amplification of CYP1B1 cDNA was log-linear for at least four orders of magnitude. The limit of quantitation (LOQ) of the assay was 100 copies. At the LOQ, the assay had an accuracy of 8% and a precision of 10%. The intraday (n=4) variability (expressed as percent coefficient of variation) was 9% for a sample with low CYP1B1 mRNA expression (cells treated with 0.1% DMSO; i.e., Sample A) and 3% for a sample with elevated CYP1B1 mRNA expression (cells treated with BNF; i.e., Sample B). The interday (n=4) variability was 16% for Sample A and 15% for Sample B. Emodin increased CYP1B1 mRNA expression in cultured MCF-7 cells (maximal effect of ninefold induction achieved at 1 microM), whereas resveratrol suppressed it.

Inhibits Genotoxicity altering CYP1B1 in Breast cancer

Genotoxicity is often caused by cytochrome P450 (CYP)–mediated oxidation of catechol estrogens to quinones that react with DNA to form depurinating estrogen-DNA adducts. CYP1B1 favors quinone formation by catalyzing estrogen 4-hydroxylation, whereas NAD(P)H quinone oxidoreductase 1 (NQO1) catalyzes the protective reduction of quinones to catechols. 2,3,7,8-Tetrachl orodi benzo-p-dioxin (TCDD) induces CYP1B1 expression through the aryl hydrocarbon receptor (AhR). Resver- atrol has anticancer effects in diverse in vitro and in vivo systems and is an AhR antagonist that decreases CYP1B1 expression but induces NQO1 expression. Resveratrol can prevent breast cancer initiation by blocking multiple sites in the estrogen genotoxicity pathway. [223]

Breast cancer: Induction of apoptosis via-p53-dependent pathways

Resveratrol has specifically demonstrated to induce apoptosis by p53-dependent pathways in murine cells. The goal of this research was to identify the role of p53-dependent or p53-independent pathways in the induction of apoptosis in human breast cancer cells by this natural product. Apoptosis induced by resveratrol was found to occur in breast cancer cells expressing wild-type p53 but not in mutant p53-expressing cells. We therefore conclude that resveratrol induces apoptosis in breast cancer cells via p53-dependent pathways. [105]

Breast cancer: Combined resveratrol, quercetin, and catechin treatment reduces breast tumor growth in a nude mouse model.

The major polyphenols of red wine (resveratrol, quercetin, and catechin) have been individually shown to have anticancer properties. However, their combinatorial effect on metastatic breast cancers has not been investigated in vivo. We tested the effect of low dietary concentrations of resveratrol, quercetin, and catechin on breast cancer progression in vitro by analyzing cell proliferation and cell cycle progression. The effects of these compounds on fluorescently tagged breast tumor growth in nude mice were assessed using in situ fluorescence image analysis. Individual polyphenols at 0.5 microM neither decreased breast cancer cell proliferation nor affected cell cycle progression in vitro. However, a combination of resveratrol, quercetin, and catechin at 0.5, 5, or 20 microM each significantly reduced cell proliferation and blocked cell cycle progression in vitro. Furthermore, using in situ image analysis, we determined that combined dietary polyphenols at 0.5, 5, or 25 mg/kg reduced primary tumor growth of breast cancer xenografts in a nude mouse model. Peak inhibition was observed at 5 mg/kg. These results indicate that grape polyphenols inhibit breast cancer progression. [224]

Drinking wine while undergoing radiation treatment for breast carcinoma may reduce the incidence of skin toxicity in breast cancer patients

Preventing radiation therapy-induced side effects is an important part of a patient's cancer treatment management. Several medications are available to help protect healthy organs from the effects of radiation, but they are often

expensive, have side effects themselves and can provide protection to tumor cells as well as healthy cells. Italian researchers conducted the study to determine if the natural antioxidants in wine would provide a radioprotective effect in preventing acute skin toxicity in patients undergoing radiation therapy after conservative surgery for breast carcinoma. Patients who drank one glass of wine per day had a 13.6 percent incidence of skin toxicity versus a 38.4 percent incidence in patients who did not drink wine. "If wine can prevent radiotherapy-induced toxicity without affecting antitumor efficacy, as we observed, it also has the potential to enhance the therapeutic benefit in cancer patients without increasing their risk of serious adverse effects," Vincenzo Valentini, M.D., a radiation oncologist at Catholic University in Rome, Italy, one of the study authors, said. "The possibility that particular dietary practices or interventions can reduce radiation-induced toxicity is very intriguing." [249]

Osteosarcoma: regulates signal kinases and p53

The chemopreventive activity of resveratrol (RSVL) has been demonstrated in several types of cancer. However, its effects and the underling mechanisms remain poorly understood. In this study, we investigated the involvement of the mitogen activated protein kinase (MAPK)/p53 signal transduction mechanism in RSVL-induced growth inhibition using a human osteosarcoma cell line. We demonstrate that RSVL reduces cell viability and growth of SJSA1 osteosarcoma cells. Morphological profiles and 4,6-diamidino-2-phenylindole nuclear staining of RSVL-treated cells indicated marked nuclear fragmentation. Cleavage of the (116-kDa) poly(ADP-ribose) polymerase protein into an 89-kDa fragment (a proapoptotic marker system) was substantially augmented by RSVL treatment. RSVL-dependent growth impairment was preceded by enhanced phosphorylation of extracellular signal-regulated kinase (ERK)1/2 (at Thr202/Tyr204). Likewise, RSVL increased the phosphorylation of p53 tumor suppressor protein (at Ser15). The effects of RSVL on ERKs and on p53 phosphorylation were abrogated by either the MAPK inhibitor PD98059 or the p53 inhibitor pifithrine-alpha. The present study indicates that RSVL antiproliferative effects on osteosarcoma cells are mediated by the activation of the ERKs/p53 signaling pathway and therefore identifies new targets for strategies to treat and/or prevent osteosarcoma. [168]

Melanoma I: Synergistic with quercetin

Resveratrol and quercetin, two structurally related and naturally occurring small polyphenols, show longer half-life in vivo. In vitro growth of highly malignant B16 melanoma F10 cells (B16M-F10) is inhibited (56%) by short-time exposure (60 min/day) to resveratrol (40 microm) and quercetin (20 microm) (approximate mean values of plasma concentrations measured within the first hour after intravenous administration of 20 mg/kg each polyphenol). Intravenous administration of resveratrol and quercetin (20 mg/kg per day) to mice inhibits (73%) metastatic growth of B16M-F10 cell in the liver, a common site for metastasis development. The anti-metastatic mechanism involves: 1) a resveratrol-induced inhibition of vascular adhesion molecule 1 expression in the hepatic sinusoidal endothelium, which consequently decreases B16M-F10 cell adhesion to the endothelium through very late activation antigen 4; and 2) a quercetin - and resveratrol -induced inhibition of Bcl-2 expression in metastatic cells, which sensitizes them to vascular endothelium-induced cytotoxicity. Our findings demonstrate that the association of resveratrol and quercetin inhibits metastatic melanoma growth and extends host survival. [131]

Melanoma II: Inhibited tumor growth in animal models of uveal melanoma

The effects of resveratrol was tested by peritumor injection around the site of the tumor (uveal melanoma). This method resulted in tumor cell death andtumor regression. In vitro experiments with multiple uveal melanoma cell lines demonstrate that resveratrol causes a decrease in cell viability, resulting at least in part from an increase in apoptosis through a mitochondrial pathway. An early event in drug action is the direct targeting of mitochondria by resveratrol, which leads to a decrease in mitochondrial membrane potential and the eventual activation of caspase-3. These data suggest that resveratrol can inhibit tumor growth and can induce apoptosis via the intrinsic mitochondrial pathway and that by further increasing bioavailability of resveratrol the potency of the drug can be increased, leading to tumor regression. The nontoxic nature of the drug at levels needed for therapy make resveratrol an attractive candidate for the treatment of uveal melanoma. [244]

Melanoma III: In vivo - Induces cell-cycle disruption and apoptosis in chemoresistant B16 melanoma, also potentiated DOX cytotoxicity in the chemoresistant B16 melanoma

In this study, we show that resveratrol (0-500 microM) inhibits the growth of a doxorubicin-resistant B16 melanoma cell subline (B16/DOX) (IC(50) = 25 microM after 72 h, $P < 0.05$). This was accomplished by imposing an artificial checkpoint at the G(1)-S phase transition, as demonstrated by cell-cycle analysis and down-regulation of cyclin D1/cdk4 and increased of p53 expression level. The G(1)-phase arrest of cell cycle in resveratrol-treated (10-100 microM) B16/DOX cells was followed by the induction of apoptosis, which was revealed by pyknotic nuclei and fragmented DNA. Resveratrol also potentiated at subtoxic dose (25 microM for 24 h) doxorubicin cytotoxicity in the chemoresistant B16 melanoma ($P < 0.01$). When administered to mice, resveratrol (12.5 mg/kg) reduced the growth of an established B16/DOX melanoma and prolonged survival (32% compared to untreated mice). All these data support a potential use of resveratrol alone or in combination with other chemotherapeutic agents in the management

of chemoresistant tumors.[262]

Melanoma IV: Increases expression of p53

In the case of cultured human melanoma cells, no one to our knowledge has investigated whether resveratrol exerts similar anti-proliferative activities in cells with different metastatic potential. Therefore, we examined the effects of this polyphenol on the growth of weakly metastatic Line IV clone 3 and on autologous, highly metastatic Line IV clone 1 cultured melanoma cells. Comparable inhibition of growth and colony formation resulted from treatment by resveratrol in both cell lines. Flow cytometric analysis revealed that resveratrol-treated clone 1 cells had a dose-dependent increase in S phase and a concomitant reduction in the G(1) phase. No detectable change in cell cycle phase distribution was found in similarly treated clone 3 cells. Western blots demonstrated a significant increase in the expression of the tumor suppressor gene p53, without a commensurate change in p21 and several other cell cycle regulatory proteins in both cell types. Chromatography of Line IV clone 3 and clone 1 cell extracts on resveratrol affinity columns revealed that the basal expression of dihydronicotinamide riboside quinone reductase 2 (NQO2) was higher in Line IV clone 1 than clone 3 cells. Levels of NQO2 but not its structural analog NQO1 were dose-dependently increased by resveratrol in both cell lines. We propose that induction of NQO2 may relate to the observed increased expression of p53 that, in turn, contributes to the observed suppression of cell growth in both melanoma cell lines.[143]

Ovarian cancer I:

In the present study, the response of ovarian cancer cells to resveratrol is explored. Resveratrol inhibited growth and induced death in a panel of five human ovarian carcinoma cell lines. The response was associated with mitochondrial release of cytochrome c, formation of the apoptosome complex, and caspase activation. Resveratrol was found to induce cell death through two distinct pathways. Consistent with resveratrols ability to kill cells via nonapoptotic processes, cells transfected to express high levels of the antiapoptotic proteins Bcl-x(L) and Bcl-2 are equally sensitive as control cells to resveratrol.

The researchers concluded: Together, these findings show that resveratrol induces cell death in ovarian cancer cells through a mechanism distinct from apoptosis, therefore suggesting that it may provide leverage to treat ovarian cancer that is chemoresistant on the basis of ineffective apoptosis. [92]

Ovarian Cancer II: Eukaryotic Elongation Factor 1A2 as a Potential Target

In the present study, the growth-inhibitory effects of resveratrol in human ovarian cancer PA-1 cells, considering eEF1A2 as a potential molecular target was examined. Pretreatment with resveratrol attenuated proliferation of serum-starved PA-1 cells stimulated with insulin or serum. Resveratrol also activated caspase-9, -7, and -3 and induced apoptosis in PA-1 cells in the presence of insulin or serum. Insulin or serum stimulation of PA-1 cells resulted in the marked induction of eEF1A2, which was suppressed by pretreatment with resveratrol. Moreover, resveratrol inhibited insulin- or serum-induced soft-agar colony formation in *eEF1A2*-transfected NIH3T3 cells. An antibody array directed to assess the phosphorylation of protein kinases revealed that treatment with insulin or serum induced the phosphorylation of Akt in PA-1 cells. Pharmacologic inhibition of Akt with LY294002 abrogated insulin- or serum-induced eEF1A2 expression and increased the caspase-3 activity.

In another experiment, i.p. administration of resveratrol retarded the growth of PA-1 cell xenograft and the expression of eEF1A2 in athymic nude mice in association with decreased bromodeoxyuridine positivity, reduced expression of proliferating cell nuclear antigen, increased the terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling and caspase-3 staining, and diminished CD31 positivity. Taken together, eEF1A2 may be considered as a potential molecular target for the antiproliferative effects of resveratrol in PA-1 ovarian cancer cells. [252]

Leukemic: Arrest AML Cells at S Phase

Resveratrol, has been reported to possess substantial antileukemic activities in different leukemia cell lines. We investigated whether resveratrol is active against fresh acute myeloid leukemia (AML) cells and its mechanism of action. Because interleukin 1beta(IL-1beta) plays a key role in proliferation of AML cells, we first tested the effect of resveratrol on the AML cell lines OCIM2 and OCI/AML3, both of which produce IL-1beta and proliferate in response to it. Resveratrol inhibited proliferation of both cell lines in a dose-dependent fashion (5-75 microM) by arresting the cells at S phase, thus preventing their progression through the cell cycle; IL-1beta partially reversed this inhibitory effect. Resveratrol significantly reduced production of IL-1beta in OCIM2 cells. It also suppressed the IL-1beta-induced activation of transcription factor nuclear factor kappaB (NF-kappaB), which modulates an array of signals controlling cellular survival, proliferation, and cytokine production. Indeed, incubation of OCIM2 cells with resveratrol resulted in apoptotic cell death. Because caspase inhibitors Ac-DEVD-CHO or z-DEVD-FMK partially reversed the antiproliferative effect of resveratrol, we tested its effect on the caspase pathway and found that resveratrol induced the activation of the cysteine protease caspase 3 and subsequent cleavage of the DNA repair enzyme poly (adenosine diphosphate [ADP]-ribose) polymerase. Finally, resveratrol suppressed colony-forming cell

proliferation of fresh AML marrow cells from 5 patients with newly diagnosed AML in a dose-dependent fashion. [67]

Anti-leukemic: proapoptotic effects stimulation of caspase-3

The pro-apoptotic ability of resveratrol was investigated in vitro on the human lymphoblastoid cell line TK6 and its p53-knockout counterpart (NH32). In both cell lines, resveratrol induced the stimulation of caspase-3. Although resveratrol induced growth inhibition and apoptosis of both cell lines, two distinct mechanisms were observed. The p53-knockout NH32 cells were shown to override the G2/M phase checkpoint with development of hyperdiploid cells, whereas TK6 cells accumulated at G2/M. As p53 function is often altered in human cancer cells, these results show that the pro-apototic effects of resveratrol against tumor cells are independent of their p53 status. [102]

Inhibits NF-kB – kills cancer cells

Marty Mayo, assistant professor of biochemistry and molecular genetics at the University of Virginia, and his team report that the compound helps to starve cancer cells by inhibiting the action of nuclear factor- kappa B (NF-kB), which is found in the nucleus of all cells and activates genes responsible for cell survival and poliferation.

"We used physiologically-relevant doses of resveratrol and found dramatic effects on human cancer cells,"

Cancer cells treated with resveratrol died because they became sensitive to a compound called Tumor Necrosis Factor alpha (TNFa). The researchers found that resveratrol initiated a reaction in the NF-kB molecule that caused the cancer cells essentially to self-destruct by inducing apoptosis.

"Current studies are using compounds similar to TNFa in conjunction with resveratrol to kill cancer cells," Mayo said. *"Clinical trials using this approach in patients are showing encouraging results. This research may explain why this combined therapy is effective and why researchers are always looking for ways to improve cancer therapy,"*

Previous studies have also shown that resveratrol can help control atherosclerosis, heart disease, arthritis, and autoimmune disorders. Mayo believes the inhibition of NF-kB may be responsible in those disorders, as well, since NF-kB can control inflammatory responses. [93]

Down-regulation of NF-kappaB, cyclooxygenase-2, and matrix metalloprotease-9 expression.

Because NF-kappaB suppression has been linked with chemoprevention, this prompted us to investigate the chemopreventive potential of resveratrol by testing it against mammary carcinogenesis induced by 7,12-dimethylbenz(a)anthracene (DMBA) in female Sprague Dawley rats. Dietary administration of resveratrol (10 ppm) had no effect on body weight gain and tumor volume but produced striking reductions in the incidence (45%; $P < 0.05$), multiplicity (55%; $P < 0.001$), and extended latency period of tumor development relative to DMBA-treated animals. Histopathological analysis of the tumors revealed that DMBA induced ductal carcinomas and focal microinvasion in situ (7 of 7), whereas treatment with resveratrol suppressed DMBA-induced ductal carcinoma. Immunohistochemistry and Western blot analysis revealed that resveratrol suppressed the DMBA-induced cyclooxygenase-2 and matrix metalloprotease-9 expression in the breast tumor. Gel shift analysis showed suppression of DMBA-induced NF-kappaB activation by resveratrol. Treatment of human breast cancer MCF-7 cells with resveratrol also suppressed the NF-kappaB activation and inhibited proliferation at S-G(2)-M phase. Overall, our results suggest that resveratrol suppresses DMBA-induced mammary carcinogenesis, which correlates with down-regulation of NF-kappaB, cyclooxygenase-2, and matrix metalloprotease-9 expression. [198]

Suppresses NF-κB activation

Piceatannol inhibits TNF-induced NF-kB activation and NF-kB-mediated gene expression through suppression of IkappaBalpha kinase and p65 phosphorylation. [237]

Resveratrol suppresses TNF-induced activation of nuclear transcription factors NF-kB, activator protein-1, and induces apoptosis. [238]

Resveratrol blocks interleukin-1β-induced activation of the NF-κB, inhibits proliferation, causes S-phase arrest, and induces apoptosis of acute myeloid leukemia cells. [239]

Cytotoxic to CML

Resveratrol (RV) exerts remarkable cytostatic and cytotoxic effects against a multitude of human cancer cell lines. Since the introduction of additional hydroxyl groups was supposed to increase the biological activity of RV, we have synthesized a number of polyhydroxylated stilbene analogues as potential antitumor agents. In this study, the activity of 3,3',4,4',5,5'-hexahydroxystilbene (M8) was investigated in HL-60 human promyelocytic leukemia cells. Employing a growth inhibition assay, incubation with M8 and RV resulted in IC50 values of 6.25 and 12 muM, respectively. Using a specific Hoechst/propidium iodide double staining method, M8 was able to induce apoptosis in concentrations significantly lower than those of RV. In addition, M8 arrested cells in the S phase and totally depleted cells in the G2-M phase of the cell cycle (143% and 0% of control after treatment with 12.5 muM M8, respectively). [176]

Cytotoxic to CML

Autophagy that is induced by starvation or cellular stress can enable cancer cell survival by sustaining energy homeostasis and eliminating damaged organelles and proteins. In response to stress, cancer cells have been reported to accumulate the protein p62/SQSTM1 (p62), but its role in the regulation of autophagy is controversial. Here, we report that the plant phytoalexin resveratrol (RSV) triggers autophagy in imatinibsensitive and imatinib-resistant chronic myelogenous leukemia (CML) cells via JNK-dependent accumulation of p62. JNK inhibition or p62 knockdown prevented RSV-mediated autophagy and antileukemic effects. RSV also stimulated AMPK, thereby inhibiting the mTOR pathway. AMPK knockdown or mTOR overexpression impaired RSV-induced autophagy but not JNK activation. Lastly, p62 expression and autophagy in CD34+ progenitors from patients with CML was induced by RSV, and disrupting autophagy protected CD34+ CML cells from RSV-mediated cell death. We concluded that RSV triggered autophagic cell death in CML cells via both JNK-mediated p62 overexpression and AMPK activation. Our findings show that the JNK and AMPK pathways can cooperate to eliminate CML cells via autophagy.[257]

Enhances the anti-tumor activity of the mTOR inhibitor rapamycin in multiple breast cancer cell lines mainly by suppressing rapamycin-induced AKT signaling.

The anti-tumor activity of rapamycin is compromised by the feedback-loop-relevant hyperactive PI3K and ERK-MAPK pathway signaling. In breast cancer cells treated with rapamycin, we observed a moderate increase of AKT phosphorylation (P-AKT) in a rapamycin resistant cell line, MDA-MB-231, as well as a slight increase of P-AKT in a rapamycin sensitive cell line, MCF-7. We found that resveratrol, a natural phytoalexin, suppressed the phosphorylation and activation of the PI3K/AKT pathway in all the three breast cancer cell lines that we tested. It also had a weak inhibitory effect on the activation of the mTOR/p70S6K pathway in two cell lines expressing wildtype PTEN, MCF-7 and MDA-MB-231. The combined use of resveratrol and rapamycin resulted in modest additive inhibitory effects on the growth of breast cancer cells, mainly through suppressing rapamycin-induced AKT activation. We, therefore, reveal a novel combination whereby resveratrol potentiates the growth inhibitory effect of rapamycin, with the added benefit of preventing eventual resistance to rapamycin, likely by suppressing AKT signaling. We also present data herein that PTEN is an important contributor to resveratrol's growth suppressive effects and its potentiation of rapamycin in this therapeutic scenario, as resveratrol's suppression of rapamycin-mediated induction of P-AKT is both PTEN-dependent and -independent. Thus, the resveratrol-rapamycin combination may have therapeutic value in treating breast cancer and perhaps other processes where mTOR is activated.[273]

Enhances the anti-tumor activity of the mTOR inhibitor rapamycin in Breast cancer (Normalizes PTEN)

Researchers from Cleveland Clinic's Lerner Research Institute have discovered that resveratrol – a compound found in red wine - when combined with rapamycin can have a tumor-suppressing effect on breast cancer cells that are resistant to rapamycin alone.

"Rapamycin has been used in clinical trials as a cancer treatment. Unfortunately, after a while, the cancer cells develop resistance to rapamycin," Eng said. "Our findings show that resveratrol seems to mitigate rapamycin-induced drug resistance in breast cancers, at least in the laboratory.

If these observations hold true in the clinic setting, then enjoying a glass of red wine or eating a bowl of boiled peanuts - which has a higher resveratrol content than red wine - before rapamycin treatment for cancer might be a prudent approach."

Rapamycin, an immunosuppressant drug used to prevent rejection in organ transplantation, has been considered for the use of anti-tumor activity against breast cancer. Resveratrol is a type of polyphenol that is found in the skin of red grapes and is a constituent of red wine, and has been considered for multiple uses regarding cellular therapies. Despite the potential for tumor suppression, rapamycin's efficacy with respect to growth inhibition differs markedly among various breast cancer cell lines. The effect of resveratrol and rapamycin, alone and in combination, on cell growth of three human breast cancer cell lines was assessed.

Rapamycin, resveratrol, and combinations of these agents inhibited cell growth in a dosedependent manner. In all three cell lines tested, the presence of low concentrations of resveratrol and rapamycin was sufficient to induce 50 percent growth inhibition. Although relatively early, these observations may suggest resveratrol as a powerful integrative medicine adjunct to traditional chemotherapy.[277]

Resveratrol and Curcumin induce apoptosis in human neuroblastoma

Neuroblastoma (NB) is an aggressive childhood cancer of the peripheral nervous system arising from neural crest sympathoadrenal progenitor cells. Despite current rigorous treatment protocols, prognosis for high stage NB patients is poor and so there remains a need for more effective, less cytotoxic treatments. Curcumin and resveratrol possess anti-tumor properties in adult cancer models and negligible toxicity in normal cells, but little is known about the effect of these agents on pediatric cancers. Stage 4 MYCN-amplified NB cell lines, with wild-type or mutant p53,

were treated with curcumin and resveratrol and analyzed for effects on proliferation, cell cycle, induction of apoptosis and p53 function. Treatment induced a dose- and time-dependent decrease in cell viability, cell cycle arrest and induction of apoptosis. Treatment transiently up-regulated p53 expression and induced nuclear translocation of p53, followed by induction of p21(WAF-1/CIP-1) and Bax expression. Observations suggest that the cytotoxicity, cell cycle arrest and apoptosis induced by curcumin and resveratrol in NB cells may be mediated via functionally activated p53 and merit further study. [94]

Gliobastoma: decreases MMP-2

Glioblastoma is a highly malignant brain tumor with a high-invasive phenotype, so the prognosis is unfavorable, even in response to multidisciplinary treatment strategies. Obviously, therefore, a better therapeutic strategy is needed. Resveratrol has been reported to be one of the most potent chemopreventive agents inhibiting the cellular processes associated with tumor development, including initiation, promotion, and progression. In this study we used RT-PCR, western blot and SDS-zymography to investigate the effect of resveratrol on the expression of genes and proteins involved in the extracellular matrix remodeling associated with tumor invasion in human cultured glioblastoma cells treated for 24, 48 and 72 h. We analyzed the expression of matrix metalloproteinase-2 (MMP-2), the main mediator of glioblastoma invasiveness, and the Secreted Protein Acidic and Rich in Cysteine (SPARC), involved in the regulation of cell-matrix interactions. Our results show a dose-related decrease of MMP-2 mRNA and protein levels 72 h after resveratrol treatment, and lower SPARC gene and protein expression 72 h after resveratrol treatment. This indicates that resveratrol may influence the two major factors in the ECM remodeling occurring with tumor invasion, suggesting it may have uses as a therapeutic agent for brain tumors.

Glioblastoma: Downregulates PI3K/Akt/mTOR signaling pathways in human U251 glioma cells.

Resveratrol (trans-3,4', 5-trihydroxystilbene) is a naturally occurring polyphenolic compound that has antiinflammatory, antioxidant, neuroprotective properties and acts as a chemopreventive agent. Resveratrol causes cell cycle arrest and induces apoptotic cell death in various types of cancer cells. In the current studies, the effect of resveratrol on phosphoinositide kinase-3 (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) signaling pathway was examined in human U251 glioma cells. Resveratrol decreased both the expression and phosphorylation of Akt. Inhibitors of PI3K (LY294002) and Akt (SH-6) enhanced resveratrol-induced LDH release and caspase-3 activation. Resveratrol reduced phosphorylation of ribosomal protein S6 and the mTOR inhibitor rapamycin further enhanced resveratrol-induced cell death. These results suggest that the downregulation of PI3K/Akt/mTOR signaling pathways may be an important mediator in resveratrol-induced apoptosis in glioma cells.[274]

Resveratrol abrogates the temozolomide-induced G2 arrest leading to mitotic catastrophe and reinforces the temozolomide-induced senescence in glioma cells.

Temozolomide (TMZ) is the most widely used drug to treat glioblastoma (GBM), which is the most common and aggressive primary tumor of the Central Nervous System and one of the hardest challenges in oncotherapy. TMZ is an alkylating agent that induces autophagy, apoptosis and senescence in GBM cells. However, therapy with TMZ increases survival after diagnosis only from 12 to 14.4 months, making the development of combined therapies to treat GBM fundamental. One candidate for GBM therapy is Resveratrol (Rsv), which has additive toxicity with TMZ in several glioma cells in vitro and in vivo. However, the mechanism of Rsv and TMZ additive toxicity, which is the aim of the present work, is not clear, especially concerning cell cycle dynamics and long term effects. Glioma cell lines were treated with Rsv and TMZ, alone or in combinations, and the induction and the role of autophagy, apoptosis, cell cycle dynamics, protein expression and phosphorylation status were measured. We further evaluated the long-term senescence induction and clonogenic capacity.

As expected, temozolomide caused a G2 cell cycle arrest and extensive DNA damage response. Rsv did not reduced this response, even increasing pATM, pChk2 and gammaH2Ax levels, but abrogated the temozolomide-induced G2 arrest, increasing levels of cyclin B and pRb(S807/811) and reducing levels of pWee1(S642) and pCdk1(Y15). This suggests a cellular state of forced passage through G2 checkpoint despite large DNA damage, a scenario that may produce mitotic catastrophe. Indeed, the proportion of cells with high nuclear irregularity increased from 6 to 26% in 48 h after cotreatment. At a long term, a reduction in clonogenic capacity was observed, accompanied by a large induction of senescence.

The presence of Rsv forces cells treated with TMZ through mitosis leading to mitotic catastrophe and senescence, reducing the clonogenic capacity of glioma cells and increasing the chronic effects of temozolomide.[297]

Colon Cancer: NK-kB inhibition/Caspase 3 induction

Several selected natural chemopreventive agents inhibit human HT-29 colon cancer cells, acting on transcription activation of nuclear factor-kappa B (NF-kappaB). The natural chemopreventive compounds isothiocyanates (ITCs)

found in cruciferous vegetables, flavonoids found in green tea, **resveratrol (RES)** and procyanidin dimers found in red wine, and curcumin (CUR) found in turmeric curry food were examined in this study. Treatments with the natural chemopreventive compounds resulted in different responses in the NF-kappaB-luciferase assay. ITCs such as phenethyl isothiocyanate (PEITC), sulforaphane (SUL), allyl isothiocyanate (AITC), and curcumin (CUR) strongly inhibited LPS-induced NF-kappaB-luciferase activations, whereas RES also inhibited activation but at a high dose, and tea flavonoids and procyanidin dimers had little or no effects. ITCs, CUR, (-)-epigallocatechin-3-gallate (EGCG), and RES reduced LPS-induced IkappaBalpha phosphorylation. Furthermore, in the MTS assay, PEITC, SUL, and CUR also potently inhibited cell growth. Caspase-3 activity was induced by chemopreventive compounds, however, the kinetics of caspase-3 activation varied between these compounds within the 48-h time period. These results suggest that natural chemopreventive agents have differential biological functions on the signal transduction pathways in the colon and/or colon cancer. [96]

Colon cancer: AP-1 Inhibition

Activator protein-1 (AP-1) has been implicated as playing important roles in apoptosis and cancer development. In this work, we studied several natural chemopreventive compounds for their potential chemopreventive properties in the modulation of AP-1 signaling pathway in HT-29 colon cancer cells. Phenethyl isothiocyanate, sulforaphane, curcumin, and **resveratrol** increased AP-1-luciferase activity dose-dependently and then decreased at higher doses in the presence or absence of an inducing agent. Allyl isothiocyanate and (-)-epigallocatechin-3-gallate (EGCG) increased AP-1-luciferase activity dose-dependently up to 50 and 100 microM. The JNK activity was induced by the isothiocyanates and EGCG. Most of the chemopreventive compounds induced cell death in a dose-dependent manner, with the exception of epicatechin (EC) and the procyanidins, which had little effect. The expression of endogenous cyclin D1 protein was well correlated with those of AP-1-luciferase assay. Taken together, these results suggest that natural chemopreventive compounds may have differential biological functions on the signal transduction pathways such as AP-1 in the intervention of colon cancer progression and carcinogenesis. [98]

Down-regulates AP-1

Resveratrol (trans-3,4',5-trihydroxystilbene) is a naturally occurring polyphenolic phytoalexin found in grapes, and has been shown to inhibit the growth of various types of cancer cells. We investigated the mechanism of the antiproliferative effect of resveratrol in A431-transformed keratinocytes harbouring mutant p53, and show that it is accompanied by G1 cell cycle arrest, which coincides with a marked inhibition of G1 cell cycle regulatory proteins, including cyclins A and D1 and cyclin-dependent kinase (CDK)6 and p53-independent induction of p21WAF1. Cell cycle arrest was also associated with the accumulation of hypophosphorylated Rb and p27KIP1. Resveratrol inhibited mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK)1 > extracellular signal-regulated protein kinase (ERK)1/2 signalling, downregulated c-Jun, and suppressed activating protein (AP)-1 DNA-binding and promoter activity. In addition, the inhibition of MEK1 > ERK1/2 signalling appears to be independent of retinoblastoma protein (pRb) hypophosphorylation in A431 cells, as PD098059 did not suppress pRb phosphorylation. Our results demonstrate that resveratrol affects multiple cellular targets in A431 cells, and that the downregulation of both AP-1 and pRb contributes to its antiproliferative activity in these cells. [167]

Suppresses IGF-1 induced human colon cancer cell proliferation and elevates apoptosis via suppression of IGF-1R/Wnt and activation of p53 signaling pathways.

Obesity is a global phenomenon and is associated with various types of cancer, including colon cancer. There is a growing interest for safe and effective bioactive compounds that suppress the risk for obesity-promoted colon cancer. Resveratrol (trans-3, 4', 5,-trihydroxystilbene), a stilbenoid found in the skin of red grapes and peanuts suppresses many types of cancers by regulating cell proliferation and apoptosis through a variety of mechanisms, however, resveratrol effects on obesity-promoted colon cancer are not clearly established. METHODS: We investigated the anti-proliferative effects of resveratrol on HT-29 and SW480 human colon cancer cells in the presence and absence of insulin like growth factor-1 (IGF-1; elevated during obesity) and elucidated the mechanisms of action using IGF-1R siRNA in HT-29 cells which represents advanced colon carcinogenesis. Resveratrol (100-150 microM) exhibited anti-proliferative properties in HT-29 cells even after IGF-1 exposure by arresting G0/G1-S phase cell cycle progression through p27 stimulation and cyclin D1 suppression. Treatment with resveratrol suppressed IGF-1R protein levels and concurrently attenuated the downstream Akt/Wnt signaling pathways that play a critical role in cell proliferation. Targeted suppression of IGF-1R using IGF-1R siRNA also affected these signaling pathways in a similar manner. Resveratrol treatment induced apoptosis by activating tumor suppressor p53 protein, whereas IGF-1R siRNA treatment did not affect apoptosis. Our data suggests that resveratrol not only suppresses cell proliferation by inhibiting IGF-1R and its downstream signaling pathways similar to that of IGF-1R siRNA but also enhances apoptosis via activation of the p53 pathway. CONCLUSIONS: For the first time, we report that resveratrol suppresses colon cancer cell proliferation and elevates apoptosis even in the presence of

IGF-1 via suppression of IGF-1R/Akt/Wnt signaling pathways and activation of p53, suggesting its potential role as a chemotherapeutic agent.[259]

Inhibits JAK/STAT-mediated gene transcription and induce the mitochondrial cell death pathway.

Resveratrol has cytotoxic effects through inhibiting cellular proliferation of A431 cells, which leads to the induction of apoptosis, as evident by an increase in the fraction of cells in the sub-G1 phase of the cell cycle and Annexin-V binding of externalized phosphatidylserine. Results revealed that inhibition of proliferation is associated with regulation of the JAK/STAT pathway, where resveratrol prevents phosphorylation of JAK, thereby inhibiting STAT1 phosphorylation. Furthermore, resveratrol treatment actively stimulated reactive oxygen species (ROS) and mitochondrial membrane depolarization. Consequently, an imbalance in the Bax/Bcl-2 ratio triggered the caspase cascade and subsequent cleavage of PARP, thereby shifting the balance in favor of apoptosis. These observations indicate that resveratrol treatment inhibits JAK/STAT-mediated gene transcription and induce the mitochondrial cell death pathway. [256]

Inhibits tumor-promting inflammatory cytokines including IL-6 and TNF-alpha

The production of inflammatory (TNF-alpha and IL-1beta) and anti-inflammatory (IL-6) cytokines was suppressed by trans-resveratrol in a concentration-dependent manner. These results support the hypothesis that the immunomodulatory effect of trans-resveratrol plays an important role in disease conditions that involve an overproduction of inflammatory cytokines. [138]

Inhibits chemical-induced (herbicides (alachlor, acetochlor) breast cancer

A study was conducted to demonstrate the preventive effect of resveratrol on the cytotoxicity of frequently used herbicides (alachlor, acetochlor). Estrogen receptor positive (ER+) MCF-7 human mammary carcinoma, HepG2 (ER+) human hepatocellular carcinoma and VERO estrogen receptor negative (ER-) non-transformed monkey fibroblast cell lines were treated with alachlor and acetochlor (2-500 microg/ml) as toxic agents, and RESV (10 microM) as preventive agent. The MTT dye reduction assay was performed to test cytotoxicity, and flow cytometry to test cell proliferation and apoptosis. RESV is not cytotoxic in the concentration range of 1-100 microM on neither cell lines examined after 24 h, but cytotoxic on Vero and MCF-7 cells at 100 microM after 48h, and on all three cell lines after 72 h. On both ER+ cell lines a stimulation of viability occurs in the low concentration range (0.5-12.5 microM) as detected by the MTT assay. Cell cycle analysis of the culture shows a significant increase of S-phase cells at low concentrations of RESV (10-50 microM) and a decrease in the 100-200 microM concentration range. The ratio of apoptotic cells significantly increases after the administration of 50 microM RESV, depending on the incubation time. The cytotoxicity of 20-65 microg/ml alachlor and 10-65 microg/ml acetochlor was significantly decreased by the addition of 10 microM RESV in Vero ER- cells whereas no significant change was detected on ER+ cell lines MCF-7 and HepG2. These results show that RESV protects non-transformed ER- cells, but has no such effect on ER+ tumor cells. [137]

Synergistic anticancer effects of curcumin and resveratrol against liver cancer

Hepatocellular carcinoma remains one of the most prevalent malignancies worldwide. Curcuma aromatica and Polygonum cuspidatum are one of the commonly used paired-herbs for liver cancer treatment. Curcumin and resveratrol are the major anticancer constituents of **Curcuma aromatica** and Polygonum cuspidatum, respectively. Curcumin and resveratrol have been found to exhibit a synergistic anticancer effect in colon cancer. However, the combined effect of curcumin and resveratrol against hepatocellular carcinoma remains unknown. In the present study, we evaluated the combined effects of curcumin and resveratrol in hepatocellular carcinoma Hepa1-6 cells. The results showed that curcumin and resveratrol significantly inhibited the proliferation of Hepa1-6 cells in a dose- and time-dependent manner. The combination treatment of curcumin and resveratrol elicited a synergistic antiproliferative effect in Hepa1-6 cells. The apoptosis of Hepa1-6 cells induced by the combination treatment with curcumin and resveratrol was accompanied by caspase-3, -8 and-9 activation, which was completely abrogated by a pan caspase inhibitor, Z-VAD-FMK. Combination of curcumin and resveratrol upregulated intracellular reactive oxygen species (ROS) levels in Hepa1-6 cells. The ROS scavenger, NAC, partially attenuated the apoptosis and caspase activation induced by the combination treatment of curcumin and resveratrol. In addition, the combination of curcumin and resveratrol downregulated XIAP and survivin expression. These data suggest that the combination treatment of curcumin and resveratrol is a promising novel anticancer strategy for liver cancer. The present study also provides new insights into the effective mechanism of paired-herbs in traditional Chinese medicine.[292]

Resveratrol potentiates grape seed extract induced human colon cancer cell apoptosis.

Colon cancer is the third leading cause of cancer deaths in men and women. Grape seed extract (GSE) and resveratrol (RSV) are potent chemopreventive agents against colon cancer both in vitro and in vivo, at relatively high concentrations. We hypothesized that RSV and GSE may act in concert with each other in potentiating their anti-cancer properties at sub-optimal doses, because they occur as complex mixtures in grapes. In this study, we

showed that RSV (~25 micromolar) potentiated GSE (≤ 35 microg/mL) induced colon cancer cell apoptosis via activation of p53 dependent pathways. Elevation of apoptosis was much more pronounced in p53 +/+ cells compared to p53 -/- cells. Apoptosis was strongly correlated with pp53 levels and Bax:Bcl-2 ratio, key players in the mitochondrial apoptotic pathway. Caspase-3 inhibition and reactive oxygen species suppression attenuated apoptosis induced by the combination. RSV-GSE combination suppressed proliferation and induced apoptosis even in the presence of mitogenic growth factor IGF-1, suggesting the importance of understanding the potentiating effects of phytonutrients in combination as they would occur in nature rather than individually.[300]

Resveratrol suppresses IGF-1 induced human colon cancer cell proliferation and elevates apoptosis via suppression of IGF-1R/Wnt and activation of p53 signaling pathways.

BACKGROUND:

Obesity is a global phenomenon and is associated with various types of cancer, including colon cancer. There is a growing interest for safe and effective bioactive compounds that suppress the risk for obesity-promoted colon cancer. Resveratrol (trans-3, 4', 5,-trihydroxystilbene), a stilbenoid found in the skin of red grapes and peanuts suppresses many types of cancers by regulating cell proliferation and apoptosis through a variety of mechanisms, however, resveratrol effects on obesity-promoted colon cancer are not clearly established.

METHODS:

We investigated the anti-proliferative effects of resveratrol on HT-29 and SW480 human colon cancer cells in the presence and absence of insulin like growth factor-1 (IGF-1; elevated during obesity) and elucidated the mechanisms of action using IGF-1R siRNA in HT-29 cells which represents advanced colon carcinogenesis.

RESULTS:

Resveratrol (100-150 microM) exhibited anti-proliferative properties in HT-29 cells even after IGF-1 exposure by arresting G0/G1-S phase cell cycle progression through p27 stimulation and cyclin D1 suppression. Treatment with resveratrol suppressed IGF-1R protein levels and concurrently attenuated the downstream Akt/Wnt signaling pathways that play a critical role in cell proliferation. Targeted suppression of IGF-1R using IGF-1R siRNA also affected these signaling pathways in a similar manner. Resveratrol treatment induced apoptosis by activating tumor suppressor p53 protein, whereas IGF-1R siRNA treatment did not affect apoptosis. Our data suggests that resveratrol not only suppresses cell proliferation by inhibiting IGF-1R and its downstream signaling pathways similar to that of IGF-1R siRNA but also enhances apoptosis via activation of the p53 pathway.

CONCLUSIONS:

For the first time, we report that resveratrol suppresses colon cancer cell proliferation and elevates apoptosis even in the presence of IGF-1 via suppression of IGF-1R/Akt/Wnt signaling pathways and activation of p53, suggesting its potential role as a chemotherapeutic agent.[301]

Anticancer Effects of Thymoquinone, Caffeic Acid Phenethyl Ester and Resveratrol on A549 Non-small Cell Lung Cancer Cells Exposed to Benzo(a)pyrene.

Background: Phytochemical compounds are emerging as a new generation of anticancer agents with limited toxicity in cancer patients. The purpose of this study was to investigate the potential effects of **thymoquinone, caffeic acid phenylester (CAPE) and resveratrol** on inflammatory markers, oxidative stress parameters, mRNA expression levels of proteins and survival of lung cancer cells in Vitro. Materials and Methods: The A549 cell line was treated with benzo(a)pyrene, benzo(a)pyrene plus caffeic acid phenylester (CAPE), benzo(a)pyrene plus resveratrol (RES), and benzo(a)pyrene plus thymoquinone (TQ). Inflammatory markers, oxidative stress parameters, mRNA expression levels of apoptotic and anti-apoptotic proteins and cell viability were assessed and results were compared among study groups. Results: **TQ treatment up-regulated Bax and down-regulated Bcl2 proteins and increased the Bax/Bcl2 ratio**. CAPE and TQ also up-regulated Bax expression. RES and TQ down-regulated the expression of Bcl-2. All three agents decreased the expression of cyclin D and increased the expression of p21. However, the most significant up-regulation of p21 expression was observed in TQ treated cells. CAPE, RES and TQ up-regulated TRAIL receptor 1 and 2 expression. **RES and TQ down-regulated the expression of NF-kappa B** and IKK1. Viability of CAPE, RES and TQ treated cells was found to be significantly decreased when compared with the control group (p=0.004). Conclusions: Our results revealed up-regulation of the key upstream signaling factors, which ultimately cause increase in their regulatory p53 levels affecting the induction of G2/M cell cycle arrest and apoptosis. Overall these results provide mechanistic insights for understanding the molecular basis and utility of the anti-tumor activity of TQ, RES and CAPE.[302]

INHIBITS ANGIOGENESIS

Resveratrol and Quercetin Inhibit Tumor Angiogenesis.

Resveratrol and quercetin are polyphenolic compounds found in grapes, red wine and other food products. In this study, we examined the effect of resveratrol and quercetin on the inhibition of angiogenesis in vitro. Resveratrol and quercetin inhibited the growth of bovine aorta endothelial (BAE) cells in a concentration-dependent manner (6-100 [mu]M). The migration of BAE was obviously inhibited by resveratrol and weakly inhibited by quercetin. When the lengths of all tubes constructed in the 3-D culture system with or without resveratrol or quercetin were measured, resveratrol was found to inhibit the tube formation of BAE cells in a concentration-dependent manner (6-100 [mu]M). On the other hand, quercetin, at concentrations above 100_M significantly inhibited the tube formation of vascular endothelial cells. From these results, we suggest that **resveratrol and quercetin may prove useful in the development of useful therapeutic agents or preventive food factors for tumor angiogenesis**. [42]

Inhibits Epidermal Growth Factor receptor: Antiviral and anticancer mechanism

Mechanism of action studies determined that resveratrol blocked virus-induced activation of the epidermal growth factor receptor (EGFR) and phosphatidylinositol-3-kinase signal transduction as well as NF-kappaB and Sp1 transcription factor activation shortly following infection. Resveratrol prevented the appearance of immediate-early, early, and late viral proteins. Human cytomegalovirus DNA replication was reduced to undetectable levels by treatment with resveratrol, as were the second (late) phases of virus-induced phosphatidylinositol-3-kinase signaling and transcription factor activation. Resveratrol lost substantial antiviral activity when its addition was delayed until 4h postinfection. Compound reversibility and preincubation studies were inconsistent with a virucidal mechanism of action. These data indicated that this compound likely operated during attachment and entry. We hypothesize that the primary molecular target for resveratrol may be blockage of epidermal growth factor receptor activation and its downstream effectors. [118]

Multiple Myloma: Inhibits angiogenesis, via VEGF, bFGF, MMP-2, MMP-9

In multiple myeloma (MM), bone marrow angiogenesis parallels tumour progression and correlates with disease activity. Recent studies have proved resveratrol possesses antiangiogenic activity in vitro and in vivo. In this study, we examined the effects of resveratrol on myeloma cell dependent angiogenesis and the effects of resveratrol on some important angiogenic factors of RPMI 8226 cells. METHODS: RPMI 8226 cells were cocultured with human umbilical vein endothelial cells (HUVECs) to evaluate the effects of myeloma cells on angiogenesis. The RPMI 8226 cells were treated with various concentrations of resveratrol (6.25 - 50.00 micromol/L) for different times (12 - 72 hours). Reverse transcriptase polymerase chain reaction (RT-PCR) was used to assay vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), metalloproteinases (MMP)-2 and MMP-9 mRNA. Gelatin zymography was used to analyze MMP-2 and MMP-9 activity. VEGF and bFGF proteins secreted by the cells in the medium were quantified by enzyme linked immunosorbent assay (ELISA). RESULTS: Cell proliferation, migration and differentiation of HUVECs markedly increased by coculture with RPMI 8226 cells. Resveratrol inhibited proliferation, migration and tube formation of HUVECs cocultured with myeloma cells in a dose dependent manner. Treatment of RPMI 8226 cells with resveratrol caused a decrease in MMP-2 and MMP-9 activity. Resveratrol inhibited VEGF and bFGF protein expression in a dose and time dependent manner. Furthermore, decreased levels of VEGF, bFGF, MMP-2 and MMP-9 mRNA from cells treated with various concentrations of resveratrol confirmed its antiangiogenic action at the level of gene expression. CONCLUSIONS: Resveratrol inhibits multiple myeloma angiogenesis by regulating expression and secretion of VEGF, bFGF, MMP-2 and MMP-9. Resveratrol may be a potential candidate for the treatment of multiple myeloma. [219]

INHIBITS TUMOR INVASION

Prevents Tumor Growth: Lung Cancer- inhibits VEGF

Resveratrol, derived from Polygonum cuspidium, at doses of 2.5 and 10 mg/kg, significantly reduced the tumor volume (42%), tumor weight (44%) and metastasis to the lung (56%) in mice bearing highly metastatic Lewis lung carcinoma (LLC) tumors, but not at a dose of 0.6 mg/kg. Resveratrol did not affect the number of CD4(+), CD8(+) and natural killer (NK)1.1.(+) T cells in the spleen. Therefore, the inhibitory effects of resveratrol on tumor growth and lung metastasis could not be explained by natural killer or cytotoxic T-lymphocyte activation. In addition, **resveratrol inhibited DNA synthesis** most strongly in LLC cells; its 50% inhibitory concentration was 6.8 micromol/L. Resveratrol at 100 micromol/L **increased apoptosis** in LLC cells, and decreased the S phase population at concentrations of 50 and 100 micromol/L, respectively. Resveratrol **inhibited tumor-induced neovascularization** at doses of 2.5 and 10 mg/kg in an in vivo model. Moreover, resveratrol significantly inhibited the formation of capillary-like tube formation from human umbilical vein endothelial cells (HUVEC) at concentrations of 10-100 micromol/L; the degree of the inhibition of capillary-like tube formation by resveratrol was 45.5% at 10 micromol/L, 50.2% at 50 micromol/L and 52.6% at 100 micromol/L. Resveratrol inhibited the binding

of vascular endothelial growth factor (VEGF) to HUVEC at concentrations of 10-100 micromol/L, but not at concentrations of 1 and 5 micromol/L. The degree of inhibition of VEGF binding to HUVEC by resveratrol was 16.9% at 10 micromol/L, 53.2% at 50 micromol/L and 47.8% at 100 micromol/L. We suggest that the antitumor and antimetastatic activities of resveratrol might be due to the inhibition of DNA synthesis in LLC cells and the inhibition of LLC-induced neovascularization and **tube formation (angiogensis)** of HUVEC by resveratrol. [6]

Lung cancer inhibition and increases effectiveness of Taxol

Resveratrol was studied on its effect on proliferation and inducing apoptosis in three lung cancer cell lines (A549, EBC-1, Lu65). Resveratrol inhibited the growth of A549, EBC-1 and Lu65 lung cancer cells by 50% (ED50) at concentrations between 5-10 microM. We also examined the combined effects in these cells of **resveratrol and paclitaxel**, an essential chemotherapeutic agent against lung cancer. Although simultaneous exposure to resveratrol plus paclitaxel did not result in significant synergy, resveratrol (10 microM, 3 days) significantly enhanced the subsequent antiproliferative effect of paclitaxel. In addition, resveratrol as well as paclitaxel induced apoptosis in EBC-1 and Lu65 cells, as measured by TUNEL and caspase assays, as well as flow cytometry. Resveratrol (10 microM, 3 days) similarly enhanced the subsequent apoptotic effects of paclitaxel. We examined the effects of resveratrol and paclitaxel on levels of p21waf1, p27kip1, E-cadherin, EGFR and Bcl-2 in EBC-1 cells. Resveratrol (10 microM, 3 days) prior to paclitaxel **induced p21waf1 expression approximately 4-fold**. These results suggest that resveratrol may be a promising alternative therapy for lung cancer and that lung cancer cells exposed to resveratrol have a lowered threshold for killing by paclitaxel. [159]

Lung cancer:

Alimta (Pemetrexed) downregulates ERCC1 expression and enhances cytotoxicity effected by resveratrol in human nonsmall cell lung cancer cells.

The multitargeted antifolate pemetrexed has demonstrated certain clinical activities against nonsmall cell lung cancer (NSCLC). Resveratrol (3,5,4-trihydroxy-trans-stilbene) is a polyphenol found in grapes and other plants and has great potential as a preventative and therapeutic agent due to its anticarcinogenic activity. The efficacy of adding resveratrol to pemetrexed to prolong the survival of patients with NSCLC still remains unclear. The excision repair cross-complementation 1 (ERCC1) is a DNA repair gene coding 5' endonuclease in nucleotide excision repair and is overexpressed in chemo- or radioresistant carcinomas. In this study, resveratrol (10-50 μM) inhibited cell survival in two NSCLC cells, H520 and H1975. Treatment with resveratrol increased ERCC1 messenger RNA and protein levels in a MKK3/6-p38 MAPK signal activation-dependent manner. Furthermore, blocking p38 MAPK activation by SB202190 or knocking down ERCC1 expression by transfection with small interfering RNA of ERCC1 enhanced the cytotoxicity of resveratrol. Combining resveratrol with pemetrexed resulted in a synergistic cytotoxic effect, accompanied with the reduction of phospho-p38 MAPK and ERCC1 protein levels, and a DNA repair capacity. Expression of constitutively active MKK6 (MKK6E) or HA-p38 MAPK vectors significantly rescued the decreased p38 MAPK activity, and restored ERCC1 protein levels and cell survival in resveratrol and pemetrexed cotreated NSCLC cells. In this study, for the first time, we have demonstrated the synergistic effect of combined treatment with resveratrol and pemetrexed in human NSCLC cells through downregulation of the MKK3/6-p38 MAPK-ERCC1 signal, suggesting a potential benefit of combining resveratrol and pemetrexed to treat lung cancer in the future.[298]

Inhibits TGF-β1-induced epithelial-to-mesenchymal transition and suppresses lung cancer invasion and metastasis.

Epithelial-to-mesenchymal transition (EMT) is a cellular process during which epithelial polarized cells become motile mesenchymal-appearing cells, which in turn promotes carcinoma invasion and metastasis. Resveratrol (trans-3,4',5-trihydroxystilbene) is a natural polyphenolic compound found in grapes, red wine and several other plants. Numerous reports in the literature indicate that resveratrol can suppress cancer invasion and metastasis. However, the underlying mechanisms of inhibiting metastasis by resveratrol are complex, not fully elucidated and the subject of intense scientific debate. Despite evidence indicating that EMT can be a target for resveratrol, little is known about the effect of resveratrol on lung cancer cells. Our previous studies demonstrated that TGF-β1 induces EMT to promote lung adenocarcinoma invasion and metastasis. To understand the repressive role of resveratrol in lung cancer invasion and metastasis, we sought to investigate the potential use of resveratrol as an inhibitor of TGF-β1-induced EMT development in A549 lung cancer cells in vitro. Here we show that when A549 cells are treated with TGF-β1 and resveratrol, the latter inhibits the initiation of TGF-β1-induced EMT. Our results show that 20 μM resveratrol increases expression of the epithelial phenotype marker E-cadherin and represses the expression of the mesenchymal phenotype markers, Fibronectin and Vimentin during the initiation of TGF-β1-induced EMT. Resveratrol also inhibits expression of EMT-inducing transcription factors Snail1 and Slug, although the expression of the Twist1 transcription factor remained unchanged. Resveratrol inhibits the TGF-β1-induced increase in cell adhesion, migration and invasion of A549 lung cancer cells. Taken together, our findings provide new evidence that

resveratrol suppresses lung cancer invasion and metastasis in vitro through inhibiting TGF-β1-induced EMT.[296]

INHIBITS METASTASIS

Activates Ceramide Synthesis Pathway: Induces Growth Inhibition and Apoptosis

Here we show that resveratrol can induce growth inhibition and apoptosis in MDA-MB-231, a highly invasive and metastatic breast cancer cell line, in concomitance with a dramatic endogenous increase of growth inhibitory/proapoptotic ceramide. We found that accumulation of ceramide derives from both de novo ceramide synthesis and sphingomyelin hydrolysis. More specifically we demonstrated that ceramide accumulation induced by resveratrol can be traced to the activation of serine palmitoyltransferase (SPT), the key enzyme of de novo ceramide biosynthetic pathway, and neutral sphingomyelinase (nSMase), a main enzyme involved in the sphingomyelin/ceramide pathway. However, by using specific inhibitors of SPT, myriocin and L-cycloserine, and nSMase, gluthatione and manumycin, we found that only the SPT inhibitors could counteract the biological effects induced by resveratrol. Thus, resveratrol seems to exert its growth inhibitory/apoptotic effect on the metastatic breast cancer cell line MDA-MB-231 by activating the de novo ceramide synthesis pathway. [21]

Reduced Hypoxia-inducible factor-1alpha (HIF-1alpha) and VEGF in human tongue squamous cell carcinomas and hepatoma cells

Hypoxia-inducible factor-1alpha (HIF-1alpha) is overexpressed in many human tumors and their metastases, and is closely associated with a more aggressive tumor phenotype. In this study, we investigated the effect of resveratrol, a natural product commonly found in grapes and various other fruits, on hypoxia-induced HIF-1alpha protein accumulation and vascular endothelial growth factor (VEGF) expression in human tongue squamous cell carcinomas and hepatoma cells. Our results showed that resveratrol significantly inhibited both basal level and hypoxia-induced HIF-1alpha protein accumulation in cancer cells, but did not affect HIF-1alpha mRNA levels. Pretreatment of cells with resveratrol significantly reduced hypoxia-induced VEGF promoter activities and VEGF expression at both mRNA and protein levels. The mechanism of resveratrol inhibition of hypoxia-induced HIF-1alpha accumulation seems to involve a gradually shortened half-life of HIF-1alpha protein caused by an enhanced protein degradation through the 26S proteasome system. In addition, resveratrol remarkably inhibited hypoxia-mediated activation of extracellular signal-regulated kinase 1/2 and Akt, leading to a marked decrease in hypoxia-induced HIF-1alpha protein accumulation and VEGF transcriptional activation. Functionally, we observed that resveratrol also significantly inhibited the hypoxia-stimulated invasiveness of cancer cells. These data suggested that HIF-1alpha/VEGF could be a promising drug target for resveratrol in the development of an effective chemopreventive and anticancer therapy in human cancers. [182]

Resveratrol chemosensitizer

Because tumors develop resistance to chemotherapeutic agents, the cancer research community continues to search for effective chemosensitizers. One promising possibility is to use dietary agents that sensitize tumors to the chemotherapeutics. In this review, we discuss that the use of resveratrol can sensitize tumor cells to chemotherapeutic agents. The tumors shown to be sensitized by resveratrol include lung carcinoma, acute myeloid leukemia, promyelocytic leukemia, multiple myeloma, prostate cancer, oral epidermoid carcinoma, and pancreatic cancer. The chemotherapeutic agents include vincristine, adriamycin, paclitaxel, doxorubicin, cisplatin, gefitinib, 5-fluorouracil, velcade, and gemcitabine. The chemosensitization of tumor cells by resveratrol appears to be mediated through its ability to modulate multiple cell-signaling molecules, including drug transporters, cell survival proteins, cell proliferative proteins, and members of the NF-κB and STAT3 signaling pathways. Interestingly, this nutraceutical has also been reported to suppress apoptosis induced by paclitaxel, vincristine, and daunorubicin in some tumor cells. The potential mechanisms underlying this dual effect are discussed. Overall, studies suggest that resveratrol can be used to sensitize tumors to standard cancer chemotherapeutics.[275]

ENHANCES IMMUNE RESPONSE/INHIBITS INFLAMMATORY MEDIATORS

Cell Mediated Cytotoxicity: Inhibits NF-[kappa]B Activation.

Resveratrol has been shown to have anti-inflammatory, antioxidant, and antitumor activities. We have investigated the effect of resveratrol on mitogen/antigen-induced proliferation of splenic lymphocytes, induction of cytotoxic T lymphocytes (CTLs) and lymphokine activated killer (LAK) cells, and the production of the cytokines interferon
(IFN)-[gamma], interleukin (IL)-2, tumor necrosis factor (TNF)-[alpha], and IL-12. We found that mitogen-, IL-2-, or alloantigen-induced proliferation of splenic lymphocytes and the development of antigen-specific CTLs were suppressed significantly at 25-50 [mu]M resveratrol. The generation of LAK cells at similar concentrations was less sensitive to the suppressive effect of resveratrol. The suppression of cell proliferation and CTL generation by resveratrol was not only reversible, but in some cases the response (mitogen/IL-2-induced proliferation and CTL generation) was actually enhanced following pretreatment of cells with resveratrol. Resveratrol blocked the

activation of the transcription factor NF-[kappa]B without affecting basal NF-[kappa]B activity. The latter result suggests that resveratrol inhibits cell proliferation, cell-mediated cytotoxicity, and cytokine production, atleast in part through the inhibition of NF-[kappa]B activation. [11]

Breast cancer: Down-regulation of Bcl-2, NF-kappaB

Resveratrol (RES), a chemopreventive molecule, inhibits the proliferation of tumor cells of different etiologies. We previously showed that RES alters the cell cycle and induces apoptosis in MCF-7 breast tumor cells by interfering with the estrogen receptor (ERaalpha)-dependent phosphoinositide 3-kinase (PI3K) pathway. Here, we analyzed signaling downstream of PI3K, to understand the mechanisms of RES-induced apoptosis. Apoptotic death by RES in MCF-7 was mediated by Bcl-2 downregulation since overexpression of this protein abolished apoptosis. Decreased Bcl-2 levels were not related to cytochrome c release, activation of caspases 3/8 or poly(ADP-ribose) polymerase proteolysis. However, RES decreased mitochondrial membrane potential and increased reactive oxygen species and nitric oxide production. NF-kappaB, a regulator of Bcl-2 expression, and calpain protease activity, a regulator of NF-kappaB, were both inhibited by RES. The patterns for NF-kappaB and calpain activities followed that of PI3K and were inhibited by LY294002. NF-kappaB inhibition coincided with diminished MMP-9 activity and cell migration. These data suggest that RES-induced apoptosis in MCF-7 could involve an oxidative, caspase-independent mechanism, whereby inhibition of PI3K signaling converges to Bcl-2 through NF-kappaB and calpain protease activity. Therefore, Bcl-2 and NF-kappaB could be considered potential targets for the chemopreventive activity of RES in estrogen-responsive tumor cells. [129]

Anti-tumor and anti-angiogenic

The antitumor and antimetastatic actions of resveratrol might be due to the inhibition of tumor-induced angiogenesis. To search for anticancer agents with stronger activity than resveratrol, we examined the antiangiogenic effects of 21 synthetic and/or natural stilbenes. Among these 21 stilbenes, 2,3-, 3,4-, and 4,4'-dihydroxystilbene inhibited the pro-matrix metalloproteinase (pro-MMP)-9 production in colon 26 cells at 5-25 microM, vascular endothelial growth factor (VEGF)-induced human umbilical vein endothelial cell (HUVEC) migration at 10 and 25 microM, and VEGF-induced angiogenesis at 5-50 microM. Resvertarol inhibited the pro-MMP-9 production and VEGF-induced angiogenesis at 25 or 50 microM. Thus, the inhibition of pro-MMP-9 production in colon 26 cells and VEGF-induced angiogenesis by three dihydroxystilbenes were greater than those of resveratrol. The three dihydroxystilbenes (8 mg/kg, intraperitoneal injection) inhibited the tumor-induced neovascularization in colon 26-packed chamber-bearing mice and the tumor growth in colon 26-bearing mice. Furthermore, the three dihydroxystilbenes inhibited VEGF-induced VEGFR-2 phosphorylation. On the other hand, the three dihydroxystilbenes had no effect on VEGFR-1 and-2 expression, and VEGF-induced VEGFR-1 phosphorylation in HUVECs. These findings suggest that the inhibition of tumor-induced neovascularization by these three dihydroxystilbenes may be due to the inhibition of VEGF-induced endothelial cell migration and VEGF-induced angiogenesis through the inhibition of VEGF-induced VEGFR-2 phosphorylation in endothelial cells and pro-MMP-9 expression in colon 26 cells. [232]

Chemopreventive

Anti-inflammatory (COX-2 inhibition)

Resveratrol functions as a plant defense molecule. It inhibits cancer by many mechanisms, including anti-inflammatory activity, anti-oxidant activity and induction of phase II detoxifying enzymes. COX-2 appears to be over-expressed, and correlates very tightly with carcinogenesis in a number of target organs — almost every target organ: head and neck cancer, bladder cancer, colon cancer, pancreatic, breast, and prostate. Dr. Ernest Hawk of the National Cancer Institute in Bethesda, Maryland, said it may be possible to intervene at every stage, from pre-invasive lesions to advanced cancers, in a broad range of organs. Dr. Hawk noted that there is additional work suggesting that over-expression of COX-2 exists among almost every other tumor, in both animal models as well as [in] human settings.

This study investigated for the first time the effects of the cis isomer of resveratrol (c-RESV) on the responses of inflammatory murine peritoneal macrophages, namely on the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during the respiratory burst; on the biosynthesis of other mediators of inflammation such prostaglandins; and on the expression of inflammatory genes such as inducible nitric oxide synthase (NOS)-2 and inducible cyclooxygenase (COX)-2. Treatment with 1-100 micro M c-RESV significantly inhibited intracellular and extracellular ROS production, and c-RESV at 10-100 micro M significantly reduced RNS production. c-RESV at 1-100 micro M was ineffective for scavenging superoxide radicals (O2(*-)), generated enzymatically by a hypoxanthine/xanthine oxidase (XO) system and/or for inhibiting XO activity. However, c-RESV at 10-100 micro M decreased nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate oxidase activity in macrophage homogenates. c-RESV at 100 micro M decreased NOS-2 and COX-2 mRNA levels in lipopolysaccharide/interferon-gamma-treated macrophages. At 10-100 micro M, c-RESV also significantly inhibited

NOS-2 and COX-2 protein synthesis and decreased prostaglandin E2 (PGE2) production. These results indicate that c-RESV at micromolar concentrations significantly attenuates several components of the macrophage response to proinflammatory stimuli (notably, production of O2(*-) and of the proinflammatory mediators NO(*) and PGE2). [71]

Inhibits COX-2 and induces apoptosis and inhibited p53 phosphorylation in breast cancer

Cyclooxygenase-2 (COX-2) is antiapoptotic and is implicated in tumorigenesis. Recent reports, however, have also ascribed a proapoptotic action to inducible COX-2. We show here for the first time that a stilbene, resveratrol, induces nuclear accumulation of COX-2 protein in human breast cancer MCF-7 and MDA-MB-231 cell cultures. The induction of COX-2 accumulation by resveratrol is mitogen-activated protein kinase (MAPK; extracellular signal-regulated kinase 1/2)- and activator protein 1- dependent. Nuclear COX-2 in resveratrol-treated cells colocalizes with Ser(15)-phosphorylated p53 and with p300, a coactivator for p53-dependent gene expression. The interaction of COX-2, p53, and p300, as well as resveratrol-induced apoptosis, was inhibited by a MAPK activation inhibitor, PD98059. A specific inhibitor of COX-2, NS398, and small interfering RNA knockdown of COX-2 were associated with reduced p53 phosphorylation and consequent decrease in p53-dependent apoptosis in resveratrol-treated cells. We conclude that nuclear accumulation of COX-2 can be induced by resveratrol and that the COX has a novel intranuclear colocalization with Ser(15)-phosphorylated p53 and p300, which facilitates apoptosis in resveratrol-treated breast cancer cells. [169]

Inhibits Head and Nck cancer: Down-regulates COX-2/p53

UMSCC-22B cells treated with resveratrol for 24 h, with or without selected inhibitors, were examined: (1) for the presence of nuclear activated ERK1/2, p53 and COX-2, (2) for evidence of apoptosis, and (3) by chromatin immunoprecipitation to demonstrate p53 binding to the p21 promoter. Stilbene-induced apoptosis was concentration-dependent, and associated with ERK1/2 activation, serine-15 p53 phosphorylation and nuclear accumulation of these proteins. These effects were blocked by inhibition of either ERK1/2 or p53 activation. Resveratrol also caused p53 binding to the p21 promoter and increased abundance of COX-2 protein in UMSCC-22B cell nuclei. Resveratrol-induced nuclear COX-2 accumulation was dependent upon ERK1/2 activation, but not p53 activation. Activation of p53 and p53-dependent apoptosis were blocked by the COX-2 inhibitor, NS398, and by transfection of cells with COX-2-siRNA. In UMSCC-22B cells, resveratrol-induced apoptosis and induction of nuclear COX-2 accumulation share dependence on the ERK1/2 signal transduction pathway. Resveratrol-inducible nuclear accumulation of COX-2 is essential for p53 activation and p53-dependent apoptosis in these cancer cells. [215]

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Myricetin, another major flavonol in red wine, down-regulates COX-2/NF-kB

Other phytochemicals might contribute to the cancer-preventive activities of red wine, and the flavonol content of red wines is about 30 times higher than that of resveratrol. Here we report that 3,3',4',5,5',7-hexahydroxyflavone (myricetin), one of the major flavonols in red wine, inhibits 12-O-tetradecanoylphorbol-13-acetate (phorbol ester)-induced COX-2 expression in JB6 P+ mouse epidermal (JB6 P+) cells by suppressing activation of nuclear factor kappa B (NF-kappaB). Myricetin at 10 and 20 microM inhibited phorbol ester-induced upregulation of COX-2 protein, while resveratrol at the same concentration did not exert significant effects. The phorbol ester-induced production of prostaglandin E 2 was also attenuated by myricetin treatment. Myricetin inhibited both COX-2 and NF-kappaB transactivation in phorbol ester-treated JB6 P+ cells, as determined using a luciferase assay. Myricetin blocked the phorbol ester-stimulated DNA binding activity of NF-kappaB, as determined using an electrophoretic mobility shift assay. Moreover, TPCK (N-tosyl-l-phenylalanine chloromethyl ketone), a NF-kappaB inhibitor, significantly attenuated COX-2 expression and NF-kappaB promoter activity in phorbol ester-treated JB6 P+ cells. In addition, red wine extract inhibited phorbol ester-induced COX-2 expression and NF-kappaB transactivation in JB6 P+ cells. Collectively, these data suggest that myricetin contributes to the chemopreventive effects of red wine through inhibition of COX-2 expression by blocking the activation of NF-kappaB. [216]

Down-regulates COX-2 60-80% in cancer cells

Research findings suggest that resveratrol (RSVL; 3,5,4'-trihydroxy-trans-stilbene) demonstrates nonselective COX-2 inhibition. We report herein that RSVL directly binds with COX-2 and this binding is absolutely required for RSVL's inhibition of the ability of human colon adenocarcinoma HT-29 cells to form colonies in soft agar. Binding of COX-2 with RSVL was compared with two RSVL analogues, 3,3',4',5',5-pentahydroxy-trans-stilbene (RSVL-2) or 3,4',5-trimethoxy-trans-stilbene (RSVL-3). The results indicated that COX-2 binds with RSVL-2 more strongly than with RSVL, but does not bind with RSVL-3. RSVL or RSVL-2, but not RSVL-3, inhibited COX-2-mediated PGE(2) production in vitro and ex vivo. HT-29 human colon adenocarcinoma cells express high levels of COX-2 and either RSVL or RSVL-2, but not RSVL-3, suppressed anchorage independent growth of these cells in soft agar. RSVL or RSVL-2 (not RSVL-3) suppressed growth of COX-2(+/+) cells by 60% or 80%, respectively. Notably, cells deficient in COX-2 were unresponsive to RSVL or RSVL-2. These data suggest that the anticancer effects of RSVL or RSLV-2 might be mediated directly through COX-2. [217]

Phytoestrogenic: A Chemopreventive Agent for Breast Cancer

Recent studies performed with MCF-7 human breast cancer cells have demonstrated superestrogenic effects with resveratrol. In contrast, studies performed using estrogen receptor-transfected cell lines have shown that resveratrol acts as a mixed agonist/antagonist. The major objective of this study was to characterize the estrogen-modulatory effects of resveratrol in a variety of in vitro and in vivo mammary models. Thus, the effect of resveratrol alone and in combination with 17beta-estradiol (E2) was assessed with MCF-7, T47D, LY2, and S30 mammary cancer cell lines. With cells transfected with reporter gene systems, the activation of estrogen response element-luciferase was studied, and using Western blot analysis, the expression of E2-responsive progesterone receptor (PR) and presnelin 2 protein was monitored. Furthermore, the effect of resveratrol on formation of preneoplastic lesions (induced by 7,12-dimethylbenz(a)anthracene) and PR expression (with or without E2) was evaluated with mammary glands of BALB/c mice placed in organ culture. Finally, the effect of p.o.administered resveratrol on N-methyl-N-nitrosourea-induced mammary tumors was studied in female Sprague Dawley rats. As a result, in transient transfection studies with MCF-7 cells, resveratrol showed a weak estrogenic response, but when resveratrol was combined with E2 (1 nM), a clear dose-dependent antagonism was observed. Similar mixed estrogenic/antiestrogenic effects were noted with S30 cells, whereas resveratrol functioned as a pure estrogen antagonist with T47D and LY2 cells. Furthermore, in MCF-7 cells, resveratrol induced PR protein expression, but when resveratrol was combined with E2, expression of PR was suppressed. With T47D cells, resveratrol significantly down-regulated steady-state and E2-induced protein levels of PR. With LY2 and S30 cells, resveratrol down-regulated presnelin 2 protein expression. Using the mouse mammary organ culture model, resveratrol induced PR when administered alone, but expression was suppressed in the presence of E2 (1 nM). Furthermore, resveratrol inhibited the formation of estrogen-dependent preneoplastic ductal lesions induced by 7,12-dimethylbenz(a)anthracene in these mammary glands (IC50 = 3.2 microM) and reduced N-methyl-N-nitrosourea-induced mammary tumorigenesis when administered to female Sprague Dawley rats by gavage. Therefore, in the absence of E2, resveratrol exerts mixed estrogen agonist/antagonist activities in some mammary cancer cell lines, but in the presence of E2, resveratrol functions as an antiestrogen. In animal models, carcinogen-induced preneoplastic lesions and mammary tumors are inhibited by resveratrol. These data suggest that resveratrol may have beneficial effects if used as a chemopreventive agent for breast cancer. [5]

Breast cancer: VEGF inhibition/increases apoptosis

Resveratrol has been shown to induce transcription via both ERa and ERb. We observed significantly lower tumor growth, decreased angiogenesis, and increased apoptotic index in ERaK ERbC MDA-MB-231 tumors in resveratrol-treated nude mice compared with controls. In vitro we found a significant increase in apoptosis in resveratrol-treated MDA-MB-231 cells in addition to significantly reduced extracellular levels of VEGF. This study supports the potential use of resveratrol as a chemotherapeutic agent in breast cancers. [163]

Colon cancer: Down-regulates telomerase

A number of studies performed in our laboratory and elsewhere, showed that resveratrol is able to prevent carcinogenesis and to impair tumor growth and progression. In order to provide additional information on the pleiotropic effects of resveratrol on malignant cells, the present study was performed to test the in vitro influence of the compound on the growth and TLMA of HT-29 and WiDr human colon cancer cell lines. The results confirmed that resveratrol has a direct, dose dependent, inhibitory effect on cell proliferation in both lines. In addition, for the first time, relatively high concentrations of this compound were found to be able to substantially down-regulate telomerase activity. These preliminary results further support the potential role of resveratrol in chemoprevention/chemotherapy of human colon tumor cells and provide the rational basis for novel strategies in cancer control. [170]

Prostate cancer: Anti-Proliferative Effect: Inhibition of D-type Cyclins and (Cdk) 4 expression

Resveratrol may have potential for the prevention and treatment of human cancer. Resveratrol inhibits the growth of human prostate carcinoma DU145 cells. Resveratrol treatment in DU145 cells resulted in a dose-dependent inhibition of cell growth and induced apoptotic cell death. The anti-proliferative effect of resveratrol was associated with the inhibition of D-type cyclins and cyclin-dependent kinase (Cdk) 4 expression, and the induction of tumor suppressor p53 and Cdk inhibitor p21. Moreover, the kinase activities of cyclin E and Cdk2 were inhibited by resveratrol without alteration of their protein levels. Resveratrol treatment also up-regulated the Bax protein and mRNA expression in a dose-dependent manner; however, Bcl-2 and Bcl-xL levels were not significantly affected. These effects were found to correlate with an activation of caspase-3 and caspase-9. Taken together, our study suggests that resveratrol has a strong potential for development as an agent for the prevention of human prostate cancer. [34]

Cytotoxic against Prostate Cancer both androgen dependant and independant: Gene protein regulation

The molecular mechanism of resveratrol-induced apoptosis and proliferation arrest in prostate derived cells PZ-

HPV-7 (non-tumorigenic line), LNCaP (androgen-sensitive cancer line) and PC-3 (androgen-insensitive cancer line) was studied. Apoptosis and cell cycle distribution were evaluated by flow cytometry and proliferation by MTT assay and direct cell counting. Caspases, bax, bcl-2, cyclins, Cdks, p53, p21 and p27 were measured by western blot and kinase activities of cyclin/Cdk complexes by immunoprecipitation followed by kinase assays with appropriate substrates. Resveratrol induced a decrease in proliferation rates and an increase in apoptosis in cancer cell lines in a dose- and time-dependent manner. These effects were coincident with cell accumulation at the G0/G1 phase. In LNCaP and PC-3, the apoptosis induced by resveratrol was mediated by activation of caspases 9 and 3 and a change in the ratio of bax/bcl-2. Expressions of cyclin D1, E and Cdk4 as well as cyclin D1/Cdk4 kinase activity were reduced by resveratrol only in LNCaP cells. In contrast, cyclin B and Cdk1 expression and cyclin B/Cdk1 kinase activity were decreased in both cell lines in presence of resveratrol. However, modulator proteins p53, p21 and p27 were increased by resveratrol only in LNCaP cells. These effects probably result in the observed proliferation arrest and disruption of cell cycle control. In addition, the specific differences found between LNCaP and PC-3 suggest that resveratrol acts through different mechanisms upon the androgen or estrogen receptor cell status. [178]

Activates p27 and PTEN

PTEN mutations have been observed in a broad range of human cancers, especially those of the breast. Active PTEN results in decreased phosphorylation of Akt and MAPK, the up-regulation of p27 and down-regulation of cyclin D1 protein levels resulting in decreased proliferation and an increase in apoptosis. MCF-7. When MCF-7 cells were stimulated with resveratrol (also quercetin and genestein) there was an increase in PTEN protein levels. Concomitantly, resveratrol stimulation resulted in decreased Akt phosphorylation and an increase in p27 protein levels, indicating active PTEN lipid phosphatase activity. In contrast, MAPK phosphorylation and cyclin D1 levels, which are regulated by PTEN's protein phosphatase activity, were not altered. This data provide evidence that a mechanism for resveratrol's protective nature is partially through increased PTEN expression, which provides a novel target for the regulation of PTEN expression and suggests that dietary changes may be adjunctive to traditional preventive and therapeutic strategies against breast cancer. [179]

Activates Anticancer Enzyme CYP1B1

Previous studies have pointed to resveratrol's cancer-fighting action, but this is the first time that scientists have gained an insight into the way the chemical's anti-cancer properties work. The researchers found that resveratrol is processed by an enzyme called CYP1B1, which is found on a variety of different types of tumors. This enzyme converts resveratrol into a toxic product called piceatannol. Previous research has shown that this process is restricted to the tumor itself, limiting the toxicity to the cancer cells and serving to selectively destroy them. The enzyme CYP1B1 has until recently been considered a cause of cancer because it is only found in tumors and not in healthy tissue.

However, far from causing cancer, scientists now think the enzyme is there to fight it. Resveratrol might be beneficial having cancer- preventative properties. This research shows just how it could prevent tumors developing by producing these anti-cancer molecules within the cancer cells themselves." [73]

Prostate cancer: Down-regulates AR and ER receptors, markedly decreased PKB/AKT

Using androgen receptor (AR)-positive LNCaP and oestrogen receptor alpha (ERalpha)-expressing PC-3 prostate tumour cells, we have analysed whether the antiproliferative activity of Resveratrol (RES) takes place by inhibition of the AR- or ERalpha-dependent PI3K pathway. Although RES treatment (up to 150 muM) decreased AR and ERalpha protein levels. Immunoprecipitation and kinase assays showed that RES inhibited AR- and ERalpha-dependent PI3K activities in LNCaP and PC-3, respectively. Consistently, lower PI3K activities correlated with decreased phosphorylation of downstream targets protein kinase B/AKT (PKB/AKT) and glycogen synthase kinase-3 (GSK-3). GSK-3 dephosphorylation could be responsible for the decreased cyclin D1 levels observed in both cell lines. Importantly, RES markedly decreased PKB/AKT phosphorylation in primary cultures from human prostate tumours, suggesting that the mechanism proposed here could take place in vivo. Thus, RES has antitumoral activity in androgen-sensitive and androgen-non-sensitive human prostate tumours by inhibiting survival pathways such as that mediated by PI3K. [193]

Breast cancer: Normalizes gene expression

Resveratrol, through its phytoestrogenic properties it regulates the expression of hormone-dependent genes, such as the oncosuppressor BRCA1, in breast cells. This gene is involved in the majority of hereditary breast cancer, as well as sporadic cancers. Methods: We used three human breast tumor cell lines (HBL100, MCF7 and MBA-MB-231) and one breast cell line (MCF10a) derived from a fibrocystic disease to study in vitro the effect of resveratrol on the transcription of a group of genes whose proteins interact in different pathways with BRCA1. BRCA1, BRCA2, ER alpha, ER beta, p53, p21(waf1/cip1), CBP/P300, RAD51, pS2 and Ki67 mRNA were quantified using real-time quantitative RT-PCR with an ABI 7700 apparatus. Results: Resveratrol modulated the expression of these genes in a pattern dependent on the status of alpha and beta estrogen receptors. These results show that resveratrol

regulates gene expression via the estrogen receptor pathway as well as other pathways. Thus, resveratrol seems to have an effect on breast tumor cell lines, on a fibrocystic cell line by affecting several factors regulating the function of BRCA1. [95]

Cancer Prevention Study Started

Scientists in Britain and the US announced plans to study a possible new cancer prevention drug based on resveratrol.

Researchers at the University of Leicester in England and the University of Michigan will begin testing tablets of pure resveratrol in healthy volunteers early next year, the British university said in a statement. The work is being funded by the US National Cancer Institute (NCI). Since resveratrol may be of value in preventing cancer, the NCI [is] funding early clinical studies of pure resveratrol capsules in healthy volunteers and patients with early cancer. The 20 or so healthy volunteers will initially be given one tablet containing 0.5 grams of resveratrol--equivalent to the amount in dozens and dozens of bottles of wine, Leicester researcher Professor Andreas Gescher told Reuters Health. Later trials will look at repeated doses.

The point of these preliminary studies is to analyse how long the compound stays in the body and how much circulates in the blood. The researchers will also look for evidence of biochemical changes that might suggest a protective effect. Several studies have found that wine drinkers seem to be less likely to develop cancer. Resveratrol has been suggested as one possible reason, but the benefits of wine may be due to a combination of compounds rather than one. [18]

Resveratrol prevents epigenetic silencing of BRCA-1 by the aromatic hydrocarbon receptor in human breast cancer cells.

The BRCA-1 protein is a tumor suppressor involved in repair of DNA damage. Epigenetic mechanisms contribute to its reduced expression in sporadic breast tumors. Through diet, humans are exposed to a complex mixture of xenobiotics and natural ligands of the aromatic hydrocarbon receptor (AhR), which contributes to the etiology of various types of cancers. The AhR binds xenobiotics, endogenous ligands, and many natural dietary bioactive compounds, including the phytoalexin resveratrol (Res). In estrogen receptor- alpha (ER alpha)-positive and BRCA-1 wild-type MCF-7 breast cancer cells, we investigated the influence of AhR activation with the agonist 2,3,7,8 tetrachlorobenzo(p)dioxin (TCDD) on epigenetic regulation of the BRCA-1 gene and the preventative effects of Res. We report that activation and recruitment of the AhR to the BRCA-1 promoter hampers 17 beta -estradiol (E2)-dependent stimulation of BRCA-1 transcription and protein levels. These inhibitory effects are paralleled by reduced occupancy of ER alpha , acetylated histone (AcH)-4, and AcH3K9. Conversely, the treatment with TCDD increases the association of mono-methylated- H3K9, DNA-methyltransferase-1 (DNMT1), and methyl-binding domain protein-2 with the BRCA-1 promoter and stimulates the accumulation of DNA strand breaks. The AhR-dependent repression of BRCA-1 expression is reversed by small interference for the AhR and DNMT1 or pretreatment with Res, which reduces TCDD-induced DNA strand breaks. These results support the hypothesis that epigenetic silencing of the BRCA-1 gene by the AhR is preventable.[266]

Cytotoxic to Adriamycin resistant breast cancer cells

To supply the scientific basis of research and development of the medicinal value of Polygonum cuspidatum. Resveratrol (trans and cis) was isolated from the roots of Polygonum cuspidatum by cytotoxicity based fractionation and identified by HPLC-MS, UV scanning and IR. The inhibition and morphology of L-02, Hep G2, SHZ-888, MCF-7, MCF-7/ADM cells growth caused by Resveratrol. Resveratrol could specifically inhibit proliferation of many cancer cells but not human normal liver cell. Resveratrol was cytotoxic to adriamycin-resistant MCF-7 cell in virtro. Resveratrol is a new anticancer composition, which has no toxicity and a high efficiency. [177]

Breast cancer Inhibition by Red wine flavonoides

Red wine is a rich source of polyphenolic components such as anthocyanins and flavonoids. The inhibitory effects of red wine polyphenolics on human breast cancer cells have been demonstrated earlier, but their effects on normal cells have not been fully established. Red wine (Merlot) was fractionated by hydrophobic interaction chromatography and different flavonoid fractions with increasing hydrophobicity were obtained. These fractions were tested for their inhibitory effect on human breast cancer cells (MCF-7), normal human mammary epithelial cells (HMEC), and a non-tumorigenic MCF-10A cell line. By contrast to the authentic flavonoids such as quercetin, naringenin and catechin which inhibited the growth of HMEC much more than that of MCF-7 cancer cells, a red wine fraction, that was comprised mainly of the flavonoid aglycones, showed maximal inhibition of the growth of breast cancer cells, with relatively low cytotoxicity towards HMEC and MCF-10A cells. In the presence of this flavonoid fraction, the normal cells grew normally, whereas the breast cancer cells underwent a change in morphology into spherical forms. Cytotoxicity analyses suggested that these cells had become apoptotic. The efficiency of inhibition of cell proliferation by various flavonoid fractions appeared to be related to their inhibition of calcium and calmodulin-promoted phosphodiesterase activity, suggesting that flavonoids may interfere with

calcium second messenger function. [108]

Anti-aromatase activity

Estrogen synthesized in situ plays a more important role in breast cancer cell proliferation than does circulating estrogen. Aromatase is the enzyme that converts androgen to estrogen and is expressed at a higher level in breast cancer tissue than in surrounding noncancer tissue. A promising route of chemoprevention against breast cancer may be through the suppression of in situ estrogen formation using aromatase inhibitors. A diet high in fruits and vegetables may reduce the incidence of breast cancer, because they contain phytochemicals that can act as aromatase inhibitors. Grapes and wine contain potent phytochemicals that can inhibit aromatase. The results of a recent study suggest that red wine or red wine extract may be a chemopreventive diet supplement for postmenopausal women who have a high risk of breast cancer. [109]

Inhibits Breast Cancer – Anti-aromatase activity

The results of another study indicate that polyphenols in grape leaf decrease cell proliferation in a dose- and a time-dependant manner. In hormone sensitive cell lines, a specific interaction of each polyphenol with steroid receptors was observed, with IC(50)s lower than previously described. Interaction of polyphenols with steroid receptors cannot fully explain their inhibitory effect on cell proliferation. In addition, discrete antioxidant action on each cell line was detected under the same concentrations, both by modifying the toxic effect of H(2)O(2), and the production of reactive oxygen species (ROS), after phorbol ester stimulation. Our results suggest that low concentrations of polyphenols, and consecutively, consumption of wine, or other polyphenol-rich foods and beverages, could have a beneficial antiproliferative effect on breast cancer cell growth. [110]

Multi-target anti-cancer effects – modulate signal-transduction pathways

Apoptosis or programmed cell death is a key regulator of tissue homeostasis during normal development and also in adult organism under various conditions including adaptive responses to cellular stress. For example, tissue homeostasis is maintained by tight control of signaling events regulating cell death and survival. Thus, uncontrolled proliferation or failure to undergo cell death is involved in pathogenesis and progression of many human diseases, for example in tumorigenesis or in cardiovascular disorders. Moreover, current cancer therapies primarily act by triggering apoptosis programs in cancer cells. THERAPEUTIC APPLICATIONS: Natural products such as resveratrol have gained considerable attention as cancer chemopreventive or cardioprotective agents and also because of their antitumor properties. Among its wide range of biological activities, resveratrol has been reported to interfere with many intracellular signaling pathways, which regulate cell survival or apoptosis. [190]

Increases endothelial progenitor cells

Recent studies have shown that resveratrol increased endothelial progenitor cells (EPCs) numbers and functional activity by activation of telomerase through the PI3K-Akt signaling pathway. Resveratrol protects EPCs against dysfunction induced by pathological factors in vivo and improve EPC functional activities in a way that is important for cell homeostasis. [234]

Activates Telomerase reparing mutant p53

Resveratrol activates, continuous telomerase activity in immortalization of mixed progenitor cells with mutant p53, repairing mutant cells, which can inhibit the initiation and progression of cancer subtypes. [235]

Grape consumption reduces prostate cancer by 50%

The study by scientists at the Fred Hutchinson Cancer Research Center, Seattle, US, found that men who consumed four or more glasses of red wine per week reduced their risk of prostate cancer by 50 per cent. Among men who consumed four or more 4-ounce glasses of red wine per week, there was a 60 per cent lower incidence of the more aggressive types of prostate cancer, The more clinically aggressive prostate cancer is where the strongest reduction in risk was observed. [122]

Red Wine consumption reduces lung cancer

The researchers from the University of Santiago de Compostela in Spain suggest that the benefits may be down to certain substances in red wine such as tannins, which have antioxidant properties, and resveratrol, shown to stifle tumor development and growth.

Dr Ruano-Ravina's team assessed the lifestyles of 132 patients with lung cancer and 187 patients requiring minor surgery at the same hospital in north-west Spain between 1999 and 2000. Most of the patients were men and in their early 60s. Everyone was asked about their diet, smoking habits, occupation, and the type and quantity of alcohol they drank every day, including whether they drank red, white, or rosé wine. One in four of the cancer patients did not drink, compared with almost one in five of the routine surgery patients. Patients with lung cancer also drank more spirits, but beer consumption was roughly the same. Both groups drank similar amounts of wine at around 3.5 glasses a day, but just over a third of the lung cancer patients drank red wine compared with over half of the other patients. Compared with non-drinkers, each daily glass of red wine afforded 13 per cent protection against lung cancer. Neither beer nor sprits seemed to affect the development of cancer.

The results held true even after taking account of the amount of tobacco smoked, job type, and the total quantity of alcohol consumed. Other studies have already demonstrated the protective effect of red wine against cancer, including ovarian and digestive cancers. [124]

Suppression of IL-8 gene transcription: Mediated, in part, through AP-1 inhibition

Resveratrol has promising anti-inflammatory and anticancer effects. To observe the modulation of interleukin-8 (IL-8) production in human monocytic cells by resveratrol and explore its mechanism at the gene transcription level, U937 cells were stimulated with phorbol 12-myristate 13-acetate (PMA) for 24h. IL-8 protein in supernatants was measured by radioimmunoassay. The cytotoxicity of PMA, dexamethasone and resveratrol was accessed by MTT cell proliferation assay. The RNA level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and IL-8 were detected by RT-PCR using specific primers. DNA binding activities of NF-kappaB and AP-1 were examined by electrophoretic mobility shift assay (EMSA). 0.01-100 nM PMA could significantly induce IL-8 production in U937 cells; 10 microM Dexamethasone and 10, 1, 0.1 microM resveratrol could inhibit PMA-induced IL-8 protein production and mRNA accumulation. The cytotoxicity did not contribute to their inhibitory effect. The DNA binding activity of AP-1 was inhibited by dexamethasone and resveratrol, but resveratrol has little effect on PMA-induced NF-kappaB activation. Resveratrol could inhibit PMA-induced IL-8 production in U937 cells at protein and mRNA levels. The suppression of IL-8 gene transcription by resveratrol was, at least partly, due to inhibition of AP-1 activation. [66]

Radio-sensitizing: Increases the effects of radiation on cancer cells

A search for new agents that can sensitize cancer cells to ionizing radiation is of continual interest and mainly due to the use of radiation in cancer therapy. Resveratrol, a powerful antioxidant has been shown to inhibit carcinogenesis in animal models. The purpose of this study was to examine whether resveratrol can sensitize cancer cells to X-irradiation. The human cancer cell lines examined were HELA (cervix carcinoma), K-562 (chronic myeloid leukemia) and IM-9 (multiple myeloma). The assays that were performed following X-irradiation (doses from 0 to 8 Gy) and/or incubation in the presence of resveratrol (concentrations ranging from 0 to 200 microM), were the following: trypan blue exclusion test to determine cell viability, cell morphology after May-Grunwald Giemsa staining, DNA profile analysis by flow cytometry to assess cell cycle distribution and the presence of the sub-G1 peak. The cell lines showed different radiation sensitivity (IM-9, high radiation sensitivity, K-562, intermediate radiation sensitivity and HELA, low radiation sensitivity) as seen by the X-irradiation dose related inhibition of cell growth and induction of apoptosis. The addition of resveratrol alone to the cell cultures induced apoptosis and inhibited cell growth from 50 (IM-9), 100 (EOL-1) or 200 microM (HELA) resveratrol concentrations. Concomitant treatment of the cells with either resveratrol and X-irradiation induced a synergistic effect at the highest dose of 200 microM. These results show that resveratrol can act as a potential radiation sensitizer. [98]

Protects normal cells from radiation damage while sensitizing radiation to cancer cells

The photoprotective effects of resveratrol, against UVB exposure-mediated damages in vitro and in vivo has been well astabolished. In addition, recently studies have shown that resveratrol can act as a sensitizer to enhance the therapeutic effects of ionizing radiation against cancer cells. Based on available literature, we suggest that resveratrol may be useful for (1) prevention of UVB-mediated damages including skin cancer and (2) enhancing the response of radiation therapies against hyperproliferative, precancerous and neoplastic conditions. [214]

Enhanced effect of resveratrol in combination with irradiation and chemotherapy: study using Merkel cell carcinoma cell lines.

BACKGROUND AND PURPOSE:

Merkel cell carcinoma (MCC) is a rare, but highly malignant tumor of the skin. In case of systemic disease, possible therapeutic options include irradiation or chemotherapy. The aim of this study was to evaluate whether the flavonoid resveratrol enhances the effect of radiotherapy or chemotherapy in MCC cell lines.

MATERIALS AND METHODS:

The two MCC cell lines MCC13 and MCC26 were treated with increasing doses of resveratrol. Combination experiments were conducted with cisplatin and etoposide. Colony forming assays were performed after sequential irradiation with 1, 2, 3, 4, 6, and 8 Gy and apoptosis was assessed with flow cytometry. Expression of cancer drug targets was analyzed by real-time PCR array.

RESULTS:

Resveratrol is cytotoxic in MCC cell lines. Cell growth is inhibited by induction of apoptosis. The combination with cisplatin and etoposide resulted in a partially synergistic inhibition of cell proliferation. Resveratrol and irradiation led to a synergistic reduction in colony formation compared to irradiation alone. Evaluation of gene expression did

not show significant difference between the cell lines.

CONCLUSION:
Due to its radiosensitizing effect, resveratrol seems to be a promising agent in combination with radiation therapy. The amount of chemosensitizing depends on the cell lines tested.[306]

Chemopreventive: inhibits chemically induced carcinogenesis

Mammographically dense breast tissue is a strong predictor of breast cancer risk, and is influenced by both mitogens and mutagens. One enzyme that is able to affect both the mitogenic and mutagenic characteristics of estrogens is cytochrome P450 1A2 (CYP1A2), which is principally responsible for the metabolism of 17É¿-estradiol. [106]

Resveratrol has in several in vitro and in vivo studies demonstrated cancer chemopreventive and chemotherapeutic potential. We investigated the in vitro effect of resveratrol on benzo[a]pyrene (B[a]P) -induced DNA adducts in human bronchial epithelial cells. This was compared to the effect of resveratrol on the expression of the cytochrome P450 (CYP) genes CYP1A1 and CYP1B1 and the formation of B[a]P metabolites. Exposure of BEAS-2B and BEP2D cells to B[a]P and increasing concentrations of resveratrol resulted in a dose- and time-dependent inhibition of DNA adduct formation quantified by (32)P-post-labeling. Supporting this result, resveratrol was shown to inhibit CYP1A1 and CYP1B1 gene expression, as measured by real-time reverse transcriptase-polymerase chain reaction. Also, a significant correlation was found between the number of DNA adducts and the mRNA levels of these genes. Using HPLC analysis, a concomitant decrease in the formation of B[a]P-derived metabolic products was detected. In conclusion, this data supports the use of resveratrol as a chemopreventive agent in polycyclic aromatic hydrocarbon-induced carcinogenesis. [100]

Blocks HIF-1alpha and VEGF

Lysophosphatidic acid (LPA) is a bioactive phospholipid that is involved in various cellular events, including tumor invasion and metastasis. In the present study, we investigated the effects of LPA and hypoxia on HIF-1alpha and VEGF expression, as well as the effect of resveratrol on LPA and hypoxia-induced HIF-1alpha and VEGF expression and human ovarian cancer cell migration. Our results show that LPA treatment under hypoxia increases HIF-1alpha protein level, which leads to increased expression of VEGF protein and mRNA. These increases in HIF-1alpha and VEGF expression are dramatically attenuated by resveratrol. The underlying mechanism of inhibition of HIF-1alpha expression by resveratrol seems to be associated with both inactivation of p42/p44 MAPK and p70S6K, as well as enhanced degradation of HIF-1alpha protein, resulting in profound decrease in VEGF expression and cell migration. Collectively, these results show that LPA under hypoxic condition enhances cell migration through the sequential induction of HIF-1alpha and VEGF, and that this enhancement is efficiently blocked by resveratrol. [202]

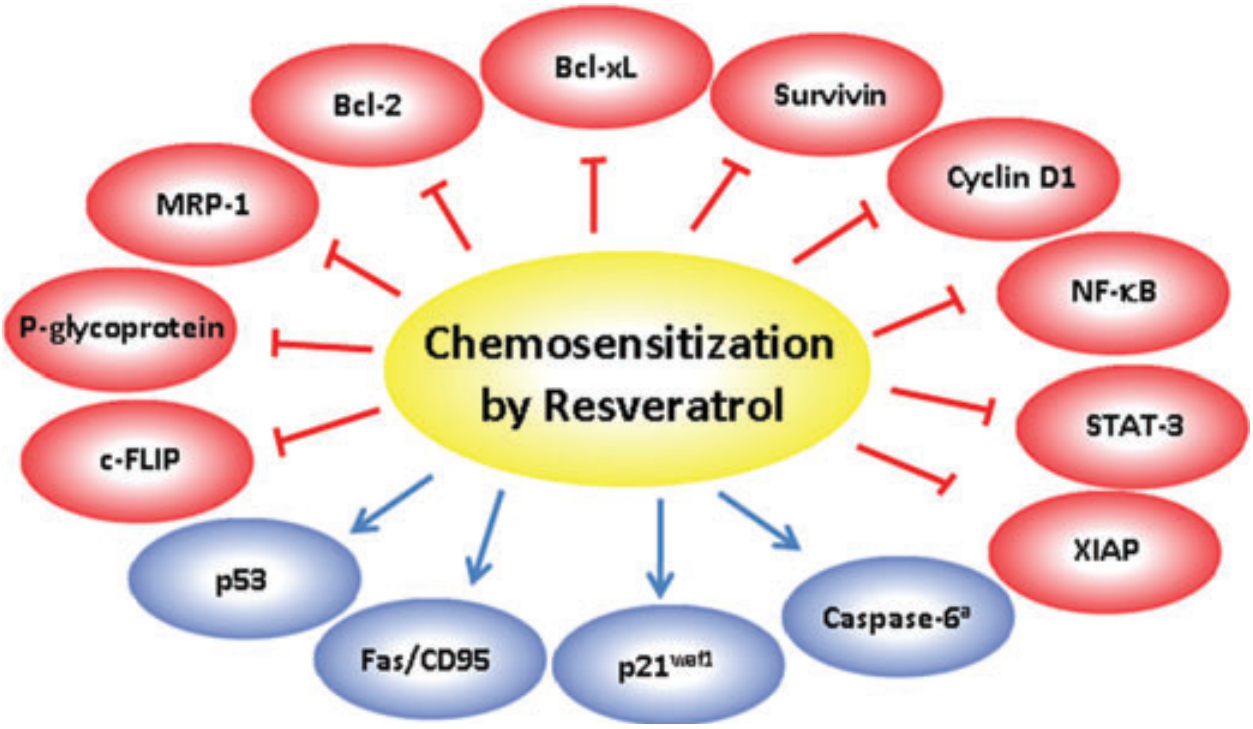
Protection from cisplatin induced kidney damage

Cisplatin is an antitumor drug widely used in the treatment of many malignant tumors. However, the most common adverse effect, nephrotoxicity, limits the use of this drug in many cancer patients. Resveratrol is a phytoalexin that presents highly efficient protection in experimental nephrotoxicity models. This study evaluated the effect of resveratrol on cisplatin-induced kidney damage. Male Wistar rats were treated with resveratrol (25 mg/kg b.w., i.p.) before the administration of cisplatin (5 mg/kg b.w., i.p.) and killed 2 or 5 days later. Blood and urine samples were collected and the kidneys were removed. Rats from the cisplatin group showed acute tubular cell necrosis and increased immunostaining for ED1 (macrophages/monocytes) and T-lymphocytes in the renal cortex and outer medulla when compared with the control group. These alterations were less intense in animals pre-treated with resveratrol. Moreover, indicators of renal injury such as increased serum creatinine levels, urinary volume and urinary protein caused by the administration of cisplatin, were also significantly reduced with resveratrol. Increased lipid peroxidation and glutathione depletion in tissue were attenuated by resveratrol. In conclusion, resveratrol attenuated the cisplatin-induced structural and functional renal changes by reducing free radicals and inhibiting inflammatory cell infiltrates. [203]

Chemosensitization of tumors by resveratrol.

Because tumors develop resistance to chemotherapeutic agents, the cancer research community continues to search for effective chemosensitizers. One promising possibility is to use dietary agents that sensitize tumors to the chemotherapeutics. In this review, we discuss that the use of resveratrol can sensitize tumor cells to chemotherapeutic agents. The tumors shown to be sensitized by resveratrol include lung carcinoma, acute myeloid leukemia, promyelocytic leukemia, multiple myeloma, prostate cancer, oral epidermoid carcinoma, and pancreatic cancer. The chemotherapeutic agents include vincristine, adriamycin, paclitaxel, doxorubicin, cisplatin, gefitinib, 5-fluorouracil, velcade, and gemcitabine. The chemosensitization of tumor cells by resveratrol appears to be mediated through its ability to modulate multiple cell-signaling molecules, including drug transporters, cell survival proteins,

cell proliferative proteins, and members of the NF-κB and STAT3 signaling pathways. Interestingly, this nutraceutical has also been reported to suppress apoptosis induced by paclitaxel, vincristine, and daunorubicin in some tumor cells. The potential mechanisms underlying this dual effect are discussed. Overall, studies suggest that resveratrol can be used to sensitize tumors to standard cancer chemotherapeutics.[284]



Mechanism of chemosensitization of tumors by resveratrol. Resveratrol sensitizes tumor cells to chemotherapeutic agents by targeting proteins involved in cell survival, cell proliferation, and drug transport. ↓, down-regulation, ↑, up-regulation, ↑ activation.

Chemotherapy: Inhibits Taxol resistances

One of the major obstacles in curing prostate cancer is the development of drug resistance to docetaxel, which is the gold standard for the treatment of this disease. It is not only imperative to discover the molecular basis of resistance but also to find therapeutic agents that can disrupt the resistant pathways. Based on initial findings that docetaxel-resistant PC3-DR and DU145-DR prostate tumor cell lines express tumor necrosis factor-related apoptosis inducing ligand (TRAIL) receptors, we examined whether TRAIL could be used as an alternative method to kill PC3-DR and DU145-DR cells. However, these tumor cells were found to be TRAIL resistant. Because PC3-DR and DU-145-DR cells were previously shown by us to be clusterin positive, we examined if clusterin could play a role in TRAIL resistance. We found that resveratrol could sensitize docetaxel-resistant tumor cells to TRAIL, and it worked by blocking clusterin expression. In particular, small interfering RNA clusterin expression in the cell lines was sufficient to produce apoptosis by TRAIL. Further analysis indicated that resveratrol functions as an effective tyrosine kinase inhibitor, similar to its analogue, piceatannol, and could inhibit Src and Jak kinases, thus resulting in loss of Stat1 activation. We have shown earlier that Stat1 is essential for gene transcription of clusterin. These results, taken together, show that resveratrol could be a useful new therapeutic agent to combat docetaxel resistance.[204]

Chemotherapy; Prevents DOX cardiotoxicity through mitochondrial stabilization and the Sirt1 pathway

Doxorubicin (DOX) is one of the most effective chemotherapeutic drugs; however, its incidence of cardiotoxicity compromises its therapeutic index. DOX-induced heart failure is thought to be caused by reduction/oxidation cycling of DOX to generate oxidative stress and cardiomyocyte cell death. Resveratrol (RV), a stilbene found in red wine, has been reported to play a cardioprotective role in diseases associated with oxidative stress. The objective of this study was to test the ability of RV to protect against DOX-induced cardiomyocyte death. We hypothesized that RV protects cardiomyocytes from DOX-induced oxidative stress and subsequent cell death through changes in

mitochondrial function. DOX induced a rapid increase in reactive oxygen species (ROS) production in cardiac cell mitochondria, which was inhibited by pretreatment with RV, most likely owing to an increase in MnSOD activity. This effect of RV caused additional polarization of the mitochondria in the absence and presence of DOX to increase mitochondrial function. RV pretreatment also prevented DOX-induced cardiomyocyte death. The protective ability of RV against DOX was abolished when Sirt1 was inhibited by nicotinamide. Our data suggest that RV protects against DOX-induced oxidative stress through changes in mitochondrial function, specifically the Sirt1 pathway leading to cardiac cell survival. [255]

Down-regulates COX-2 by targeting aromatic hydrocarbon receptor

The role of the aromatic hydrocarbon receptor (AhR) in transcriptional regulation of the human cyclooxygenase-2 (COX-2) gene remains elusive. We report that the AhR-ligands benzo[a]pyrene and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induced transcription activity of COX-2 in breast cancer MCF-7 cells. The TCDD-dependent activation of the COX-2 promoter was abrogated by mutation of 2 xenobiotic response elements (XREs) = CGTG). We found that TCDD stimulated the binding of the AhR to COX-2 and cytochrome P4501A1 (CYP1A1) oligonucleotides containing consensus XREs. Conversely, the cotreatment with TCDD plus a mixture of conjugated linoleic acid (CLA) or selected CLA isomers prevented (CLAmix = t10,c12-CLA > c9,t11-CLA) the induction of transcription from the COX-2 promoter. The TCDD-induced binding of the AhR to COX-2 and CYP1A1 oligonucleotides was repressed by cotreatment with CLA (t10,c12-CLA > c9,t11-CLA), and the AhR antagonists, 3-methoxy-4-naphthoflavone, and resveratrol. We conclude that the AhR may be a suitable target for prophylactic strategies that target COX-2 expression. [205]

Enhances the sensitivity of cholangiocarcinoma to chemotherapeutic agents.

Cholangiocarcinomas are devastating cancers that are resistant to chemotherapies. Resveratrol can regulate the expression of cytochrome p450 1b1 (Cyp1b1), which may confer chemoresistance in various cancers. Our aims were to assess the effects of resveratrol on the sensitivity of cholangiocarcinoma cells to chemotherapeutic agents and show an association between Cyp1b1 expression and chemosensitivity. Cholangiocarcinoma cell lines were treated with resveratrol before the addition of 5-fluorouracil (5-FU), gemcitabine, or mitomycin C. Cell proliferation and apoptosis were assessed by MTS assays and Annexin staining.

Resveratrol effects on cholangiocarcinoma tumor sensitivity to 5-FU was assessed in an in vivo xenograft model using Mz-ChA-1 cells. After resveratrol treatment, Cyp1b1 expression was assessed by real-time PCR and immunoblotting. Stable-transfected cell lines with Cyp1b1 expression knocked down (Mz-Cyp1b1) were used to assess sensitivity to chemotherapeutic agents by MTS assays and Annexin staining and in a xenograft model using Mz-ChA-1 and Mz-Cyp1b1 cells, respectively. For each chemotherapeutic agent, co-treatment with resveratrol in vitro decreased cell proliferation and increased apoptosis to a greater extent than with the chemotherapeutic agent alone. In vivo, 5-FU+resveratrol decreased tumor size and increased TUNEL staining to a greater extent than 5-FU alone. In parallel, resveratrol decreased Cyp1b1 expression in Mz-ChA-1 cells and in cholangiocarcinoma tumors. Mz-Cyp1b1 cells were more sensitive to chemotherapeutic agents in vitro than mock-transfected cells, and Mz-Cyp1b1-induced tumors were more susceptible to 5-FU treatment. We suggest that **resveratrol treatment may be a useful adjunct therapy to improve chemosensitivity in cholangiocarcinoma**.[263]

Attenuates the anticancer efficacy of paclitaxel in human breast cancer cells *in vitro* and *in vivo*

It was reported recently that resveratrol could sensitise a number of cancer cell lines to the anticancer actions of several other cancer drugs, including paclitaxel. In the present study, we further investigated whether resveratrol could sensitise human breast cancer cells to paclitaxel-induced cell death. Unexpectedly, we found that resveratrol strongly diminished the susceptibility of MDA-MB-435s, MDA-MB-231 and SKBR-3 cells to paclitaxel-induced cell death in culture, although this effect was not observed in MCF-7 cells. Using MDA-MB-435s cells as a representative model, a similar observation was made in athymic nude mice. Mechanistically, the modulating effect of resveratrol was partially attributable to its inhibition of paclitaxel-induced G2/M cell cycle arrest, together with an accumulation of cells in the Sphase. In addition, resveratrol could suppress paclitaxel-induced accumulation of reactive oxygen species (ROS) and subsequently the inactivation of anti-apoptotic Bcl-2 family proteins. These observations suggest that the strategy of concomitant use of resveratrol with paclitaxel is detrimental in certain types of human cancers. Given the widespread use of resveratrol among cancer patients, this study calls for more preclinical and clinical testing of the potential benefits and harms of using resveratrol as a dietary adjuvant in cancer patients.[264]

Clinical Pharmacology of Resveratrol and Its Metabolites in Colorectal Cancer Patients.

Resveratrol is a phytochemical with chemopreventive activity in preclinical rodent models of colorectal carcinogenesis. Antiproliferation is one of the many chemopreventive modes of action it has been shown to engage in. Concentrations of resveratrol, which can be achieved in human tissues after p.o. administration, have not yet

been defined. The purpose of this study was to measure concentrations of resveratrol and its metabolites in the colorectal tissue of humans who ingested resveratrol. Twenty patients with histologically confirmed colorectal cancer consumed eight daily doses of resveratrol at 0.5 or 1.0 g before surgical resection. Resveratrol was found to be well tolerated. Normal and malignant biopsy tissue samples were obtained before dosing. Parent compound plus its metabolites resveratrol-3-O-glucuronide, resveratrol-4'-O-glucuronide, resveratrol-3-O-sulfate, resveratrol-4'-O-sulfate, resveratrol sulfate glucuronide, and resveratrol disulfate were identified by high-performance liquid chromatography (HPLC) with UV or mass spectrometric detection in colorectal resection tissue. Quantitation was achieved by HPLC/UV. Cell proliferation, as reflected by Ki-67 staining, was compared in preintervention and postintervention tissue samples. Resveratrol and resveratrol-3-Oglucuronide were recovered from tissues at maximal mean concentrations of 674 and 86.0 nmol/g, respectively. Levels of resveratrol and its metabolites were consistently higher in tissues originating in the right side of the colon compared with the left. Consumption of resveratrol reduced tumor cell proliferation by 5% (P = 0.05). The results suggest that daily p.o.doses of resveratrol at 0.5 or 1.0 g produce levels in the human gastrointestinal tract of an order of magnitude sufficient to elicit anticarcinogenic effects. Resveratrol merits further clinical evaluation as a potential colorectal cancer chemopreventive agent.[265]

Inhibits TNF-alpha induction of MMP-9

Resveratrol is an active polyphenol found in red wine that has anti-cancer effects, but the molecular mechanisms of resveratrol on tumor invasion inhibition have not been well documented. The aim of this study was to elucidate the effects of resveratrol on invasion ability of human hepatocellular carcinoma cells and TNF-alpha-mediated MMP-9 expression. The expression activity of MMP-9 was measured by zymography, RT-PCR and western blot analysis. The expression of NF-kappa B was measured by EMSA and western blot analysis. TNF-alpha induced the MMP-9 expression in HepG2 cells. Resveratrol significantly inhibited TNF-alpha-mediated MMP-9 expression in HepG2 cells. NF-kappa B inhibitor induced a marked reduction in MMP-9 expression, and it suggested that NF-kappa B could play an important role in TNF-alpha-mediated MMP-9 expression. Furthermore, resveratrol significantly suppressed TNF-alpha-mediated NF-kappa B expression and invasion of HepG2 cells. Our results showed that resveratrol inhibited TNF-alpha-mediated MMP-9 expression and invasion of human hepatocellular carcinoma cells. The inhibitory effects are partly associated with the downregulation of the NF-kappa B signaling pathway. [206]

Liver cancer: Effects of resveratrol on matrix metalloproteinase-9 expression in hepatoma cells

To observe the effects of resveratrol on proliferation of human hepatocellular carcinoma cell line SMMC-7721 cells and expression of matrix metalloproteinase-9 (MMP-9) in vitro. SMMC-7721 cells were treated with different concentrations of resveratrol for 24, 48 and 72 h, respectively. The effect of resveratrol on proliferation of SMMC-7721 cells was assessed with methyl thiazolyl tetrazolium (MTT). The expression of MMP-9 mRNA was determined by reverse transcription polymerase chain reaction (RT-PCR). MMP-9 protein was identified by Western blot analysis. Resveratrol could inhibit the proliferation of SMMC-7721 cells with dose- and time-dependent effects. Moreover, both MMP-9 mRNA expression and MMP-9 protein production were markedly reduced after resveratrol treatment. Resveratrol can inhibit the proliferation of SMMC-7721 cells and down-regulate MMP-9 expression. It is presumed that resveratrol may suppress the invasion and metastasis of hepatocellular carcinoma. [236]

Induces apoptosis in prostate cancer

Resveratrol (RV), a naturally occurring phytoalexin, exerts manifold biological effects against a variety of human tumor cell lines. In this study, the cytotoxic and biological effects of novel RV derivatives were investigated in prostate cancer cells. MATERIALS AND METHODS: Cytotoxicity of the compounds was assessed by clonogenic assays in PC-3, LNCaP and DU-145 human prostate cancer cell lines. Induction of apoptosis was studied by Hoechst-propidium-iodide double staining. Cell cycle phase distribution of prostate cancer cells was analyzed using flow cytometry. RESULTS: Methoxy- and hydroxy-substituted RV derivatives exerted cytotoxic effects against all three cell lines. The most potent compounds, 3,3',4,4',5,5'-hexahydroxy-stilbene and 3,4,4',5-tetramethoxystilbene, induced apoptosis at concentrations lower than RV and caused cell cycle arrest in the cell lines investigated. Introducing additional hydroxy- and methoxymoieties to the stilbene ring of RV is capable of enhancing its cytotoxic and pro-apoptotic effects in hormone-responsive and non-responsive prostate cancer cells. [207]

Regulates telomerase

Within the hierarchy of epithelial stem cells, normal progenitor cells may express regulated telomerase during renewal cycles of proliferation and differentiation. Discontinuous telomerase activity may promote increased renewal capacity of progenitor cells, while deregulated/continuous telomerase activity may promote immortalization when differentiation and/or senescent pathways are compromised. In the present work, we show that resveratrol activates, while progesterone inactivates, continuous telomerase activity within 24 h in subpopulations of human Li-

Fraumeni syndrome-derived breast epithelial cells. Resveratrol results in immortalization of mixed progenitor cells with mutant p53, but not human epithelial cells with wild type p53. Our results demonstrate the potential for renewing progenitor cells with mutant p53 to immortalize after continuous telomerase expression when exposed to certain environmental compounds. Understanding the effects of telomerase modulators on endogenous telomerase activity in progenitor cells is relevant to the role of immortalization in the initiation and progression of cancer subtypes. [208]

Colon cancer: induces pro-apoptosis

Resveratrol-induced dose-dependent apoptotic cell death in colon carcinoma cells, was measured by FACS analysis. Treatment of HT29 human colon carcinoma cells with resveratrol was found to induce a number of signature ER stress markers; phosphorylation of eukaryotic initiation factor-2alpha (eIF-2alpha), ER stress-specific XBP1 splicing and CCAAT/enhancer-binding protein-homologous protein (CHOP). In addition, resveratrol induced up-regulation of glucose-regulated protein (GRP)-78, suggesting the induction of ER stress. Furthermore, the inhibition of caspase-4 activity by z-LEVD-fmk significantly reduced resveratrol-induced apoptosis. Taken together, the present study therefore provides strong evidence to support an important role of ER stress response in mediating the resveratrol-induced apoptosis. [209]

Anti-aromatase activity

Aromatase, an enzyme of the cytochrome P450 family, is a very important pharmacological target, particularly for the treatment of breast cancer. The anti-aromatase activity of a set of natural polyphenolic compounds was evaluated in vitro. Strong aromatase inhibitors including flavones, flavanones, resveratrol, and oleuropein, with activities comparable to that of the reference anti-aromatase drug aminoglutethimide, were identified. Through the application of molecular modeling techniques based on grid-independent descriptors and molecular interaction fields, the major physicochemical features associated with inhibitory activity were disclosed, and a putative virtual active site of aromatase was proposed. Docking of the inhibitors into a 3D homology model structure of the enzyme defined a common binding mode for the small molecules under investigation. The good correlation between computational and biological results provides the first rationalization of the anti-aromatase activity of polyphenolic compounds. Moreover, the information generated in this approach should be further exploited for the design of new aromatase inhibitors. [210]

Anti-inflammatory/anti-ageing

Recent studies have documented that resveratrol has various health benefits, such as cardiovascular and cancer preventive properties. However, the experimental basis for such health benefit is not fully understood. One of the possible mechanisms for its protective activities is by down regulation of the inflammatory responses. That includes the inhibition of synthesis and release of pro-inflammatory mediators, modifications of eicosanoid synthesis, inhibition of some activated immune cells, or inhibiting the enzymes, such as cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2), which are responsible for the synthesis of pro-inflammatory mediators through the inhibitory effect of resveratrol on transcription factors like nuclear factor kappaB (NFkappaB) or activator protein-1 (AP-1). Being a phenolic compound, resveratrol certainly possesses a low bioavailability and most importantly, a rapid clearance from the plasma. Recent growing interest in varying protective nature of resveratrol may clinically also hold a respectable position as a better alternative for anti-inflammatory drugs. The purpose of this review is to provide evidence that resveratrol exhibits potent anti-inflammatory activity and also to explain the underling mechanism for both resveratrol- induced cardioprotective and anti-inflammatory properties. While it is true that the cardioprotective properties of resveratrol are likely attributable, at least in part, to its anti-inflammatory properties, the mechanisms discussed address foremost mechanisms for the anti-inflammatory activity which, in turn, is responsible for cardioprotection. [211]

Restores cellular homostasis

Resveratrol (3,5,4'-trihydroxy-trans-stilbene; RV), a dietary constituent found in grapes and wine, exerts a wide variety of pharmacological activities. Because the grape skins are not fermented in the production process of white wines, only red wines contain considerable amounts of this compound. RV is metabolized into sulfated and glucuronidated forms within approximately 15min of entering the bloodstream, and moderate consumption of red wine results in serum levels of RV that barely reach the micromolar concentrations. In contrast, its metabolites, which may be the active principle, circulate in serum for up to 9h. RV has been identified as an effective candidate for cancer chemoprevention due its ability to block each step in the carcinogenesis process by inhibiting several molecular targets such as kinases, cyclooxygenases, ribonucleotide reductase, and DNA polymerases. In addition, RV protects the cardiovascular system by a large number of mechanisms, including defense against ischemic-reperfusion injury, promotion of vasorelaxation, protection and maintenance of intact endothelium, anti-atherosclerotic properties, inhibition of low-density lipoprotein oxidation, and suppression of platelet aggregation, thereby strongly supporting its role in the prevention of coronary disease. Promising data within the use of RV have

also been obtained regarding progressive neurodegenerative maladies such as Alzheimer's, Huntington's, and Parkinson's diseases. Because neurotoxicity is often related to mitochondrial dysfunction and may be ameliorated through the inclusion of metabolic modifiers and/or antioxidants, RV may provide an alternative (and early) intervention approach that could prevent further damage. RV induces a multitude of effects that depend on the cell type (e.g., NF-kappaB modulation in cancer cells vs. neural cells), cellular condition (normal, stressed, or malignant), and concentration (proliferative vs. growth arrest), and it can have opposing activities. RV affects whole pathways and sets of intracellular events rather than a single enzyme and, therefore, may be an effective therapy to restore homoestasis. [212]

Cardioprotective: The French Paradox

In some Mediterranean countries, including France, the incidence of smoking and eating fatty foods is high. Also, the practice of regular exercise in many of these countries is low compared to the US. Yet. The French have one-third the incidence of heart attacks compared to Americans. This has been called "The French Paradox". Epidemiologists have for some years suggested that the moderate consumption of wine and to a lesser extent beer and other alcoholic beverage (2-3 drinks/day) may have a protective effect against the development of coronary artery disease. It may do this by acting as both an anti-oxidant and inhibiting platelet aggregation, both of which may also make it useful in cancer treatment. **[35]**

Grapeseed/skin improve Cardiovasular Health

Drinking a bottle of wine a week can help reduce a further narrowing of blood vessels in men who have undergone heart surgery, report German researchers this week, providing further evidence to support the beneficial impact of moderate drinking on cardiovascular health. [114]

Five-year prospective study conducted among 9750 men (7352 in France and 2398 in Northern Ireland) free of CHD at entry. Outcomes were angina pectoris, myocardial infarction or CHD death. RESULTS: In all, 90% of subjects in In France, after adjusting for other CHD risk factors, subjects in the highest quartile of alcohol consumption, mostly in form of red wine, had a significantly lower risk of developing angina pectoris relative to non-drinkers. For myocardial infarction and all CHD events, the risk also decreased. [115]

The beneficial effects of red wine is mainly attributed to the occurrence of polyphenol compounds such as anthocyanosides (ACs), catechins, proanthocyanidins (PAs), stilbenes and other phenolics in red wine. This review focuses on the vascular effects of red wine polyphenols (RWPs), with emphasis on anthocyanosides and proanthocyanidins. From in vitro studies, the effect of red wine polyphenols on the vascular tone is thought to be due to short- and long-term mechanisms. NO-mediated vasorelaxation represents the short-term response to wine polyphenols, which exert the effect by increasing the influx of extracellular Ca(2+), and the mobilization of intracellular Ca(2+) in endothelial cells. Polyphenolic compounds may also have long-term properties, as they increase endothelial NO synthase expression acting on the promoter activity. In addition, they decrease the expression of adhesion molecules and growth factors, involved in migration and proliferation of vascular smooth muscle cells. Moreover, they inhibit platelet aggregation. [116]

Cardiology Department of the University of Athens Medical School in the Hippokrateion reviewed 329 patients with electrocardiographically confirmed first coronary infarct or a first positive coronary arteriogram, or both (participation fraction 93%). Controls were 570 patients admitted to the same hospital for minor conditions unrelated to nutrition (participation fraction 95%). All cases and controls were interviewed in the hospital wards by experienced interviewers, and a 110-item food frequency questionnaire was administered. RESULTS: There was statistically significant evidence (P approximately 0.03) for an inverse association between intake of flavan-3-ols and CHD risk, an increase of about 21 mg per day corresponding to a 24% decrease in CHD risk. The inverse association between flavan-3-ols and CHD risk was largely accounted for by the intake of wine and to a lesser extent tea. Flavan-3-ols, which are largely found in wine and tea, are inversely associated with a protective against, coronary heart disease. [117]

Boost to women's heart health

In the current study, however, the researchers used a lyophilized grape powder consisting of 92 percent carbohydrate and rich in flavans, anthocyanins, quercetin, myricetin, kaempferol, and resveratrol. They set out to evaluate the effects of grape polyphenols on key risk factors for coronary heart disease in pre- and post-menopausal women.

Heart disease is traditionally thought of as affecting more men than women, but in fact it is the number one killer of women in America, according to the American Heart Association. In 2002 493,690 women in the US died of heart disease, compared with 256,503 for all forms of cancer combined. The study, involved 24 pre-menopausal women and 20 post-menopausal women, who were assigned to one of two groups. For the first four-week period, one group consumed 36g of lyophilized grape powder, and the other received a placebo with an equal ratio of fructose and dextrose and energy content similar to the grape powder (554KJ). After a three-week washout, the two

groups were switched for a second four-week period. Plasma triglyceride concentrations were reduced by 15 percent in the pre-menopausal women after they had taken the grape powder, and 6 percent in the post-menopausal women. Plasma LDL ('bad') cholesterol and apolipoproteins B and E were also lower with the grape powder, and cholesterol ester transfer protein activity was decreased by around 15 percent.

The grape powder significantly reduced oxidative stress, and inflammation, by reducing levels of plasma tumor necrosis factor-alpha. These observations caused the researchers to conclude: "Through alterations in lipoprotein metabolism, oxidative stress, and inflammatory markers, grape powder intake beneficially affected key risk factors for coronary heart disease in both pre- and postmenopausal women. [141]

Study Shows That Grapes Decrease Risk the of Atherosclerosis

A new study shows that eating fresh grapes may prevent the accumulation of harmful oxidized cholesterol as well as the development of atherosclerotic lesions. Naturally occurring antioxidants in fresh grapes known as polyphenols are believed to be responsible for this beneficial impact.

In order to ensure the scientific validity of grape health studies, a representative sample of fresh California grapes was collected and freeze-dried into an edible grape powder. The grape powder used in this study contains all of the biologically active compounds found in fresh grapes. This study found a remarkable reduction in the development of atherosclerosis following consumption of grape powder.

This study showed that grape polyphenols reduced oxidative stress, increased serum antioxidant capacity, reduced cell uptake of oxidized LDL cholesterol and decreased the oxidation of LDL in general. These processes eventually reduce the accumulation of cholesterol in the cells and prevent foam cell formation, thus inhibiting the development of atherosclerosis. This study furthers the mounting evidence that grapes exert a protective role in heart health and this work provides insight that grapes impact a number of mechanisms that may lead to a reduction in atherosclerosis. [142]

Protects Myocardial Ischemia-Reperfusion Injury

We previously showed that resveratrol stimulates NO production and is cardioprotective in rat heart subjected to ischemia-reperfusion (I/R rat heart). We now show that in I/R rat heart, inducible nitric oxide synthase (iNOS) expression is markedly induced, while expression of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS) is unchanged. In animals preconditioned with resveratrol (0.5 to 1 mg/kg body wt), I/R-induced iNOS induction is abrogated; however, expression of eNOS and nNOS is greatly upregulated. The protective effects of resveratrol on I/R rat heart include reduced rhythm disturbances, reduced cardiac infarct size, and decreased plasma levels of lactate dehydrogenase (LDH) and creatine kinase (CK). Among these, the reductions in LDH/CK levels and infarct size are NO-dependent as the co-administration of N(omega)-nitro-l-arginine methyl ester (l-NAME, 1 mg/kg body wt) with resveratrol abolishes the resveratrol effect. **[36]**

Improves Venous Insufficiency

A prospective, randomized, double-blind, placebo-controlled clinical trial of parallel-group design properly planned, implemented, quality-assured, analysed and reported in accordance with the current state of our knowledge, confirmed that the phytotherapeutic agent red wine leaf extract reduces or prevents edema in CVI, reduces the diameter of the calf and ankle, and ameliorates the cardinal symptoms. The effects reach statistical significance, are physiologically comprehensible, and may be adjudged to be clinical relevant. This study thus provides the necessary evidence for the edema-protective effect of the preparation, it therefore lends support to the implicit recommendations of the relevant guidelines by making the usefulness of edemaprotective agents for the treatment of chronic venous insufficiency comprehensible. **[37]**

Anti-Platelet Activity/Anti-Thrombin: Lowers Fibrinogen

Compounds with potential anti-platelet activity can be used FOR cardiovascular disorders. The effects of three different antioxidants with carcinostatic properties: resveratrol, Trolox (a water-soluble analog of vitamin E), and inorganic selenocompounds (sodium selenite and selenate), were studied on blood platelet adhesion to fibrinogen. Resveratrol significantly inhibited adhesion, the initial step of platelet activation, of both thrombin- and ADP-activated platelets to fibrinogen. After incubation of platelets with resveratrol at the concentration of 100 mug/ml above 40% inhibition of adhesion was achieved. The inhibition of platelet adhesion of fibrinogen caused by Trolox was lower than by resveratrol and a higher concentration (1 mM) reached maximum 12%. It was demonstrated that neither sodium selenite nor selenate significantly altered platelet adhesion to fibrinogen.
It was concluded that changed adhesion of blood platelets to fibrinogen in the presence of resveratrol and Trolox, but not selenium may be the result of different anti-oxidative activities of tested compounds. **[7]**

Improves blood flow in the brain by 30 per cent, thereby reducing the risk of stroke

Rats with induced reduction of blood flow (ischemia) in the brain experienced an improved blood flow from a single dose of resveratrol. Resveratrol administration… led to cerebral blood flow elevation and protected animals from ischemia-induced neuron loss. The researchers also found that the concentration of nitric oxide (NO) in the

affected part of the brain was 25 per cent higher than for both the control and ischemia-only group. NO is a molecule used by lining of blood vessels (endothelium) to signal to the surrounding muscle to relax – this dilates the blood vessel and increases the blood flow. [153]

Cardio-protective

Moderate consumption of red wine is associated with a reduced risk of coronary heart disease (CHD). This phenomenon is based on data from epidemiological observations known as the French paradox, and has been attributed to CHD-protective phytochemicals, e.g. resveratrol in red wine. Since red wine also contains alcohol, it is conceivable that alcohol interacts with resveratrol to elicit the observed cardioprotective effects. To determine whether resveratrol has alcohol-independent affects, we compared cardioprotective properties of dealcoholized Chinese red wine with alcohol-containing Chinese red wine having comparable amounts of resveratrol, using a hypercholesterolemic rabbit model and resveratrol as a reference. Animals fed a high cholesterol (1.5%) diet were simultaneously given water containing resveratrol (3 mg/kg/day) or red wine (4 ml/kg/day) containing 3.98 mg/l and 3.23 mg/l resveratrol for regular and dealcoholized red wine, respectively, for a 12-week duration. Total, HDL- and LDL-cholesterol and triglyceride levels in the plasma were measured before and after the cholesterol challenge. Atherosclerotic plaques in the thoracic aorta were evaluated using histochemical methods. Vascular and endothelial functions in the femoral artery were also assessed by ultrasonographic image analysis. High cholesterol-fed animals showed a significant increase in plasma levels of total, HDL- and LDL-cholesterol, but not triglycerides, compared to those fed a regular diet. Dietary cholesterol-elicited lipid changes were similarly observed in animals concurrently fed dealcoholized red wine, red wine or resveratrol. In contrast, whereas atherosclerotic lesions were clearly evident in specimens prepared from the thoracic aorta of high cholesterol-fed animals, the size, density, and mean area of atherosclerotic plaques, and thickness of the intima layer were significantly reduced in rabbits given dealcoholized red wine, red wine, or resveratrol. These results were in agreement with data obtained by an ultrasound analysis of endothelial function, which showed a 25% reduction in flow-mediated dilation (FMD) in rabbits fed a high cholesterol diet compared to animals on control diet. This decrease was effectively prevented by the simultaneous exposure to dealcoholized red wine, red wine, or resveratrol. Our study shows that animals given dealcoholized red wine exhibited cardio-active effects comparable to those of animals orally administered resveratrol, and suggests that wine polyphenolics, rather than alcohol present in red wine, suffice in exerting cardioprotective properties. The results also provide support for the notion that resveratrol and phytochemicals in red wine can suppress atherosclerosis. [162]

Cardio-protective – raises GSH, inhibits oxidative endothelial damage

Atherosclerosis, the main cause of cardiovascular disease (CD), is a chronic inflammatory condition associated with an overproduction of oxidant species, namely peroxynitrite, which is a powerful oxidant that reacts directly with all biomolecules. Glutathione is an efficient scavenger of peroxynitrite, so, modulation of glutathione synthesis may provide a strategy to selectively protect cells from this oxidant. Here, we investigated the ability of **resveratrol**, a component of red wine, to prevent peroxynitrite-mediated endothelial cells toxicity and the underlying mechanism. Bovine aortic endothelial cells (BAEC) in primary cultures were treated with authentic peroxynitrite and the cell viability and intracellular glutathione contents were assessed. Our results demonstrate that a long pre-incubation (14h) of BAEC with resveratrol (1-50muM) leads to the endothelial cells rescue from injury triggered by authentic peroxynitrite by a mechanism of up-regulation of the intracellular GSH content, for the highest resveratrol concentration tested. Considering the importance of GSH in regulation of cell life, this capacity of resveratrol provides a new mechanism for its cardioprotective effects and may contribute to the development of novel therapeutic strategies. [180]

Inhibits Stroke

Since matrix metalloproteinase 9 (MMP-9) has been postulated to be the major contributor of neuronal injury during reperfusion, inhibition of MMP-9 could be a potential approach in maintaining the viability of neurons. Trans-resveratrol (resveratrol), a polyphenolic compound has recently been shown to have neuroprotective activity against cerebral ischemia. Therefore, the aim of the present study was to evaluate the effect of resveratrol on MMP-9 induced by cerebral ischemia-reperfusion in vivo. Male Balb/C mice were treated with resveratrol for 7 days (50 mg/kg, gavage). Thereafter, middle cerebral artery occlusion (MCAo) was performed for 2 h with the help of intraluminal thread. Drug-treated mice showed improvement in necrotic changes in cortex and basal ganglia. Detection of MMP-9 activity and gene expression was performed at various time points after MCAo. The elevated levels of MMP-9 were significantly attenuated in the resveratrol-treated mice as compared to the vehicle MCAo mice. The study suggests that resveratrol has protective effects against acute ischemic stroke, which could be attributed to its property against MMP-9. Thus, resveratrol may be a potential agent for the treatment of neuronal injury associated with stroke. [154]

Inhibition of Platelet Aggregation: **Synergistic Effect of Grape Seed and Grape Skin Extracts**

When grape seed extract and grape skin extract was tested, researchers found that, individually, their potency for inhibiting the clotting system was quite low, but when they were combined – as they might be in purple grape juice or red wine, for example – the two components together displayed a significant added effect. In other words, when they were combined they were much more potent.

Researchers looked at the ability of grape seed extract and a grape skin extract to inhibit platelet aggregation when incubated in whole blood drawn from healthy volunteers. Grape seed extract inhibited platelet aggregation by only 9%. Grape skin extract showed no measurable effect on platelet aggregation. Yet, when the two were incubated together at the same concentration, inhibition of platelet aggregation soared to 91%.

While we may be able to isolate certain biologically active components of seeds and skins of grapes, when we separated them from the whole, we saw a significant falling off of effect in this in test. Clearly there was much gained through the synergy of these two contributors to grape beverages. The best way for people to get that synergy is to drink whole grape juice or red wine, both of which contain both seed and skin proponents, rather than taking commercially available extracts of one or the other. Significant positive effects on platelet aggregation, endothelial function and LDL oxidation in humans were found in those that drank about two glasses of purple grape juice a day. [38]

Inhibits Platelet Function And Enhances Nitric Oxide Release.

Moderate red wine consumption is inversely associated with coronary ischemia, and both red wine and purple grape juice (PGJ) contain flavonoids with antioxidant and anti-platelet properties believed to be protective against cardiovascular events. Acute cardiac events are also associated with decreased platelet-derived nitric oxide (NO) release. In this study, the effects of PGJ and PGJ-derived flavonoids on platelet function and platelet NO production were determined.

Incubation of platelets with dilute PGJ led to inhibition of aggregation, enhanced release of platelet-derived NO, and decreased superoxide production. To confirm the in vivo relevance of these findings, 20 healthy subjects consumed 7 mL/kg of PGJ for 14 days. Platelet aggregation was inhibited after PGJ supplementation, platelet-derived NO production increased from 3.5+/-1.2 to 6.0+/-1.5 pmol/10(8) platelets, and superoxide release decreased from 29.5+/-5.0 to 19.2+/-3.1 arbitrary units. alpha-Tocopherol levels increased significantly after PGJ consumption (from 15.6+/-0.7 to 17.6+/-0.9 micromol/L, and the plasma protein-independent antioxidant activity increased by 50.0%. Last, incubation of platelets with select flavonoid fractions isolated from PGJ consistently attenuated superoxide levels but had variable effects on whole-blood aggregation, platelet aggregation, and NO release. CONCLUSIONS: Both in vitro incubation and oral supplementation with PGJ decrease platelet aggregation, increase platelet-derived NO release, and decrease superoxide production. These findings may be a result of antioxidant-sparing and/or direct effects of select flavonoids found in PGJ. The suppression of platelet-mediated thrombosis represents a potential mechanism for the beneficial effects of purple grape products, independent of alcohol consumption, in cardiovascular disease. [40]

Another recent study confirmed that wine, and wine phenolics in particular, could have a more significant inhibitory effect on platelet aggregation and could explain, in part, the hypothesis that red wine is more protective against atherosclerosis and coronary heart disease. [125]

Cardio-protective

Resveratrol is a potent cardioprotective agent. Its cardioprotective effects may be due to a reduction of atrial natriuretic peptide and transforming growth factor-beta1, which are known to protect the heart from detrimental remodeling. [227]

Resveratrol: Anti-inflammatory and antioxidant effects in healthy smokers a randomized, double-blind, placebo-controlled, cross-over trial.

Smokers are characterized by a low-grade systemic inflammatory state and an oxidant-antioxidant imbalance. Few human studies were conducted on the effects of resveratrol, a natural compound with anti-inflammatory and antioxidant properties, and no trial on smokers has been performed to date. We evaluated whether resveratrol has beneficial effects on markers of inflammation and oxidative stress in smokers.

METHODS AND RESULTS:

A randomized, double- blind, cross-over trial was performed in 50 healthy adult smokers: 25 were randomly allocated to "resveratrol-first" (30-days: 500mg resveratrol/day, 30-days wash-out, 30-days placebo) and 25 to "placebo-first" (30-days placebo, 30-days wash-out, 30-days 500mg resveratrol/day). Resveratrol significantly reduced C-reactive protein (CRP) and triglyceride concentrations, and increased Total Antioxidant Status (TAS) values. After analyzing data with general linear models to assess period and carry-over effects, the ratios of the

values after resveratrol to those after placebo were respectively: 0.47 (95%CI 0.38-0.59) -CRP- and 0.71 (95%CI 0.65-0.78) -triglycerides-, while TAS increased by 74.2 µmol/L (95%CI 60.8-87.6). Uric acid, glucose, insulin, cholesterol, liver enzyme concentrations, and weight, waist circumference, and blood pressure values did not significantly change after resveratrol supplementation.

CONCLUSIONS:
Because resveratrol has anti-inflammatory, anti-oxidant, and hypotriglyceridemic effects, its supplementation may beneficially affect the increased cardiovascular risk of healthy smokers.[304]

Nutritional Supplement-5 with a Combination of Proteasome Inhibitors (Resveratrol, Quercetin, Pterostilbene, δ-Tocotrienol) Modulate Age-Associated Biomarkers and Cardiovascular Lipid Parameters in Human Subjects.

BACKGROUND:
Age-associated altered redox imbalances and dysregulated immune function, contribute to the development of a variety of age associated diseases. Inflammatory markers and lipid profiles are useful prognostic indicators of a variety of age-associated and cardiovascular diseases. We have previously studied the impact of several proteasome inhibitors on several markers of inflammation and lipid profiles *in vitro, in vivo*, in cell lines, animal models, and in human subjects. The current study represents an extension of this work. Our main hypothesis is that a combination of various naturally-occurring proteasome inhibitors, which inhibits nitric oxide (NO), and C-reactive protein (CRP) mediated inflammation, will have better efficacy in the prevention and treatment of age-associated disorders including cardiovascular disease.

METHODS:
Two double blind, randomized, placebo-controlled cross-over trials were conducted to determine the impact of a mixture of NS-5 (resveratrol, pterostilbene, quercetin, δ-tocotrienol, nicotinic acid) on serum NO, CRP, γ-glutamyl-transferase (γ-GT) activity, total antioxidant status (TAS), total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides levels. Healthy seniors (Group-1; $n = 32$) free-living (A, B; 16/group), and hypercholesterolemic (Group-2; $n = 64$) subjects on AHA-Step-1-diet were divided into two groups (C, D; 32/group). Baseline levels were established for parameters as mentioned above. Groups A, C were administered 4-capsules/d of NS-5 and groups B, D, placebo (starch) for 6-weeks. Groups were crossed-over, followed by a 2-week wash-out period. Groups A, C were given 4-capsules/d of placebo and groups B, D, 4-capsules/d of NS-5 for 6-weeks. Groups C, D were continued on AHA-Step-1-diet.

RESULTS:
All the subjects completed each phase in both studies without any complaints. There were significant ($\boldsymbol{P} < 0.01$ - 0.05) decreases in the serum levels of NO (30%, 26%), CRP (29%, 21%), γ-GT activity (14%, 17%), and blood pressure (systolic/diastolic, 3/6%, 3/3%) of Groups A and B, respectively, of free-living healthy seniors without affecting the total, HDL-, LDL-cholesterol or triglycerides compared to their respective baseline values. However, serum levels of NO (36%, 43%), CRP (31%, 48%), γ-GT (17%, 20%), total cholesterol (19%, 15%), LDL-cholesterol (28%, 20%), triglycerides (11%, 18%) of Groups C and D were significantly ($\boldsymbol{P} < 0.01$-0.05) decreased with NS-5 treatment of hypercholesterolemic subjects compared to baseline values, without affecting the serum HDL-cholesterol levels. The serum levels of total antioxidant status (TAS) were increased (10%, 14%; $\boldsymbol{P} < 0.05$) in Groups A and B, increased (19%, 24%; $\boldsymbol{P} < 0.02$), and blood pressure (systolic/diastolic, 5/6%, 3/5%) in Groups C and D with NS-5 treatment, compared to respective baseline values.

CONCLUSIONS:
The consumption of NS-5 mixture decreased significantly serum NO, CRP and γ-GT levels, improved TAS and lipid profiles at risk cardiovascular and hold promise for delaying onset of age-associated diseases.[305]

Resveratrol treatment as an adjunct to pharmacological management in Type 2 diabetes mellitus - systematic review and meta-analysis.

The red wine polyphenol, resveratrol, is highly effective in treating type 2 diabetes mellitus (T2DM) in animal models, but there is no consensus regarding its efficacy in humans. We conducted a systematic review, which included searches in nine scholarly databases and six clinical trial registries, and identified randomized controlled clinical trials whereby resveratrol was used as an adjunct to pharmaceutical interventions in T2DM. Meta-analysis on clinical parameters was performed for available data. Of 764 articles originally identified, data from 6 unique

data sets, examining a total of 196 T2DM patients (104 resveratrol, 92 control/placebo) ultimately met inclusion criteria. Statistically significant ($p<0.05$) positive effects, indicating that resveratrol supplementation was more effective than placebo/control, were identified for systolic blood pressure, hemoglobin A1c, and fasting glucose and creatinine, but not for fasting glucose, HOMAIR, diastolic blood pressure, insulin, triglycerides, LDL, or HDL cholesterol. No major adverse events were reported and side effects of resveratrol were not different than placebo/control. Though limitations in sample size and treatment duration preclude definitive changes in clinical practice, significant improvements in multiple cardiometabolic biomarkers and an excellent safety profile support resveratrol as a leading candidate as an adjunct to pharmacological management of T2DM.[308]

Neuroprotective: Inhibition of ischemia-reperfusion, upregulates NO

Recently, it has been reported in some literature that resveratrol protects the spinal cord, kidney, and heart from ischemia-reperfusion injury through upregulation of nitric oxide (NO). Therefore, this study was designed to investigate the role of nitric oxide on the neuroprotective mechanisms of resveratrol on rats after FCI injury. METHODS: The FCI injury was induced by the middle cerebral artery (MCA) occlusion for 1 hour and then a 24-hour reperfusion followed in the anesthetized Long-Evans rats. Resveratrol was intravenously injected after 1 hour MCA occlusion. RESULTS: Treatment of resveratrol (0.1 and 1 microg/kg) decreased the lactate dehydrogenase (LDH) in plasma and malondialdehyde (MDA) in FCI injury brain tissue, whereas the level of NO in plasma was increased. In addition, resveratrol downregulated protein and mRNA expression of inducible nitric oxide synthase (iNOS), and upregulated protein and mRNA expression of endothelial nitric oxide synthase (eNOS), while the expression of protein and mRNA of neuronal nitric oxide synthase (nNOS) was unchanged. Pretreatment with N(G)-nitro-L-arginine methyl ester (L-NAME, the nonselective NOS inhibitor) or L-N(5)-(1-iminoethyl)-ornithine (L-NIO, the eNOS selective inhibitor) completely blocked the effect of resveratrol in decreasing infarction volumes. CONCLUSIONS: This study demonstrated the important role of NO in the neuroprotective effect of resveratrol in FCI injury. [211]

Protects against endothelial dysfunction caused by high glucose

Endothelial dysfunction secondary to persistent hyperglycemia plays a key role in the development of type 2 diabetic vascular disease. The aim of the present study was to examine the protective effect of resveratrol against hyperglycemia-induced endothelial dysfunction. In cultured human umbilical vein endothelial cells (HUVECs), resveratrol (10 muM - 100 muM) concentration dependently enhanced phosphorylation of endothelial nitric oxide synthesis (eNOS) at Ser1177 and nitric oxide (NO) production. In addition, resveratrol can increase the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) at Thr172 and suppress high glucose-induced generation of superoxide anion. In mouse aortic rings, resveratrol (1 muM - 100 muM) elicited endothelium-dependent vasodilatations and alleviated high glucose-mediated endothelial dysfunction. All these beneficial effects of resveratrol on the endothelium were abolished by pharmacological antagonism of AMPK by compound C. These results provide new insight into the protective properties of resveratrol against endothelial dysfunction caused by high glucose, which is attributed to the AMPK mediated reduction of superoxide level. [248]

Protection against Recurrent Stroke with Resveratrol: Endothelial Protection

Despite increased risk of a recurrent stroke following a minor stroke, information is minimal regarding the interaction between injurious mild cerebral ischemic episodes and the possible treatments which might be effective. The aim of the current study was to investigate recurrent ischemic stroke and whether resveratrol, a nutritive polyphenol with promising cardio- and neuro- protective properties, could ameliorate the associated brain damage. Experiments in adult rats demonstrated that a mild ischemic stroke followed by a second mild cerebral ischemia exacerbated brain damage, and, daily oral resveratrol treatment after the first ischemic insult reduced ischemic cell death with the recurrent insult ($P<0.002$). Further investigation demonstrated reduction of both inflammatory changes and markers of oxidative stress in resveratrol treated animals. The protection observed with resveratrol treatment could not be explained by systemic effects of resveratrol treatment including effects either on blood pressure or body temperature measured telemetrically. Investigation of resveratrol effects on the blood-brain barrier in vivo demonstrated that resveratrol treatment reduced blood-brain barrier disruption and edema following recurrent stroke without affecting regional cerebral blood flow. Investigation of the mechanism in primary cell culture studies demonstrated that resveratrol treatment significantly protected endothelial cells against an in vitro 'ischemia' resulting in improved viability against oxygen and glucose deprivation (39.6±6.6% and 81.3±9.5% in vehicle and resveratrol treated cells, respectively). An inhibition of nitric oxide synthesis did not prevent the improved cell viability following oxygen glucose deprivation but SIRT-1 inhibition with sirtinol partially blocked the protection ($P<0.001$) suggesting endothelial protection is to some extent SIRT-1 dependent. Collectively, the results support that oral resveratrol treatment provides a low risk strategy to protect the brain from enhanced damage produced by recurrent stroke which is mediated in part by a protective effect of resveratrol on the endothelium of the cerebrovasculature.[289]

Resveratrol increases metabolic rate, insulin sensitivity, mitochondrial biogenesis and physical endurance, and reduces fat accumulation in mice via activation of AMPK pathways and pro-survival routes such as SIRT1 in animal studies.[291]

Drinking grape juice significantly increased good cholesterol and significantly lowered two markers of inflammation in people with stable coronary artery disease.

In addition to an increase in HDL (good cholesterol) levels, they saw a significant decrease in the production of superoxide, a free radical, and soluble CD40 ligand, an inflammatory marker that is provoking growing interest *"Platelet release of soluble CD40 ligand is thought to contribute to the development of atherosclerosis and vascular inflammation,"* noting that previous studies of healthy subjects have shown that drinking grape juice decreases superoxide production and inhibits platelet aggregation. However, its impact on the inflammatory properties of platelets had not been previously studied. *The soluble CD40 ligand information is new and particularly interesting, given the growing interest in the link between this inflammatory marker and cardiovascular disease.* [126]

Pterostilbene, a natural methoxylated analogue of resveratrol, was evaluated for antioxidative potential. The peroxyl-radical scavenging activity of pterostilbene was the same as that of resveratrol, having total reactive antioxidant potentials of 237 +/- 58 and 253 +/- 53 microM, respectively. [119] Pterostilbene is yet another antioxidant identified in grapes and red wine that is also believed to lower cholesterol. [120]

Red Grape juice concentrate improves lipids, lowers inflammatory biomarkers, and reduces CVD

Patients treated with hemodialysis frequently experience cardiovascular complications attributed, among other causes, to dyslipidemia, increased oxidative stress, and inflammation. OBJECTIVE: The aim of the study was to study the effects of dietary supplementation with concentrated red grape juice (RGJ), a source of polyphenols, on lipoprotein profile, antioxidant capacity, LDL oxidation, and inflammatory biomarkers. DESIGN: Twenty-six patients receiving hemodialysis and 15 healthy subjects were instructed to drink 100 mL RGJ/d for 14 d. Blood was drawn at baseline, twice during RGJ supplementation, and twice during the 6-mo follow-up period. As a control, 12 other randomly recruited hemodialysis patients not receiving RGJ were studied. Lipids, apolipoproteins, oxidized LDL, and antioxidant vitamins were measured in plasma. The bioavailability of RGJ polyphenols was assessed in healthy subjects. RESULTS: The maximum plasma concentration of quercetin was achieved 3 h after RGJ ingestion, which indicates that supplement-derived polyphenols are rapidly absorbed. In both healthy subjects and hemodialysis patients, RGJ consumption increased the antioxidant capacity of plasma without affecting concentrations of uric acid or ascorbic acid; reduced the concentration of oxidized LDL; and increased the concentration of cholesterol-standardized alpha-tocopherol. RGJ supplementation also caused a significant decrease in LDL-cholesterol and apolipoprotein B-100 concentrations, while increasing the concentrations of HDL cholesterol and apolipoprotein A-I. In a further study in hemodialysis patients, RGJ supplementation for 3 wk significantly reduced plasma monocyte chemoattractant protein 1, an inflammatory biomarker associated with cardiovascular disease risk. CONCLUSION: Dietary supplementation with concentrated RGJ improves the lipoprotein profile, reduces plasma concentrations of inflammatory biomarkers and oxidized LDL, and may favor a reduction in cardiovascular disease risk. [213]

Lowers Homocysteine

Inflammation, immune activation and oxidative stress play a major role in the pathogenesis of cardiovascular disorders. In addition to markers of inflammation, moderate hyperhomocysteinemia is an independent risk factor for cardiovascular disease, and there is a link between the activation of immunocompetent cells and the enhanced formation of homocysteine in vitro. Likewise, anti-inflammatory drugs and nutrients rich in antioxidant vitamins are able to reduce cardiovascular risk and to slow down the atherogenic process. Resveratrol, a phenolic antioxidant synthesized in grapes and vegetables and present in wine, has also been supposed to be beneficial for the prevention of cardiovascular events. Apart from its strong antioxidant properties, resveratrol has also been demonstrated to act as an anti-inflammatory agent. In this study the influence of resveratrol on the production of homocysteine by stimulated human peripheral blood mononuclear cells (PBMCs) was investigated. Results were compared to earlier described effects of the anti-inflammatory compounds aspirin and salicylic acid and of the lipid-lowering drug atorvastatin. Stimulation of PBMCs with the mitogens concanavalin A and phytohemagglutinin induced significantly higher homocysteine accumulation in supernatants compared with unstimulated cells. Treatment with 10-100 muM resveratrol suppressed homocysteine formation in a dose-dependent manner. Resveratrol did not influence the release of homocysteine from resting PBMCs. The data suggest that resveratrol may prevent homocysteine accumulation in the blood by suppressing immune activation cascades and the proliferation of mitogen-driven T-cells. The effect of resveratrol to down-regulate the release of homo-cysteine was comparable to the decline of neopterin concentrations in the same experiments. The suppressive effect of resveratrol was very similar to results obtained earlier with aspirin, salicylic acid and atorvastatin; however, it appeared that doses of compounds needed to reduce homocysteine levels to 50% of stimulated cells were always slightly lower than those

necessary to achieve the same effect on neopterin concentrations. The influence of resveratrol and of all the other compounds on homocysteine production appears to be independent of any direct effect on homocysteine biochemistry. [145]

Inhibits Brain Damage From Strokes

Researchers have found that a compound in red wine or grapes may help to minimize brain damage from strokes. Many Americans have suffered from a stroke, whether it's themselves or family members. As many people know, strokes can be very unexpected and the results can be permanent. Research has discovered that resveratrol--a compound found in grapes--can absorb the free radicals, stopping them from doing any more damage to the brain. [60]

Inhibits Alzheimer's lowers amyloid-beta plaque

Resveratrol, lowers levels of the amyloid-beta peptides that cause the plaques in the brain leading to Alzheimer's disease. It could help to explain the large body of epidemiological evidence linking wine consumption to lower risk of dementia.

By adding resveratrol to cells which produce human amyloid-beta the levels of amyloid-beta in the treated cells were much lower than those in untreated cells. The deposition of amyloid-beta peptides in the brain is one of the characteristic features of Alzheimer's disease. *Resveratrol in grapes may never reach the concentrations required to obtain the effect observed in our studies, so supplementation would be required. Grapes and wine however contain more than 600 different components. Therefore, you cannot exclude the possibility that several compounds work in synergy with small amounts of resveratrol to slow down the progression of the neurodegenerative process in humans. It is therefore important to combine concentrated sources of resveratrol with whole extracts of grape seed and skin.* The researchers believe that resveratrol acts by stimulating the degradation of amyloid-beta peptides by the proteasome, a barrel-shaped multi-protein complex that can specifically digest proteins into short polypeptides and amino acids. Resveratrol may also be effective in fighting other human amyloid-related diseases such as Huntington's, Parkinson's and prion diseases. Studies by a group at the Institute National de la Santé et de la Recherche Médicale in Paris, France headed by Christian Néri have recently shown that resveratrol may protect neurons against amyloid-like polyglutamines, a hallmark of Huntington's disease. [148]

Neuroprotective effect (Parkinson's Disease)

The present study was undertaken to investigate the neuroprotective effects of resveratrol on 6-hydroxydopamine (6-OHDA)-induced Parkinson's disease in rats. 6-OHDA-induced Parkinson's disease rat model involves chronic inflammation, mitochondrial dysfunction, and oxidative stress, and the loss of the dopaminergic neurons in the substantia nigra is the predominant lesion. Resveratrol has been shown to have anti-inflammatory actions, and thus was tested for its beneficial effects using 6-OHDA-induced Parkinson's disease rat model. Adult Sprague-Dawley (SD) rats were unilaterally injected with 6-OHDA (5 microg/2 microl) into the right striatum, and the striatum damage was assessed by rotational test, ultrahistopathology, and molecular alterations. Resveratrol (10, 20 and 40 mg/kg) was then given orally to Parkinson's disease rats, daily for 10 weeks to examine the protective effects. Rotational test (turns of rats) showed that resveratrol significantly attenuated apomorphine-induced turns of rats in 6-OHDA-injuried Parkinson's disease rat model as early as two weeks of administration. Ultrastructural analysis showed that resveratrol alleviated 6-OHDA-induced chromatin condensation, mitochondrial tumefaction and vacuolization of dopaminergic neurons in rat substantia nigra. Furthermore, resveratrol treatment also significantly decreased the levels of COX-2 and TNF-alpha mRNA in the substantia nigra as detected by real-time RT-PCR. COX-2 protein expression in the substantia nigra was also decreased as evidenced by Western blotting. These results demonstrate that resveratrol exerts a neuroprotective effect on 6-OHDA-induced Parkinson's disease rat model, and this protection is related to the reduced inflammatory reaction. [246]

Inhibits Alzheimer Disease: protection from beta-amyloid cytoxicity

Resveratrol has been reported to exert a variety of important pharmacological effects including anti-inflammatory, cardio-protective and cancer chemopreventive properties; however, its mechanisms of action are not completely under-stood. beta-amyloid protein is considered to be responsible for the formation of senile plaques that accumulate in the brains of patients with Alzheimer disease. In the present study, we investigated the protective effect of resveratrol on beta-amyloid-induced cytoxicity in cultured rat astroglioma C6 cells. Preincubation of C6 cells with resveratrol concentration-dependently protected the cells from the growth inhibition induced by beta-amyloid treatment. beta-amyloid treatment led to increased nitric oxide (NO) synthesis and inducible nitric oxide synthase (iNOS) expression; however, cells pretreated with resveratrol showed a dose-dependent inhibition of NO production and iNOS expression following beta-amyloid treatment. Resveratrol also attenuated beta-amyloid-induced prostaglandin E2 (PGE2) release, which was associated with the inhibition of cyclooxygenase (COX)-2 expression. Furthermore, beta-amyloid treatment induced nuclear translocation of NF-kappaB, which was suppressed by resveratrol pretreatment. Collectively, the present results indicate that modulation of nuclear factor-kappaB (NF-kappaB) activity is involved in the neuroprotective action of resveratrol against beta-amyloid-induced toxicity. [157]

Neuron-Protective: Inhibits are-related neurological diseases

Resveratrol has been shown to have significant antioxidant properties in a variety of in vitro and in vivo models. It can reduce ischemic damage in heart ischemia reperfusion injury and also in brain ischemia/reperfusion in rodent models. Due to the high rate of oxygen consumption in the brain, and especially low levels of antioxidant defense enzymes, this organ is particularly susceptible of free radical damage. Most of the protective biological actions associated with resveratrol have been associated with its intrinsic radical scavenger properties. We have investigated the possibility of other indirect pathways by which resveratrol can exert its neuroprotective abilities. We have specifically tested whether heme oxygenase neuroprotective enzyme could be stimulated after resveratrol treatment. Using primary neuronal cultures, resveratrol was able to significantly induce heme oxygenase 1, whereas vehicle control showed no effect. No detectable toxicity was quantified. It is well established that after stroke significant levels of intracellular heme levels increase. The source of free heme comes mainly from several heme-containing enzymes. Heme (iron-protoporphyrin IX) is a pro-oxidant and its rapid degradation by heme oxygenase is believed to be protective. Moreover, the generation of heme metabolites can also have their own intrinsic cellular properties. All together, increased heme oxygenase activity by resveratrol is a unique pathway by which this compound can exert its neuroprotective actions. [158]

Red wine inhibits cognitive deterioration

Recent studies suggest that moderate red wine consumption reduces the incidence of Alzheimer's disease (AD) clinical dementia. Using Tg2576 mice, which model AD-type amyloid beta-protein (Abeta) neuropathology, a test was conducted to assess whether moderate consumption of the red wine Cabernet Sauvignon modulates AD-type neuropathology and cognitive deterioration. The wine used in the study was generated using Cabernet Sauvignon grapes from Fresno, California, and was delivered to Tg2576 in a final concentration of approximately 6% ethanol. This study found that Cabernet Sauvignon significantly attenuated AD-type deterioration of spatial memory function and Abeta neuropathology in Tg2576 mice relative radto control Tg2576 mice that were treated with either a comparable amount of ethanol or water alone. This study supports epidemiological evidence indicating that moderate wine consumption, within the range recommended by the FDA dietary guidelines of one drink per day for women and two for men, may help reduce the relative risk for AD clinical dementia. [181]

Grape juice may 'reverse' brain aging

Drinking Concord grape juice appears to reverse the course of neuronal and behavioral aging in rats, an effect that is proposed to be due to the complex mix of polyphenols.

Concord grape juice is a rich source of polyphenols, potent antioxidants that 'mop up' harmful reactive oxygen species that have been identified as key to the aging process. Previous research has linked polyphenols, such as catechins, epicatechins, and anthocyanins to protecting against various cancers and heart disease.

The new study supports previous work on the subject. The researchers, from the Human Nutrition Research Center on Aging at Tufts University, studies the effect of 10 and 50 per cent concord grape juice on the behavioural and neuronal functions of 45 mature male rats. The scientists used 10 per cent grape juice in order to have an equivalent amount of Concord grape juice per body weight per day as previous human clinical trials, making the research comparable with human trials. The total polyphenol concentration of the 10 and 50 per cent juices were 255 and 1,098 mg per litre of gallic acid equivalents. The rats were randomly assigned to one of three groups: placebo/control group (only drinking water); the 10 per cent juice group; and the 50 per cent juice group. Diets were calorically equivalent for the three groups. After six weeks the rats were tested for motor skills, and after eight weeks cognitive testing occurred. *The rats that consumed the 10 per cent and 50 per cent grape juices performed significantly better on the behavioral tests than did control rats*. In terms of cognitive performance, the researchers observed that the 10 per cent group performed better than the control and 50 per cent juice group. *These findings suggest that it may take a higher concentration of grape juice to enhance motor performance, whereas lower concentrations may be sufficient to alter cognitive performance*.[150]

Resveratrol Attenuates High-Fat Diet-Induced Disruption of the Blood-Brain Barrier and Protects Brain Neurons from Apoptotic Insults.

The blood-brain barrier (BBB) maintains brain microenvironment. Our previous study showed that oxidized low-density lipoprotein (oxLDL) can damage the BBB by inducing apoptosis of cerebrovascular endothelial cells. This study was aimed to evaluate the effects of resveratrol on high-fat diet-induced insults to the BBB and brain neurons. Exposure of mice to a high-fat diet for 8 weeks increased levels of serum total cholesterol (146 ± 13) and LDL (68 ± 8), but resveratrol decreased such augmentations (119 ± 6; 45 ± 8). Permeability assays showed that a high-fat diet induced breakage of the BBB (88 ± 21). Meanwhile, resveratrol alleviated this interruption (16 ± 6). Neither resveratrol nor a high-fat diet caused the death of cerebrovascular endothelial cells. Instead, exposure to a high-fat

diet disrupted the polymerization of occludin and zonula occludens (ZO)-1, but resveratrol significantly attenuated those injuries. Neither a high-fat diet nor resveratrol changed the levels of occludin or ZO-1 in brain tissues. Resveratrol protected brain neurons against high-fat diet-induced caspase-3 activation and genomic DNA fragmentation. This study shows that resveratrol can attenuate the high-fat diet-induced disruption of the BBB via interfering with occludin and ZO-1 tight junctions, and protects against apoptotic insults to brain neurons.[307]

Inhibition of Inflammatory Cytokine Release in COPD

The pathophysiology of chronic obstructive pulmonary disease (COPD) features pulmonary inflammation with a predominant alveolar macrophage involvement. Bronchoalveolar macrophages from patients with COPD release increased amounts of inflammatory cytokines in vitro, an effect that is not inhibited by the glucocorticosteroid dexamethasone. Resveratrol is a component of red wine extract that has anti-inflammatory and antioxidant properties. A study was undertaken to determine whether or not resveratrol would inhibit cytokine release in vitro by alveolar macrophages from patients with COPD.

Alveolar macrophages were isolated from bronchoalveolar lavage (BAL) fluid from cigarette smokers and from patients with COPD (n=15 per group). The macrophages were stimulated with either interleukin (IL)-1beta or cigarette smoke media (CSM) to release IL-8 and granulocyte macrophage-colony stimulating factor (GM-CSF). The effect of resveratrol was examined on both basal and stimulated cytokine release. RESULTS: Resveratrol inhibited basal release of IL-8 in smokers and patients with COPD by 94% and 88% respectively, and inhibited GM-CSF release by 79% and 76% respectively. Resveratrol also inhibited stimulated cytokine release. Resveratrol reduced IL-1beta stimulated IL-8 and GM-CSF release in both smokers and COPD patients to below basal levels. In addition, resveratrol inhibited CSM stimulated IL-8 release by 61% and 51% respectively in smokers and COPD patients, and inhibited GM-CSF release by 49% for both subject groups.

Resveratrol inhibits inflammatory cytokine release from alveolar macrophages in COPD. Resveratrol or similar compounds may be effective pharmacotherapy for macrophage pathophysiology in COPD.[43]

Skin Protectant

Wound Care

A treatment that combines anti-microbial and anti-inflammatory actions may be desirable for alleviating many skin conditions that range in severity. Anti-microbial activity of resveratrol against bacteria and dermatophytes that are major etiologic agents of human skin infections has been evaluated. Resveratrol and its analogs may have wide application to skin conditions that afflict a significant portion of our population, and may also have promising clinical potentials in diabetic wounds. [12]

Inhibition of Matrix Metalloproteinase (MMP)-9: Topically Applied Resveratrol

AIM: To investigate the expression of matrix metalloproteinase-9 (MMP-9) in mouse ears induced with croton oil and the inhibitory effect of dexamethasone, indomethacin and resveratrol on MMP-9 expression, and further explore the relationship between anti-inflammation and MMP-9 inhibition of these three medicines. METHODS: Immuno-histochemistry was used to detect the expression of MMP-9 in mouse ears. Expression of MMP-9 in U937 cells was analyzed by gelatin zymography. RESULTS: Mouse ear edema was inhibited significantly by dexamethasone and indomethacin at the dose of 10 mg.kg-1 and resveratrol at 50 mg.kg-1 administered subcutaneously. The inhibitory rate was 76.2%, 56.7% and 36.9% respectively. The MMP-9 expression increased in mouse ears and was inhibited by dexamethasone, indomethacin and resveratrol at above doses. Gelatin zymography results showed that MMP-9 expression in U937 cells increased significantly after exposed to phorbol myristate acetate at 1 x 10(-8) mol.L-1; MMP-9 expression induced with phorbol myristate acetate was inhibited by dexamethasone at 1 x 10(-9), 1 x 10(-7) and 1 x 10(-5) mol.L-1, indomethacin at 1 x 10(-6) and 1 x 10(-5) mol.L-1 and resveratrol at 1 x 10(-6) and 1 x 10(-5) mol.L-1. CONCLUSION: The inhibition of MMP-9 expression may be one of the anti-inflammatory mechanisms resveratrol. [23] Reseveratrol, applied topically, inhibited the expression of a cancer-causing protein normally caused by exposure to UVB rays. [24]

Powerful COX-2 Inhibitor

Resveratrol has shown to have anti-inflammatory properties, and it has been ascribed as having health benefits that help to prevent cancer and coronary heart disease. It is one of the strongest natural COX-2 inhibitors known.

Inhibition of COX-2 and Inducible Nitric Oxide Synthase.

The anti-inflammatory activity of alpha-viniferin, a trimer of resveratrol, has been demonstrated in an animal model of carrageenin-induced paw edema, and inhibitory effects of the compound on cyclooxygenase and inducible nitric oxide synthase (iNOS) have been investigated in order to understand the mode of the observed action. alpha-Viniferin at doses > 30 mg/kg (oral) or > 3 mg/kg (IV) showed significant anti-inflammatory activity on paw edema in mice. alpha-Viniferin showed an inhibitory effect with an IC (50) value of 4.9 microM on COX-2 activity but a

very weak inhibitory effect with 55.2 +/- 2.1 % of the control (100 %) at 100 microM on COX-1 activity. alpha-Viniferin at doses of 3 microM to 10 microM inhibited the synthesis of COX-2 transcript in lipopolysaccharide (LPS)-activated murine macrophages Raw264.7. alpha-Viniferin showed an IC (50) value of 2.7 microM on nitric oxide (NO) production in LPS-activated Raw264.7 cells when alpha-viniferin and LPS were treated simultaneously, but did not inhibit the NO production when alpha-viniferin was treated at 12 h after LPS stimulation. alpha-Viniferin inhibited synthesis of iNOS transcript with an IC (50) value of 4.7 microM. Consequently, the inhibitory effect of alpha-viniferin on the release of prostanoids and NO could play an important role to show anti-inflammatory action. [23]

Inhibits COX-2 and PG_2

This study investigated for the first time the effects of the cis isomer of resveratrol (c-RESV) on the responses of inflammatory murine peritoneal macrophages, namely on the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) during the respiratory burst; on the biosynthesis of other mediators of inflammation such prostaglandins; and on the expression of inflammatory genes such as inducible nitric oxide synthase (NOS)-2 and inducible cyclooxygenase (COX)-2. Treatment with 1-100 microM c-RESV significantly inhibited intracellular and extracellular ROS production, and c-RESV at 10-100 microM significantly reduced RNS production. c-RESV at 1-100 microM was ineffective for scavenging superoxide radicals (O2(*-)), generated enzymatically by a hypoxanthine/xanthine oxidase (XO) system and/or for inhibiting XO activity. However, c-RESV at 10-100 microM decreased nicotinamide adenine dinucleotide/nicotinamide adenine dinucleotide phosphate oxidase activity in macrophage homogenates. c-RESV at 100 microM decreased NOS-2 and COX-2 mRNA levels in lipopolysaccharide/interferon-gamma-treated macrophages. At 10-100 microM, c-RESV also significantly inhibited NOS-2 and COX-2 protein synthesis and decreased prostaglandin E2 (PGE2) production. These results indicate that c-RESV at micromolar concentrations significantly attenuates several components of the macrophage response to pro-inflammatory stimuli (notably, production of O2(*-) and of the pro-inflammatory mediators NO(*) and PGE2). [39]

Inhibits Oxidative Stress and Inflammation, via COX-2 Suppression

Oxidative stress, neutrophil infiltration, proinflammatory cytokines and eicosanoid generation are clearly involved in the pathogenesis of intestinal bowel disease. We have investigated the effects of resveratrol on the production of prostaglandin (PG)E(2) and PGD(2) in colon mucosa and the expression of cyclo-oxygenases (COX)-1 and -2 immunohistochemically. The inflammatory response was assessed by histology and myeloperoxidase activity, as an index of neutrophil infiltration. Interleukin-1beta production, histological and histochemical analysis of the lesions were also carried out. Finally, since resveratrol has been found to modulate apoptosis we intended to elucidate its effects on colonic mucosa under early acute inflammatory conditions. Resveratrol (5-10mg/kg/day) significantly reduced the degree of colonic injury, the index of neutrophil infiltration and the levels of the cytokine. Resveratrol did not revert the increased PGE(2) levels but produced a significant fall in the PGD(2) concentration. Compared with inflamed colon, no changes in staining for COX-1 were observed in colon of resveratrol and TNBS-treated rats. In contrast, COX-2 expression was decreased. Furthermore, resveratrol enhanced apoptosis compared with already high level induced by TNBS. In conclusion, resveratrol reduces the damage in experimentally induced colitis, alleviates the oxidative events and stimulates apoptosis. [72]

Powerful Anti-Oxidant

Inhibits Lipid Peroxidation of Cell Membrane

The effects of stilbene derivatives, including resveratrol, diethylstilboestrol and stilbene, as anti-oxidants or pro-oxidants were examined. Resveratrol and diethylstilboestrol, but not stilbene, strongly inhibited NADPH- and adenosine 5'-diphosphate (ADP)-Fe3+-dependent lipid peroxidation at the initial and propagation stages. In addition, phenolic stilbenes also inhibited ultraviolet light-induced lipid peroxidation. Resveratrol and diethylstilboestrol efficiently scavenged 2,2'-azobis-(2-amidinopropane)-dihydrochloride peroxyl radicals. However, 2,2'-diphenyl-p-picrylhydrazyl radicals were trapped only by resveratrol, but not by diethylstilboestrol. These results suggest that the inhibitory effect of phenolic stilbenes on lipid peroxidation was due to their scavenging ability of lipid peroxyl and/or carbon-cantered radicals. Resveratrol efficiently reduced ADP-Fe3+, but not EDTA-Fe3+. Stilbenes and diethylstilboestrol did not reduce either ADP-Fe3+ or EDTA-Fe3+. The strand breaks of DNA were stimulated during the interaction of resveratrol with ADP-Fe3+ in the presence of H2O2. These results suggest that phenolic stilbenes act as anti-oxidants of membrane lipids and that resveratrol has a pro-oxidative effect DNA damage during interaction with ADP-Fe3+ in the presence of H2O2. [1]

Inhibits and reverses fatty liver

Resveratrol is a potent inducer of SIRT1. [231] The new study, performed with mice, found that resveratrol may activate two molecules that play a role in cell signaling and the breakdown of fats in the liver: AMP-activated protein kinase (AMPK) and SIRT1. These molecules are reportedly inhibited by alcohol, leading to fat build-up and

fatty liver. Although expert advice is clearly to avoid excessive alcoholic consumption altogether, the results suggest alcoholics could benefit from upping their intake of resveratrol-rich foods.

Study details

The researchers studied the effects of resveratrol at a molecular level. Mice were divided into groups and all of them were fed a low-fat diet. One group of the mice had their diets supplemented with resveratrol, one group was supplemented with resveratrol plus alcohol (ethanol), one group with only ethanol, and one group consumed only the diet (control group). The researchers used two different dose levels of resveratrol. At the end of the experiment, resveratrol increased the expression of SIRT1 and stimulated the activity of AMPK in the livers of alcohol-fed mice. Furthermore, these increases were associated with changes in the levels of other molecules that control fat metabolism, including adiponectin, a hormone produced by fat cells, which helps control obesity.

Such changes are reported to prevent the accumulation of fat in the mouse liver by both reducing the production of fat and increasing the burning of the fat already present. The study's senior author, Dr. Min You, said that it was interesting that the combination of alcohol with resveratrol appeared to enhance the positive effects of resveratrol. *"Our study suggests that resveratrol may serve as a promising agent for preventing … human alcoholic fatty liver disease,"* concluded the authors. [225]

Antioxidant: Makes Fruit Stay Fresher Longer

The antioxidant, trans-resveratrol, is one of the components of red wine that is thought to combat heart disease and cancer by neutralizing oxidizing agents including free radicals. The substance has previously been found to kill fungi on fruit and is known to fight diseases caused by yeast and mould that wilt many fruits, vegetables and cereals. Modern agriculture is plagued by fruit losses due to microbial infections and natural aging during storage. Moreover, synthetic pesticides pose health risks to humans and can cause negative environmental effects. Researchers wondered whether trans-resveratrol's antioxidant properties would help to conserve fruit and found that a coating of the substance protected fruit from Botrytis cinerea, a fungus that causes fruit such as apples and grapes to shrivel. Apples dipped in a trans-resveratrol solution had a greatly increased shelf life, from two weeks to three months. Similarly, the shelf life of grapes doubled to two weeks after being dipped in the substance. As little as four micrograms of trans-resveratrol per grape was needed to produce the effect, researchers say. This amount is only 50 percent more than naturally found in grape skins. In apples, the same low-level amount of trans-resveratrol kept 90 percent of apples that had been exposed to B. cinerea fresh after 60 days. Some remained fresh even after 75 days, whereas untreated apples shriveled within two weeks. Researchers found that the substance also protected tomatoes, avocados and green peppers. They are looking for less-expensive methods of producing trans-resveratrol and plan to develop a commercial fruit-preservation system in about 18 months. [19]

Glutathione Sparing

Resveratrol has been identified as a potential cancer chemopreventative agent and it has been sugested that its' presence in red wine to be linked to the low incidence of heart disease in some regions of France. Recently, however, resveratrol was reported to promote DNA fragmentation in the presence of copper ions1[27], prompting us to investigate this phenomenon in mechanistic detail. By acting as a reducing agent, resveratrol was found to promote hydroxyl-radical (*OH) formation by DNA-bound Cu(H) ions. **However**, in the presence of either ascorbic acid or glutathione (i.e., **under more physiological conditions**), the phenolic lost this property and behaved as an antioxidant. In the ascorbate system, resveratrol had no effect on the rate of *OH formation, but protected DNA from damage by acting as a radical-scavenging antioxidant. In contrast, in the glutathione system, resveratrol inhibited *OH formation via a novel mechanism involving the inhibition of glutathione disulfide formation. We have concluded, therefore, that the DNA-damaging properties of resveratrol, identified recently by Fukuhara and Miyata, will be of no significance under physiological conditions. To the contrary, we have demonstrated that the phenolic behaves as a powerful anti-oxidant, both via classical, hydroxyl-radical scavenging and via a novel, glutathione-sparing mechanism. [26]

ENHANCE IMMUNE CELL RESPONSE

Modulates Cytokine Production by CD4 and CD8

Resveratrol has been reported to exhibit a wide range of biological and pharmacological activities both in vitro and in vivo. Because many of the biological activities of resveratrol, like the inhibition of cyclooxygenase, induction of CD95 signaling-dependent apoptosis, effects on cell division cycle and modulation of NF-kB activation, suggest a possible effect on the immune system, we evaluated the in vitro effects of resveratrol in three immune response models: i) development of cytokine-producing CD4+ and CD8+ T cells induced by stimulation of peripheral blood mononuclear cells with anti-CD3/anti-CD28; ii) specific antigen-induced generation of cytotoxic T lymphocytes; iii) natural killer (NK) activity of PBMC. The results showed that in vitro exposure to resveratrol produces a biphasic effect on the anti-CD3/anti-CD28-induced development of both IFN-[gamma]- IL2- and IL4-producing CD8+ and CD4+ T cells, with stimulation at low resveratrol concentrations and suppression at high

concentrations. Similarly, the compound was found to induce a significant enhancement at low concentrations and suppression at high concentrations of both CTL and NK cell cytotoxic activity. On the whole, the results of the study indicate that resveratrol modulates several human immune cell functions and suggest that this activity may be related to its effects on cytokine production by both CD4+ and CD8+ T cells. [44]

Antibacterial:

Inhibits H Pylori, the intestinal bacteria that is associated with ulcers and stomach cancer. [65]

Grape Skin Compounds Synergistically Inhibit COX-2 enzyme

Saponin compounds and resveratrol found together in grape skin inhibited the COX-2 enzyme. [75, 111]

Grape Skin Saponins and Resveratrol Lower Lipids & Prevent LDL Oxidation

Andrew Waterhouse, professor of Oenology, said saponins could be just as important as resveratrol, thought to be responsible for the so-called French Paradox — the association between red wine and decreased heart disease. According to the scientist, red wines contain about the same amount of saponin as they do resveratrol. But while resveratrol is thought to block cholesterol oxidation (LDL oxidation) by its antioxidant action, saponins are believed to work by binding to and preventing the absorption of cholesterol. [69,70]

Chronic venous insufficiency: Grape Leaf treats ankle edema

A prospective, randomized, double-blind, placebo-controlled clinical trial of parallel-group design properly planned, implemented, quality-assured, analysed and reported in accordance with the current state of our knowledge, confirmed that the phytotherapeutic agent grape leaf extract reduces or prevents edema in CVI, reduces the diameter of the calf and ankle, and ameliorates the cardinal symptoms. The effects reach statistical significance, are physiologically comprehensible, and may be adjudged to be clinical relevant. This study thus provides the necessary evidence for the edema-protective effect of the preparation, it therefore lends support to the implicit recommendations of the relevant guidelines by making the usefulness of edemaprotective agents for the treatment of chronic venous insufficiency comprehensible. [107]

Eye Health: Inhibits Macular Degeneration

Age-related macular degeneration is the most common cause of vision loss among people over the age of 60, affecting millions of older adults every year. Among the antioxidants of edible vegetables, resveratrol has been shown to be a potent angiostatic compound in vitro as well as in vivo and in particular administered orally. Resveratrol's angiostatic mechanism, or that which stops the development of new blood vessels, appears to both inhibit production of VEGF, one of a number of genes associated with angiogenesis, aswell as the effects seen after activation of VEGF receptors. This unique profile of resveratrol activity represents a scientific basis for a novel nutritional supplement to slow the evolution of AMD to the neovascular form. The neovascular, or 'wet' form, of AMD affects only 10 per cent of those diagnosed with the disease yet it accounts for almost 90 per cent of the severe vision loss associated with the condition. [139]

Inhibits oxidative damage to the eye

Epidemiological evidence suggests that moderate wine consumption and antioxidant-rich diets may protect against agerelated macular degeneration (AMD), the leading cause of vision loss among the elderly. To test this hypothesis, the antioxidant and antiproliferative effects of resveratrol were examined in a human retinal pigment epithelium (RPE) cell line (designated ARPE-19). Cell proliferation was determined using the bromodeoxyuridine (BrdU) assay, intracellular oxidation was assessed by dichlorofluorescein fluorescence, and activation of the mitogen-activated protein kinase (MAPK) cascade was measured by immunoblotting. Treatment with 50 and 100 micromol/L resveratrol significantly reduced proliferation of RPE cells by 10% and 25%, respectively ($P<0.05$). This reduction in proliferation was not associated with resveratrol-induced cytotoxicity. Resveratrol (100 micromol/L) inhibited basal and H2O2-induced intracellular oxidation and protected RPE cells from H2O2-induced cell death. The observed reduction in cell proliferation was associated with inhibition of mitogen activated protein kinase/ERK (MEK) and extracellular signal-regulated kinase (ERK 1/2) activities at concentrations of resveratrol as low as 5 micromol/L. These results suggest that resveratrol can reduce oxidative stress and hyperproliferation of the RPE. [140]

Kidney health – inhibits chemical-induced toxcitiy- induction on nitric oxide:

Cyclosporine A (CsA) is a potent and effective immunosuppressive agent, but its use is frequently accompanied by severe renal toxicity. In this study eight groups of animals were employed, group 1 served as control, group 2 were treated with olive oil (vehicle for CsA), group 3 were treated with CsA (20 mg/kg, s.c. for 21 days), groups 4, 5 and 6 received CsA along with resveratrol (2, 5 and 10 mg/kg, p.o. 24 h before and 21 days concurrently), respectively, group 7 were treated with NOS inhibitor, CsA administration for 21 days resulted in a marked renal oxidative stress, significantly deranged the renal functions, reduced the tissue and urine nitrite levels and markedly altered the renal morphology. Treatment with resveratrol (5 and 10 mg/kg) significantly improved the renal dysfunction; tissue and urine total nitric oxide levels, renal oxidative stress and prevented the alterations in renal morphology.

Resveratrol exerts its protective effect by releasing nitric oxide. These results clearly demonstrate the pivotal role of nitric oxide in etiology of CsA nephrotoxicity and indicate the renoprotective potential of resveratrol in CsA nephrotoxicity. [151]

Activates SIRT1 protecting pancreatic beta-cell damage

SIRT1, a class III histone/protein deacetylase, is known to interfere NF-kappaB signaling pathway and thereby has an anti-inflammatory function. Due to the central role of NF-kappaB in cytokine-mediated pancreatic beta-cell damage, we postulated that SIRT1 might work in pancreatic beta-cell damage model. Research Design and Methods: RINm5F (RIN) cells or isolated rat islets were treated with IL-1beta and IFN-gamma. SIRT1 was activated by resveratrol, a pharmacological activator, or ectopic overexpression. The underlying mechanisms of SIRT1 against cytokine toxicity were further explored. Results: Treatment of RIN cells with cytokines induced cell damage, and this damage was well correlated with the expression of inducible form of NO synthase (iNOS) and nitric oxide production. However, SIRT1 overexpression completely prevented cytokine-mediated cytotoxicity, as well as nitric oxide production and iNOS expression. The molecular mechanism by which SIRT1 inhibits iNOS gene expression appeared to involve the inhibition of NF-kappaB signaling pathway through deacetylation of p65. In addition, SIRT1 activation by either resveratrol or adenoviral-directed overexpression of SIRT1 could prevent cytokine toxicity and maintain normal insulin secreting responses to glucose in isolated rat islets. Conclusions: This study will provide valuable information not only into the mechanisms underlying beta-cell destruction but also into the regulation of SIRT1 as a possible target to attenuate cytokine-induced beta-cell damage. [229]

Extends Lifespan: inhibition of insulin responses in a SirT1-independent pathway

Resveratrol mimics calorie restriction to extend lifespan of certain species, and may in humans as well, in part through the activation of Sir2 (silent information regulator 2), a NAD+-dependent histone deacetylase. In this study, resveratrol is shown to inhibit the insulin-signaling pathway in several cell lines and rat primary hepatocytes in addition to its broad-spectrum inhibition of several signalling pathways. Resveratrol effectively inhibits insulin-induced Akt and MAPK (mitogen-activated protein kinase) activation mainly through disruption of the interactions between insulin receptor substrates and its downstream binding proteins including p85 regulatory subunit of phosphoinositide 3-kinase and Grb2 (growth factor receptor-bound protein 2). The inhibitory effect of resveratrol on insulin-signaling is also demonstrated at mRNA level, where resveratrol reverses insulin effects on phosphoenolpyruvate carboxykinase, glucose-6-phosphatase, fatty acid synthase and glucokinase. In addition, RNA interference experiment shows that the inhibitory effect of resveratrol on insulin-signaling pathway is not weakened in cells with reduced expression of SirT1, the mammalian counterpart of Sir2. These observations raise the possibility that resveratrol may additionally modulate lifespan through inhibition of insulin-signaling pathway, independently of its activation of SirT1 histone deacetylase. [183]

Extends lifespan of obese rats

Resveratrol (3,5,4'-trihydroxystilbene) extends the lifespan of diverse species including Saccharomyces cerevisiae, Caenorhabditis elegans and Drosophila melanogaster. In these organisms, lifespan extension is dependent on Sir2, a conserved deacetylase proposed to underlie the beneficial effects of caloric restriction. Here we show that resveratrol shifts the physiology of middle-aged mice on a high-calorie diet towards that of mice on a standard diet and significantly increases their survival. Resveratrol produces changes associated with longer lifespan, including increased insulin sensitivity, reduced insulin-like growth factor-1 (IGF-I) levels, increased AMP-activated protein kinase (AMPK) and peroxisome proliferator-activated receptor-gamma coactivator 1alpha (PGC-1alpha) activity, increased mitochondrial number, and improved motor function. Parametric analysis of gene set enrichment revealed that resveratrol opposed the effects of the high-calorie diet in 144 out of 153 significantly altered pathways. These data show that improving general health in mammals using small molecules is an attainable goal, and point to new approaches for treating obesity-related disorders and diseases of ageing. [184]

Normalizes gene-regulator PPARalpha decreasing cardiovascular disease

The aim of this study was to investigate whether resveratrol (RES), an antioxidant polyphenol of red wine, can influence the activity of PPARalpha in the rat hepatoma cell line McArdle-RH7777. PPARalpha is a transcriptional factor that regulates gene expression when activated by endogenous or exogenous long-chain fatty acids. Its activation results in significant protection from cardiovascular diseases in humans. By means of the electromobility shift assay (EMSA), we observed that PPARalpha is redox-sensitive as it displays reduced DNA-binding activity following in vivo treatment of the cells with 1mmol/L diethylmaleate (DEM), a glutathione-depleting agent. This finding could be relevant considering the important role of redox balance in pathological and physiological processes. We also observed a dual effect of 100mumol/L RES on PPARalpha activity: it was able to prevent, to a large extent, the DEM-induced reduction of DNA-binding activity at earlier time points, when the effect of DEM was stronger, but it depressed PPARalpha activity at later time points, when the effect of DEM was greatly reduced. [185]

Improves lipid metabolism by altering beneficial gene expression

Resveratrol, also improves metabolic diseases, ameliorated dyslipidemia and steatohepatitis induced by the atherogenic diet, and its beneficial effects were associated with the altered expression of hepatic genes involved in lipid metabolism. [230]

Pleiotrophic cardiovascular benefits: Inhibition of cholesterol uptake, 5-LOX inhibition

This study aimed to determine whether the reported pleiotropic effects of several polyphenolic extracts from grape seed products or red wine would also include inhibition of cholesterol uptake and cell proliferation, and inhibit a known specific target of the inflammatory process, that is, 5-lipoxygenase (5-LOX). Incubation of HT29, Caco2, HepG2, or HuTu80 cells in a medium containing [(3)H]cholesterol in the presence of a grape seed extract (GSE) or red wine polyphenolic compounds (RWPCs) inhibited [(3)H]cholesterol uptake by up to 66% (which appeared maximal). The estimated IC(50) values were 60 and 83 microg/mL for RWPC and GSE, respectively. Similar cholesterol uptake inhibitory effects were observed using the fluorescent cholesterol analogue NBD cholesterol. The inhibition of cholesterol uptake was independent of the sample's (GSE and RWPC) potent antioxidative capacity. Red wine polyphenolic compound and GSE dose dependently inhibited HT29 colon adenocarcinoma cell proliferation, which was accompanied by an increase in apoptosis. In addition, RWPC and GSE inhibited 5-LOX activity with the IC(50) values being 35 and 13 microg/mL, respectively. Two of 3 other GSEs tested also significantly inhibited 5-LOX activity. Inhibition of cholesterol uptake and proinflammatory 5-LOX activity may be beneficial in preventing the development of chronic degenerative diseases such as cardiovascular disease and cancer. [240]

Regulates Circadian clock

Circadian clocks, especially peripheral clocks, can be strongly entrained by daily feedings, but few papers have reported the effects of food components on circadian rhythm. The effects of resveratrol, a natural polyphenol, on circadian clocks of Rat-1 cells were analyzed. A dose of 100 muM resveratrol, which did not show cytotoxicity, regulated the expression of clock genes Per1, Per2, and Bma1l. [228]

Grape Seed Extract

Into and Summary

Grape seeds contain lipid, protein, carbohydrates, and 5-8% polyphenols depending on the variety. Polyphenols in grape seeds are mainly flavonoids, including gallic acid, the monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin, and epicatechin 3-O-gallate, and procyanidin dimers, trimers, and more highly polymerized procyanidins. Grape seed extract (GSE) is known as a powerful antioxidant that protects the body from premature aging, disease, and decay. Grape seeds contain mainly phenols such as proanthocyanidins (oligomeric proanthocyanidins). Scientific studies have shown that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C. Extensive research suggests that grape seed extract is beneficial in many areas of health because of its antioxidant effect to bond with collagen, promoting youthful skin, cell health, elasticity, and flexibility. Studies have shown that proanthocyanidins help to protect the body from sun damage, to improve vision, to improve flexibility in joints, arteries, and body tissues such as the heart, and to improve blood circulation by strengthening capillaries, arteries, and veins. The most abundant phenolic compounds isolated from grape seed are catechins, epicatechin, procyanidin, and some dimers and trimers. [112] GSE contains a important group phenolic compounds referred to as oligomeric proanthocyanidin complexes (OPCs) which are primarily known for their antioxidant activity. However, these compounds have also been reported to demonstrate antibacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-allergic, and vasodilatory actions. In addition, they have been found to inhibit lipid peroxidation, platelet aggregation, capillary permeability and fragility, and to affect enzyme systems including phospholipase A2, cyclooxygenase, and lipoxygenase. Based on these reported findings, OPCs may be a useful component in the treatment of a number of conditions. [49, 61]

GSE protects the body from premature aging, disease, and decay. Grape seeds contains mainly phenols such as proanthocyanidins (oligomeric proanthocyanidins). Scientific studies have shown that the antioxidant power of proanthocyanidins is 20 times greater than vitamin E and 50 times greater than vitamin C. Extensive research suggests that grape seed extract is beneficial in many areas of health because of its antioxidant effect to bond with collagen, promoting youthful skin, cell health, elasticity, and flexibility. Other studies have shown that proanthocyanidins help to protect the body from sun damage, to improve vision, to improve flexibility in joints, arteries, and body tissues such as the heart, and to improve blood circulation by strengthening capillaries, arteries, and veins. [64, 86]

Actions of Grape Seed Extract

- Anti-Carcinogenic: inhibits NF Kappa B in prostate cancer; up-regulates IGF-Binding Protein; selective induction of apoptosis; anti-angiogenic; inhibits Vascular Endothelial Growth Factor (VEGF), modulation of inflammatory cytokines, anti-oxidative
- Ameliorates effects of and/or protects against chemotherapy-related toxicity – DOX induced heart damage
- Cardioprotective: Anti-Atherosclerosis (reduction in foam cells), lowers cholesterol, inhibits LDL oxidation, lowers blood pressure, inhibits platelet aggregation, inhibits vascular inflammation, strengthens vascular tissue and heart
- Improves Visual Health, prevents cataracts
- Anti-Aging/Antioxidant - scavenge oxygen free radicals 20-50 greater than that of vitamin C or E
- Stabilizes Collagen, promotes health connective tissue (tendons, cartilage, skin etc)
- Beneficial to Connective Tissue, promotes wound healing, protects skin from sun damage and cancer and prevents skin aging - inhibit UV radiation-induced peroxidation.
- Promotes Healing of Skin: cellular protection; post-radiation scarring
- Antiinflamatory/ Anti-arthritic
- Regulates tumor suppressor genes: bcl-2; downregulates oncogene c-myc;
- Protects against acetaminophen induced liver and kidney damage' ameliorates chronic pancreatitis
- Cytoprotective: Removes toxins and heavy metals from the body
- Anti-obesity

Grape Skin Extract synergistic with Green tea against cancer: inhibition of tNOX, and more potent than seed extract

Grapes and grape extracts were compared for inhibition of a growth-related and cancer-specific form of cell surface NADH oxidase with protein disulfide-thiol interchange activity designated tNOX from human cervical carcinoma (HeLa) cells and growth of HeLa and mouse mammary 4T1 cells in culture and transplanted tumors in mice. Grapes and grape extracts of several varieties had activity. With an extracted grape preparation provided by the California Table Grape Commission, an active fraction was eluted with methanol from a Diaion HP-20 column after removal

of inactive water-soluble materials. Grape skins were a much more potent source than either grape pulp, juice or seeds. Ethanol extracts of the ground freeze-dried pomace was an excellent source. The grape extracts interacted, often synergistically, with decaffeinated green tea extracts both in the inhibition of tNOX activity and in the inhibition of cancer cell growth. Intratumoral injections of a 25:1 mixture of a green tea extract plus ground freeze-dried pomace was nearly as effective as standard synergistic green tea–Capsicum mixtures in inhibiting growth of 4T1 mammary tumors in situ in mice. [189]

Anti-Carcinogenic

Inhibits NF-kappaB pathway to Induce Apoptosis

The alarmingly high rate of prostate cancer (PCA) mortality as well as the limited success in the treatment of advanced PCA suggest that additional approaches are needed to control PCA growth and its metastatic potential. A constitutive activation of NF-kappaB family of transcription factors is known to play a major role in chemotherapy resistance in advanced PCA. In recent studies we showed that grape seed extract (GSE) inhibits advanced human PCA growth and induces apoptosis in cell culture and in nude mice. Accordingly, here we assessed the effect of GSE on constitutive and TNFalpha-induced NF-kappaB DNA binding activity and apoptotic death in advanced human prostate carcinoma DU145 cells. Constitutive and TNFalpha-induced NF-kappaB DNA binding activity was inhibited by GSE at doses > or =50 microg/ml and treatments for > or =12 h. This was accompanied by inhibition of IkappaBalpha phosphorylation and IKKalpha kinase activity. A strong induction of apoptosis ($P<0.01$) was also observed following GSE treatment, while a combination with TNFalpha strongly potentiated apoptosis induction. Our results indicate the potential of developing GSE as an effective cancer therapeutic agent, both alone and in combination with TNFalpha-based chemotherapy of advanced human prostate carcinoma that might prove to be a more effective and less toxic alternative in clinical therapy of PCA. **[46]**

Prevents nuclear factor-kappaB activation – cancer and Cardiovascular protective

BACKGROUND: Several epidemiological studies have demonstrated the beneficial effect of red wine intake in reducing total and cardiovascular mortality. This effect has been attributed in part to its antioxidant properties. Because the monocytes/macrophages and the nuclear transcription factor kappaB (NF-kappaB) are implicated in the pathogenesis of atherosclerotic lesions, we examined the effect of red wine intake on the activation of NF-kappaB in peripheral blood mononuclear cells.

METHODS AND RESULTS: Sixteen healthy volunteers were studied 3 times each: after a moderate dose, a low dose, and no wine with a fat-enriched breakfast. Lipid profile and NF-kappaB activation (electrophoretic mobility shift assay) were examined in blood samples taken before and 3, 6, and 9 hours after wine intake. In addition, mononuclear cells were incubated with VLDL in the presence of some antioxidants (quercetin and alpha-toco-pherol succinate) contained in red wine to study their effects on NF-kappaB activity even though it induced a certain increase in serum lipids, particularly VLDL, that did not increase after the fat ingestion alone. However, another form of alcohol intake (volka) did not modify the NF-kappaB activation provided by postprandial lipemia. In cultured mononuclear cells, isolated human VLDL caused NF-kappaB activation in a time-dependent manner that did not occur in the presence of the red wine antioxidants quercetin and alpha-tocopherol.

CONCLUSIONS: Our results provide a new potential mechanism to explain the beneficial effects of red wine intake in the reduction of cardiovascular mortality. [62]

Inhibits tumor growth, anti-angiogeneic via VEGF inhibition; upregulates IGF binding protein-3

Dietary intake of many fruits and vegetables has been shown to be associated with reduced risk of cancer. We investigated the in vivo efficacy of grape seed extract (GSE, patented as Traconol) against prostate cancer (PCA) and associated molecular events. Athymic nude mice were implanted with hormone-refractory human prostate carcinoma DU145 cells and fed with 100 and 200 mg/kg/day (5 days/week) doses of GSE for 7 weeks. At the end of experiment, tumors were immunohistochemically analyzed for cell proliferation, apoptosis and angiogenesis. Our data show that GSE feeding strongly inhibited tumor growth that accounted for 59-73% inhibition in tumor volume and 37-47% ($p < 0.05$) decrease in tumor weight at the end of the experiment. It did not show any significant change in body weight gain profile and diet consumption. Immunohistochemical analysis of tumors showed that GSE decreases proliferation index by 51-66% ($p < 0.001$) and increases apoptotic index by 3-4-fold. CD31 staining for endothelial cells, showed decrease in intratumoral microvasculature in GSE-fed group of mice. Control tumors showed 64.0 +/- 1.6 CD31 positive cells/400x field compared to 23.2 +/- 0.9 and 15.7 +/- 0.08 CD31 positive cells in 100 and 200 mg/kg doses of GSE-treated tumors, respectively. **GSE strongly inhibited vascular endothelial growth factor (VEGF) secretion in conditioned medium by DU145 cells**. Recently, the circulating level of insulin-like growth factor binding protein (IGFBP)-3 is shown to inversely related with PCA risk, growth and prognosis. Consistent with this, we observed 6-7-fold increase in tumor-secreted levels of IGFBP-3 after GSE feeding. In other immunohistochemical studies, compared to controls, tumor xenografts from GSE-fed groups of mice showed a moderate decrease in VEGF but an increase in IGFBP-3 levels. These findings suggest that GSE

possesses in vivo anti-cancer efficacy against hormone-refractory human PCA, which is associated with its antiproliferative, proapoptotic and antiangiogenic activities together with upregulation of IGFBP-3. [47]

Anti-angiogenic: MMP-2 inhibition

The present study is focused on the investigation of in vitro angiogenic potential of grape seed extract (GSE). Human umbilical vein endothelial cells (HUVEC) in culture were used to assess the effect of GSE on proliferation, survival, matrix metalloproteinases (MMPs) secretion and capillary tube formation. Our data show that GSE significantly inhibited cell growth and cell viability of HUVEC. Further studies by BrdU incorporation and annexin V staining showed that GSE strongly inhibits DNA synthesis and induces apoptotic cell death in HUVEC, respectively. Similar GSE treatment decreased secreted levels of MMP-2 from HUVEC. GSE also inhibited capillary tube formation on Matrigel by endothelial cells in a dose-dependent manner. These findings suggest that GSE possesses an anti-angiogenic potential, which is associated with its antiproliferative, proapoptotic and inhibition of MMP-2 secretion in endothelial cells. Further studies are warranted to evaluate the in vivo anti-angiogenic efficacy of GSE for its possible usefulness in the inhibition of tumor angiogenesis. [52]

Antitumor Activity related to in part antioxidative effects

Since most of these events are associated with the tumor promotion stage of carcinogenesis, these studies suggest that grape seed polyphenols and the procyanidins present therein could be anticarcinogenic and/or anti-tumor-promoting agents. Therefore, we assessed the anti-tumor-promoting effect of a polyphenolic fraction isolated from grape seeds (GSP) employing the 7,12-dimethylbenz[a]anthracene (DMBA)-initiated and 12-O-tetradecanoylphorbol 13-acetate (TPA)-promoted SENCAR mouse skin two-stage carcinogenesis protocol as a model system. Following tumor initiation with DMBA, topical application of GSP at doses of 0.5 and 1.5 mg/mouse/application to the dorsal initiated mouse skin resulted in a highly significant inhibition of TPA tumor promotion. The observed anti-tumor-promoting effects of GSP were dose dependent and were evident in terms of a reduction in tumor incidence (35 and 60% inhibition), tumor multiplicity (61 and 83% inhibition) and tumor volume (67 and 87% inhibition) at both 0.5 and 1.5 mg GSP, respectively. Based on these results, we directed our efforts to separate and identify the individual polyphenols present in GSP and assess their antioxidant activity in terms of inhibition of epidermal lipid peroxidation. Employing HPLC followed by comparison with authentic standards for retention times in HPLC profiles, physiochemical properties and spectral analysis, nine individual polyphenols were identified as catechin, epicatechin, procyanidins B1-B5 and C1 and procyanidin B5-3'-gallate. Five of these individual polyphenols with evident structural differences, namely catechin, procyanidin B2, procyanidin B5, procyanidin C1 and procyanidin B5-3'-gallate, were assessed for antioxidant activity. All of them significantly inhibited epidermal lipid peroxidation, albeit to different levels. A structure-activity relationship study showed that with an increase in the degree of polymerization in polyphenol structure, the inhibitory potential towards lipid peroxidation increased. In addition, the position of linkage between inter-flavan units also influences lipid peroxidation activity; procyanidin isomers with a 4-6 linkage showed stronger inhibitory activity than isomers with a 4-8 linkage. A sharp increase in the inhibition of epidermal lipid peroxidation was also evident when a gallate group was linked at the 3'-hydroxy position of a procyanidin dimer. Procyanidin B5-3'-gallate showed the most potent antioxidant activity with an IC(50) of 20 microM in an epidermal lipid peroxidation assay. Taken together, for the first time these results show that grape seed polyphenols possess high anti-tumor-promoting activity due to the strong antioxidant effect of procyanidins present therein. [89]

Anti-cancer: Induction of apoptosis and increases chemotherapy-killing effects

Current attempts to improve the survival of cancer patients largely depend on strategies to target tumor cell resistance. Naturally occurring dietary compounds such as resveratrol have gained considerable attention as cancer chemopreventive agents. Here, we report that resveratrol acts as potent sensitizer for anticancer drug-induced apoptosis by inducing cell cycle arrest, which in turn resulted in survivin depletion. Concomitant analysis of cell cycle and apoptosis revealed that pretreatment with resveratrol resulted in cell cycle arrest in S phase and apoptosis induction preferentially out of S phase upon subsequent drug treatment. Likewise, cell cycle arrest in S phase by cell cycle inhibitors enhanced drug-induced apoptosis. Resveratrol-mediated cell cycle arrest sensitized for apoptosis by downregulating survivin expression through transcriptional and post-transcriptional mechanisms. Similarly, downregulation of survivin expression using survivin antisense oligonucleotides sensitized for drug-induced apoptosis. Importantly, downregulation of survivin and enhanced drug-induced apoptosis by resveratrol occurred in various human tumor cell lines irrespective of p53 status. Thus, this combined sensitizer (resveratrol)/inducer (cytotoxic drugs) concept may be a novel strategy to enhance the efficacy of anticancer therapy in a variety of human cancers. [127]

Ameliorates the toxic effects associated with chemotherapeutic agents

Anticancer chemotherapeutic agents are effective in inhibiting growth of cancer cells in vitro and in vivo, however, toxicity to normal cells is a major problem. In this study, we assessed the effect of a novel IH636 **grape**

seed proanthocyanidin extract (GSPE) to ameliorate chemotherapy-induced toxic effects in cultured Chang epithelial cells, established from nonmalignant human tissue. These cells were treated in vitro with idarubicin (Ida) (30 nM) or 4-hydroxyperoxycyclophosphamide (4HC) (1 microg/ml) with or without GSPE (25 microg/ml). The cells were grown in vitro and the growth rate of the cells was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; thiazolyl blue] assay. Our results showed that GSPE decreased the growth inhibitory and cytotoxic effects of Ida as well as 4HC on Chang epithelial cells in vitro. Because these chemotherapeutic agents are known to induce apoptosis in the target cells, we analyzed the Chang epithelial cells for apoptotic cell population by flow cytometry. There was a significant decrease in the number of cells undergoing apoptosis following treatment with GSPE. Thus, these results indicate that GSPE can be a potential candidate to ameliorate the toxic effects associated with chemotherapeutic agents and one of the mechanisms of action of GSPE includes protection of healthy cells. [57]

Grape seed selectively induced apoptosis in human mammary cancer cells [59]

Grape seed extract and Genistein inhibit chemically induced breast cancer

Evidence suggests that the proanthocyanidins in grape seed extract (GSE) may be metabolized to the monomeric catechins. A study was carried out to determine whether GSE added to rodent diets protected against carcinogen-induced mammary tumorigenesis in rats and whether this was affected by the composition of the whole diet. Female rats were begun on 5%, 1.25%, or 0% (control) GSE-supplemented diets at age 35 d. At age 50 d they were administered 7,12-dimethylbenz[a]anthracene (DMBA) in sesame oil at 80 mg/kg body weight. They were weighed and monitored weekly for tumor development until 120 d after DMBA administration. Administration of GSE in AIN-76A diet did not show any protective activity of GSE against DMBA-induced breast cancer. However, administration of GSE in a laboratory dry food diet (Teklad 4% rodent diet) resulted in a 50% reduction in tumor multiplicity. In similar experiments, genistein administered in AIN-76A diet also failed to show chemopreventive activity against the carcinogen N-methyl-N-nitrosourea; however, when administered at the same dose in the Teklad 4% rodent diet, genistein exhibited significant chemopreventive activity (44-61%). These results demonstrate that GSE is chemopreventive in an animal model of breast cancer; moreover, the diet dependency of the chemopreventive activity for both GSE and genistein suggests that whether or not a compound is chemopreventive may depend on the diet in which the agent is administered. [128]

Inhibits Melanoma metastasis to the lung

Melanoma is one of the neoplasias that most frequently metastasize, especially in the lung, where represents a challenge in oncology since current treatment is ineffective, and mortality is high. Swiss mice (n = 52) were inoculated with 0.5 x 106 B16F10 cell lines and, later, given an oral administration of grape-seed extract, red wine or ethanol. Metastatic nodules on the lung surface were counted and, after processing for microscopy, five sections were selected for image analysis and the invasion index was calculated. RESULTS: Macroscopic analysis showed that grape-seed extract and red wine reduced the number of metastatic nodules by 26.07 and 20.81%, respectively, compared with a control group treated with ethanol. Microscopically, the reduction in the invasion index was 31.65 for grape-seed extract and 17.57% for red wine. CONCLUSION: Ethanol administration significantly increased pulmonary metastasis while grape-seed extract and red wine led to their reduction. [134]

Inhibits Melanoma celll migration via COX-2 and PG-2 suppression

Melanoma is the leading cause of death from skin disease due, in large part, to its propensity to metastasize. We have examined the effect of grape seed proanthocyanidins (GSPs) on melanoma cancer cell migration and the molecular mechanisms underlying these effects using highly metastasis-specific human melanoma cell lines, A375 and Hs294t. Using in vitro cell invasion assays, we observed that treatment of A375 and Hs294t cells with GSPs resulted in a concentrationdependent inhibition of invasion or cell migration of these cells, which was associated with a reduction in the levels of cyclooxygenase (COX)-2 expression and prostaglandin (PG) E2 production. Treatment of cells with celecoxib, a COX-2 inhibitor, or transient transfection of melanoma cells with COX-2 small interfering RNA, also inhibited melanoma cell migration. Treatment of cells with 12-O-tetradecanoylphorbol-13-acetate, an inducer of COX-2, enhanced the phosphorylation of ERK1/2, a protein of mitogen-activated protein kinase family, and subsequently cell migration whereas both GSPs and celecoxib significantly inhibited 12-O-tetradecanoylphorbol-13-acetate -promoted cell migration as well as phosphorylation of ERK1/2. Treatment of cells with UO126, an inhibitor of MEK, also inhibited the migration of melanoma cells. Further, GSPs inhibited the activation of NF-kB/p65, an upstream regulator of COX-2, in melanoma cells, and treatment of cells with caffeic acid phenethyl ester, an inhibitor of NF-kB, also inhibited cell migration. Additionally, inhibition of melanoma cell migration by GSPs was associated with reversal of epithelial-mesenchymal transition process, which resulted in an increase in the levels of epithelial biomarkers (E-cadherin and cytokeratins) while loss of mesenchymal biomarkers (vimentin, fibronectin and N-cadherin) in melanoma cells. Together, these results indicate that GSPs

have the ability to inhibit melanoma cell invasion/migration by targeting the endogenous expression of COX-2 and reversing the process of epithelial-to-mesenchymal transition.[280]

Breast cancer inhibition: IGF-II regulation

IGF-II is a potent mitogen and inhibitor of apoptosis in breast cancer. Regulation of IGF-II is complex and includes inhibition by tumor suppressors, stimulation by oncogenes, and imprinting and hormonal regulation by estrogens. Resveratrol (RSV) is a phytoestrogen that displays estrogen-like agonistic and antagonistic activity. Recent studies have shown that RSV inhibits the growth of breast cancer cells and may represent a potent agent in chemopreventive therapy. Because 17beta-estradiol regulates IGF-II, we hypothesized that RSV may have a similar effect on IGF-II. The present study was designed to examine whether: 1) RSV modulates IGF-II in breast cancer cells; 2) regulation of IGF-II by RSV is dependent on the ER status; and 3) IGF-II (not IGF-I) mediates RSV effects on breast cancer cells. Treatment of MCF-7 and T47D cells with RSV (10(-6) M) caused stimulation of precursor IGF-II mRNA and protein; this effect was blocked by coincubation with 17beta-estradiol (10(-9) M). Cell growth stimulated by RSV (10(-6) M) was blocked by addition of a blocking IGF-I receptor antibody, or the antiestrogen tamoxifen (10(-7) M). In contrast, RSV treatment (10(-4) M) inhibited IGF-II secretion and cell growth in MCF-7 and T47D cells. No increase in IGF-II levels is seen in estrogen receptor (-) MCF-10 cells, even though cell growth was inhibited by RSV 10(-4) M and precursor IGF-II blocked the inhibitory effect of resveratrol. No change in IGF-I was observed with RSV treatment (10(-6) to 10(-4) M). Our study demonstrates that RSV regulates IGF-II and that IGF-II mediates RSV effect on cell survival and growth in breast cancer cells. [144]

Inhibits prostate cancer

Recently, grape seed extract (GSE), has been identified in laboratory studies, which could be useful in the management of PCa. In vivo pre-clinical studies have indicated chemopreventive effect of many such agents in PCa xenograft and transgenic mouse models. The molecular targets include cell signaling, cell-cycle regulators, and survival/apoptotic molecules, which are implicated in uncontrolled PCa growth and progression. Furthermore, angiogenic and metastatic targets, including vascular endothelial growth factor, hypoxia-inducing factor-1alpha, matrix metalloproteinase, and urokinase-type plasminogen activator are also modulated by GSE to suppress the growth and invasive potential of PCa. [172]

Inhibits Prostate Cancer

Recently it has been shown that grape seed extract (GSE) inhibits growth and induces apoptotic death of advanced human prostate cancer DU145 cells in culture and xenograft. Because prostate cancer is initially an androgen-dependent malignancy, here we used LNCaP human prostate cancer cells as a model to assess GSE efficacy and associated mechanisms. GSE treatment of cells led to their detachment within 12 hours, as occurs in anoikis, and caused a significant decrease in live cells mostly due to their apoptotic death. GSE-induced anoikis and apoptosis were accompanied by a strong decrease in focal adhesion kinase levels, but an increase in caspase-3, caspase-9, and poly(ADP-ribose) polymerase cleavage; however, GSE caused both caspase-dependent and caspase-independent apoptosis as evidenced by cytochrome c and apoptosis-inducing factor release into cytosol. Additional studies revealed that GSE causes DNA damage-induced activation of ataxia telangiectasia mutated kinase and Chk2, as well as p53 Ser(15) phosphorylation and its translocation to mitochondria, suggesting this to be an additional mechanism for apoptosis induction. GSE-induced apoptosis, cell growth inhibition, and cell death were attenuated by pretreatment with N-acetylcysteine and involved reactive oxygen species generation. Together, these results show GSE effects in LNCaP cells and suggest additional in vivo efficacy studies in prostate cancer animal models. [174]

Prostate cancer: Gallic acid, an active constituent of grape seed extract, exhibits antiproliferative, pro-apoptotic and anti-tumorigenic effects against prostate carcinoma

Gallic acid, a natural agent present in a wide-range of fruits and vegetables, has been of potential interest as an anti-cancer agent; herein, we evaluated its efficacy in androgen-independent DU145 and androgendependent-22Rv1 human prostate cancer (PCa) cells.

Gallic acid decreased cell viability in a dose-dependent manner in both DU145 and 22Rv1 cells largely via apoptosis induction. In tumor studies, gallic acid feeding inhibited the growth of DU145 and 22Rv1 PCa xenografts in nude mice. Immunohistochemical analysis revealed significant inhibition of tumor cell proliferation, induction of apoptosis, and reduction of microvessel density n tumor xenografts from gallic acid-fed mice as compared to controls in both DU145 and 22Rv1 models. [253]

Fights against colon cancer: Increase Cip1/p21

Interest in grape seed extracts has been increasing, particularly in Europe where half of the world's grape seed extract is said to end up. The health benefits of its grape seed extract have mostly focused on heart health, but there is also evidence of benefits of grape seed extract against skin and prostate cancer. Moreover, the NCI is currently conducting a trial on grape seed extract and women who are disposed to have a higher risk of breast cancer.

This new study presents data of in vitro and in vivo studies and suggests the extracts may also have benefits in the prevention of colorectal cancer, which accounts for nine per cent of new cancer cases every year worldwide. The value of this preclinical study is that it shows grape seed extract can attack cancer, and how it works, but much more investigation will be needed before these chemicals can be tested as a human cancer… preventive. The researchers used two cell lines to model human colorectal cancer, LoVo and HT29, and found a dose- and time-dependent inhibition of cell growth. The latter cell-line is representative of relatively late-stage colorectal cancer, said the researchers.

The in vitro data showed that cell growth of the LoVo cells was inhibited by 13 to 58 per cent for grape seed extract doses of 25, 50 and 100 micrograms per millilitre after 24 hours. In HT29 cells, doses on 50 and 100 micrograms per millilitre decreased cell numbers by 36 and 43 per cent, respectively. The authors also report that the grape seed extract lead to increases in the availability of a critical protein, Cip1/p21, in the tumors. Indeed, levels were found to be more than 150 times higher after 12 hours of grape seed extract exposure. The protein is involved in effectively freezing the cell cycle, and often promotes programmed cell death (apoptosis).

Decreases in a number of different cyclin proteins and associated cyclin-dependent kinases (CDKs) were also measured by the researchers, a result that did not surprise Prof. Agarwal since Cip1/p21 is said to be powerful enough to inhibit the activity of CDKs and can also control apoptosis.

This protein physically interacts with CDKs," he explained. "In normal cells, it attaches to CDKs to inhibit growth, but if a cell wants to grow, as it does in cancer, levels of Cip1/p21 are reduced, or non-functional. Based on the encouraging in vitro anticancer efficacy of grape seed extract against colorectal cancer, we further studied its efficacy in a preclinical animal model by… implantation of HT29 xenograft in… mice.

Mice were fed a dose of 200 milligrams per kilogram of body weight (a dose larger than a human would reasonably use) and the researchers found that, after eight weeks, the volume of the grafted tumor had decreased by 44 per cent, compared to control mice. No toxic side effects were observed in treated mice, despite the high dose. The researchers also report that, as was observed in the cell culture studies, Cip1/p21 protein levels increased in the tumors of the grape seed extract-fed mice. [175]

Increases Cip/p21 protein level and inhibits growth and induces apoptosis in human colon carcinoma HT29 cells both in vitro and in vivo.

GSE treatment of HT29 cells resulted in a strong dose- and time-dependent phosphorylation of extracellular signal regulated kinase 1/2 (ERK1/2), consistent with p21 induction. The inhibition of sustained ERK1/2 activation by GSE using pharmacological inhibitors abrogated GSE-induced p21 upregulation. Furthermore, pretreatment of cells with N-acetylcysteine inhibited GSE-induced ERK1/2 phosphorylation as well as p21 upregulation, suggesting the involvement of GSE-induced oxidative stress as an upstream event. Consistent with this, GSE also decreased intracellular level of reduced glutathione. Next, we determined whether GSE-induced signaling regulates p21 expression at transcriptional and/or translational levels. GSE was found to increase the stability of p21 message with resultant increase in p21 protein level, but it did not alter the protein stability to a great extent. Importantly, knock-down of p21 abrogated GSE-induced G(1) arrest suggesting that p21 induction by GSE is essential for its G(1) arrest effect. Together, our results for the first time identify a central role of p21 induction and associated mechanism in GSE-induced cell cycle arrest in HT29 cells.[276]

Inhibition of prostate cancer: upregulates p21

We recently reported that gallic acid is a major active agent responsible for grape seed extract activity in DU145 human prostate carcinoma cells. The present study was conducted to examine its efficacy and associated mechanism. Gallic acid treatment of DU145 cells resulted in a strong cell growth inhibition, cell cycle arrest, and apoptotic death in a dose- and time-dependent manner, together with a decrease in cyclin-dependent kinases and cyclins but strong induction in Cip1/p21. Additional mechanistic studies showed that gallic acid induces an early Tyr(15) phosphorylation of cell division cycle 2 (cdc2). Further upstream, gallic acid also induced phosphorylation of both cdc25A and cdc25C via ataxia telangiectasia mutated (ATM)-checkpoint kinase 2 (Chk2) activation as a DNA damage response evidenced by increased phospho-histone 2AX (H2A.X) that is phosphorylated by ATM in response to DNA damage. Time kinetics of ATM phosphorylation, together with those of H2A.X and Chk2, was in accordance with an inactivating phosphorylation of cdc25A and cdc25C phosphatases and cdc2 kinase, suggesting that gallic acid increases cdc25A/C-cdc2 phosphorylation and thereby inactivation via ATM-Chk2 pathway following DNA damage that induces cell cycle arrest. Caffeine, an ATM/ataxia telangiectasia-rad3-related inhibitor, reversed gallic acid-caused ATM and H2A.X phosphorylation and cell cycle arrest, supporting the role of ATM pathway in gallic acid-induced cell cycle arrest. Additionally, gallic acid caused caspase-9, caspase-3, and poly(ADP)ribose polymerase cleavage, but pan-caspase inhibitor did not reverse apoptosis, suggesting an additional caspase-independent apoptotic mechanism. Together, this is the first report identifying gallic acid efficacy and

associated mechanisms in an advanced and androgen-independent human prostate carcinoma DU145 cells, suggesting future in vivo efficacy studies with this agent in preclinical prostate cancer models. [187]

Grape seed extract inhibits prostate cancer invasion via NF-kB/uPA regulation
Tumor invasion and metastasis present major obstacles to successful control of androgen-independent prostate cancer. Cell migration is a fundamental aspect of cancer cell metastasis. Urokinase plasminogen activator (uPA) system is implicated in cell migration and cancer metastasis and has potential to be developed as therapeutic target. In recent years, efficacy of dietary nutrients in preventing and curing cancer has gained increasing attention. One such promising candidate is proanthocyanidin-rich grape seed extract (GSE). We investigated the efficacy of GSE in regulating uPA expression and cell migration using highly metastatic androgen-independent PC3 prostate cancer cells as a model. GSE down-regulated uPA as a function of concentration. Additional studies showed that GSE inhibited DNA-binding activity of the transcription factor nuclear factor kappa B (NFkappaB), which in turn decreased NFkappaB-dependent uPA transcription. Invasion assays revealed the inhibitory effect of GSE on PC3 cell migration. These in-vitro experiments demonstrate the therapeutic property of GSE as an antimetastatic agent by targeting uPA.[267]

Inhibits colon cancer: upregulates p21
Accumulating evidences suggest the beneficial effects of fruit-and-vegetable consumption in lowering the risk of various cancers, including colorectal cancer. Herein, we investigated the in vitro and in vivo anticancer effects and associated mechanisms of grape seed extract (GSE), a rich source of proanthocyanidins, against colorectal cancer. EXPERIMENTAL DESIGN: Effects of GSE were examined on human colorectal cancer HT29 and LoVo cells in culture for proliferation, cell cycle progression, and apoptosis. The in vivo effect of oral GSE was examined on HT29 tumor xenograft growth in athymic nude mice. Xenografts were analyzed by immunohistochemistry for proliferation and apoptosis. The molecular changes associated with the biological effects of GSE were analyzed by Western blot analysis. RESULTS: GSE (25-100 microg/mL) causes a significant dose- and time-dependent inhibition of cell growth with concomitant increase in cell death. GSE induced G1 phase cell cycle arrest along with a marked increase in Cip1/p21 protein level and a decrease in G1 phase-associated cyclins and cyclin-dependent kinases. GSE-induced cell death was apoptotic and accompanied by caspase-3 activation. GSE feeding to mice at 200 mg/kg dose showed time-dependent inhibition of tumor growth without any toxicity and accounted for 44% decrease in tumor volume per mouse after 8 weeks of treatment. GSE inhibited cell proliferation but increased apoptotic cell death in tumors. GSE-treated tumors also showed enhanced Cip1/p21 protein levels and poly(ADP-ribose) polymerase cleavage. CONCLUSIONS: GSE may be an effective chemopreventive agent against colorectal cancer, and that growth inhibitory and apoptotic effects of GSE against colorectal cancer could be mediated via an up-regulation of Cip1/p21. [188]

Inhibits Aromatase (Breast and other cancers)
Aromatase is the enzyme that converts androgen to estrogen. It is expressed at higher levels in breast cancer tissues than normal breast tissues. Grape seed extract (GSE) contains high levels of procyanidin dimers that have been shown to be potent inhibitors of aromatase. In this study, GSE was found to inhibit aromatase activity in a dose-dependent manner and reduce androgen-dependent tumor growth in an aromatase-transfected MCF-7 (MCF-7aro) breast cancer xenograft model, agreeing with our previous findings. We have also examined the effect of GSE on aromatase expression. Reverse transcription-PCR experiments showed that treatment with 60 mug/mL of GSE suppressed the levels of exon I.3-, exon PII-, and exon I.6-containing aromatase mRNAs in MCF-7 and SK-BR-3 cells. The levels of exon I.1-containing mRNA, however, did not change with GSE treatment. Transient transfection experiments with luciferase-aromatase promoter I.3/II or I.4 reporter vectors showed the suppression of the promoter activity in a dose-dependent manner. The GSE treatment also led to the down-regulation of two transcription factors, cyclic AMP-responsive element binding protein-1 (CREB-1) and glucocorticoid receptor (GR). CREB-1 and GR are known to up-regulate aromatase gene expression through promoters I.3/II and I.4, respectively. We believe that these results are exciting in that they show GSE to be potentially useful in the prevention/treatment of hormone-dependent breast cancer through the inhibition of aromatase activity as well as its expression. [173]

Prostate cancer: Ups p21 & 27 favorable changes Bcl-2/Bax ratio
Oligomeric proanthocyanidin complexes (OPC) extracted from grape seeds inhibit prostate cancer of expression of cyclin-dependent kinases and cyclins and stimulation of tumor suppressors p21 and p27, were seen in LNCaP and PC3 cells. Favorable changes in the Bcl-2/Bax ratio were observed in LNCaP and PC3 cells after the treatment with OPC. OPC caused an increase of phosphorylated mitogen-activated protein kinase p44 and p42, thus suggesting induction of cellular differentiation. OPC is a candidate that fulfills criteria for chemopreventive strategies in prostate cancer. [226]

Grape seed/skin inhibit cancer growth

Grapes and grape extracts were compared for inhibition of a growth-related and cancer-specific form of cell surface NADH oxidase with protein disulfide-thiol interchange activity designated tNOX from human cervical carcinoma (HeLa) cells and growth of HeLa and mouse mammary 4T1 cells in culture and transplanted tumors in mice. Grapes and grape extracts of several varieties had activity. With an extracted grape preparation provided by the California Table Grape Commission, an active fraction was eluted with methanol from a Diaion HP-20 column after removal of inactive water-soluble materials. Grape skins were a much more potent source than either grape pulp, juice or seeds. Ethanol extracts of the ground freeze-dried pomace was an excellent source. The grape extracts interacted, often synergistically, with decaffeinated green tea extracts both in the inhibition of tNOX activity and in the inhibition of cancer cell growth. Intratumoral injections of a 25:1 mixture of a green tea extract plus ground freeze-dried pomace was nearly as effective as standard synergistic green tea-Capsicum mixtures in inhibiting growth of 4T1 mammary tumors in situ in mice. [146]

Inhibits Post-radiation Scarring in Breast cancer Patients: Seed & Skin extract The humble grape has been unveiled as a weapon against a painful side effect of breast cancer treatment.

Doctors believe grape seeds have healing properties that can counter radiation fibrosis caused by high doses of radiotherapy. Antioxidants in grape seed and skin extracts prevent scarring caused by radiation fibrosis. The condition in which tissue around the breasts becomes hard and stiff can occur as a result of radiotherapy years after it has been carried out. **[51]**

Procyanidin B2 has anti- and pro-oxidant effects on metal-mediated DNA damage

Procyanidin B2 (epicatechin-(4beta-8)-epicatechin), present in grape seeds, as well as other foods including apples, and cacao beans, has antioxidant properties. We investigated the mechanism of preventive action of procyanidin B2 against oxidative DNA damage in human cultured cells and isolated DNA. Procyanidin B2 inhibited the formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) in the human leukemia cell line HL-60 treated with an H(2)O(2)-generating system. In contrast, a high concentration of procyanidin B2 increased the formation of 8-oxodG in HL-60 cells. Experiments with calf thymus DNA also revealed that procyanidin B2 decreased 8-oxodG formation by Fe(II)/H(2)O(2), whereas procyanidin B2 induced DNA damage in the presence of Cu(II), and H(2)O(2) extensively enhanced it. An electron spin resonance spin trapping study utilizing 3,3,5,5-tetramethyl-1-pyrroline-N-oxide (M(4)PO) demonstrated that procyanidin B2 decreased the signal of M(4)PO-OH from H(2)O(2) and Fe(II), whereas procyanidin B2 enhanced the signal from H(2)O(2) and Cu(II). As an antioxidant mechanism, UV-visible spectroscopy showed that procyanidin B2 chelated Fe(II) at equivalent concentrations. As a pro-oxidant property, we examined DNA damage induced by procyanidin B2, using (32)P-labeled DNA fragments obtained from genes relevant to human cancer. Our results raise the possibility that procyanidin B2 exerts both antioxidant and pro-oxidant properties by interacting with H(2)O(2) and metal ions. [149]

Antioxidant/AntiCancer/Cardio-Protective: Kidney and Liver Protective as Well

Oligomeric proanthocyanidins, naturally occurring antioxidants widely available in fruits, vegetables, nuts, seeds, flowers and bark, have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress. We have assessed the concentration- or dose-dependent free radical scavenging ability of grape seed proanthocyanidin extract (GSPE) both in vitro and in vivo models, and compared the free radical scavenging ability of GSPE with vitamins C, E and beta-carotene. These experiments demonstrated that GSPE is highly bioavailable and provides significantly greater protection against free radicals and free radical-induced lipid peroxidation and DNA damage than vitamins C, E and beta-carotene. GSPE was also shown to demonstrate cytotoxicity towards human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal human gastric mucosal cells. The comparative protective effects of GSPE, vitamins C and E were examined on tobacco-induced oxidative stress and apoptotic cell death in human oral keratinocytes. Oxidative tissue damage was determined by lipid peroxidation and DNA fragmentation, while apoptotic cell death was assessed by flow cytometry. GSPE provided significantly better protection as compared to vitamins C and E, singly and in combination. GSPE also demonstrated excellent protection against acetaminophen overdose-induced liver and kidney damage by regulating bcl-X(L) gene, DNA damage and presumably by reducing oxidative stress. GSPE demonstrated excellent protection against myocardial ischemia-reperfusion injury and myocardial infarction. [56]

Prostate cancer: MMP-2 and -9, and NF-kB inhibition

The recognition that matrix metalloproteinases (MMPs) facilitate tumor cell invasion and metastasis of PCA has led to the development of MMP inhibitors as cancer therapeutic agents. As part of our efforts to develop newer and effective chemopreventive agents for PCA, we evaluated the effect of proanthocyanidins from grape seeds (GSP) on metastasis-specific MMP-2 and -9 in human prostate carcinoma DU145 cells by employing western blot and gelatinolytic zymography. Treatment of GSP dose-dependently inhibited cell proliferation (15-100% by 5-80

microg/ml of GSP), viability (30-80% by 20-80 microg/ml of GSP) and fibroblast conditioned medium (FCM)-induced expression of MMP-2 and -9 in DU145 cells. Since the signaling cascade of mitogen-activated protein kinases (MAPK) have been shown to regulate the expression of MMPs in tumor cells, we found that the treatment of DU145 cells with GSP (20-80 microg/ml) resulted in marked inhibition of FCM-induced phosphorylation of extracellular signal regulated kinase (ERK)1/2 and p38 but had little effect on c-Jun N-terminal kinase under similar experimental conditions. GSP treatment (20-80 microg/ml) to DU145 cells also dose-dependently inhibited FCM-induced activation of NF kappa B concomitantly with inhibition of MMP-2 and -9 expression in the same system. Additionally, the treatment of inhibitors of MEK (PD98059) and p38 (SB203580) to DU145 cells resulted in the reduction of FCM-induced phosphorylation of ERK1/2 and p38 concomitantly marked reduction in MMP-2 and -9 expressions. In further studies, treatment of androgen-sensitive LNCaP cells with a synthetic androgen R1881, resulted in an increase of MMP-2 and -9, which were completely abrogated in the presence of GSP (20-60 microg/ml). These data suggest that inhibition of metastasis-specific MMPs in tumor cells by GSP is associated with the inhibition of activation of MAPK and NF kappa B pathways, and thus provides the molecular basis for the development of GSP as a novel chemopreventive agent for both androgen-sensitive and -insensitive prostate cancer therapies. [121]

GSE Procyanidin B2-3,3'-di-O-gallate potent inhibitors of prostate cancer

The focus of this study was to purify 14 procyanidins from the fractions and to identify those with highest activity toward growth inhibition, cell death and apoptosis in DU145 cells. The most active procyanidin was identified by mass spectrometry and enzymatic hydrolysis as the 3,3'-di-O-gallate ester of procyanidin dimer B2 (Epi-Epi). B2-digallate exhibited dose-dependent effects on DU145 cells over the range 25-100 muM, whereas GA exhibited comparable activity at lower doses but was highly lethal at 100 muM. Structure-activity studies demonstrated that all three hydroxyl groups of GA are necessary for activity, but a free carboxylic acid group is not required even though esterification reduced the activity of GA. These data, and the fact that non-esterified B2 exhibited little or no activity, suggest that the galloyl groups of B2-digallate are primarily responsible for its effects on DU145 cells. Taken together, these data identify procyanidin B2-3,3'-di-O-gallate as a novel biologically active agent in GSE that should be studied in greater detail to determine its effects against prostate cancer. [191]

Suppress bladder cancer inducing programmed cancer cell death through oxidative stress

In present study, we evaluated grape seed extract (GSE) efficacy against bladder cancer and associated mechanism in two different bladder cancer cell lines T24 and HTB9. A significant inhibitory effect of GSE on cancer cell viability was observed, which was due to apoptotic cell death. Cell death events were preceded by vacuolar appearance in cytoplasm, which under electron microscopy was confirmed as swollen mitochondrial organelle and autophagosomes. Through detailed in vitro studies, we established that GSE generated oxidative stress that initiated an apoptotic response as indicated by the reversal of GSE-mediated apoptosis when the cells were pre-treated with antioxidants prior to GSE. However, parallel to a strong apoptotic cell death event, GSE also caused a pro-survival autophagic event as evidenced by tracking the dynamics of LC3-II within the cells. Since the pro-death apoptotic response was stronger than the pro-survival autophagy induction within the cells, cell eventually succumbed to cellular death after GSE exposure. Together, the findings in the present study are both novel and highly significant in establishing, for the first time, that GSE-mediated oxidative stress causes a strong programmed cell death in human bladder cancer cells, suggesting and advocating the effectiveness of this non-toxic agent against this deadly malignancy.[299]

Anit-leukemic: Induces cancer cell apoptosis

A study was conducted to characterize the functional role of c-Jun NH_2-terminal kinase (JNK) and other apoptotic pathways in **grape seed** extract (GSE)-induced apoptosis in human leukemia cells by using pharmacologic and genetic approaches. Jurkat cells were treated with various concentrations of GSE for 12 and 24 h or with 50 μg/mL GSE for various time intervals, after which apoptosis, caspase activation, and cell signaling pathways were evaluated. Parallel studies were done in U937 and HL-60 human leukemia cells. Exposure of Jurkat cells to GSE resulted in dose- and time-dependent increase in apoptosis and caspase activation, events associated with the pronounced increase in Cip1/p21 protein level. Furthermore, treatment of Jurkat cells with GSE resulted in marked increase in levels of phospho-JNK. Conversely, interruption of the JNK pathway by pharmacologic inhibitor (e.g., SP600125) or genetic (e.g., small interfering RNA) approaches displayed significant protection against GSE-mediated lethality in Jurkat cells. The result of the present study showed that GSE induces apoptosis in Jurkat cells through a process that involves sustained JNK activation and Cip1/p21 up-regulation, culminating in caspase activation. [241]

Inhibits in vitro and in vivo human non-small cell lung cancer cells by inhibiting the prostaglandin E(2) and prostaglandin E(2) receptors via COX-2 suppression

Overexpression of cyclooxygenase-2 (COX-2) and prostaglandins (PG) is linked to a wide variety of human cancers. Here, we assessed whether the chemotherapeutic effect of grape seed proanthocyanidins (GSP) on non-small cell lung cancer (NSCLC) cells is mediated through the inhibition of COX-2 and PGE(2)/PGE(2) receptor expression. The effects of GSPs on human NSCLC cell lines in terms of proliferation, apoptosis, and expression of COX-2, PGE(2), and PGE(2) receptors were determined using Western blotting, fluorescence-activated cell sorting analysis, and reverse transcription-PCR. In vitro treatment of NSCLC cells (A549, H1299, H460, H226, and H157) with GSPs resulted in significant growth inhibition and induction of apoptosis, which were associated with the inhibitory effects of GSPs on the overexpression of COX-2, PGE(2), and PGE(2) receptors (EP1 and EP4) in these cells. Treatment of cells with indomethacin, a pan-COX inhibitor, or transient transfection of cells with COX-2 small interfering RNA, also inhibited cell growth and induced cell death. The effects of a GSP-supplemented AIN76A control diet fed to nude mice bearing tumor xenografts on the expression of COX-2, PGE(2), and PGE(2) receptors in the xenografts were also evaluated. The growth-inhibitory effect of dietary GSPs (0.5%, w/w) on the NSCLC xenograft tumors was associated with the inhibition of COX-2, PGE(2), and PGE(2) receptors (EP1, EP3, and EP4) in tumors. This preclinical study provides evidence that the chemotherapeutic effect of GSPs on lung cancer cells in vitro and in vivo is mediated, at least in part, through the inhibition of COX-2 expression and subsequently the inhibition of PGE(2) and PGE(2) receptors.[278]

Anti-angiogenic suppresses VEGF

Blockade of angiogenesis is an important approach for cancer treatment and prevention. Vascular endothelial growth factor (VEGF) is one of the most critical factors that induce angiogenesis and has thus become an attractive target for antiangiogenesis treatment. However, most current anti-VEGF agents often cause some side effects when given chronically. Identification of naturally occurring VEGF inhibitors derived from diet would be one alternative approach with an advantage of known safety. Grape seed extract (GSE), a widely used dietary supplement, is known to have antitumor activity. In this study, we have explored the activity of GSE on VEGF receptor and angiogenesis. We found that GSE could directly inhibit the kinase activity of purified VEGF receptor 2, a novel activity of GSE that has not been characterized. GSE could also inhibit the VEGF receptor/mitogen-activated protein kinase-mediated signaling pathway in endothelial cells. As a result, GSE could inhibit VEGF-induced endothelial cell proliferation and migration as well as sprout formation from aorta ring. In vivo assay further showed that GSE could inhibit tumor growth and tumor angiogenesis of MDA-MB-231 breast cancer cells in mice. Consistent with the in vitro data, GSE treatment of tumor-bearing mice led to concomitant reduction of blood vessel density and phosphorylation of mitogen-activated protein kinase. Depletion of polyphenol with polyvinylpyrrolidone abolished the antiangiogenic activity of GSE, suggesting a water-soluble fraction of polyphenol in GSE is responsible for the antiangiogenic activity. Taken together, this study indicates that GSE is a well-tolerated and inexpensive natural VEGF inhibitor and could potentially be useful in cancer prevention or treatment. [242]

Anti-angiogenic suppresses VEGF and HIF-1 alpha

Grape Seed Extract (GSE) is a widely consumed dietary supplement that has anti-tumor activity. Here we have investigated the inhibitory effect of GSE on the expression of vascular endothelial growth factor (VEGF) and the mechanism underlying this action. We found that GSE inhibited VEGF mRNA and protein expression in U251 human glioma cells and MDA-MB-231 human breast cancer cells. GSE inhibited transcriptional activation of the VEGF gene through reducing protein but not mRNA expression of hypoxia-inducible factor 1alpha (HIF-1alpha). The inhibitory effect of GSE on HIF-1alpha expression was mainly through inhibiting HIF-1alpha protein synthesis rather than promoting protein degradation. Consistent with this result, GSE suppressed phosphorylation of several important components involved in HIF-1alpha protein synthesis, such as Akt, S6 kinase and S6 protein. Furthermore, in the MDA-MB-231 tumor, we found that GSE treatment inhibited the expression of VEGF and HIF-1alpha and the phosphorylation of S6 kinase without altering the subcellular localization of HIF-1alpha, correlating with reduced vessel density and tumor size. Depletion of polyphenol with polyvinylpyrrolidone (PVPP) abolished the inhibitory activity of GSE, suggesting a water soluble fraction of polyphenol in GSE is responsible for the inhibitory activity. Taken together, our results indicate that GSE inhibits VEGF expression by reducing HIF-1alpha protein synthesis through blocking Akt activation. This finding provides new insight into the mechanisms of anti-cancer activity of GSE and reveals a novel molecular mechanism underlying the anti-angiogenic action of GSE. [243]

Protects against the toxicity from chemotherapy (Cisplatin)

Cisplatin is one of the most potent chemotherapeutic antitumor drugs. Oxidative stress has been proven to be involved in cisplatin-induced toxicity. Therefore, the present study was undertaken to examine the antioxidant potential of grape seed proanthocyanidin extract (GSPE) against the toxicity of cisplatin in male rats. Cisplatin treated animals revealed a significant elevation in plasma, heart, kidney and liver thiobarbituric acid reactive substances (TBARS), while the activities of antioxidant enzymes (GST, SOD, CAT and GSH-Px, and the levels of glutathione (GSH) were decreased. Aspartate and alanine transaminases (AST and ALT), creatine kinase and lactate

dehydrogenase were significantly increased in plasma, while liver AST and ALT were significantly decreased. Cisplatin significantly increased the levels of plasma total lipid, cholesterol, urea and creatinine, and the relative weight of kidney. On the other hand, plasma total protein and albumin, and body weight were significantly decreased. GSPE reduced cisplatininduced the levels of TBARS in plasma, heart, kidney and liver, TL, cholesterol, urea and creatinine, and liver AST and ALT. Moreover, it ameliorated cisplatin-induced decrease in the activities of antioxidant enzymes, and GSH, total protein and albumin. Therefore, the present results revealed that GSPE exerts a protective effect by antagonizing cisplatin toxicity. [250]

Protects against the toxicity from chemotherapy (Cisplatin) - 2

The clinical use of cisplatin is highly limited, because of its renal toxicity. In this study, the protective effect of grape seed proanthocyanidin extract (GSPE) against cisplatin-induced nephrotoxicity is investigated in rats. Results showed that DNA qualitative analysis indicated an increase in the instability of the DNA purified from the cisplatin exposed kidney cells. Agarose gel electrophoresis revealed DNA damage in the form of smearing as well as ladder like fragmentation of the kidney genomic DNA. Cisplatin produced different RAPD patterns compared to control. Deletion of bands for the amplified DNA extracted from cisplatin treated rats was the most common outcome. Treatment with cisplatin decreased albumin, and increased urea and creatinine. Cisplatin significantly increased the level of kidney free radicals, and decreased the glutathione content and the activities of the antioxidant enzymes. The presence of GSPE with cisplatin significantly alleviated its nephrotoxicity. In conclusion, the present study showed that cisplatin induced damage in the kidney genomic DNA, lipid peroxidation, inhibition of antioxidant enzymes and alterations of biochemical parameters in plasma and kidney of rats. While, GSPE treatment protected against the toxic effects induced by cisplatin. Thus, GSPE may be used to prevent toxicity during chemotherapeutic treatment with cisplatin. [251]

Grape seed proanthocyanidins inhibit the invasive potential of head and neck cancer by targeting EGFR expression and epithelial-to-mesenchymal transition.

Head and neck squamous cell carcinoma (HNSCC) is responsible for over 20,000 deaths every year in United States. Most of the deaths are due, in large part, to its propensity to metastasize. We have examined the effect of bioactive component grape seed proanthocyanidins (GSPs) on human cutaneous HNSCC cell invasion and the molecular mechanisms underlying these effects using SCC13 cell line as an in vitro model.

METHODS:

The therapeutic effects of GSPs on cancer cell invasion were studied using Boyden chamber and wound healing assays. The effects of GSPs on the levels of various proteins related with cancer cell invasion were determined using western blot analysis.

RESULTS:

Using in vitro cell invasion assays, we observed that treatment of SCC13 cells with GSPs resulted in a concentration-dependent inhibition of cell invasion of these cells, which was associated with a reduction in the levels of epidermal growth factor receptor (EGFR). Treatment of cells with gefitinib and erlotinib, inhibitors of EGFR, or transient transfection of SCC13 cells with EGFR small interfering RNA, also inhibited invasion of these cells. The inhibition of cell invasion by GSPs was associated with the inhibition of the phosphorylation of ERK1/2, a member of mitogen-activated protein kinase family. Treatment of cells with UO126, an inhibitor of MEK, also inhibited the invasion potential of SCC13 cells. Additionally, inhibition of human cutaneous HNSCC cell invasion by GSPs was associated with reversal of epithelial-to-mesenchymal transition (EMT) process, which resulted in an increase in the levels of epithelial biomarker (E-cadherin) while loss of mesenchymal biomarkers (vimentin, fibronectin and N-cadherin) in cells. Similar effect on EMT biomarkers was also observed when cells were treated with erlotinib.[286]

CONCLUSION:

The results obtained from this study indicate that grape seed proanthocyanidins have the ability to inhibit the invasion of human cutaneous HNSCC cells by targeting the EGFR expression and reversing the process of epithelial-to-mesenchymal transition. These data suggest that GSPs can be developed as a complementary and alternative medicine for the prevention of invasion/metastasis of HNSCC cells.

GSE enhanced antiproliferative effect of interferon on bladder cancer cells.

Although interferon (IFN) has been often used as immunotherapy for bladder cancer, its efficacy is rather unsatisfactory, demanding further improvement. Combination therapy is one of viable options, and grape seed proanthocyanidin (GSP) could be such an agent to be used with IFN because it has been shown to have anticancer activity. We thus investigated whether combination of IFN and GSP might enhance the overall antiproliferative effect on bladder cancer cells in vitro. Human bladder cancer T24 cells were employed and treated with the varying concentrations of recombinant IFN-α(2b) (0-100,000 IU/ml), GSP (0-100 μg/ml), or their combinations. IFN-α(2b) alone led to a ~50% growth reduction at 20,000 (20K) IU/ml, which further declined to ~67% at ≥50K IU/ml.

Similarly, GSP alone induced a ~35% and ~100% growth reduction at 25 and ≥50 μg/ml, respectively. When IFN-α(2b) and GSP were then combined, combination of 50K IU/ml IFN-α(2b) and 25 μg/ml GSP resulted in a drastic >95% growth reduction. Cell cycle analysis indicated that such an enhanced growth inhibition was accompanied by a G(1) cell cycle arrest. This was further confirmed by Western blot analysis revealing that expressions of G(1)-specific cell cycle regulators (CDK2, CDK4, cyclin E and p27/Kip1) were distinctly modulated with such IFN-α(2b)/GSP treatment. Therefore, these findings support the notion that combination of IFN-α(2b) and GSP is capable of additively enhancing antiproliferative effect on T24 cells with a G(1) cell cycle arrest, implying an adjuvant therapeutic modality for superficial bladder cancer.[287]

Anticancer activity of grape and grape skin extracts alone and combined with green tea infusions.
Grapes and grape extracts were compared for inhibition of a growth-related and cancer-specific form of cell surface NADH oxidase with protein disulfide-thiol interchange activity designated tNOX from human cervical carcinoma (HeLa) cells and growth of HeLa and mouse mammary 4T1 cells in culture and transplanted tumors in mice. Grapes and grape extracts of several varieties had activity. With an extracted grape preparation provided by the California Table Grape Commission, an active fraction was eluted with methanol from a Diaion HP-20 column after removal of inactive water-soluble materials. Grape skins were a much more potent source than either grape pulp, juice or seeds. Ethanol extracts of the ground freeze-dried pomace was an excellent source. The grape extracts interacted, often synergistically, with decaffeinated green tea extracts both in the inhibition of tNOX activity and in the inhibition of cancer cell growth. Intratumoral injections of a 25:1 mixture of a green tea extract plus ground freeze-dried pomace was nearly as effective as standard synergistic green tea-Capsicum mixtures in inhibiting growth of 4T1 mammary tumors in situ in mice.[292]

Grape seed extract attenuates arsenic-induced nephrotoxicity in rats
Oxidative stress is a recognized factor in nephrotoxicity induced by chronic exposure to inorganic arsenic (As). Grape seed extract (GSE) possesses antioxidant properties. The present study was designed to evaluate the beneficial effects of GSE against arsenic-induced renal injury. Healthy, male Sprague-Dawley rats were exposed to As in drinking water (30 ppm) with or without GSE (100 mg/kg) for 12 months. The serum proinflammatory cytokine levels and mRNA expression levels of fibrogenic markers in the renal tissues were evaluated using enzyme-linked immunosorbent assay and quantitative polymerase chain reaction, respectively. The protein expression levels of nicotinamide adenine dinucleotide phosphate (NADPH) subunits, transforming growth factor-β1 (TGF-β1) and phosphorylated Smad2/3 (pSmad2/3) were assessed using western blot analysis. The results demonstrated that cotreatment with GSE significantly improved renal function, as demonstrated by the reductions in relative kidney weight (% of body weight) and blood urea nitrogen, and the increase in the creatinine clearance capacity. GSE attenuated the As-induced changes in the serum levels of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-1β and the mRNA levels of TGF-β1, α-smooth muscle actin (α-SMA), connective tissue growth factor (CTGF) and fibronectin (FN) in renal tissue. Furthermore, administration of GSE markedly reduced As-stimulated reactive oxygen species (ROS) production and Nox activity, as well as the protein expression levels of the NADPH subunits (Nox2, p47phox and Nox4). In addition, GSE cotreatment was correlated with a significant reduction in TGF-β/Smad signaling, as demonstrated by the decreased protein levels of TGF-β1 and pSmad2/3 in renal tissue. This study indicated that GSE may be a useful agent for the prevention of nephrotoxicity induced by chronic exposure to As. GSE may exert its effects through the suppression of Nox and inhibition of TGF-β/Smad signaling activation.[303]

Cardio-protective

Free radicals and oxidative stress play a crucial role in the pathophysiology of a broad spectrum of cardiovascular diseases including congestive heart failure, valvular heart disease, cardiomyopathy, hypertrophy, atherosclerosis and ischemic heart disease. We have demonstrated that IH636 grape seed proanthocyanidin extract (GSPE) provides superior antioxidant efficacy as compared to Vitamins C, E and beta-carotene. A series of studies were conducted using GSPE to demonstrate its cardioprotective ability in animals and humans. GSPE supplementation improved cardiac functional assessment including post-ischemic left ventricular function, reduced myocardial infarct size, reduced ventricular fibrillation (VF) and tachycardia, decreased the amount of reactive oxygen species (ROS) as detected by ESR spectroscopy and reduced malondialdehyde (MDA) formation in the heart perfusate. Cardiomyocyte apoptosis detected by terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) staining. In concert, the proapoptotic signals mediated by JNK-l and c-fos proteins were also reduced suggesting that the novel cardioprotective properties of GSPE may be at least partially attributed to its ability to block anti-death signaling mediated through the proapoptotic transcription factors and genes such as JNK-1 and c-JUN. In a separate study, GSPE pretreatment significantly inhibited doxorubicin-induced cardiotoxicity as demonstrated by reduced serum creatine kinase (CK) activity, DNA damage and histopathological changes in the

cardiac tissue of mice. Concentration-dependent efficacy of GSPE was also assessed in a hamster atherosclerosis model. Approximately 49 and 63% reduction in foam cells, a biomarker of early stage atherosclerosis, were observed following supplementation of 50 and 100 mg GSPE/kg body weight, respectively. A human clinical trial was conducted on hypercholesterolemic subjects. GSPE supplementation significantly reduced oxidized LDL, a biomarker of cardiovascular diseases. Finally, a cDNA microarray study demonstrated significant inhibition of inducible endothelial CD36 expression, a novel cardioregulatory gene, by GSPE. These results demonstrate that GSPE may serve as a potential therapeutic tool in promoting cardiovascular health via a number of novel mechanisms. [80]

Grape seed proanthocynadin extract (GSPE) improve thoracic aorta of hyperlipemia rats

Four groups were randomly selected from 32 male Sprague-Dawley rats: control group (C), the hyperlipemia group (HL highlipid diets), the pioglitazone group (PI highlipid diets), the procyanidins group (PC highlipid diets). The PI is positive to PC. At the same time, rats were treated with either 10 mg/kg body weight pioglitazone, 100 mg/kg body weight procyanidins or distilled water by gavage daily till the end of the experiment. At 0 week, 3th week, 6th week, blood was gotten by tail vein. Rats were sacrificed at 6th week. The levels of serum TG, TC, HDL-C were tested by kits, PPARgamma mRNA were measured by RT-PCR and the activities of NF-kappaB by EMSA. RESULTS: (1) At the 6th week, the levels of TG, TC in HL rats is higher than C rats ($P< 0.05$). Procyanidins and reduced the levels of TG, TC ($P < 0.05$), but there has no statistical difference between PI, C and PC. (2) Compared with C rats, the levels of PPARgamma mRNA of thoracic aorta were lower, the activities of the NF-kappaB of thoracic aorta were higher ($P < 0.05$) in HL and showed to be statistical difference ($P < 0.05$). (3) Compared with HL rats, the levels of PPARgamma mRNA of thoracic aorta were improved were reduced in PI rats ($P < 0.05$). (4) Compared with HL rats, the levels of PPARgamma mRNA of thoracic aorta were improved and the activities of the NF-kappaB of thoracic aorta were reduced in PC rats ($P < 0.05$). CONCLUSION: PC can reduce the levels of TG, TC and PC can reduce the activities of NF-kappaB of thoracic aorta of hyperlipemia rats by preventing the expressions of PPARgamma mRNA down-regulating. [254]

Grape seed extract and Niacin bound Chromium Decrease LDL

Hypercholesterolemia, a significant cardiovascular risk factor, is prevalent in the American population. Many drugs lower circulating cholesterol levels, but they are not infrequently associated with severe side effects. Accordingly, natural means to lower cholesterol levels safely would be welcomed. We examined 40 hypercholesterolemic subjects (total cholesterol 210-300 mg/dL) in a randomized, double-blind, placebo-controlled study. The four groups of ten subjects received either placebo bid, chromium polynicotinate (Cr) 200 microg bid, grape seed extract (GSE) 100 mg bid, or a combination of Cr and GSE at the same dosage bid. Over two months, the average percent change +/- SEM in the total cholesterol from baseline among groups was: placebo -3.5% +/- 4, GSE -2.5% +/- 2, Cr -10% +/- 5, and combination -16.5% +/- 3. The decrease in the last group was significantly different from placebo. The major decrease in cholesterol concentration was in the LDL levels: placebo -3.0% +/- 4, GSE -1.0% +/- 2.0, Cr -14% +/- 4.0, and the combination -20% +/- 6.0. Again, the combination of Cr and GSE significantly decreased LDL when compared to placebo ($p<0.01$). HDL levels essentially did not change among the groups. Also, there was no significant difference in the triglyceride concentrations among the groups; and no statistically significant differences were seen in the levels of autoantibodies to oxidized LDL (Ox-LDL). However, the trend was for the two groups receiving GSE to have greater decreases in the latter parameter, i.e., -30.7% and -44.0% in the GSE and combined groups in contrast to -17.3% and -10.4% in the placebo and chromium groups. We determined the number of subjects in each group who decreased autoantibodies to oxidized LDL greater than 50% over eight weeks and found these ratios among groups: placebo = 2/9, Cr = 1/10, GSE = 6/10, and combined = 3/8. Thus, 50% of subjects (9/18) receiving GSE had a greater than 50% decrease in autoantibodies compared to 16% (3/19) in the two groups not receiving GSE. No significant changes occurred in the levels of circulating homocysteine and blood pressure among the four groups. We conclude that a combination of Cr and GSE can decrease total cholesterol and LDL levels significantly. Furthermore, there was a trend to decrease the circulating autoantibodies to oxidized LDL in the two groups receiving GSE. **[48]**

Anti-atherosclerosis

The link between flavonoids and atherosclerosis is based partly on the evidence that some flavonoids possess antioxidant properties and have been shown to be potent inhibitors of LDL oxidation in vitro. Hypercholesterolemia, a significant cardiovascular risk factor is prevalent in the American population. Grape seed proanthocyanidin extracts are known to exhibit a broad spectrum of chemopreventive and cardioprotective properties against oxidative stress. A recent study has shown that a combination of IH636 grape seed proanthocyanidin extract (GSPE) and a niacin-bound chromium (NBC) can decrease total cholesterol, LDL and oxidized LDL levels in hypercholesterolemic human subjects. In this study, we assessed the efficacy of GSPE supplementation in hamsters, singly and in combination with NBC, since these animals have a similar lipid profile to hypercholesterolemic

humans when fed a hypercholesterolemic diet of 0.2% cholesterol and 10% coconut oil (HCD). After 10 weeks of feeding HCD, these animals developed foam cells, which is a biomarker of early stages of atherosclerosis. Atherosclerosis (% of aorta covered with foam cells) was reduced by approximately 50% and 63% following supplementation of these animals with 50 mg/kg and 100 mg/kg of GSPE, respectively, in conjunction with a HCD, while approximately 32% reduction was observed following supplementation of GSPE plus NBC. A range of 7-9 animals was used in each study group. GSPE alone and in combination with NBC exerted a pronounced effect on the cholesterol, and triglyceride levels, as well as oxidative lipid damage as demonstrated by the formation of thiobarbituric acid reactive substances (TBARS). This data demonstrates that GSPE and NBC may provide significant health benefits by dramatically ameliorating the incidence of atherosclerosis as demonstrated by reducing the formation of foam cells. [90]

Red grape seed extract improves lipid profiles and decreases oxidized low-density lipoprotein in patients with mild hyperlipidemia.

Abstract Hyperlipidemia can lead to atherosclerosis by lipoprotein deposition inside the vessel wall and oxidative stress induction that leads to the formation of atherosclerotic plaque. Oxidized low-density lipoprotein particles (Ox-LDL) have a key role in the pathogenesis of atherosclerosis. The lipid-lowering properties and antioxidants of the grape seed can be beneficial in atherosclerosis prevention. We conducted a randomized double-blind placebo-controlled crossover clinical trial. Fifty-two mildly hyperlipidemic individuals were divided into two groups that received either 200 mg/day of the red grape seed extract (RGSE) or placebo for 8 weeks. After an 8-week washout period, the groups were crossed over for another 8 weeks. Lipid profiles and Ox-LDL were measured at the beginning and the end of each phase. RGSE consumption reduced total cholesterol (-10.68±26.76 mg/dL, P=.015), LDL cholesterol (-9.66±23.92 mg/dL, P=.014), and Ox-LDL (-5.47±12.12 mg/dL, P=.008). While triglyceride and very low-density lipoprotein cholesterol were decreased and high-density lipoprotein cholesterol was increased by RGSE, the changes were not statistically significant. RGSE consumption decreases Ox-LDL and has beneficial effects on lipid profile-consequently decreasing the risk of atherosclerosis and cardiovascular disorders-in mild hyperlipidemic individuals.[295]

Stop arteries from hardening

Recent studies demonstrated that GSPE stopped cholesterol from building up in the arteries of guinea pigs, which in turn leads to the thickening and hardening of the vessels and the resulting condition, atherosclerosis. This limits the ability of the arteries to expand and contract as blood passes through them and can caused strokes and heart attacks. Researchers fed the guinea pigs a diet rich in coconut fat. One group was supplemented GSPE provided by US-based Polyphenolics. After 12 weeks, the cholesterol accumulation in the animals' tissues was significantly lower in the group that received the grape seed extract.

The findings confirm previous evidence from trials done on grape powder and grape juice. In April, Israeli researchers reported that consumption of grape powder reduced the atherosclerotic lesion area in mice by 41 per cent compared to a control group. Polyphenolics claims however that the product used in the new studies contains an oxygen radical absorbance capacity, or ORAC value, 1000 times higher than that of grape juice. The antioxidant activity of the flavonoids in grapes is thought to be responsible for reducing the cholesterol build-up. [132]

Antihypertensive

Hypertension, or a blood pressure higher than 140/90 mm Hg, is the most common risk factor for cardiovascular and cerebrovascular morbidity and mortality. Purpose: The aim of the study was to test a possible protective effect of a oral OPC supplement called Pycnogenol, administrated for eight weeks to non-smoking, mildly hypertensive patients. Methods: Pycnogenol, 200 mg/day, or placebo was provided to eleven subjects (seven men and four women) with systolic blood pressure of 140-159 mm Hg in a double blind, randomized, cross-over study and/or diastolic blood pressure of 90-99 mm Hg for eight weeks. The subject's blood pressure was taken during supplementation, and the serum level of thromboxane was measured.

Results: A significant decrease in the systolic blood pressure was observed during Pycnogenol supplementation. However, Pycnogenol's lowering of diastolic blood pressure did not reach statistical significance when compared to placebo. Serum thromboxane concentration was significantly ($p < 0.05$) decreased during Pycnogenol supplementation. [59]

Lowers blood pressure in metabolic syndrome

A recent human study (2009) testing grape seed extract (GSE) phenolic compounds could lower blood pressure in subjects with the metabolic syndrome. The subjects were randomized into 3 groups-(a) placebo, (b) 150 mg GSE per day, and (c) 300 mg GSE per day-and treated for 4 weeks. Serum lipids and blood glucose were measured at the beginning of the study and at the end. Blood pressure was recorded using an ambulatory monitoring device at the start of the treatment period and at the end. Both the systolic and diastolic blood pressures were lowered after

treatment with GSE as compared with placebo.[269]
An earlier animal study tested GSE in young, estrogen-depleted salt-sensitive hypertensive animals. GSE at .5% added to the daily food, decreased arterial pressure.[270]
GSE also possesses profound redox/anti-oxidant effects and recently demonstrated in a human trial to inhibit LDL cholesterol oxidation (vascular damage).[271]
A study published in the July 16th issue of Metabolism Clinical and Experimental indicates that, when taken in conjunction with lifestyle modification, MegaNatural BP GSE, may effectively lower blood pressure in patients with metabolic syndrome. The placebo-controlled trial involved 24 subjects with metabolic syndrome and documented the blood pressure lowering effect of MegaNatural BP GSE. Two dosage levels (150 mg/day and 300 mg/day) and a placebo were given to 3 groups of 8 patients, respectively. After 1 month of treatment, there was a significant reduction in blood pressure in both groups given MegaNatural BP. There was no change in the group given the placebo. Also, subjects given 300 mg of MegaNatural BP had a significant decrease in the concentration of oxidized LDL in plasma.[272]

Ant-inflammatory within Lung Tissue

The inhibitory properties of procyanidins from Vitis vinifera L. seeds on the respiratory burst and on the release of granule components myeloperoxidase, beta-glucuronidase and elastase were studied in activated human neutrophils. Procyanidins strongly inhibit superoxide generation through a direct scavenging of superoxide and prevent the release from calcium ionophore activated neutrophils of beta-glucuronidase, myeloperoxidase and elastase. In addition they dose-dependently inhibit the activity of myeloperoxidase released from calcium ionophore-stimulated cells. The monomeric constitutive unit (+)-catechin was far less active than procyanidins in all the models tested. These results evidence that procyanidins efficiently restrain the inflammatory response of activated neutrophils in vitro and whenever absorbed in vivo can prevent their oxidative discharge at the site(s) of their adhesion. [55]

Anti-aging/ Antioxidant

Young and aged male rats were fed a diet enriched with procyanidins complexed (1:3 w/w) with soybean lecithin (2.4%); control animals (CTR-young and CTR-aged) received an equal amount of lecithin and 2 additional groups of animals the standard diet. At the end of the treatment, the total plasma antioxidant defense (TRAP), vitamin E, ascorbic acid and uric acid were determined in plasma and the hearts from all groups of animals subjected to moderate ischemia (flow reduction to 1 ml/min for 20 min) and reperfusion (15 ml/min for 30 min). In both young and aged rats supplemented with procyanidins the recovery of left ventricular developed pressure (LVDP) at the end of reperfusion was 93% ($p < 0.01$) and 74% ($p < 0.01$) of the preischemic values and the values of coronary perfusion pressure (CPP) were maintained close to those of the preischemic period. Also creatine kinase (CK) outflow was restrained to baseline levels, while a 2-fold increase in prostacyclin (6-keto-PGF1alpha) in the perfusate from hearts of young and aged rats was elicited during both ischemia and reperfusion. In parallel, procyanidins significantly increased the total antioxidant plasma capacity (by 40% in young and by 30% in aged rats) and the plasma levels of ascorbic acid, while tend to reduce vitamin E levels; no significant differences were observed in uric acid levels. The results of this study demonstrate that procyanidins supplementation in the rat (young and aged) makes the heart less susceptible to ischemia/reperfusion damage and that this is positively associated to an increase in plasma antioxidant activity. [75]

Anti-Oxidative

The protective effects of grape seed procyanidin extract on the repair of H2O2-induced DNA lesions were tested using Fao cells. Cells were exposed to 600 microM H2O2 for 3 or 21 h. A procyanidin extract from grape seed (PE) was incubated or preincubated (1 h) during the exposure to H2O2. The ability of procyanidins to protect against the genotoxicity of H2O2 was compared with those of the monomeric flavanols (+)-catechin and (-)-epicatechin and the flavonol quercetin. After treatment, DNA damage was monitored using alkaline single-cell gel electrophoresis (the comet assay) (Aherne, S. A.; O'Brien, N. M. Nutr. Cancer 1999, 34, 160-166). At the end of the experiment, PE significantly decreased the damage caused by H2O2. The results also showed that quercetin was the most effective of the flavonoids tested, which is consistent with its powerful antioxidant character. The results indicate that procyanidins are more effective than the corresponding individual monomers, catechin and epicatechin, at preventing DNA lesions in hepatocytes and that this protection is higher after preincubation than after co-incubation. [53]

The anti-oxidative effect of proanthocyanidins (PC), from grape seed extract, was evaluated in D-galactose-induced murine model. Male KUNMING mice were divided into different groups at random. The mice were induced by subcutaneous injection of D-galactose on the back of mice daily for 60 days and simultaneously PC and VE were administered to the mice by oral feeding. Results indicate that the lipid peroxide in blood, liver and brain

significantly increased respectively because of the D-galactose injection, when compared to the control. The activity of SOD in RBC and liver significantly decreased while that of GSH-PX in blood and liver decreased. MAO-B in brain was significantly increased while MAO-B in liver doesn't change significantly. Oral feeding PC markedly decreased the formation of MDA in blood, liver and brain, increased the activities of SOD in blood and liver and decreased the activity of MAO-B in brain. In summary, treatment with PC at all the three tested doses were effective in exerting a protective effect against oxidative stress. [88]

Antioxidative

Oxidative stress is considered as a major risk factor that contributes to age-related increase in lipid peroxidation and declined antioxidants in the central nervous system during aging. Grape seed extract, one of the bioflavonoid, is widely used for its medicinal properties. In the present study, we evaluated the role of grape seed extract on lipid peroxidation and antioxidant status in discrete regions of the central nervous system of young and aged rats. Male albino rats of Wistar strain were divided into four groups: Group I-control young rats, Group II-young rats treated with grape seed extract (100mg/kg body weight) for 30 days, Group III-aged control rats and Group IV-aged rats supplemented with grape seed extract (100mg/kg body weight) for 30 days. Age-associated increase in lipid peroxidation was observed in the spinal cord, cerebral cortex, striatum and the hippocampus regions of aged rats (Group III). Activities of antioxidant enzymes like superoxide dismutase, catalase, glutathione peroxidase and levels of non-enzymic antioxidants like reduced glutathione, Vitamin C and Vitamin E were found to be significantly decreased in all the brain regions studied in aged rats when compared to young rats. However, normalized lipid peroxidation and antioxidant defenses were reported in the grape seed extract-supplemented aged rats. These findings demonstrated that grape seed extract enhanced the antioxidant status and decreased the incidence of free radical-induced lipid peroxidation in the central nervous system of aged rats. [133]

Inhibits oxidative damage and atherosclerosis

Grape seed extract (GSE), rich in OPCs reduces free radical damage of blood vessel cells by 85 per cent and protect against heart disease, Oxidative stress of endothelial cells can cause inflammation, hardening of the walls of the arteries (atherosclerosis), and cardiovascular disease (CVD). The results of the new *ex vivo* study show that endothelial cells, the cells that line the inner walls of blood vessels, grown in the presence of OPCs were more resistant to oxidative stress due to hydrogen peroxide. A time- and dose-dependent attenuation of cell death was observed. Pre-incubation with OPCs (60 micrograms per millilitre for 24 hours resulted in a reduction of hydrogen peroxide cell lysis from 70 to 10 per cent. [151]

Reduces lung fibrosis (silica-induced – scavenges ROS)

Due to the production of reactive oxygen species (ROS), oxidative stress has been implicated in the pathogenesis of silica-induced lung fibrosis. So it is hypothesized that grape seed extract (GSE) or vitamin E (Vit E) as antioxidants may ameliorate some symptoms of the disease. Male Wistar albino rats were divided into 7 groups: rats in group I instilled intratracheally (IT) with a single dose of silica suspension (50mg/rat) as positive control (PC). Treatment groups (II-IV) received Vit E (20IU/kg/day), GSE (150mg/kg/day), or Vit E+GSE simultaneously orally 1 day after instillation of silica. Groups V and VI were given oral GSE or Vit E after instillation of the equivalent volume of saline (IT) as controls for GSE or Vit E. Rats of group VII only instilled saline (IT) as negative control. After 90 days animals were sacrificed and plasma-malondialdehyde (p-MDA) and lung tissue hydroxyproline (HP) were quantified. The lungs were also investigated for histopathological changes. The mean concentrations of p-MDA and HP in studied groups (I-VII) were 1.95, 2.77, 0.72, 0.81, 0.64, 0.94, 1.02mumolMDA/L(plasma) and 28.476, 27.85, 22.83, 22.64, 15.40, 18.31, 18.51mgHP/g(tissue), respectively. Silica caused a significant increase in HP content of lungs and MDA levels in the plasma except in GSE-treated groups (III and IV). According to the results of this study GSE could reduce the fibrogenic effect of silica. However; no synergistic effect was observed after co-administration of GSE and Vit E. [221]

Attenuates Airway Inflammation and Hyperresponsiveness in a Murine Model of Asthma by Downregulating Inducible Nitric Oxide Synthase.

Allergic asthma is characterized by hyperresponsiveness and inflammation of the airway with increased expression of inducible nitric oxide synthase (iNOS) and overproduction of nitric oxide (NO). Grape seed proanthocyanidin extract (GSPE) has been proved to have antioxidant, antitumor, anti-inflammatory, and other pharmacological effects. The purpose of this study was to examine the role of GSPE on airway inflammation and hyperresponsiveness in a mouse model of allergic asthma. BALB/c mice, sensitized and challenged with ovalbumin (OVA), were intraperitoneally injected with GSPE. Administration of GSPE remarkably suppressed airway resistance and reduced the total inflammatory cell and eosinophil counts in BALF. Treatment with GSPE significantly enhanced the interferon (IFN)- γ level and decreased interleukin (IL)-4 and IL-13 levels in BALF and total IgE levels in serum. GSPE also attenuated allergen-induced lung eosinophilic inflammation and mucus-producing goblet cells in the airway. The elevated iNOS expression observed in the OVA mice was significantly

inhibited by GSPE. In conclusion, GSPE decreases the progression of airway inflammation and hyperresponsiveness by downregulating the iNOS expression, promising to have a potential in the treatment of allergic asthma.[279]

Improves Visual Health/Prevention of Cataracts/Antioxidative

This paper investigates the anticataract activity of grape seed extract (GSE, which contains 38.5% procyanidins) in hereditary cataractous rats (ICR/f rats). The ICR/f rats were fed a standard diet containing 0 or 0.213% GSE [0.082% procyanidins in the diet (w/w)] for 27 days. The GSE significantly prevented and postponed development of cataract formation by evaluation of slit lamp observations of the rats' eyes. Lens weight and malondialdehyde concentration in the lens and plasma cholesteryl ester hydroperoxide (ChE-OOH) level induced by CuSO4 were significantly lower in the GSE group compared with the control group. The rats were also fed for 14 days either the diet containing 0.085% procyanidin dimer to tetramer fraction (0.085% as the procyanidins), the diet containing 0.090% procyanidin pentamer to heptamer fraction (0.085% as the procyanidins), or the diet containing 0.093% procyanidin oligomers more than decamer fraction (0.085% as the procyanidins). The ChE-OOH levels in the procyanidin pentamer to heptamer and procyanidin oligomers more than decamer groups were significantly lower than in the procyanidin dimer to tetramer group. These results suggested that procyanidins and their antioxidative metabolites prevented the progression of cataract formation by their antioxidative action. The larger molecular procyanidins in the GSE might contribute this anticataract activity. [76]

Cytoprotective: Liver, Kidney, Heart and Lung Protective

Grape seed extract, primarily a mixture of proanthocyanidins, has been shown to modulate a wide-range of biological, pharmacological and toxicological effects which are mainly cytoprotective. This study assessed the ability of IH636 grape seed proanthocyanidin extract (GSPE) to prevent acetaminophen (AAP)-induced nephrotoxicity, amiodarone (AMI)-induced lung toxicity, and doxorubicin (DOX)-induced cardiotoxicity in mice. Experimental design consisted of four groups: control (vehicle alone), GSPE alone, drug alone and GSPE+drug. For the cytoprotection study, animals were orally gavaged 100 mg/Kg GSPE for 7-10 days followed by i.p. injections of organ specific three drugs (AAP: 500 mg/Kg for 24 h; AMI: 50 mg/Kg/day for four days; DOX: 20 mg/Kg for 48 h). Parameters of study included analysis of serum chemistry (ALT, BUN and CPK), and orderly fragmentation of genomic DNA (both endonuclease-dependent and independent) in addition to microscopic evaluation of damage and/or protection in corresponding PAS stained tissues. Results indicate that GSPE preexposure prior to AAP, AMI and DOX, provided near complete protection in terms of serum chemistry changes (ALT, BUN and CPK), and significantly reduced DNA fragmentation. Histopathological examination of kidney, heart and lung sections revealed moderate to massive tissue damage with a variety of morphological aberrations by all the three drugs in the absence of GSPE preexposure than in its presence. GSPE+drug exposed tissues exhibited minor residual damage or near total recovery. Additionally, histopathological alterations mirrored both serum chemistry changes and the pattern of DNA fragmentation. Interestingly, all the drugs, such as, AAP, AMI and DOX induced apoptotic death in addition to necrosis in the respective organs which was very effectively blocked by GSPE. Since AAP, AMI and DOX undergo biotransformation and are known to produce damaging radicals in vivo, the protection by GSPE may be linked to both inhibition of metabolism and/or detoxification of cytotoxic radicals. In addition, its' presumed contribution to DNA repair may be another important attribute, which played a role in the chemoprevention process. [79]

Grape seed proanthocyanidin and ginkgo biloba extract protect against doxorubicin-induced cardiac injury in rats, via antioxidant and antiapoptotic effects

Grape seed proanthocyanidins (GSPE) and ginkgo biloba extract (EGb761) are considered to have protective effects against several diseases. The cardiotoxicity of doxorubicin (DOX) has been reported to be associated with oxidative damage. This study was conducted to evaluate the cardioprotective effects of GSPE and EGb761 against DOX-induced heart injury in rats. DOX was administered as a single i.p. dose (20 mg kg(-1)) to adult male rats. DOX-intoxicated rats were orally administered GSPE (200 mg kg(-1) day(-1)) or EGb761 (100 mg kg(-1) day(-1)) for 15 consecutive days, starting 10 days prior DOX injection. DOX-induced cardiotoxicity was evidenced by a significant increase in serum aspartate transaminase (AST), creatine phosphokinase isoenzyme (CK-MB), lactate dehydrogenase (LDH), total cholesterol (TC) and triglyceride (TG) activities and levels. Increased oxidative damage was expressed by the depletion of cardiac reduced glutathione (GSH), elevation of cardiac total antioxidant (TAO) level and accumulation of the lipid peroxidation product, malondialdehyde (MDA). Significant rises in cardiac tumour necrosis factor-alpha (TNF-α) and caspase-3 levels were noticed in DOX-intoxicated rats. These changes were ameliorated in the GSPE and EGb761-treated groups. Histopathological analysis confirmed the cardioprotective effects of GSPE and EGb761. In conclusion, GSPE and EGb761 mediate their protective effect against DOX-induced cardiac injury through antioxidant, anti-inflammatory and antiapoptotic mechanisms.[290]

Protection From cadmium chloride

Grape seed proanthocyanidin extract (GSPE) provides excellent concentration- and dose-dependent protection

against toxicities induced by diverse agents, such as acetaminophen, hydrogen peroxide, 12-O-tetradecanoylphorbol-13-acetate (TPA), smokeless-tobacco extract, idarubicin and 4-hydroxyperoxycyclophosphamide in both in vitro and in vivo models. In some models, GSPE proved to be a better cytoprotectant than vitamins C, E and beta-carotene. The purpose of this investigation was three fold: (i) to indirectly assess the bioavailability of GSPE in multiple target organs, (ii) quantify GSPE's capacity to avert cadmium chloride (CdCl2)-induced nephrotoxicity, dimethylnitrosamine (DMN)-induced splenotoxicity and O-ethyl-S,S-dipropyl phosphorodithioate (MOCAP)-induced neurotoxicity, and lastly (iii) to evaluate possible mechanisms of protection in mice. Results indicate that GSPE preexposure prior to cadmium chloride and DMN provided near complete protection in terms of serum chemistry changes (ALT, BUN and CK) and inhibition of both forms of cell death. e.g., apoptosis and necrosis. DNA damage, a common denominator usually associated with both apoptosis and necrosis was significantly reduced by GSPE treatment. Histopathological examination of organs correlated strongly with the changes in serum chemistry and the DNA modification data. Surprisingly, MOCAP exposure showed symptoms of neurotoxicity coupled with serum chemistry changes in the absence of any significant genomic DNA damage or brain pathology. Although, GSPE appeared to partially protect the neural tissue, it powerfully antagonized MOCAP-induced mortality. Taken together, this study suggests that in vivo GSPE-preexposure may protect multiple target organs from a variety of toxic assaults induced by diverse chemical entities. [81]

Restores Normal Permeability to the Blood Brain Barrier (BBB)

Blood-brain barrier (BBB) is the site of regulatory mechanisms, which control the exchange of substances between the brain and the blood through the wall of 'true' brain capillaries with tight junctions between endothelial cells. In some pathological situations the permeability of the BBB is increased because of a partial proteolytic degradation of some constituents of the capillary basement lamina. In such cases it is important to restore normal permeability. The effect of procyanidolic oligomers (PCO) on the BBB was investigated in vivo with quantitative morphologic procedures. We also investigated the action of this drug on collagen and basement lamina constituents (Matrigel) in vitro. Collagenase injected in lateral brain ventricles was shown to increase BBB permeability. Per os administration of PCO to rats greatly increased the resistance of brain capillaries to bacterial collagenase, as shown by the inhibition of the diffusion of fluorescein-isothiocyanate-marked dextran particles from the blood-stream into the brain tissues. Calf skin collagen pretreated in vitro with PCO became more resistant to the hydrolytic action of collagenase. Similar, even more intense protective effect was seen when basal lamina constituents containing type IV collagen was incubated with PCO before exposure to pronase. These in vitro effects may partly explain the in vivo protective effect of PCO against the alteration of brain capillaries by i.v. injected bacterial collagenase.[58]

Reduces cognitive decline in animal model of Alzheimer's disease

The June 18, 2008 issue of The Journal of Neuroscience published the discovery of researchers at Mount Sinai School of Medicine in New York that administering grape seed polyphenols reduces amyloid beta aggregation in the brain and slows cognitive impairment in a mouse model of Alzheimer's disease. Accumulation of soluble high-molecular weight amyloid beta compounds in the brains of Alzheimer's disease patients leads to the formation of plaques that are believed to be responsible for the memory loss and dementia that occurs with the disease.For the current study, Giulio Pasinetti, MD, PhD, of Mount Sinai's Departments of Psychiatry and Neuroscience, and his associates used mice that were genetically modified to develop Alzheimer's disease. The animals were divided to receive a polyphenolic grape seed extract or a placebo for five months prior to the usual age at which signs of the disease develop. The dose of extract used in the study was equivalent to the daily amount of polyphenolics consumed by the average person.At the end of the treatment period, beta-amyloid accumulation was significantly reduced in the brains of animals that received the polyphenolic extract compared with the placebo group. The animals also demonstrated improved spatial memory compared with those that did not receive the extract, indicating less cognitive decline. In previous experimentation conducted by Dr Pasinetti, red wine was found to limit cognitive decline in the Alzheimer's disease mouse model. Research carried out by Dr Pasinetti's team has sought to identify the compounds in red wine's nearly 5,000 molecules that are responsible for its benefits. "Our intent is to develop a highly tolerable, nontoxic, orally available treatment for the prevention and treatment of Alzeheimer's dementia," Dr Pasinetti stated. Future research may determine whether grape polyphenols can be used to treat human Alzheimer's disease patients. [220]

Inhibition of protein glycation by skins and seeds of the muscadine grape

The formation of advanced glycation end products (AGEs) leading to protein glycation and cross-linking is associated with the pathogenesis of diabetic complications. The inhibition of protein glycation by phenolic and flavonoid antioxidants demonstrates that the process is mediated, in part, by oxidative processes. In this study, the

effects of seed and skin extracts of the muscadine grape on AGEs formation were examined. Seeds and skins were extracted (10% w/v) with 50% ethanol and incubated at 37 degrees C with a solution containing 250 mM fructose and 10 mg/ml albumin. After 72 h, fluorescence was measured at the wavelength pair of 370 and 440 nm as an index of the formation of AGEs. Both seed and skin extracts were found to be efficacious inhibitors of AGE formation. A 1:300 dilution of the seed extract decreased fluorescence by approximately 65%, whereas muscadine grape skin extract produced a 40% lowering. This difference correlates with the greater antioxidant activity found in muscadine seeds in comparison to skins, however, on a mass basis, the inhibitory activities of the seeds and skins were found to be nearly equivalent. Gallic acid, catechin and epicatechin, the three major polyphenols in the seeds, all significantly decreased the AGE product related fluorescence at a concentration of 50 muM. Neither muscadine seed extract nor skin extract inhibited the methylglyoxal-mediated glycation of albumin. These results suggest that consumption of the muscadine grape may have some benefit in altering the progression of diabetic complications. [222]

Beneficial to Connective Tissue

GSE and quercetin and their potential roles in treating musculoskeletal conditions

There is evidence to suggest that flavonoids may be beneficial to connective tissue for several reasons, which include the limiting of inflammation and associated tissue degradation, the improvement of local circulation, as well as the promoting of a strong collagen matrix. [49]

Promotes wound healing

The wound site is rich in oxidants such as hydrogen peroxide mostly contributed by neutrophils and macrophages. Proanthocyanidins or condensed tannins are a group of biologically active polyphenolic bioflavonoids that are synthesized by many plants. This study provides first evidence showing that natural extracts such as grape seed proanthocyanidin extract containing 5000 ppm resveratrol (GSPE) facilitates oxidant-induced VEGF expression in keratinocytes. The current results suggest that GSPE may have beneficial therapeutic effects in promoting dermal wound healing and other related skin disorders. [85]

In another study GSPE topical treatment was associated with a more well-defined hyperproliferative epithelial region, higher cell density, enhanced deposition of connective tissue, and improved histological architecture. [86]

Promotes Healing of Skin

Flavonoids are a family of polyphenols that display a remarkable ability to inhibit cellular damage to the skin, inhibit aging of the skin, and promote healing of the skin.

Cellular Protection

Grape seed proanthocyanidins have been reported to possess a broad spectrum of pharmacological and medicinal properties against oxidative stress. Grape seed proanthocyanidins extract (GSPE) provides excellent protection against free radicals in both in vitro and in vivo models. GSPE had significantly better free radical scavenging ability than vitamins C, E, and beta-carotene and demonstrated significant cytotoxicity towards human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal cells. GSPE protected against tobacco-induced apoptotic cell death in human oral keratinocytes and provided protection against cancer chemotherapeutic drug-induced cytotoxicity in human liver cells by modulating cell cycle/apoptosis regulatory genes such as bc12, p53 and c-myc. Recently, the bioavailability and mechanistic pathways of cytoprotection by GSPE were examined on acetaminophen-induced hepatotoxicity and nephrotoxicity, amiodarone-induced pulmonary toxicity, doxorubicin-induced cardiotoxicity, DMN-induced immunotoxicity and MOCAP-induced neurotoxicity in mice. Serum chemistry changes, integrity of genomic DNA and histopathology were assessed. GSPE pre-exposure provided near complete protection in terms of serum chemistry changes and DNA damage, as well as abolished apoptotic and necrotic cell death in all tissues. Histopathological examination reconfirmed these findings. GSPE demonstrated concentration-/dose-dependent inhibitory effects on the drug metabolizing enzyme cytochrome P450 2E1, and this may be a major pathway for the anti-toxic potential exerted by GSPE. Furthermore, GSPE treatment significantly decreased TNFalpha-induced adherence of T-cells to HUVEC by inhibiting VCAM-1 expression. These results demonstrate that GSPE is highly bioavailable and may serve as a potential therapeutic tool in protecting multiple target organs from structurally diverse drug- and chemical-induced toxicity. [50]

Antiinflammatory

To investigate the anti-inflammatory effect and mechanism of proanthocyanidins (PA) from grape seeds. PA inhibited carrageenan-induced paw edema in rats and croton oil-induced ear swelling in mice in a dose-dependent manner. PA 10 mg/kg reduced MDA content in inflamed paws, inhibited beta-NAG and NOS activity, and lowered the content of NO, IL-1beta, TNFalpha, and PGE2 in exudate from edema paws of rats induced by carrageenan. The inhibitory effect of PA on all above indices was more evident than that of dexamethasone 2 mg/kg. CONCLUSION:

PA has anti-inflammatory effect on experimental inflammation in rats and mice. Its mechanisms of anti-inflammatory action are relevant to oxygen free radical scavenging, anti-lipid peroxidation, and inhibition of the formation of inflammatory cytokines. [91]

Protects Against Oxidative Stress

Regulates bcl-2 gene; downregulates oncogene c-myc; protects against acetaminophen induced liver and kidney damage; ameliorates chronic pancreatitis

Free radicals have been implicated in over a hundred disease conditions in humans, including arthritis, hemorrhagic shock, atherosclerosis, advancing age, ischemia and reperfusion injury of many organs, Alzheimer and Parkinson's disease, gastrointestinal dysfunctions, tumor promotion and carcinogenesis, and AIDS. Antioxidants are potent scavengers of free radicals and serve as inhibitors of neoplastic processes. A large number of synthetic and natural antioxidants have been demonstrated to induce beneficial effects on human health and disease prevention. However, the structure-activity relationship, bioavailability and therapeutic efficacy of the anti-oxidants differ extensively. Oligomeric proanthocyanidins, naturally occurring anti-oxidants widely available in fruits, vegetables, nuts, seeds, flowers and bark, have been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress. We have assessed the concentration- or dose-dependent free radical scavenging ability of a novel IH636 grape seed proanthocyanidin extract (GSPE) both in vitro and in vivo models, and compared the free radical scavenging ability of GSPE with vitamins C, E and beta-carotene. These experiments demonstrated that GSPE is highly bioavailable and provides significantly greater protection against free radicals and free radical-induced lipid peroxidation and DNA damage than vitamins C, E and beta-carotene. GSPE was also shown to demonstrate cytotoxicity towards human breast, lung and gastric adenocarcinoma cells, while enhancing the growth and viability of normal human gastric mucosal cells. The comparative protective effects of GSPE, vitamins C and E were examined on tobacco-induced oxidative stress and apoptotic cell death in human oral keratinocytes. Oxidative tissue damage was determined by lipid peroxidation and DNA fragmentation, while apoptotic cell death was assessed by flow cytometry. GSPE provided significantly better protection as compared to vitamins C and E, singly and in combination. GSPE also demonstrated excellent **protection against acetaminophen overdose-induced liver and kidney damage by regulating bcl-X(L) gene, DNA damage** and presumably by reducing oxidative stress. GSPE demonstrated excellent protection against myocardial ischemia-reperfusion injury and myocardial infarction in rats. GSPE was also shown to **regulate bcl-2 gene and downregulate the oncogene c-myc.** Topical application of GSPE enhances sun protection factor in human volunteers, as well as supplementation of GSPE **ameliorates chronic pancreatitis** in humans. These results demonstrate that GSPE provides excellent protection against oxidative stress and free radical-mediated tissue injury. **56**

Ameliorate chemotherapy

Chemotherapeutic agents are effective in inhibiting growth of cancer cells in vitro and in vivo, however, toxicity to normal cells is a major problem. In this study, grape seed proanthocyanidin extract (GSPE) was found to ameliorate chemotherapy-induced toxic effects in cultured Chang epithelial cells, established from nonmalignant human tissue. Thus, these results indicate that GSPE can be a potential candidate to ameliorate the toxic effects associated with chemotherapeutic agents. [84]

Synergistic with chemotherapy

With an approach to enhance the efficacy of chemotherapy agents against breast cancer treatment, here we investigated the anticancer effects of grape seed extract (GSE) and doxorubicin (Dox), either alone or in combination, in estrogen receptor-positive MCF-7 and receptor-negative MDA-MB468 human breast carcinoma cells.

GSE (25-200 mcg/ml) treatment of cells resulted in 16%-72% growth inhibition and 9%-33% cell death, in a dose- and a time-dependent manner. In other studies, Dox (10-100 nM) treatment showed 23%-96% growth inhibition and 10%-55% cell death. Based on these results, several combinations of GSE (25-100 mcg/ml) with Dox (10-75 nM) were next assessed for their synergistic, additive and/or antagonistic efficacy towards cell growth inhibition and death.

In both MCF-7 and MDA-MB468 cells, a combination of 100 mcg/ml GSE with 25-75 nM Dox treatment for 48 h showed a strong synergistic effect [combination index (CI)<0.5] in cell growth inhibition, but mostly an additive effect (CI approximately 1) in cell death. In cell-cycle progression studies, GSE plus Dox combination resulted in a moderate increase in G1 arrest in MCF-7 cells compared to each agent alone. GSE plus Dox combination showed a very strong and significant G1 arrest in MDA-MB468 cells when compared with Dox alone, however, it was less than that observed with GSE alone.

In quantitative apoptosis studies, GSE and Dox alone and in combination showed comparable apoptotic death of MCF-7 cells, however, a combination of the two was inhibitory to Dox induced apoptosis in MDA-MB468 cells.

This was further confirmed in another estrogen receptor-negative MDA-MB231 cell line, in which GSE and Dox combination strongly inhibited cell growth but did not show any increase in apoptotic cell death caused by Dox. Together, these results suggest a strong possibility of synergistic efficacy of GSE and Dox combination for breast cancer treatment, independent of estrogen receptor status of the cancer cell. [101]

Anticancer activity of grape and grape skin extracts alone and combined with green tea infusions.

Grapes and grape extracts were compared for inhibition of a growth-related and cancer-specific form of cell surface NADH oxidase with protein disulfide-thiol interchange activity designated tNOX from human cervical carcinoma (HeLa) cells and growth of HeLa and mouse mammary 4T1 cells in culture and transplanted tumors in mice. Grapes and grape extracts of several varieties had activity. With an extracted grape preparation provided by the California Table Grape Commission, an active fraction was eluted with methanol from a Diaion HP-20 column after removal of inactive water-soluble materials. Grape skins were a much more potent source than either grape pulp, juice or seeds. Ethanol extracts of the ground freeze-dried pomace was an excellent source. The grape extracts interacted, often synergistically, with decaffeinated green tea extracts both in the inhibition of tNOX activity and in the inhibition of cancer cell growth. Intratumoral injections of a 25:1 mixture of a green tea extract plus ground freeze-dried pomace was nearly as effective as standard synergistic green tea-Capsicum mixtures in inhibiting growth of 4T1 mammary tumors in situ in mice.[293]

Liver Protective: Prevents Acetaminophen (Tylenol) Toxicity

A remarkable counteraction of **Acetaminophen**-toxicity was demonstrated by GSE and substantial inhibition of both apoptotic and necrotic liver cell death. [83]

Inhibits Receptor for advanced glycation endproducts, up-regulates PPARgamma: important target for the prevention and treat of obesity, heart disease, cancer, and age-related decline

GSPE) can selectively inhibit cell adhesion molecule expression induced by advanced glycation end products (AGEs), through activation of PPARgamma. [233]

Anti-obesity effects

Grape seed procyanidin extract (GSPE) effects on adipose metabolism affecting peroxisome proliferator-activated receptor-gamma (PPARgamma) plays a central role in the lipolytic effects of GSPE on adipocytes. Since PPARgamma2 is a main regulator of the differentiation process of adipocytes, we investigated whether GSPE affects the adipogenesis of 3T3-L1 cells: We performed a time point screening by treating 3T3-L1 cells with GSPE during the differentiation process for 24 h.MEASUREMENTS: Differentiation markers and differential gene expression due to GSPE treatment (using the microarray technique).RESULTS: Twenty four hour-GSPE treatment at the onset of differentiation reduces adipose-specific markers and maintains the expression of preadipocyte marker preadipocyte factor-1 (Pref-1) significantly elevated. These effects were not found in other time points. Microarray analysis of gene expression after GSPE treatment at the early stage of differentiation showed a modified gene expression profile in which cell cycle and growth-related genes were downregulated by GSPE. <u>These results suggest that GSPE affects adipogenesis, mainly at the induction of differentiation, and that procyanidins may have a new role in which they impede the formation of adipose cells</u>. [135]

Grape seed and skin extract alleviates high-fat diet-induced renal lipotoxicity and prevents copper depletion in rat.

Obesity is a public health problem that contributes to morbidity and mortality from diabetes, heart disease, stroke, and cancers. The purpose of this investigation was to analyse the link between obesity-induced oxidative stress, renal steatosis, and kidney dysfunction, as well as the protective effect of grape seed and skin extract. Rats were fed a standard diet or a high-fat diet for 6 weeks and were either treated or not treated with grape seed and skin extract. Fat-induced oxidative stress was evaluated in the kidney with a special emphasis on transition metals. High-fat diet induced triglyceride deposition and disturbances in kidney function parameters, which are linked to an oxidative stress status and depletion of copper from the kidney. Grape seed and skin extract abrogated almost all fat-induced kidney disturbances. Grape seed and skin extract exerted potential protection against fat-induced kidney lipotoxicity and should find potential application in other kidney-related diseases.[294]

The topical effect of grape seed extract 2% cream on surgery wound healing.

BACKGROUND:

Reducing the wound healing time is crucial in wound as it lowers the chance of infection and decreases complications and cost. Grape seed extract has the ability to release endothelial growth factor and its topical application results in contraction and closure of the skin wound. Furthermore, it possesses antioxidant and antibacterial properties. In several studies it has been proved effective in animals. Therefore, due to low side effects

and recognition of herbal medicine, we decided to evaluate the effect of grape seed extract 2% herbal cream on human skin lesions.

MATERIALS:
This study is a double blind clinical trial conducted on two groups of treatment and placebo. Surgery was performed on skin lesions such as skin tags and moles which were found on the neck, trunk and limbs (except for face). After enrollment and obtaining informed consent from participants, they were randomized into two groups of treatment and placebo. Excision of the lesions was done by surgical scissors. The lesions got restored by secondary intention method. After the first day of treatment, the patients were visited on the 3rd, 7th, 10th, 14th, and 21st day. Grape seed extract cream 2% was produced and coded by the Faculty of Pharmacy, Ahvaz University of Medical Sciences. In order to compare the two groups, T-test was used. For time assessing, analysis of variance with repeated measures was employed.

RESULTS:
The results showed complete repair of wounds averagely on day 8 for the treatment group and on day 14 for the placebo group, which was clearly significant in terms of statistical difference (p=0.00).

CONCLUSION:
Proanthocyanidins in grape seed extract trigger the release of vascular endothelial growth factor and its topical application causes wound contraction and closure. Curing skin lesions with grape seed extract caused proliferation areas with protected boundaries in epithelium, increased cell density and increased deposition of connective tissue at the wound site which in general improves cellular structure in wound. In addition, its anti-inflammatory and anti-microbial properties are effective in wound healing.[309]

Grape Seed Proanthocyanidin Extract Ameliorates Diabetic Bladder Dysfunction via the Activation of the Nrf2 Pathway.

Diabetes Mellitus (DM)-induced bladder dysfunction is predominantly due to the long-term oxidative stress caused by hyperglycemia. Grape seed proanthocyanidin extract (GSPE) has been reported to possess a broad spectrum of pharmacological and therapeutic properties against oxidative stress. However, its protective effects against diabetic bladder dysfunction have not been clarified. This study focuses on the effects of GSPE on bladder dysfunction in diabetic rats induced by streptozotocin. After 8 weeks of GSPE administration, the bladder function of the diabetic rats was improved significantly, as indicated by both urodynamics analysis and histopathological manifestation. Moreover, the disordered activities of antioxidant enzymes (SOD and GSH-Px) and abnormal oxidative stress levels were partly reversed by treatment with GSPE. Furthermore, the level of apoptosis in the bladder caused by DM was decreased following the administration of GSPE according to the Terminal Deoxynucleotidyl Transferase (TdT)-mediated dUTP Nick-End Labeling (TUNEL) assay. Additionally, GSPE affected the expression of apoptosis-related proteins such as Bax, Bcl-2 and cleaved caspase-3. Furthermore, GSPE showed neuroprotective effects on the bladder of diabetic rats, as shown by the increased expression of nerve growth factor (NGF) and decreased expression of the precursor of nerve growth factor (proNGF). GSPE also activated nuclear erythroid2-related factor2 (Nrf2), which is a key antioxidative transcription factor, with the concomitant elevation of downstream hemeoxygenase-1 (HO-1). These findings suggested that GSPE could ameliorate diabetic bladder dysfunction and decrease the apoptosis of the bladder in diabetic rats, a finding that may be associated with its antioxidant activity and ability to activate the Nrf2 defense pathway.[310]

Grape Seed Proanthocyanidin Extract Alleviates Arsenic-induced Oxidative Reproductive Toxicity in Male Mice.

OBJECTIVE:
To determine the ability of grape seed proanthocyanidin extract (GSPE) in alleviating arsenic-induced reproductive toxicity.

METHODS:
Sixty male Kunming mice received the following treatments by gavage: normal saline solution (control); arsenic trioxide (ATO; 4 mg/kg); GSPE (400 mg/kg); ATO+GSPE (100 mg/kg); ATO+GSPE (200 mg/kg) and ATO+GSPE (400 mg/kg). Thereafter, the mice were sacrificed and weighed, and the testis was examined for pathological changes. Nuclear factor (erythroid-derived 2)-like 2 (Nrf2), heme oxygenase 1 (HO1), glutathione S-transferase (GST), NAD(P)H dehydrogenase, and quinone 1 (NQO1) expression in the testis was detected by real-time PCR.

Superoxide dismutase (SOD), glutathione (GSH), total antioxidative capability (T-AOC), malondialdehyde (MDA), 8-hydroxydeoxyguanosine (8-OHdG), and reproductive indexes were analyzed.

RESULTS:
ATO-treated mice showed a significantly decreased sperm count and testis somatic index and activity levels of SOD, GSH, and T-AOC than control group. Compared to the ATO-treated group, ATO +GSPE group showed recovery of the measured parameters. Mice treated with ATO+high-dose GSPE showed the highest level of mRNA expression of Nrf2, HO, NQO1, and GST.

CONCLUSION:
GSPE alleviates oxidative stress damage in mouse testis by activating Nrf2 signaling, thus counteracting arsenic-induced reproductive toxicity.[311]

Safety

Grape seed proanthocyanidins are known to possess a broad spectrum of pharmacological, medicinal and therapeutic properties. Previous studies in our laboratories have demonstrated the various protective abilities of a novel grape seed proanthocyanidin extract (GSE) against various pathologic conditions. This study demonstrates the acute and chronic safety studies on GSE. These acute studies demonstrated that GSE is safe and did not cause any detrimental effects in vivo under the conditions investigated in this study. [77] No evidence of acute oral toxicity at dosages of 2 and 4 g/kg, and no evidence of mutagenicity in the above tests was found. Administration of GSE as a dietary admixture at levels of 0.02, 0.2 and 2% (w/w) to the rats for 90 days did not induce noticeable signs of toxicity. The no-observed-adverse-effect level (NOAEL) of GSE in the subchronic toxicity study was 2% in the diet (equal to 1410 mg/kg body weight/day in males and 1501 mg/kg body weight/day in females). The results of our studies indicate a lack of toxicity and support the use of proanthocyanidin-rich extract from grape seeds for various foods. [78] Neither grape seed extract (GSE) and grape skin extract (GSKE) have demonstrated any toxicity. [82]

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