QUERCETIN MONOGRAPH

Courtesy of Donald R. Yance

Introduction

Quercetin is a flavone, a sub category of a class of water-soluble plant pigments called flavonoids. It is found in apples, onions, broccoli, eucalyptus, green, black and red tea, and blue-green algae. Apples contain a variety of phytochemicals, including quercetin, which contribute to apple's overall health benefits. [38, 39] Flavonoids are reported to exhibit a wide variety of biological effects, including antioxidant and free radical scavenging activities. Quercetin is not only useful in preventing allergic reactions, but also as a useful agent for the prevention and treatment of cancer, heart disease, inflammatory condition, such as psoriasis, urticaria. Quercetin possesses anti-viral, antioxidant, anti-inflammatory, and anticancer abilities.

Quercetin protects LDL cholesterol (the "bad" cholesterol) from becoming damaged. Quercetin blocks an enzyme that leads to accumulation of sorbitol, which has been linked to nerve, eye, and kidney damage in those with diabetes.

Allergies

Quercetin acts as an antihistamine and has anti-inflammatory properties. Quercetin prevents allergic reactions by stabilizing mast cells. Mast cells are the body's main storage unit for histamines. When quercetin stabilizes these cell membranes it prevents histamines from spilling out of mast cells into the bloodstream and surrounding tissues. Quercetin has been shown to prevent mast cell degranulation and histamine release more effectively than the often prescribed anti-allergy drug, Cromoglycate. Also, quercetin helps inhibit the action of two enzymes - phospholipase A2 and lipoxygenase - which act on arachidonic acid (a key fatty acid constituent of many cell membranes) to create leukotrienes. By inhibiting the release of histamines and leukotrienes into our bloodstream, quercetin can leave us free to enjoy the natural world and the need for anti-histamines or anti-inflammatory medication may be reduced or eliminated. Quercetin, by blocking pro-inflammatory reactions in the body that release arachidonic acid into the cells, acts as a powerful inhibitor of the pro-inflammatory, tumor-promoting prostaglandin PGE-2. It also stabilizes mast cell walls by prolonging the health of lipids, by blocking lipoxygenase activity and through stabilization of capillary beds by decreasing capillary fragility. [68]

Mast cells participate in allergies, and also in immunity and inflammation by secreting proinflammatory cytokines. Quercetin inhibits histamine and some cytokine release from basophils and mast cells. Quercetin has shown to inhibit histamine release by 52-77%. [62]

Quercetin, is the most effective natural remedy for chronic allergy rhinitis, but really needs to be taken in a sub-lingual form. [69]

Quercetin inhibits histamine and some cytokine release from basophils and mast cells; and is therefore suitable for the treatment of allergic and inflammatory diseases. [70]

Quercetin is has been found to be useful allergy induced asthma. [71]

In a recent study, the anti-inflammatory effect of quercetin and isoquercitrin in a murine model of asthma was studied. METHODS: BALB/c mice were immunized (ovalbumin/aluminum hydroxide, s. c.), followed by two intranasal ovalbumin challenges. From day 18 to day 22 after the first immunization, the mice received daily gavages of isoquercitrin (15 mg/kg) or quercetin (10 mg/kg). Dexamethasone (1 mg/kg, s. c.) was administered as a positive control. Leucocytes were analyzed in bronchoalveolar lavage fluid (BALF), blood and pulmonary parenchyma at 24 h after the last ovalbumin challenge. Interleukin-5 (IL-5) was analyzed in BALF and lung homogenates. RESULTS: In animals receiving isoquercitrin or quercetin, eosinophil counts were lower in the BALF, blood and lung parenchyma. Neutrophil counts in blood and IL-5 levels in lung homogenate were lower only in isoquercitrin-treated mice. No alterations in mononuclear cell numbers were observed. CONCLUSION: Quercetin and isoquercitrin are effective eosinophilic inflammation suppressors, suggesting a potential for treating allergies. [80]

Quercetin inhalation inhibits the asthmatic responses

Effects of quercetin inhalation on immediate (IAR), late-phase (LAR) and late late-phase (LLAR) asthmatic responses by exposure to aerosolized-ovalbumin (AOA) (2w/v% in saline, inhalation for 3 min) were studied in conscious guinea-pigs sensitized with AOA. We measured specific airway resistance (sRaw), and recruitment of leukocytes, histamine and protein contents and phospholipase A2 (PLA2) activity in bronchoalveolar lavage fluid (BALF). Effects of quercetin (10 mg/mL, inhalation for 2 min) compared with cromolyn sodium, salbutamol, and dexamethasone inhalations, respectively. Quercetin inhalation decreased sRaw by 57.15 +/- 3.82% in IAR, 57.72 +/- 7.28% in LAR, and 55.20 +/- 5.69% in LLAR compared with AOA-inhaled control. Salbutamol inhalation (5 mg/mL) significantly inhibited sRaw in IAR, but inhalations of cromolyn sodium (10 mg/mL) and dexamethasone (5 mg/mL) significantly inhibited sRaw in IAR, LAR and LLAR, respectively. Inhibitory activity of quercetin inhalation on sRaw was similar to effect of its oral administration (10 mg/kg) in asthmatic responses. Quercetin (10 mg/mL, inhalation for 2 min) significantly decreased histamine and protein contents, PLA2 activity, and recruitments of leukocytes in BALF and also improved slightly infiltration of eosinophils and neutrophils in histopathological survey. Its anti-asthmatic activity was similar to cromolyn sodium and dexamethasone.[97]

Mechanisms involved in anti-allergenic actions

Quercetin is a natural compound that blocks substances involved in allergies and is able to act as an inhibitor of mast cell secretion, causes a decrease in the release of tryptase, MCP-1 and IL-6 and the down-regulation of histidine decarboxylase (HDC) mRNA from few mast cell lines. Quercetin is a safe, natural therapy that may be used as primary therapy or in conjunction with conventional methods. [80]

Quercitin inhibits the growth of certain malignant cells in vitro, and histamine and most cyclin-dependent kinases and also displays unique anticancer properties. Quercetin is a natural compound that blocks substances involved in allergies and is able to act as an inhibitor of mast cell secretion, causes a decrease in the release of tryptase, MCP-1 and IL-6 and the down-regulation of histidine decarboxylase (HDC) mRNA from few mast cell lines. Quercetin is a safe, natural therapy that may be used as primary therapy or in conjunction with conventional methods.[96]

Note: I have found quercetin to be useful in a number of conditions and it has become a favorite supplement of mine. These include asthma, sinusitis, hives, eczema, certain types of allergic headaches, irritable bowel syndrome, poison oak and poison ivy, and many chronic and acute viral conditions.

Supplementation reduces upper respiratory tract infection by 36%: A randomized community clinical trial.

A few small-scale human quercetin supplementation studies have produced conflicting results regarding quercetin's effects on upper respiratory tract infection rates, and little is known regarding the appropriate human dose. The purpose of this randomized, double-blinded, placebo-controlled trial was to measure the influence of two quercetin doses (500 and 1000 mg/day) compared to placebo on upper respiratory tract infection (URTI) rates in a large community group (N=1002) of subjects varying widely in age (18-85 years). Subjects ingested supplements for 12 weeks and logged URTI symptoms on a daily basis using the Wisconsin Upper Respiratory Symptom Survey (WURSS). No significant group differences were measured for URTI outcomes for all subjects combined, or when analyzing separately by gender, body mass index, and age categories. Regression analysis revealed that the strongest interaction effect with group status was self-reported fitness level. A separate analysis of subjects 40 years of age and older rating themselves in the top half of the entire group for fitness level (N=325) showed lower URTI severity (36% reduction, P=0.020) and URTI total sick days (31% reduction, P=0.048) for the Q-1000 group compared to placebo. In summary, for all subjects combined, quercetin supplementation over 12 weeks had no significant influence on URTI rates or symptomatology compared to placebo. A reduction in URTI total sick days and severity was noted in middle aged and older subjects ingesting 1000 mg quercetin/day for 12

weeks who rated themselves as physically fit.[95]

Quercetin inhibits transcriptional up-regulation of histamine H1 receptor via suppressing protein kinase C-δ/extracellular signal-regulated kinase/poly(ADP-ribose) polymerase-1 signaling pathway in HeLa cells.

It has been reported that the histamine H1 receptor (H1R) gene is up-regulated in patients with allergic rhinitis and H1R expression level strongly correlates with the severity of allergy symptoms. Accordingly compounds that suppress the H1R gene expression are promising as useful anti-allergic medications. Recently, we demonstrated that histamine or phorbol-12-myristate-13-acetate (PMA) stimulation induced the up-regulation of H1R gene expression through the protein kinase Cδ (PKCδ)/extracellular signal-regulated kinase/poly(ADP-ribose) polymerase-1 signaling pathway in HeLa cells expressing H1R endogenously. Quercetin is one of the well-characterized flavonoids and it possesses many biological activities including anti-allergic activity. However, effect of quercetin on H1R signaling is remained unknown. In the present study, we examined the effect of quercetin on histamine- and PMA-induced up-regulation of H1R gene expression in HeLa cells. We also investigated its in vivo effects on the toluene-2,4-diisocyanate (TDI)-sensitized allergy model rats. Quercetin suppressed histamine- and PMA-induced up-regulation of H1R gene expression. Quercetin also inhibited histamine- or PMA-induced phosphorylation of Tyr(311) of PKCδ and translocation of PKCδ to the Golgi. Pre-treatment with quercetin for 3weeks suppressed TDI-induced nasal allergy-like symptoms and elevation of H1R mRNA in the nasal mucosa of TDI-sensitized rats. These data suggest that quercetin suppresses H1R gene expression by the suppression of PKCδ activation through the inhibition of its translocation to the Golgi.[111]

Quercetin is more effective than cromolyn in blocking human mast cell cytokine release and inhibits contact dermatitis and photosensitivity in humans.

Mast cells are immune cells critical in the pathogenesis of allergic, but also inflammatory and autoimmune diseases through release of many pro-inflammatory cytokines such as IL-8 and TNF. Contact dermatitis and photosensitivity are skin conditions that involve non-immune triggers such as substance P (SP), and do not respond to conventional treatment. Inhibition of mast cell cytokine release could be effective therapy for such diseases. Unfortunately, disodium cromoglycate (cromolyn), the only compound marketed as a mast cell "stabilizer", is not particularly effective in blocking human mast cells. Instead, flavonoids are potent anti-oxidant and anti-inflammatory compounds with mast cell inhibitory actions. Here, we first compared the flavonoid quercetin (Que) and cromolyn on cultured human mast cells. Que and cromolyn (100 μM) can effectively inhibit secretion of histamine and PGD(2). Que and cromolyn also inhibit histamine, leukotrienes and PGD(2) from primary human cord blood-derived cultured mast cells (hCBMCs) stimulated by IgE/Anti-IgE. However, Que is more effective than cromolyn in inhibiting IL-8 and TNF release from LAD2 mast cells stimulated by SP. Moreover, Que reduces IL-6 release from hCBMCs in a dose-dependent manner. Que inhibits cytosolic calcium level increase and NF-kappa B activation. Interestingly, Que is effective prophylactically, while cromolyn must be added together with the trigger or it rapidly loses its effect. In two pilot, open-label, clinical trials, Que significantly decreased contact dermatitis and photosensitivity, skin conditions that do not respond to conventional treatment. In summary, Que is a promising candidate as an effective mast cell inhibitor for allergic and inflammatory diseases, especially in formulations that permit more sufficient oral absorption.[112]

Quercetin effectively quells peanut-induced anaphylactic reactions in the peanut sensitized rats.

Peanut allergy is the major leading cause of fatal or life-threatening anaphylactic reactions to foods. At present, there is no remedy for this condition. The applied pharmaceutical cares are merely palliative, while their deleterious side effects have already been established. Hence, many sufferers search for complementary and alternative medicines. A versatile-, "flavonol" subgroup-member of the flavonoid family, quercetin, is of paramount interest to investigators. In this study the effects of quercetin on peanut-induced anaphylactic reactions were investigated in a rat model of peanut allergy. Wistar rats were sensitized with crude peanut extract in the presence of Cholera toxin and Aluminium hydroxide. Sensitized rats were then allotted into three groups; Positive control, Quercetin-treatment and Sham, (n=7, each). Naive rats (n=7) served as negative controls. One week post-sensitization period, the rats in treatment

group were treated with quercetin at a dose of 50 mg/kg(Body Weight)/mL Di-methyl-sulfoxide 5%/rat, over a period of four weeks. Subsequently, rats were challenged, and anaphylactic reaction parameters including variations in plasma histamine levels, vascular permeability, systemic anaphylaxis scores, and total serum Immunoglobulin E levels were measured. After daily-gavaging for four weeks, quercetin completely abrogated peanut-induced anaphylactic reactions following challenges, so that the mean of plasma histamine levels in the quercetin-treated rats, were lower significantly (p=0.004) as compared with positive control group. Our findings suggest that the flavonoid quercetin is potent enough to suppress the on-going Immunoglobulin E responses against peanut proteins, and can be propounded as an alternative medicine to protect against Immunoglobulin E-mediated food allergies.[113]

Quercetin as a potential anti-allergic drug

Flavonoids polyphenolic compounds that exert many anti-inflammatory and anti-microbial effects, and exhibit an anti-allergic action. Quercetin is a flavonoids that recently has raised many issues and shown evidence about its action as a potential drug to allergy. A Chinese herbal formula, known as Food Allergy Herbal Formula (FAHF) has been related with blocking of anaphylaxis to peanuts (PNA) in mouse models. Quercetin appears to possess the same potential of FAHF as a safe anti-allergic substance but it opens only a wide perspective, at the moment, due to several complex issues that hamper the possibility to use natural medicine and phytochemicals as true drugs.[116]

Prostatitis

In a double-blind trial, 67% of patients taking quercetin had an improvement of prostatitis symptoms, compared to a 20% response rate in the placebo group. [6]

Interstitial cystitis

Interstitial cystitis (IC) is a disorder of unknown etiology with few effective therapies. Oral bioflavonoid therapy utilizing quercetin recently proved to be clinically effective in men with chronic pelvic pain syndrome, a disorder with similarities to IC. We therefore tested in an open-label trial a quercetin-based supplement in patients with clinically proven IC. The conclusion of this study found oral therapy with the quercetin supplement was well-tolerated and provided significant symptomatic improvement in patients with IC. [29] Other research has confirmed that quercetin painful bladder syndrome/interstitial cystitis (PBS/IC). [72]

Anti-diarrhea effects

Quercetin inhibited the contraction of guinea pig ileum in vitro and the peristaltic motion of mouse small intestine, and reduced the permeability of abdominal capillaries. Quercetin can inhibit the intestinal movement and reduce capillary permeability in the abdominal cavity. [19]

Antiinflammatory

The anti-inflammatory activities of quercetin include: inhibitory effects on the chemical mediators released from mast cells, neutrophils, and macrophages cells; strongly inhibits the release of beta-glucuronidase and lysozyme from neutrophils; inhibition of superoxide anion formation, and a potent inhibitory effect on tumor-necrosis factor-alpha (TNF-alpha). [28]

Improves bone health

Many plant-derived substances have estrogenic activities. Due to their ability to bind the estrogen receptor (ER), these compounds have the potential to counteract the deleterious effects of estrogen deficiency on bone. aken together, these results suggest that quercetin can stimulate osteoblastic activity. [31]

Dietary quercetin inhibits bone loss

In the present study, we demonstrate for the first time the effects of dietary quercetin on bone loss and uterine weight loss by ovariectomy in vivo. Female mice were ovariectomized (OVX) and were randomly allocated to 3 groups: a control diet or a diet with 0.25% (LQ) or 2.5% quercetin (HQ). After 4 weeks, dietary quercetin had no effects on uterine weight in OVX mice, but bone mineral density of the lumbar spine L4 and femur measured by peripheral quantitative computed tomography (pQCT) was higher in both the sham and the HQ groups than in the OVX group. Histomorphometric analysis showed that the HQ group restored bone volume (BV/TV) completely in distal femoral cancellous bone, but did not reduce the osteoclast surface area and osteoclast number when compared with the OVX group. In in-vitro experiments using mouse monocyte/macrophage cell line RAW264.7 cells, however, quercetin and its conjugate, quercetin-3-O-beta-D: -glucuronide dose-dependently inhibited the receptor activator of nuclear factor-kappa B ligand (RANKL)-induced osteoclast differentiation, and the RANKL-stimulated expression of osteoclast related genes was also inhibited by quercetin. The luciferase reporter assay showed that quercetin did not appear to have estrogenic activity through estrogen receptors. These results suggest that dietary quercetin inhibits bone loss without effect on the uterus in OVX mice and does not act as a potent inhibitor of osteoclastogenesis or as a selective estrogen receptor modulator in vivo. [84]

Improves mitochondrial biogenesis and enhances exercise performance

We examined the effects of 7 days of quercetin feedings in mice on markers of mitochondrial biogenesis in skeletal muscle and brain, and on endurance exercise tolerance. Mice were randomly assigned to one of the following three treatment groups: placebo, 12.5 mg/kg quercetin, or 25 mg/kg quercetin. Mice underwent a treadmill performance run to fatigue or were placed in voluntary activity wheel cages, and their voluntary activity (distance, time, and peak speed) was recorded. Quercetin increased mRNA expression of PGC-1alpha and SIRT1 ($P < 0.05$), mtDNA ($P < 0.05$) and cytochrome c concentration ($P < 0.05$). These changes in markers of mitochondrial biogenesis were associated with an increase in both maximal endurance capacity ($P < 0.05$) and voluntary wheel-running activity ($P < 0.05$). These benefits of querectin on fitness without exercise training may have important implications for enhancement of athletic and military performance and may also extend to prevention and/or treatment of chronic diseases. [85]

Neuroprotection

Quercetin significantly decreased the brain ischemic lesion. It is concluded that when administered in liposomal preparations, that include flavonoides, and specifically quercetin, could become leads for the development of a new generation of molecules to be clinically effective in human brain ischemia. [33, 37]

Quercetin appears to protect brain cells against oxidative stress, a tissue-damaging process associated with Alzheimer's and other neurodegenerative disorders. A new study showed that brain cells treated with the quercetin had significantly less damage than those treated with vitamin C or not exposed to antioxidants. [48]

In ischemic animals, quercetin revealed protective effect by decreasing of delayed neuronal death and reducing reactive astrogliosis after ischemia-reperfusion. [64]

Antioxidative: inhibits cholesterol oxidation

Quercetin inhibits free radical damage including the inhibition of lipid peroxidation of low density lipoprotein (LDL) cholesterol. [34]

Dietary quercetin attenuates oxidant-induced endothelial dysfunction and atherosclerosis in apolipoprotein E knockout mice fed a high-fat diet: a critical role for heme oxygenase-1.

Several lines of evidence indicate that quercetin, a polyphenol derived in the diet from fruit and vegetables, contributes to cardiovascular health. We aimed to investigate the effects of dietary quercetin on endothelial function and atherosclerosis in mice fed a high-fat diet. Wild-type C57BL/6 (WT) and apolipoprotein E gene knockout (ApoE(-/-)) mice were fed: (i) a high-fat diet (HFD) or (ii) a HFD supplemented with 0.05%

w/w quercetin (HFD+Q), for 14 weeks. Compared with animals fed HFD, HFD+Q attenuated atherosclerosis in ApoE(-/-) mice. Treatment with the HFD+Q significantly improved endothelium-dependent relaxation of aortic rings isolated from WT but not ApoE(-/-) mice and attenuated hypochlorous acid-induced endothelial dysfunction in aortic rings of both WT and ApoE(-/-) mice. Mechanistic studies revealed that HFD+Q significantly improved plasma F2-isoprostanes, 24h urinary nitrite, and endothelial nitric oxide synthase activity, and increased heme oxygenase-1 (HO-1) protein expression in the aortas of both WT and ApoE(-/-) mice ($P<0.05$). HFD+Q also resulted in small changes in plasma cholesterol ($P<0.05$ in WT) and plasma triacylglycerols ($P<0.05$ in ApoE (-/-)mice). In a separate experiment, quercetin did not protect against hypochlorite-induced endothelial dysfunction in arteries obtained from heterozygous HO-1 gene knockout mice with low expression of HO-1 protein. Quercetin protects mice fed a HFD against oxidant-induced endothelial dysfunction and ApoE(-/-) mice against atherosclerosis. These effects are associated with improvements in nitric oxide bioavailability and are critically related to arterial induction of HO-1.[115]

Quercetin in Oncology: Anticancer effects and mechanisms

Quercetin is one of the most powerful anticancer agents present in nature. Quercetin has been shown to inhibit the growth of several human cancer cell lines including breast (Estrogen receptor positive and ER-negative), prostate, ovarian, squamous cell, cervical, bladder and gastric cancers, acute myeloid and acute lymphoid leukemia, Moloney murine leukemia (by inhibiting reverse transcriptase) and some lymphomas. Quercetin, as well as some other flavonoids of been found to be potent inhibitors of cyclin-dependent kinases, but in addition also inhibit the activity of angiogenic mediators and induce apoptosis by mechanisms that are still not fully understand. [1-5, 24]

Quercetin, by blocking pro-flammatory reactions in the body that release arachidonic acid into the cells, acts as a powerful inhibitor of the tumor-promoting prostaglandin PG E-2. It also stabilizes mast cell walls by prolonging the health of lipids, by blocking lipoxygenase activity and through stabilization of capillary beds by decreasing capillary fragility.

Quercetin's and cancer inhibition: pleotrophic/multi-tasking in review:

Redox/antioxidative
Modulates inflammatory pathways including COX and LOX pathways
Inhibits cancer-related angiogenesis
Down-regulates tumor promoting growth factors including EGF and Her II neu
Down regulates NFkB and AP-1
Activates PTEN
Down regulates mutant p53
Down regulates Bcl-2
Down regulates TNF-alpha
Suppresses COX-2
Activated caspase-3, Bax, and Bak
Elevates p21 and p27
ER regulation - down-regulating estrogen binding/inhibits angiogenesis in TAM resistance breast cancer
Reduces circulating IGF, increasing IGFBP
Down-regulates MMP-2 and 9
Inhibits VEGF-2
Inhibits HIF-1alpha
Down-regulates cyclin D and E
Down regulates Topo II alpha
Inhibits raf and MEK protein
Inhibits PI3-K
Down-regulated the expression of heat shock protein 70
Chemosensitize
Inhibits chemo resistance
Radiosensitize

Redox antioxidant effects

The cytotoxicity of the flavonoids quercetin, rutin, apigenin and luteolin and their ability to protect DNA molecules against free radical damage (H_2O_2-induced damage) was recently studied. Cytotoxicity of studied of these flavonoids was studied and quercetin was found to possess the highest protective effect among the flavonoids studied (45%). The protective activity determined was lower for luteolin (40%). Protective effect of apigenin (600 micromol/l) was only marginal (2%). However, at the higher concentration of apigenin (1200 micromol/l), this flavonoid induced DNA single strand breaks. This indicates the ability of apigenin to serve as a pro-oxidant. Rutin had no protective effect on DNA single strand breaks induced by H_2O_2. [21]

Redox antioxidant effects and down-regulated PARP and Bcl-2 proteins, and activated caspase-3, Bax, and Bak

A recent study focused on the relationship between the influence of flavonoids on cell population growth and their antioxidant activity. The results showed that the inhibition of flavonoids (naringenin, rutin, hesperidin, resveratrol, naringin and quercetin) on 3T3-L1 pre-adipocytes was 28.3, 8.1, 11.1, 33.2, 5.6 and 71.5%, respectively. In oxygen radical absorbance capacity (ORAC) assay, quercetin had the highest ORAC(ROO) value among the six flavonoids tested. Apoptosis assays showed that quercetin increased apoptotic cells in time- and dose-dependent manner. Treatment of cells with quercetin decreased the mitochondrial membrane potential in the courses of time and dose. The cell apoptosis/necrosis assay showed that quercetin increased the number of apoptotic cells, but not necrotic cells. Quercetin treatment of

cells caused a significant time- and dose-dependent increase in the caspase-3 activity. Western analysis indicated that treatment of quercetin markedly down-regulated PARP and Bcl-2 proteins, and activated caspase-3, Bax, and Bak proteins. [60]

Breast Cancer

Binds to estrogen receptor (ER) sites inhibiting capacity of ER cancer cell adhesion:
Quercetin binds to type II estrogen-binding sites more effectively than the antiestrogen drug, tamoxifen, which is used so often to treat and inhibit the recurrence of estrogen positive breast cancer. By securing these binding sites with weak plant estrogen-like compounds such as quercetin, the true estrogens have nowhere to bind to, which stops the promotion of cancer growth. Type II estrogen binding sites are present in a variety of human cancers including breast cancer and melanoma. [7-10]

Quercetin, along with curcumin was found to inhibit chemically induced colon and breast cancer. [11]

Inhibits the growth of transplanted breast cancer
To investigate the effects of quercetin on tumor growth, cell proliferation and apoptosis in transplantation tumor of breast cancer cell line MCF-7 in nude mice. METHODS: MCF-7 cells were inoculated into the mammary fatty pad of nude mice to establish breast cancer model, then twenty-four BALB/c nude mice with xenograft tumor were randomized into four groups: Control group, Quercetin group, 5-Fu group and Quercetin + 5-Fu group. After 15 days treatment, samples of tumor were collected. The sections of tumor were observed under light microscope and electron microscope. Cell apoptosis in situ was examined by a Tunel assay, and the expressions of ki67 antigen and B-cell lymphoma/leukemia-2 (Bcl-2) were detected by immunohistochemistry. RESULTS: 1. The tumor weight of Control group was significantly higher than those of Quercetin group, 5-Fu group and Quercetin + 5-Fu group ($P < 0.05$). 2. Immunohistochemical staining of ki67 showed that the ki67 label index (ki67-LI) displayed significant difference between Control group and Quercetin group, 5-Fu group, Quercetin + 5-Fu group, and so did the staining of Bcl-2. 3. Detection of apoptosis in situ showed that apoptosis index (AI) was significantly higher in Quercetin group, 5-Fu group and Quercetin + 5-Fu group than in control group ($P < 0.01$), and higher AI was observed in Quercetin + 5-Fu group as compared with the AI in Quercetin group and 5-Fu group ($P < 0.05$). CONCLUSION: Quercetin can inhibit the growth of transplantation tumor of breast cancer cell line MCF-7 in nude mice. [24]

Inhibition of angiogenesis in tamoxifen-resistant breast cancer cells.
Acquired resistance to tamoxifen (TAM) is a serious therapeutic problem among breast cancer patients. Previously, we have reported that TAM-resistant MCF-7 cells (TAMR-MCF-7 cells) showed increased angiogenic intensity through Pin1-dependent vascular endothelial growth factor (VEGF) production. Among six flavonoids tested in the current study, VEGF gene transcription in MCF-7 cells with stable Pin1 overexpression was inhibited most effectively by quercetin. Reporter gene assays using minimal reporters containing hypoxia response elements and activator protein-1 (AP-1) elements revealed that the activities of hypoxia inducible factor-1α (HIF-1α) and AP-1, key transcription factors for VEGF gene transcription, were suppressed by quercetin. Western blot analyses confirmed that the increased nuclear levels of c-Jun and HIF-1α in TAMR-MCF-7 cells were blocked by quercetin. Moreover, quercetin inhibited the enhanced VEGF secretion and Pin1 expression in TAMR-MCF-7 cells, which was dependent on its phosphatidyl inositol 3-kinase inhibiting effect. Chick chorioallantoic membrane assays demonstrated that the enhanced angiogenesis intensity of TAMR-MCF-7 cells was also suppressed significantly by quercetin. These results demonstrate that quercetin may have therapeutic potential for the treatment of TAM-resistant breast cancer via Pin1 inhibition.[98]

Down-regulating Epidermal Growth Factor (EGF) expression
Characterization of intracellular signaling pathways should lead to a better understanding of ovarian epithelial carcinogenesis and provide an opportunity to interfere with signal transduction targets involved in ovarian tumor cell growth, survival, and progression. Challenges toward such an effort are significant because many of these signals are part of cascades within an intricate and likely redundant intracellular

signaling network. The EGF-specific tyrosine kinase inhibitor ZD 1839 (Iressa) may have a beneficial therapeutic effect on ovarian epithelial cancer combined with quercetin and genistein. [12]

To glean insights into the mechanism of their action, we assessed the effects of two flavonoids, quercetin (Qu) and luteolin (Lu), on the growth and epidermal growth factor receptor (EGFR) tyrosine kinase activity of MiaPaCa-2 cancer cells. Exposure of these EGFR-expressing cells to 20 microM Qu or Lu resulted in concomitant decreases in cellular protein phosphorylation and growth. On the cellular level, Qu and Lu sensitivity correlated with EGFR levels and rapid cell proliferation, indicating the possibility of targeting those cells most prone to neoplastic progression. Cell treatment with the flavonoids markedly diminished the extent of cellular protein phosphorylation, by effectively modulating protein tyrosine kinase (PTK) activities, including that of EGFR. Immunocomplex kinase assay revealed that both Qu and Lu inhibited the PTK activities responsible for the autophosphorylation of EGFR as well as for the transphosphorylation of enolase. Treatment of the cells with Qu or Lu also reduced the phosphotyrosyl levels of 170-, 125-, 110-, 65-, 60-, 44-, 30- and 25-kDa proteins. We identified the 170-kDa phosphotyrosylprotein as EGFR. Qu and Lu exhibited a specific action in hampering the levels of phosphorylation of this and the aforementioned proteins, while having no discernible effect on their synthesis. A time-dependent attenuation of the phosphorylation of the above proteins was demonstrable. Treatment of the cells with Qu or Lu for 6 hours showed little inhibition, but prolonging the cell treatment for 24 hours caused the suppression of phosphorylation. Further continuation of the cell treatment culminated in the induction of apoptosis, characteristically exhibiting shrinkage of the cell morphology, DNA fragmentation and poly(ADP-ribose)polymerase (PARP) degradation. The onset of apoptosis and associated events occurred in a time-dependent fashion. The data clearly demonstrate that MiaPaCa-2 cells respond to Qu and Lu by a parallel reduction in cellular protein phosphorylation and cellular proliferation. The flavonoid-evoked attenuation of the phosphorylation of EFGR and of other proteins appeared to be transient, since removal of the flavonoid from the cell growth medium after 24 hours of incubation followed by exposure to 10 nm EGF, restored protein phosphorylation and cellular proliferation. Such an addition of EGF was also able to reverse Qu- or Lu-induced cell growth inhibition and diminish nuclear digestion evoked by 20 microM Qu or Lu. Both Qu and Lu were able to reverse the effect of EGF biochemically as well as functionally. Based on the evidence accrued, the above proteins could be implicated in growth signal transduction and the subtle changes in their phosphorylation, as effected by flavonoids, utilized as a reliable guide to predict growth response. The antiproliferative effect of flavonoids might result, at least in part, from the modulation of the EGF-mediated signaling pathway. The results indicate that the blockade of the EGFR-signaling pathway by the PTK inhibitors Qu and Lu significantly inhibits the growth of MiaPaCa-2 cells and induces apoptosis. The modulation of EGFR kinase appears to be a critically important, intrinsic component of Qu- and Lu-induced growth suppression, even though other mechanisms could also have contributed to the net effect. [49]

The polyphenol-rich extract of a consumer-relevant apple juice blend was found to potently inhibit the growth of the human colon cancer cell line HT29 in vitro. The epidermal growth factor receptor (EGFR) and its subsequent signaling cascade play an important role in the regulation of cell proliferation in HT29 cells. The protein tyrosine kinase activity of an EGFR preparation was effectively inhibited by the polyphenol-rich apple juice extract. Treatment of intact cells with this extract resulted in the suppression of the subsequent mitogen-activated protein kinase cascade. Amongst the so far identified apple juice constituents, the proanthocyanidins B1 and B2 as well as quercetin-3-glc (isoquercitrin) and quercetin-3-gal (hyperoside) were found to possess substantial EGFR-inhibitory properties. However, as to be expected from the final concentration of these potential EGFR inhibitors in the original polyphenol-rich extract, a synthetic mixture of the apple juice constituents identified and available so far, including both proanthocyanidins and the quercetin glycosides, showed only marginal inhibitory effects on the EGFR. These results permit the assumption that yet unknown constituents contribute substantially to the potent EGFR-inhibitory properties of polyphenol-rich apple juice extract. In summary, the polyphenol composition of apple juice possesses promising growth-inhibitory properties, affecting proliferation-associated signaling cascades in colon tumor cells.[50]

Inhibits ErbB-2 (Her II neu) and ErbB-3 expression

Because ErbB-2 receptor is involved in hormone-independency for growth and metastasis of prostate cancer cells, the aim was to investigate the effects of quercetin on ErbB-2 and ErbB-3 expression and its

critical components such as MAP kinase and PI-3 kinase. Hemocytometric counts and [3H]-thymidine incorporation were used to determine the effects of quercetin, EGF and TGF-alpha on cell proliferation and DNA synthesis in PC-3 and LnCap cells. Changes in ErbB-2, ErbB-3 and components of MAPK and PI-3K pathways were analyzed by Western blot analysis. Treatment of PC-3 and LnCap cells with quercetin resulted in a dose-dependent growth inhibition. The rate of DNA synthesis was decreased by 40, 55 and 65% on treatment with 14.5, 29.0 and 58.0 microM of quercetin, respectively. Concomitantly, these treatments led to a dose-dependent decrease in ErbB-2, ErbB-3 and their basal autophosphorylation levels as compared to controls. Cyclin D1 expression and basal phosphorylation of c-Raf, MAPK, Elk-1 and Akt-1 in PC-3 cells was also inhibited by quercetin treatment. Co-treating PC-3 cells with quercetin significantly attenuated EGF- and TGF-alpha-induced growth and phosphorylation of ErbB-2, ErbB-3, c-Raf, MAPK kinase 1/2 (MEK1/2), MAPK, Elk-1 and Akt-1. Since ErbB receptor is important for growth, metastasis and drug resistance, inhibition of ErbB-2 and ErbB-3 by pharmacological doses of quercetin may provide a new approach for treatment of prostate cancers. [14]

In another study quercetin decreased the level of Her-2/neu protein in time- and dose-dependent manners and also inhibited the downstream survival PI3K-Akt signaling pathway in Her-2/neu-overexpressing breast cancer SK-Br3 cells. We also observed that quercetin induced polyubiquitination of Her-2/neu. When the proteasome pathway was blocked by MG-132 during quercetin treatment, accumulation of the NP-40 insoluble form of Her-2/neu occurred. Interestingly, data from immunocomplex studies revealed that quercetin promoted interaction between Her-2/neu and Hsp90 which is a molecular chaperone involved in stabilization of Her-2/neu. In this condition, inhibition of Hsp90 activity by a specific inhibitor, geldanamycin (GA), or intracellular ATP depletion caused dissociation of Hsp90 from Her-2/neu and promoted ubiquitination and down-regulation of Her-2/neu protein. In addition, the carboxyl terminus of Hsc70-interacting protein (CHIP), a chaperone-dependent E3 ubiquitin ligase, played a crucial role in the quercetin-induced ubiquitination of Her-2/neu. Inhibition of tyrosine kinase activity of Her-2/neu by quercetin could indicate an lateration in the Her-2/neu structure which promotes CHIP recruitments and down-regulation of Her-2/neu. [82]

Quercetin induces growth inhibition in the human breast carcinoma cell line MCF-7 through at least two different mechanisms; by inhibiting cell cycle progression through transient M phase accumulation and subsequent G2 arrest, and by inducing apoptosis.[44]

Inhibits environmental estrogens binding capacity.[45]

Quercetin inhibits noradrenaline-promoted invasion of human breast cancer cells by blocking β2-adrenergic signaling.

Endogenous catecholamines such as adrenaline (A) and noradrenaline (NA) are released from the adrenal gland and sympathetic nervous system during exposure to stress. The adrenergic system plays a central role in stress signaling, and excessive stress was found to be associated with increased production of reactive oxygen species (ROS). Overproduction of ROS induces oxidative damage in tissues and causes the development of diseases such as cancer. In this study, we investigated the effects of quercetin-3-O-glucuronide (Q3G), a circulating metabolite of quercetin, which is a type of natural flavonoid, on the catecholamine-induced β2-adrenergic receptor (β2-AR)-mediated response in MDA-MB-231 human breast cancer cells expressing β2-AR. Treatment with A or NA at concentrations above 1μM generated significant levels of ROS, and NA treatment induced the gene expression of heme oxygenase-1 (HMOX1), and matrix metalloproteinase-2 (MMP-2) and -9 (MMP9). Inhibitors of p38 MAP kinase (SB203580), cAMP-dependent protein kinase (PKA) (H-89), activator protein-1 (AP-1) transcription factor (SR11302), and NF-κB and AP-1 (Tanshinone IIA) decreased MMP2 and MMP9 gene expression. NA also enhanced cAMP induction, RAS activation and phosphorylation of ERK1/2. These results suggested that the cAMP-PKA, MAPK, and ROS-NF-κB pathways are involved in β2-AR signaling. Treatment with 0.1μM Q3G suppressed ROS generation, cAMP and RAS activation, phosphorylation of ERK1/2 and the expression of HMOX1, MMP2, and MMP9 genes. Furthermore, Q3G (0.1μM) suppressed invasion of MDA-MB-231 breast cancer cells and MMP-9 induction, and inhibited the binding of [(3)H]-NA to β2-AR. These results suggest that Q3G may function to suppress invasion of breast cancer cells by controlling β2-adrenergic signaling, and may be a dietary chemopreventive factor for stress-related breast cancer.[127]

Anti-leukemic effects

In a current study by Liesveld et al., quercetin demonstrated a antiproliferative and proapoptotic effect in leukemic cells. The implications of the results of this study on the activity of quercetin, and other flavonoids in leukemias and their future development should be explored. [24]

Quercetin and flavopiridol, both influence oxidative milieu, proliferation, and apoptosis of various cell types, were examined for their effects on acute myelogenous leukemic cells and normal progenitors. Both quercetin and flavopiridol inhibited the growth and viability of various acute myelogenous leukemia (AML) cell lines and AML blasts isolated afresh from patients with AML of various subtypes. The effects on inhibition of proliferation and decreased viability were also significant in normal CD34+ cells isolated from normal marrow donors. These flavonoid compounds might find use in various therapeutic settings in AML. [16]

Anti-leukemic: causes G2/M arrest, down-regulates cyclin D and E and induces caspase-dependant apotosis

Quercetin induces anti-proliferation and arrests G2/Mphase in U937 cells. The G2/Mphase accumulation was accompanied by an increase in the level of the cyclin B. In contrast, the level of the cyclin D, cyclin E, E2F1, and E2F2 was marked decreased in quercetin-treated U937 cells. Removal of quercetin from the culture medium stimulates U937 cells to synchronously re-enter the cell cycle, decrease expression level of cyclin B, and increased the expression level of cyclin D and cyclin E. These data demonstrate that quercetin causes reversible G2/M phase arrest, which was related with dramatic changes in the level of cyclin B, cyclin D, and cyclin E. Quercetin-induced down-regulation of cyclin D and cyclin E was associated with suppression of transcriptional levels but not protein stability. In addition, quercetin-treated U937 cells showed DNA fragmentation, increased sub-G1 population, and generated a 60 kDa cleavage product of PLC-g1 in a dose-dependent manner, which were significantly inhibited by z-VAD-fmk. These data clearly indicate that quercetin-induced apoptosis is associated with caspase activation. In summary, the growth inhibition of the quercetin is highly related to cell cycle arrest at the G2/M phase and induction of caspase-dependent apoptosis in human promonocytic U937 cells. [59]

Enhances TRAIL-induced cytotoxicity by activating caspases and inhibiting phosphorylation of Akt

TNF-related apoptosis-inducing ligand (TRAIL) is a promising cancer therapy that preferentially induces apoptosis in cancer cells. However, many neoplasms are resistant to TRAIL by mechanisms that are poorly understood. Here we demonstrated that human prostate cancer cells, but not normal prostate cells, are dramatically sensitized to TRAIL-induced apoptosis and caspase activation by quercetin. Quercetin, a ubiquitous bioactive plant flavonoid, has been shown to inhibit the proliferation of cancer cells. We have shown that quercetin can potentiate TRAIL-induced apoptotic death. Human prostate adenocarcinoma DU-145 and LNCaP cells were treated with various concentrations of TRAIL (10-200 ng/ml) and/or quercetin (10-200 microM) for 4 h. Quercetin, which caused no cytotoxicity by itself, promoted TRAIL-induced apoptosis. The TRAIL-mediated activation of caspase, and PARP (poly(ADP-ribose) polymerase) cleavage were both enhanced by quercetin. Western blot analysis showed that combined treatment with TRAIL and quercetin did not change the levels of TRAIL receptors (death receptors DR4 and DR5, and DcR2 (decoy receptor 2)) or anti-apoptotic proteins (FLICE-inhibitory protein (FLIP), inhibitor of apoptosis (IAP), and Bcl-2). However, quercetin promoted the dephosphorylation of Akt. Quercetin-induced potent inhibition of Akt phosphorylation. Taken together, the present studies suggest that quercetin enhances TRAIL-induced cytotoxicity by activating caspases and inhibiting phosphorylation of Akt. [61]

Inhibition of P53 gene mutation:

A mechanism by which quercetin shows its antitumor effects is by inhibiting the expression of certain gene mutations. Quercetin inhibits the mutation of the tumor suppressor protein gene p53. The mutation, or defect of this suppressor is involved in more than half of all cancer cell lines including breast, ovarian and prostate cancers. [17]

This study investigated the anti-cancer effect of combined quercetin and a recombinant adenovirus vector expressing the human p53, GM-CSF and B7-1 genes (designated BB-102) on human hepatocellular carcinoma (HCC) cell lines in vitro. METHODS: The sensitivity of HCC cells to anticancer agents was

evaluated by 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The viability of cells infected with BB-102 was determined by trypan blue exclusion. The expression levels of human wild-type p53, GM-CSF and B7-1 genes were determined by Western blot, enzyme-linked immunosorbent assay (ELISA) and flow cytometric analysis, respectively. The apoptosis of BB-102-infected or quercetin-treated HCC cells was detected by terminal deoxynucleotidyl transferase (TdT) assay or DNA ladder electrophoresis. RESULTS: Quercetin was found to suppress proliferation of human HCC cell lines BEL-7402, HuH-7 and HLE, with peak suppression at 50 micromol/L quercetin. BB-102 infection was also found to significantly suppress proliferation of HCC cell lines. The apoptosis of BB-102-infected HCC cells was greater in HLE and HuH-7 cells than in BEL-7402 cells. Quercetin did not affect the expression of the three exogenous genes in BB-102-infected HCC cells (P>0.05), but it was found to further decrease proliferation and promote apoptosis of BB-102-infected HCC cells. CONCLUSION: BB-102 and quercetin synergetically suppress HCC cell proliferation and induce HCC cell apoptosis, suggesting a possible use as a combined anti-cancer agent. [18]

Quercetin strongly inhibited, in a time- and dose-dependent fashion, the expression of the mutated p53 protein, in breast cancer. [27]

Chemopreventive effect of quercetin, a natural dietary flavonoid on prostate cancer in in vivo model.

Prostate cancer is one of the frequently diagnosed cancers in men. Increased Growth factor IGF-1/IGF-1R axis activation mediated by both PI3K/Akt or RAF/MEK/ERK system and AR expression remains important in the development and progression of prostate cancer. Targeting such system by dietary agents quercetin in vivo model could aid its application in both treatment as well as prevention of prostate cancer.

In our study the rats were divided into four groups; Group I: control (propylene glycol-vehicle), Group II: cancer-induced (MNU and Testosterone treated) rats, Group III: cancer-induced + Quercetin (200 mg/kg body wt/orally) and Group IV: Quercetin (200 mg/kg body wt) thrice a week. After the treatment period rats were sacrificed and the ventral and dorsolateral prostate lobes were dissected.

Antioxidant enzymes and apoptotic proteins were significantly decreased in cancer-induced animal and upon quercetin supplement its level was increased. The IGFIR, AKT, AR, cell proliferative and anti-apoptotic proteins were increased in cancer-induced group whereas supplement of quercetin decreased its expression.

Quercetin down regulates the cell survival, proliferative and anti-apoptotic proteins thereby prevents prostate cancer, by acting as a chemopreventive agent in preclinical model.[118]

Quercetin inhibits lapatinib-sensitive and -resistant breast cancer cell growth by inducing G(2)/M arrest and apoptosis.

Lapatinib, an oral, small-molecule, reversible inhibitor of both EGFR and HER2, is highly active in HER2 positive breast cancer as a single agent and in combination with other therapeutics. However, resistance against lapatinib is an unresolved problem in clinical oncology. Recently, interest in the use of natural compounds to prevent or treat cancers has gained increasing interest because of presumed low toxicity. Quercetin-3-methyl ether, a naturally occurring compound present in various plants, has potent anticancer activity. Here, we found that quercetin-3-methyl ether caused a significant growth inhibition of lapatinib-sensitive and -resistant breast cancer cells. Western blot data showed that quercetin-3-methyl ether had no effect on Akt or ERKs signaling in resistant cells. However, quercetin-3-methyl ether caused a pronounced G(2)/M block mainly through the Chk1-Cdc25c-cyclin B1/Cdk1 pathway in lapatinib-sensitive and -resistant cells. In contrast, lapatinib produced an accumulation of cells in the G(1) phase mediated through cyclin D1, but only in lapatinib-sensitive cells. Moreover, quercetin-3-methyl ether induced significant apoptosis, accompanied with increased levels of cleaved caspase 3, caspase 7, and poly(ADP-ribose) polymerase (PARP) in both cell lines. Overall, these results suggested that quercetin-3-methyl ether might be a novel and promising therapeutic agent in lapatinib-sensitive or -resistant breast cancer patients.[128]

Anti-leukemic (AML) inhibits VEGF

To investigate the effects of quercetin on cell morphology and VEGF expression of acute myeloblastic leukemia cells NB4 in vitro. METHODS: The cytomorphology of NB4 cells was assessed by Wright-stain,

apoptosis rate by apoptotic marker Annexin V, and VEGF secretion level by ELISA. RESULTS: Typical apoptosis was found in NB4 cells after treatment with quercetin. Apoptotic marker Annexin V analysis showed that the apoptotic rate of NB4 cells was increased after treatment with quercetin. The secretion of VEGF of NB4 cells was significantly decreased after treatment with quercetin. Quercetin can induce apoptosis and inhibit secretion of VEGF in NB4 leukemia cells. [86]

Anti-leukemic in vivio

The purpose of the present studies was to focus on the in vivo effects of quercetin on leukemia WEHI-3 cells. The effects of quercetin on WEHI-3 cells injected into BALB/c mice were examined. Quercetin decreased the percentage of Mac-3 and CD11b markers, suggesting that the differentiation of the precursors of macrophages and T cells was inhibited. There was no effect on CD3 levels but increased CD19 levels. Quercetin decreased the weight of the spleen and liver compared with the olive oil treated animals. Quercetin stimulated macrophage phagocytosis of cells isolated from peritoneum. Quercetin also promoted natural killer cell activity. Based on pathological examination, an effect of quercetin was observed in the spleen of mice previously injected with WEHI-3 cells. Apparently, quercetin affects WEHI-3 cells in vivo. [90]

Elevates cancer inhibiting gene p27

Quercetin, in another study arrested cells in G1 and G2/M, in correlation with p53 activation. Arrest was linked to an elevation of the cyclin-dependent kinase inhibitor p27. [22]

Inhibits colon cancer: elevates p21 gene expression

In vivo anti-tumor study indicates that quercetin exhibits ability to inhibit tumor formation elicited by s.c. injection of COLO205 cells in nude mice, and apoptotic cells and an increase in p21 protein were observed in tumor tissues derived from quercetin -treated group. Additionally, quercetin induced apoptosis in primary colon carcinoma cells COLO205-X with appearance of DNA ladders, caspase 3 protein procession, PARP protein cleavage, and an increase in p21 protein. These data provide evidence to suggest that quercetin is an effective agent to induce apoptosis in colorectal carcinoma cells in vitro and in vivo. [43]

Inhibits Raf and MEK protein kinases

Considerable attention has focused on the health-promoting effects of red wine and its nonflavonoid polyphenol compound resveratrol. However, the underlying molecular mechanisms and molecular target(s) of red wine or other potentially active ingredients in red wine remain unknown. Here, we report that red wine extract (RWE) or the red wine flavonoid quercetin inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced transformation of JB6 promotion-sensitive mouse skin epidermal (JB6 P+) cells. The activation of activator protein-1 and nuclear factor-kappaB induced by TPA was dose dependently inhibited by RWE or quercetin treatment. Western blot and kinase assay data revealed that RWE or quercetin inhibited mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) kinase (MEK) 1 and Raf1 kinase activities and subsequently attenuated TPA-induced phosphorylation of ERK/p90 ribosomal S6 kinase. Although either RWE or quercetin suppressed Raf1 kinase activity, they were more effective in inhibiting MEK1 activity. Importantly, quercetin exerted stronger inhibitory effects than PD098059, a well-known pharmacologic inhibitor of MEK. Resveratrol did not affect either MEK1 or Raf1 kinase activity. Pull-down assays revealed that RWE or quercetin (but not resveratrol) bound with either MEK1 or Raf1. RWE or quercetin also dose dependently suppressed JB6 P+ cell transformation induced by epidermal growth factor or H-Ras, both of which are involved in the activation of MEK/ERK signaling. Docking data suggested that quercetin, but not resveratrol, formed a hydrogen bond with the backbone amide group of Ser(212), which is the key interaction for stabilizing the inactive conformation of the activation loop of MEK1.[104]

Quercetin inhibits migration and invasion of SAS human oral cancer cells through inhibition of NF-κB and matrix metalloproteinase-2/-9 signaling pathways.

Quercetin, a principal flavanoid compound in onions, has been shown to possess a wide spectrum of pharmacological properties, including anticancer activities. Our earlier study showed that quercetin induced cytotoxic effects on SAS human oral cancer cells. In this study, we found that quercetin significantly reduced wound closure of SAS cells in culture plates after 12- and 24-h treatments. Results indicated that

quercetin inhibited the expression and activity of matrix metalloproteinase (MMP)-2 and MMP-9, as measured by western blotting and gelatin zymography. The results from western blotting also showed that quercetin reduced the protein levels of MMP-2, -7, -9 and -10, vascular endothelial growth factor (VEGF), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) p65, inductible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), urokinase-type plasminogen activator (uPA), phosphatidylinositide-3 kinases (PI3K), nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha (IKBα), IKB-α/β, phosphorylated nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor kinase, alpha/beta (p-IKKα/β), focal adhesion kinase (FAK), son of sevenless homolog-1 (SOS1), growth factor receptor-bound protein-2 (GRB2), mitogen-activated protein kinase kinase kinase-3 (MEKK3), MEKK7, extracellular-signal-regulated kinase 1/2 (ERK1/2), p-ERK1/2, c-Jun N-terminal kinase 1/2 (JNK1/2), p38, p-p38, Jun proto-oncogene (c-JUN) and p-c-JUN but it did not affect Ras homolog gene family, member A (RhoA), Protein kinase C (PKC) and rat sarcoma viral oncogene homolog (RAS) in SAS cells. Confocal laser microscopy also showed that quercetin promoted the expressions of RhoA and Rho-associated, coiled-coil containing protein kinase-1 (ROCK1), but inhibited the expression of NF-κB p65 in SAS cells. It is concluded from these data that inhibition of migration and invasion of SAS cells by quercetin is associated with the down-regulation of PKC and RhoA by blocking MAPK and PI3K/AKT signaling pathways and NF-κB and uPA, resulting in inhibition of MMP-2 and MMP-9 signaling.[121]

Potentiates chemotherapy and inhibits multi-drug resistance:

Multidrug resistance is associated with overexpression of a membrane protein called P-glycoprotein. Heat shock factor (HSF) is produced within the cell in response to heat or various forms of stress (for example, chemotherapy). HSF can cause P-glycoprotein to be over-expressed which can then lead to chemotherapy resistance within the tumor cell. The result is that what was once an effective cytotoxic drug is no longer effective. Quercetin can inhibit this process in a variety of neoplastic cell lines, as well as in many chemotherapeutic agents that might be used in treating that particular cancer. Quercetin enhances the cytotoxic effects of many chemotherapeutic drugs, including Adriamyacin and Cytoxin. It also potentiates the cytotoxicity of Adriamycin against Adriamycin-resistant human breast cancer cells.

Sensitization of human tumor cells (with heat) to chemotherapy

Quercetin has been found to be useful in cancer therapy as a thermosensitizer by increasing the cell killing effect of hyperthermia and chemotherapy because of its ability to suppress heat-shock protein expression. We investigated the effect of quercetin combined with two cytotoxic agents, cDDP (cis-diamminedichloroplatinum II) and VP-16 (etoposide), under various heat-shock conditions in two Ewing's tumor cell lines SK-ES-1 and RD-ES, using XTT-assay and Western blot analysis. Quercetin (> or = 50 microM), alone as well as in combination with thermochemotherapy, inhibited the expression of both HSP70 and HSP27. CONCLUSION: <u>These data suggest that quercetin potentially may be useful in clinical trials for optimizing the efficacy of hyperthermia in combination with chemotherapy</u>. [23]

Heat shock protein 70 (HSP70) has been thought to inhibit apoptosis and reduce the effectiveness of hyperthermal therapy and chemotherapy on cancer. However, the relationship between HSP70 and the drug resistance to chemotherapy has not been definited. P-glycoprotein (P-gp) was a kind of protein that could decrease the effectiveness of chemotherapy. In order to explore the relationship between HSP70 and P-gp, the human hepatocarcinoma line HepG2 cells was induced by heat shock in vitro, and the inhibiting effect of quercetin on them was observed at the same time to seek the method increasing the effectiveness of hyperthermal therapy on hepatocarcinoma. The overexpression of HSP70 and P-gp induced by heat shock could be inhibited effectively by quercetin in a dose-dependent manner, especially by quercetin at the concentrations of 100- 200 micromol/L($P < 0.01$). CONCLUSION: (1) The overexpression of HSP70 and P-gp in HepG2 cells can be induced by heat shock and P-gp maybe has a correlation with HSP70. (2) <u>The overexpression of HSP70 and P-gp in HepG2 cells induced by heat shock can be inhibited by quercetin</u>. [20]

Greatly improved therapeutic index of doxorubicin against 4T1 breast cancer by its opposing effects on HIF-1alpha in tumor and normal cells

The anthracycline antibiotic doxorubicin (DOX) has been used successfully for treating various types of cancers. However, the therapeutic efficacy of DOX was greatly restricted by its cumulative dose-related cardiotoxicity and common side effects such as bone marrow and immune suppression. Quercetin had better cardioprotective and hepatoprotective activities. The present study was to observe whether quercetin

could improve therapeutic index of DOX and explore its mechanisms. METHODS: Effects of quercetin on doxorubicin (DOX)-induced cytotoxicity were investigated in 4T1 cells and murine spleen cells by methylthiazoletetrazolium assay, flow cytometry and single cell gel electrophoresis. Influences of quercetin on therapeutic efficacy and systemic toxicity of DOX were evaluated in BALB/c mice with 4T1 breast cancer. Hypoxia-inducible factor-1 alpha (HIF-1alpha) in tumor and normal cells was examined to explore mechanisms of quercetin by Western blot and enzyme-linked immunosorbent assay. RESULTS: In vitro, quercetin at dose less than 100 muM had only slight effects on cell viability and DOX-induced cytotoxicity in 4T1 cells under normoxia, but it could reverse 4T1 cell resistance to DOX under hypoxia and protect spleen cells against DOX-induced cytotoxicity. In vivo, quercetin suppressed tumor growth and prolonged survival in BALB/c mice bearing 4T1 breast cancer. Importantly, quercetin enhanced therapeutic efficacy of DOX and simultaneously reduced DOX-induced toxic side effects. Further study showed that quercetin suppressed intratumoral HIF-1alpha in a hypoxia-dependent way but increased its accumulation in normal cells. HIF-1alpha siRNA abolished effects of quercetin on both tumor and normal cells. CONCLUSIONS: These results suggested that quercetin could improve therapeutic index of DOX by its opposing effects on HIF-1alpha in tumor and normal cells, and was a promising candidate as anticancer agents. [89]

Quercetin and tamoxifen sensitize human melanoma cells to hyperthermia

Hyperthermia produces regression of human cancer. Because hyperthermia has produced only limited results, attention has focused on searching for substances able to sensitize tumor cells to the effects of hyperthermia. The flavonoid quercetin has been reported to be a hyperthermic sensitizer in ovarian and uterine cervical tumors and in leukemia. Quercetin and tamoxifen inhibit melanoma cells to hyperthermia. We observed that both quercetin and tamoxifen synergize with hyperthermia (42.5 degrees C) in reducing the clonogenic activity of M14 and MNTI and in inducing apoptotic cell death in all three cell lines. As revealed by flow cytometric and Northern blot analyses, quercetin and tamoxifen reduced heat shock protein -70 expression at both protein and mRNA levels. Our results suggest that quercetin and tamoxifen can be usefully combined with hyperthermia in the therapy of recurrent and/or metastatic melanoma. [26]

Inhibits melanoma

Tyrosinase is expressed in melanoma cells and catalyzes the formation of 3,3',4',5,7-pentahydroxyflavone (quercetin) into reactive quinone species and subsequent glutathionyl adducts. Therefore, we examined the effect of quercetin metabolism on the glutathione (GSH) bioreduction pathway and cell viability in DB-1 melanoma cells that express varying levels of tyrosinase (Tyr+). In a cell-free system, GSH was significantly decreased by quercetin, which coincided with the formation of glutathionyl adducts. In Tyr+ clones, quercetin decreased bioreduction capacity and increased reactive oxygen species (ROS) to a greater degree compared to control cells. The antioxidant/electrophile response element-induced enzymes, glutathione-S-transferase (GST), and nicotinamide adenine dinucleotide phosphate:quinone oxidoreductase 1 were expressed at high levels in Tyr+ cells and contributed to pro-oxidant quercetin metabolism. The basal level of ROS and apoptosis was higher in Tyr+ cells and were selectively increased after exposure to quercetin. The increase in apoptosis following quercetin exposure was p53/Bax mediated and correlated with a decrease in GST-driven bioreduction capacity and an increase in ROS. In conclusion, quercetin can selectively sensitize Tyr+ expressing melanoma cells to apoptosis and may serve as an adjuvant to chemotherapy by enhancing cell death and interfering with GST-mediated drug resistance. [78]

Abrogates chemoresistance in melanoma cells by modulating deltaNp73.

The alkylating agent dacarbazine (DTIC) has been used in the treatment of melanoma for decades, but when used as a monotherapy for cancer only moderate response rates are achieved. Recently, the clinical use of temozolomide (TMZ) has become the more commonly used analog of DTIC-related oral agents because of its greater bioavailability and ability to cross the blood brain barrier. The response rates achieved by TMZ are also unsatisfactory, so there is great interest in identifying compounds that could be used in combination therapy. We have previously demonstrated that the bioflavonoid quercetin (Qct) promoted a p53-mediated response and sensitized melanoma to DTIC. Here we demonstrate that Qct also sensitizes cells to TMZ and propose a mechanism that involves the modulation of a truncated p53 family member, deltaNp73.

DB-1 melanoma (p53 wildtype), and SK Mel 28 (p53 mutant) cell lines were treated with TMZ (400 microM) for 48 hrs followed by Qct (75 microM) for 24 hrs. Cell death was determined by Annexin V-FITC staining and immunocytochemical analysis was carried out to determine protein translocation.

After treatment with TMZ, DB-1 cells demonstrated increased phosphorylation of ataxia telangiectasia mutated (ATM) and p53. However, the cells were resistant to TMZ-induced apoptosis and the resistance was associated with an increase in nuclear localization of deltaNp73. Qct treatment in combination with TMZ abolished drug insensitivity and caused a more than additive induction of apoptosis compared to either treatment alone. Treatment with Qct, caused redistribution of deltaNp73 into the cytoplasm and nucleus, which has been associated with increased p53 transcriptional activity. Knockdown of deltaNp73 restored PARP cleavage in the TMZ treated cells, confirming its anti-apoptotic role. The response to treatment was predominantly p53 mediated as the p53 mutant SK Mel 28 cells showed no significant enhancement of apoptosis.

This study demonstrates that Qct can sensitize cells to TMZ and that the mechanisms of sensitization involve modulation of p53 family members.[109]

Reduces oxidative stress/NF Kappa Beta

Increasing evidence in both experimental and clinical studies suggests that oxidative stress is involved in the pathogenesis and progression of diabetic tissue damage. This study investigated the protective effects of quercetin treatment on oxidative stress, nuclear factor (NF)-kappaB activation and expression of inducible nitric oxide synthase (iNOS) in streptozotocin-induced diabetic rats. Male Wistar rats were divided into 4 groups: control rats, control rats treated daily with quercetin (150 mumol/kg, i.p.), untreated diabetic rats, and diabetic rats treated with quercetin. Diabetes was induced by a single i.p. injection of streptozotocin (70 mg/kg). Eight weeks later we measured TBARS and hydroperoxide-initiated chemiluminescence (QL) in liver as markers of oxidative stress, and activities of the antioxidant enzymes catalase, superoxide dismutase (SOD), and glutathione peroxidase, NF-kappaB activation by an electrophoretic mobility shift assay and expression of IkappaB kinases (IKKalpha and IKKbeta), the inhibitor IkappaB (IkappaBalpha and IkappaBbeta), and iNOS by Western blot. The plasma glucose concentration was significantly increased in diabetic rats and was not changed by quercetin. Streptozotocin administration induced significant increases in hepatic TBARS concentration, QL, and SOD and catalase activities that were prevented by quercetin. Activation of NF-kappaB, induction of IKKalpha and iNOS protein levels, and increased degradation of IkappaBalpha were also observed in streptozotocin-treated rats. All of those effects were abolished by quercetin. These findings suggest that quercetin treatment, by abolishing the IKK/NF-kappaB signal transduction pathway, may block the production of noxious mediators involved in the development of early diabetes tissue injury and in the evolution of late complications. [51]

Anti-inflammatory: Inhibits COX-2

Cyclooxygenase-2 (COX-2)-catalysed synthesis of prostaglandin E2 plays a key role in inflammation and its associated diseases, such as cancer and cardiovascular disease. Quercetin, reduces COX-2 mRNA expression in both unstimulated and interleukin-1beta stimulated colon cancer (Caco2) cells. Quercetin and quercetin 3'-sulfate, also inhibited COX-2 activity. [36]

Quercetin, in another study was found to modulates COX- catalyzed prostaglandin E-2 (PGE-2) generation, thus suppress tumor growth. [58]

Potentiates chemotherapy

Effects of quercetin on cisplatin (cis-Pt)-induced apoptosis of human promyelocytic leukemia HL-60 cells and murine leukemia L1210 cells were investigated. Quercetin enhanced the apoptotic DNA damage induced by cis-Pt, displaying a significant synergistic effect. Quercetin is a pro-apoptotic agent with important potential in chemotherapy treatment protocols. [13]

Melanoma: Temozolomide and Quercetin - abrogates chemoresistance in melanoma cells by modulating deltaNp73.

BACKGROUND: The alkylating agent dacarbazine (DTIC) has been used in the treatment of melanoma

for decades, but when used as a monotherapy for cancer only moderate response rates are achieved. Recently, the clinical use of temozolomide (TMZ) has become the more commonly used analog of DTIC-related oral agents because of its greater bioavailability and ability to cross the blood brain barrier. The response rates achieved by TMZ are also unsatisfactory, so there is great interest in identifying compounds that could be used in combination therapy. We have previously demonstrated that the bioflavonoid quercetin (Qct) promoted a p53-mediated response and sensitized melanoma to DTIC. Here we demonstrate that Qct also sensitizes cells to TMZ and propose a mechanism that involves the modulation of a truncated p53 family member, deltaNp73.
METHODS: DB-1 melanoma (p53 wildtype), and SK Mel 28 (p53 mutant) cell lines were treated with TMZ (400 microM) for 48 hrs followed by Qct (75 microM) for 24 hrs. Cell death was determined by Annexin V-FITC staining and immunocytochemical analysis was carried out to determine protein translocation.
RESULTS: After treatment with TMZ, DB-1 cells demonstrated increased phosphorylation of ataxia telangiectasia mutated (ATM) and p53. However, the cells were resistant to TMZ-induced apoptosis and the resistance was associated with an increase in nuclear localization of deltaNp73. Qct treatment in combination with TMZ abolished drug insensitivity and caused a more than additive induction of apoptosis compared to either treatment alone. Treatment with Qct, caused redistribution of deltaNp73 into the cytoplasm and nucleus, which has been associated with increased p53 transcriptional activity. Knockdown of deltaNp73 restored PARP cleavage in the TMZ treated cells, confirming its anti-apoptotic role. The response to treatment was predominantly p53 mediated as the p53 mutant SK Mel 28 cells showed no significant enhancement of apoptosis.
CONCLUSION: This study demonstrates that Qct can sensitize cells to TMZ and that the mechanisms of sensitization involve modulation of p53 family members.[94]

Ellagic acid significantly potentiated the effects of quercetin (at 5 and 10 micro mol/L each) in the reduction of proliferation and viability and the induction of apoptosis. Significant alterations in cell cycle kinetics were also observed. The synergy was confirmed by an isobolographic analysis of the cell proliferation data. The interaction of ellagic acid and quercetin demonstrated an enhanced anticarcinogenic potential of polyphenol combinations, which was not based solely on the additive effect of individual compounds, but rather on synergistic biochemical interactions. [15]

Chemo-protective
Quercetin protects against anthracycline-induced toxicity. [41]

Decreases intracellular GSH content and potentiates the apoptotic action of the anti-leukemic drug arsenic trioxide in human leukemia cell lines
Arsenic trioxide (ATO) is an effective therapeutic agent for the treatment of acute promyelocytic leukemia, but successful application of this agent may occasionally require the use of sensitizing strategies. The present work demonstrates that the flavonoids quercetin and chrysin cooperate with ATO to induce apoptosis in U937 promonocytes and other human leukemia cell lines (THP-1, HL-60). Co-treatment with ATO plus quercetin caused mitochondrial transmembrane potential dissipation, stimulated the mitochondrial apoptotic pathway, as indicated by cytochrome c and Omi/Htra2 release, XIAP and Bcl-X(L) down-regulation, and Bax activation, and caused caspase-8/Bid activation. Bcl-2 over-expression abrogated cytochrome c release and apoptosis, and also blocked caspase-8 activation. Quercetin and chrysin, alone or with ATO, decreased Akt phosphorylation as well as intracellular GSH content. GSH depletion was regulated at the level of L-buthionine-(S,R)-sulfoximine (BSO)-sensitive enzyme activity, and N-acetyl-L-cysteine failed both to restore GSH content and to prevent apoptosis. Treatment with BSO caused GSH depletion and potentiated ATO-provoked apoptosis, but did not affect apoptosis induction by ara-C and cisplatin. As an exception, ATO plus quercetin failed to elicit Akt de-phosphorylation and GSH depletion in NB4 acute promyelocytic leukemia cells, and correspondingly exhibited low cooperative effect in inducing apoptosis in this cell line. It is concluded that GSH depletion explains at least in part the selective potentiation of ATO toxicity by quercetin, and that this flavonoid might be used to increase the clinical efficacy of the antileukemic drug. [81]

Inhibits heat shock transcription factors
Quercetin's action is cell-type specific, and in breast cancer cells may involve regulation of heat shock

transcription factors (HSF) transcriptional activity, rather than regulation of its DNA-binding activity. [46]

Decreases IGF levels, improves signaling and IGFBP-3

This study was designed to investigate its effects on insulin-like growth factors (IGFs) and their binding protein-3 (IGFBP-3) proteins secretion and also apoptosis induction in the human prostate cancer cell line, PC-3. METHODS: We evaluated the secretion of IGF-I, -II and IGFBP-3 in quercetin treated cells by immunoradiometric (IRMA) method. Apoptosis was studied in quercetin treated cells by TUNEL and DNA fragmentation. Protein expressions of Bcl-2, Bcl-xL, Bax and caspase-3 were studied by western blot. RESULTS: At a dose of 100 M concentration, we observed increased IGFBP-3 accumulation in PC-3 cells conditioned medium with a dose dependent increase with 2 fold over a base line, and significantly reduced the both IGF-I and IGF-II levels. Apoptosis induction was also confirmed by TUNEL assay. Bcl-2 and Bcl-xL protein expressions were significantly decreased and Bax and caspase-3 were increased. These results suggest that the decreased level of IGFs could be due to the increased levels of IGFBP-3, because of the high binding affinity towards IGFs, thereby decreasing the cell proliferation. The increased level of IGFBP-3 was associated with increased pro-apoptotic proteins and apoptosis in response to quercetin, suggesting it may be a p53-independent effector of apoptosis in prostate cancer cells. [57]

Downregulation of COX-2 and iNOS in human lung cancer

In the present study, we demonstrate the potential effects of different flavonoids on cytokines mediated cyclooxygenase-2 and inducible nitric oxide synthase expression and activities in A549 cell line using quercetin, amentoflavone and flavanone. Our data revealed that quercetin, at 50 micro M concentration inhibited PGE(2) biosynthesis by A549 very strongly with little effect on COX-2 mRNA and protein expression. Unlike quercetin, amentoflavone inhibited both PGE(2) biosynthesis and COX-2 mRNA and protein expression strongly. In another set of experiment, quercetin inhibited iNOS protein expression completely without affecting iNOS mRNA expression. In contrast, amentoflavone although exerted no inhibitory effect on iNOS mRNA expression, did inhibit weakly iNOS protein expression. Flavanone had no inhibitory effect on either enzyme at the same concentration. Taken together, our data indicated that amentoflavone and quercetin differentially exerted supression of PGE(2) biosynthesis via downregulation of COX-2/iNOS expression. [30]

Another animal study found the quercetin decreased COX-2 induced inflammation by 50%. [42]

Inhibition of Prostate cancer

In the present investigation we studied the effect of quercetin on the ability of prostate cancer cell lines with various degrees of aggressive potential to form colonies in vitro. Specifically, we examined the molecular mechanisms underlying this effect, including the expression of cell cycle and tumor suppressor genes as well as oncogenes. We observed that quercetin at concentrations of 25 and 50 micro M significantly inhibited the growth of the highly aggressive PC-3 prostate cancer cell line and the moderately aggressive DU-145 prostate cancer cell line, whereas it did not affect colony formation by the poorly aggressive LNCaP prostate cancer cell line or the normal fibroblast cell line BG-9. Using the gene array methodology, we found that quercetin significantly inhibited the expression of specific oncogenes and genes controlling G(1), S, G(2), and M phases of the cell cycle. Moreover, quercetin reciprocally up-regulated the expression of several tumor suppressor genes. In conclusion, our results demonstrate that the antitumor effects of quercetin directly correlate with the aggressive potential of prostate cancer cells and that the mechanism(s) of quercetin-mediated antitumor effects may involve up-regulation of tumor suppressor genes and reciprocal down-regulation of oncogenes and cell cycle genes. The results of these studies provide a scientific basis for the potential use of flavonoids as nutraceuticals in the chemoprevention of cancer. [32]

Down-regulates MMP2 and 9

Quercetin is a flavonoid and widely used as an antioxidant and recent studies have revealed its pleiotropic anticancer and antiproliferative capabilities. Gelatinases A and B (matrixmetalloproteinases 2 and 9) are enzymes known to involve in tumor invasion and metastases. In this study, we observed the precise involvement of quercetin role on these proteinases expression and activity. Design and methods: PC-3 cells were treated with quercetin at various concentrations (50 and 100 muM), for 24 h period and then subjected to western blot analysis to investigate the impact of quercetin on matrix metalloproteinase-2 (MMP-2) and

9 (MMP-9) expressions. Conditioned medium and cell lysate of quercetin-treated PC-3 cells were subjected to western blot analysis for proteins expression of MMP-2 and MMP-9. Gelatin zymography was also performed in quercetin treated PC-3 cells. Results: The results showed that quercetin treatment decreased the expressions of MMP-2 and MMP-9 in dose-dependent manner. The level of pro-MMP-9 was found to be high in the 100 muM quercetin-treated cell lysate of PC-3 cells, suggesting inhibitory role of quercetin on pro-MMP-9 activation. Gelatin zymography study also showed the decreased activities of MMP-2 and MMP-9 in quercetin treated cells. Conclusion: Hence, we speculated that inhibition of metastasis-specific MMPs in cancer cells may be one of the targets for anticancer function of quercetin, and thus provides the molecular basis for the development of quercetin as a novel chemopreventive agent for metastatic prostate cancer. [54]

Inhibits breast cancer cells via down-regulation of MMP-9

Increases in the protein, messenger RNA and enzyme activity levels of matrix metalloproteinase (MMP)-9 were observed in 12-O-tetradecanoylphorbol-13-acetate (TPA)-treated MCF-7 cells, and these were blocked by QUE, but not by quercitrin or rutin. A translocation of protein kinase C (PKC)delta from the cytosol to the membrane followed by activation of extracellular signal-regulated kinase (ERK) and c-Jun/activator protein-1 (AP-1) by TPA was demonstrated, and TPA-induced MMP-9 activation and migration were inhibited by the pan PKC inhibitor, GF109203X, the specific PKCdelta inhibitor, rottlerin, an ERK inhibitor (PD98059) and an AP-1 inhibitor (curcumin). Application of QUE significantly suppressed TPA-induced activation of the PKCdelta/ERK/AP-1-signaling cascade. To elucidate the importance of hydroxyl (OH) substitutions to QUE's inhibition of tumor migration, several structurally related flavones of QUE including 3',4'-diOH, 3',4'-diOCH(3), 3,5,7-triOH, 3,4',4'-triOH, 3,3',4'-triOCH(3), luteolin and fisetin were used. Results suggested that OH groups at both C3' and C4' play central roles in QUE's inhibition of TPA-induced MMP-9 activation and migration, and an additional OH at C3, C5 or C7 may increase the inhibitory potency of the 3',4'-diOH flavone against TPA-induced MMP-9 activity and migration. The antitumor invasion and migration effects of breast carcinoma cells induced by QUE with the structure-activity relationship analysis were identified. [83]

Prostate Cancer: Inhibits Benzo(a)pyrene toxicity

Benzo(a)pyrene (BaP)-mediated toxicity is prostate cancer and the chemopreventative potential of quercetin was studued. Quercetin inhibited both BaP-mediated effects on peroxiredoxin (Prx) I and II, in 22Rv1 human prostate cancer cells. In Prostate cancer cells, quercetin inhibited BaP-mediated upregulation of Prx I and had tendency to neutralize BaP-mediated downregulation of Prx II. Quercetin also inhibited BaP-induced concentrations of reactive oxygen species as well. These results suggest that Prx I and II may be involved in BaP-mediated toxicity and the potential chemopreventative mechanisms of quercetin. [65]

Quercetin increased the antiproliferative activity of green tea polyphenol (-)-epigallocatechin gallate in prostate cancer cells

This study investigated the combination of quercetin, a natural inhibitor of catechol-O-methyl transferase (COMT), would synergize with EGCG enhancing antiproliferative activity of EGCG in prostate cancer cells. Incubation with both quercetin and EGCG for 2 h increased the cellular concentrations of EGCG by 4- to 8-fold and 6- to 10-fold in androgen-independent PC-3 cells and androgen-dependent LNCaP cells, respectively. Concurrently, the percent of 4″-MeEGCG in the total EGCG was decreased from 39% to 15% in PC-3 cells and from 61% to 38% in LNCaP cells. Quercetin and EGCG in combination synergistically inhibited cell proliferation, caused cell cycle arrest, and induced apoptosis in PC-3 cells. In LNCaP cells, EGCG and quercetin exhibited a stronger antiproliferative activity leading to an additive effect. The synergistic effect of these 2 agents in PC-3 cells could be based on the fact that EGCG primarily inhibited COMT activity, whereas quercetin reduced the amount of COMT protein. In summary, quercetin combined with EGCG demonstrated enhanced inhibition of cell proliferation by increasing the intracellular concentration of EGCG and decreasing EGCG methylation.[103]

Induces c-Jun inhibiting androgen receptor activity

Prostate cancer cells treated with quercetin or without treatment were used for checking protein expression levels of c-Jun and cAMP response element binding protein (CREB)-binding protein (CBP). Regulatory effects of c-Jun and CBP on the function of androgen receptor (AR) were examined by cotransfection experiment. Quercetin dramatically induced the protein expression of c-Jun which in turn inhibited the AR function.

Overexpression of c-Jun induced by quercetin had inhibitory effect on the function of AR protein, leading to an inhibitory effect on prostate cancer.[66]

Enhanced inhibition of prostate cancer xenograft tumor growth by combining quercetin and green tea.

The chemopreventive activity of green tea (GT) is limited by the low bioavailability and extensive methylation of GT polyphenols (GTPs) in vivo. We determined whether a methylation inhibitor quercetin (Q) will enhance the chemoprevention of prostate cancer in vivo. Androgen-sensitive LAPC-4 prostate cancer cells were injected subcutaneously into severe combined immunodeficiency (SCID) mice one week before the intervention. The concentration of GTPs in brewed tea administered as drinking water was 0.07% and Q was supplemented in diet at 0.2% or 0.4%. After 6-weeks of intervention tumor growth was inhibited by 3% (0.2% Q), 15% (0.4% Q), 21% (GT), 28% (GT+0.2% Q) and 45% (GT+0.4% Q) compared to control. The concentration of non-methylated GTPs was significantly increased in tumor tissue with GT+0.4% Q treatment compared to GT alone, and was associated with a decreased protein expression of catechol-O-methyltransferase and multidrug resistance-associated protein (MRP)-1. The combination treatment was also associated with a significant increase in the inhibition of proliferation, androgen receptor and phosphatidylinositol 3-kinase/Akt signaling, and stimulation of apoptosis. The combined effect of GT+0.4% Q on tumor inhibition was further confirmed in another experiment where the intervention started prior to tumor inoculation. These results provide a novel regimen by combining GT and Q to improve chemoprevention in a non-toxic manner and warrant future studies in humans.[114]

Combinational Treatment of Curcumin and Quercetin against Gastric Cancer MGC-803 Cells in Vitro.

Gastric cancer remains a major health problem worldwide. Natural products, with stronger antitumor activity and fewer side effects, are potential candidates for pharmaceutical development as anticancer agents. In this study, quercetin and curcumin were chosen for testing and were applied separately and in combination to human gastric cancer MGC-803 cells. The MTT assay was used to evaluate cell growth inhibition. Annexin V-FITC/PI was carried out to measure apoptosis rate. Flow cytometry was performed to analyze mitochondrial membrane potential levels. Western blots were applied to detect expression of cytochrome c, total and phosphorylated ERK and AKT. Combined treatment with curcumin and quercetin resulted in significant inhibition of cell proliferation, accompanied by loss of mitochondrial membrane potential ($\Delta\Psi m$), release of cytochrome c and decreased phosphorylation of AKT and ERK. These results indicate that the combination of curcumin and quercetin induces apoptosis through the mitochondrial pathway. Notably, effect of combined treatment with curcumin and quercetin on gastric cancer MGC-803 cells is stronger than that of individual treatment, indicating that curcumin and quercetin combinations have potential as anti-gastric cancer drugs for further development.[130]

Potentiate TNF-related apoptosis-inducing ligand (TRAIL)

TNF-related apoptosis-inducing ligand (TRAIL) is a promising cancer therapy that preferentially induces apoptosis in cancer cells. However, many neoplasms are resistant to TRAIL by mechanisms that are poorly understood. Human prostate cancer cells, but not normal prostate cells, are dramatically sensitized to TRAIL-induced apoptosis and caspase activation by quercetin. Quercetin can potentiate TRAIL-induced apoptotic death. Human prostate adenocarcinoma DU-145 and LNCaP cells were treated with various concentrations of TRAIL (10-200 ng/ml) and/or quercetin (10-200 microM) for 4 h. Quercetin, which caused no cytotoxicity by itself, promoted TRAIL-induced apoptosis. Quercetin-induced potent inhibition of Akt phosphorylation. Quercetin enhances TRAIL-induced cytotoxicity by activating caspases and inhibiting phosphorylation of Akt.[67]

Chemosensitizer

This study was designed to investigate the enhancement of the chemoresponse to cisplatin by quercetin in human lung cancer H-520 cells and to elucidate the role of mitochondria in the induction of apoptosis. Apoptosis was detected by flow cytometry. The protein expressions of Bcl-XL, Bcl-2, Bax, cytochrome c and AIF were studied by Western blotting. The transcription of antioxidant enzymes was quantitated by RT-PCR. The findings suggested that priming H-520 cells with quercetin increased the cisplatin-induced

apoptosis by 30.2%. This was accompanied by down-regulation of Bcl-XL and Bcl-2 and up-regulation of Bax. Both cytochrome c and AIF were implicated in the apoptotic process. There was no significant change in the transcription level of antioxidant enzymes in quercetin-mediated apoptosis. Based on these findings, it can be concluded that quercetin might act as an effective chemosensitizer in the chemotherapy of lung cancer by regulating the expression of various apoptosis-related genes. [55]

Decreases intracellular GSH content and potentiates the apoptotic action of the antileukemic drug arsenic trioxide in human leukemia cell lines

Arsenic trioxide (ATO) is an effective therapeutic agent for the treatment of acute promyelocytic leukemia, but successful application of this agent may occasionally require the use of sensitizing strategies. The present work demonstrates that the flavonoids quercetin and chrysin cooperate with ATO to induce apoptosis in U937 promonocytes and other human leukemia cell lines (THP-1, HL-60). Co-treatment with ATO plus quercetin caused mitochondrial transmembrane potential dissipation, stimulated the mitochondrial apoptotic pathway, as indicated by cytochrome c and Omi/Htra2 release, XIAP and Bcl-X(L) down-regulation, and Bax activation, and caused caspase-8/Bid activation. Bcl-2 over-expression abrogated cytochrome c release and apoptosis, and also blocked caspase-8 activation. Quercetin and chrysin, alone or with ATO, decreased Akt phosphorylation as well as intracellular GSH content. GSH depletion was regulated at the level of L-buthionine-(S,R)-sulfoximine (BSO)-sensitive enzyme activity, and N-acetyl-L-cysteine failed both to restore GSH content and to prevent apoptosis. Treatment with BSO caused GSH depletion and potentiated ATO-provoked apoptosis, but did not affect apoptosis induction by ara-C and cisplatin. As an exception, ATO plus quercetin failed to elicit Akt de-phosphorylation and GSH depletion in NB4 acute promyelocytic leukemia cells, and correspondingly exhibited low cooperative effect in inducing apoptosis in this cell line. It is concluded that GSH depletion explains at least in part the selective potentiation of ATO toxicity by quercetin, and that this flavonoid might be used to increase the clinical efficacy of the antileukemic drug. [81]

Inhibits NFKB & AP-1: may reduces risk of metastatic bone cancer

The effects of quercetin on osteoclast differentiation were studied, which is a critical determinant step of in vivo bone resorption. Two in vitro models of osteoclast differentiation were used in this study: a murine one, involving the culture of RAW 264.7 cells in presence of receptor activator of NF kappa B ligand (RANKL), and a human model consisting of differentiating peripheral blood monocytic cells (PBMC) isolated from peripheral blood in presence of RANKL and macrophage-colony stimulating factor (M-CSF). Osteoclastogenesis was assessed by osteoclast-like number, tartrate resistant acid phosphatase (TRAP) activity, and bone resorbing activity. We showed that quercetin (0.1-10 microM) decreased osteoclastogenesis in a dose dependent manner in both models with significant effects observed at low concentrations, from 1 to 5 microM. The IC(50) value was about 1 microM. Analysis of protein-DNA interaction by electrophoretic mobility shift assay (EMSA) performed on RAW cells showed that a pre-treatment with quercetin inhibited RANKL-induced nuclear factor kB (NF kappa B) and activator protein 1 (AP-1) activation. NF kappa B and AP-1 are transcription factors highly involved in osteoclastic differentiation and their inhibition could play an important role in the decrease of osteoclastogenesis observed in the presence of quercetin. In conclusion, the present results demonstrate for the first time that quercetin is a potent inhibitor of in vitro osteoclastic differentiation, via a mechanism involving NF kappa B and AP-1. [35]

Colon cancer: induces apoptosis and increases p21

New data provides evidence to suggest that quercetin is an effective agent to induce apoptosis in colorectal carcinoma cells in vitro and in vivo; activation of caspase 3, ROS production, and increasing p21 protein are involved. [47]

Controls cell signaling and cell behavior

Quercetin downregulated expression of cell cycle genes (for example CDC6, CDK4 and cyclin D1), downregulated cell proliferation and induced cell cycle arrest in Caco-2 cells. After exposure to 50 micro M quercetin cell proliferation decreased to 51.3% of control, and further decrease of the percentage of cells in the G1 phase coincided with an increase of the percentage of cells in the sub-G1 phase. Quercetin upregulated expression of several tumor suppressor genes. In addition, genes involved in signal

transduction pathways like beta catenin/TCF signalling and MAPK signal transduction were influenced by quercetin. [40]

Activates PTEN

The tumor suppressor gene PTEN, mutated in 40-50% of patients with brain tumors, especially those with glioblastomas, maps to chromosome 10q23.3 and encodes a dual-specificity phosphatase. PTEN exerts its effects partly via inhibition of protein tyrosine kinase B (Akt/Protein Kinase B), which is involved in the phosphatidylinositol (PtdIns) 3-kinase (PI3K)-mediated cell-survival pathway. The naturally occurring bioflavonoid Quercetin (Qu) shares structural homology with the commercially available selective PI3K inhibitor, LY 294002 (LY). Here, the effects of Qu on the Akt/PKB pathway were evaluated. MATERIALS AND METHODS: The human breast carcinoma cell lines, HCC1937, with homozygous deletion of the PTEN gene, and T47D, with intact PTEN, were time-treated with Qu or LY and analyzed for activated levels of Akt by measuring phospho-Akt (p-Akt) levels using immunoblotting analysis. To detect p-Akt, the T47D cells were treated with EGF prior to treatment with or without Qu or LY Cell proliferation after 24-h treatment with Qu or LY was quantified by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. RESULTS: Treatment with Qu (25 microM) for 0.5, 1 and 3 h completely suppressed constitutively activated Akt/PKB phosphorylation at Ser-473 in HCC1937 cells. Pre-exposing T47D cells to Qu (25 microM) or LY (10 microM) abrogated EGF-induced Akt/PKB phosphorylation at Ser-473. Both Qu (100 microM) and LY (50 microM) treatments for 24 h significantly decreased cell proliferation, as shown by the MTT assay. CONCLUSION: Pharmacologically safe doses of the naturally occurring bioflavonoid Qu inhibit the PI3K-Akt/PKB pathway, in a manner similar to that of the commercially available LY. Overall, our results indicated that Qu inhibited the constitutively activated-Akt/PKB pathway in PTEN-null cancer cells, and suggest that this compound may have therapeutic benefit against tumorigenesis and cancer progression. [52]

Activates PTEN and inhibits P13K

Quercetin (Qu) shares structural homology with the commercially available selective PI3K inhibitor, LY 294002 (LY). Here, the effects of Qu on the Akt/PKB pathway were evaluated. MATERIALS AND METHODS: The human breast carcinoma cell lines, HCC1937, with homozygous deletion of the PTEN gene, and T47D, with intact PTEN, were time-treated with Qu or LY and analyzed for activated levels of Akt by measuring phospho-Akt (p-Akt) levels using immunoblotting analysis. To detect p-Akt, the T47D cells were treated with EGF prior to treatment with or without Qu or LY Cell proliferation after 24-h treatment with Qu or LY was quantified by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. RESULTS: Treatment with Qu (25 microM) for 0.5, 1 and 3 h completely suppressed constitutively activated Akt/PKB phosphorylation at Ser-473 in HCC1937 cells. Pre-exposing T47D cells to Qu (25 microM) or LY (10 microM) abrogated EGF-induced Akt/PKB phosphorylation at Ser-473. Both Qu (100 microM) and LY (50 microM) treatments for 24 h significantly decreased cell proliferation, as shown by the MTT assay. CONCLUSION: Pharmacologically safe doses of the naturally occurring bioflavonoid Qu inhibit the PI3K-Akt/PKB pathway, in a manner similar to that of the commercially available LY. Overall, our results indicated that Qu inhibited the constitutively activated-Akt/PKB pathway in PTEN-null cancer cells, and suggest that this compound may have therapeutic benefit against tumorigenesis and cancer progression. [56]

Inhibits EGFR

To glean insights into the mechanism of their action, we assessed the effects of two flavonoids, quercetin (Qu) and luteolin (Lu), on the growth and epidermal growth factor receptor (EGFR) tyrosine kinase activity of MiaPaCa-2 cancer cells. Exposure of these EGFR-expressing cells to 20 microM Qu or Lu resulted in concomitant decreases in cellular protein phosphorylation and growth. On the cellular level, Qu and Lu sensitivity correlated with EGFR levels and rapid cell proliferation, indicating the possibility of targeting those cells most prone to neoplastic progression. Cell treatment with the flavonoids markedly diminished the extent of cellular protein phosphorylation, by effectively modulating protein tyrosine kinase (PTK) activities, including that of EGFR. Immunocomplex kinase assay revealed that both Qu and Lu inhibited the PTK activities responsible for the autophosphorylation of EGFR as well as for the transphosphorylation of enolase. Treatment of the cells with Qu or Lu also reduced the phosphotyrosyl levels of 170-, 125-, 110-, 65-, 60-, 44-, 30- and 25-kDa proteins. We identified the 170-kDa phosphotyrosylprotein as EGFR. Qu and Lu exhibited a specific action in hampering the levels of phosphorylation of this and the aforementioned

proteins, while having no discernible effect on their synthesis. A time-dependent attenuation of the phosphorylation of the above proteins was demonstrable. Treatment of the cells with Qu or Lu for 6 hours showed little inhibition, but prolonging the cell treatment for 24 hours caused the suppression of phosphorylation. Further continuation of the cell treatment culminated in the induction of apoptosis, characteristically exhibiting shrinkage of the cell morphology, DNA fragmentation and poly(ADP-ribose)polymerase (PARP) degradation. The onset of apoptosis and associated events occurred in a time-dependent fashion. The data clearly demonstrate that MiaPaCa-2 cells respond to Qu and Lu by a parallel reduction in cellular protein phosphorylation and cellular proliferation. The flavonoid-evoked attenuation of the phosphorylation of EFGR and of other proteins appeared to be transient, since removal of the flavonoid from the cell growth medium after 24 hours of incubation followed by exposure to 10 nm EGF, restored protein phosphorylation and cellular proliferation. Such an addition of EGF was also able to reverse Qu- or Lu-induced cell growth inhibition and diminish nuclear digestion evoked by 20 microM Qu or Lu. Both Qu and Lu were able to reverse the effect of EGF biochemically as well as functionally. Based on the evidence accrued, the above proteins could be implicated in growth signal transduction and the subtle changes in their phosphorylation, as effected by flavonoids, utilized as a reliable guide to predict growth response. The antiproliferative effect of flavonoids might result, at least in part, from the modulation of the EGF-mediated signaling pathway. The results indicate that the blockade of the EGFR-signaling pathway by the PTK inhibitors Qu and Lu significantly inhibits the growth of MiaPaCa-2 cells and induces apoptosis. The modulation of EGFR kinase appears to be a critically important, intrinsic component of Qu- and Lu-induced growth suppression, even though other mechanisms could also have contributed to the net effect.[53]

Quercetin-4'-O-β-D-glucopyranoside (QODG) inhibits angiogenesis by suppressing VEGFR2-mediated signaling in zebrafish and endothelial cells.

Angiogenesis plays an important role in many physiological and pathological processes. Identification of small molecules that block angiogenesis and are safe and affordable has been a challenge in drug development. Hypericum attenuatum Choisy is a Chinese herb medicine commonly used for treating hemorrhagic diseases. The present study investigates the anti-angiogenic effects of quercetin-4'-O-β-D-glucopyranoside (QODG), a flavonoid isolated from Hypericum attenuatum Choisy, in vivo and in vitro, and clarifies the underlying mechanism of the activity.

METHODOLOGY/PRINCIPAL FINDINGS:
Tg(fli1:EGFP) transgenic zebrafish embryos were treated with different concentrations of quercetin-4'-O-β-D-glucopyranoside (QODG) (20, 60, 180 μM) from 6 hours post fertilisation (hpf) to 72 hpf, and adult zebrafish were allowed to recover in different concentrations of QODG (20, 60, 180 μM) for 7 days post amputation (dpa) prior morphological observation and angiogenesis phenotypes assessment. Human umbilical vein endothelial cells (HUVECs) were treated with or without VEGF and different concentrations of QODG (5, 20, 60, 180 μM), then tested for cell viability, cell migration, tube formation and apoptosis. The role of VEGFR2-mediated signaling pathway in QODG-inhibited angiogenesis was evaluated using quantitative real-time PCR (qRT-PCR) and Western blotting.

CONCLUSION/SIGNIFICANCE:
Quercetin-4'-O-β-D-glucopyranoside (QODG) was shown to inhibit angiogenesis in human umbilical vein endothelial cells (HUVECs) in vitro and zebrafish in vivo via suppressing VEGF-induced phosphorylation of VEGFR2. Our results further indicate that QODG inhibits angiogenesis via inhibition of VEGFR2-mediated signaling with the involvement of some key kinases such as c-Src, FAK, ERK, AKT, mTOR and S6K and induction of apoptosis. Together, this study reveals, for the first time, that QODG acts as a potent VEGFR2 kinase inhibitor, and exerts the anti-angiogenic activity at least in part through VEGFR2-mediated signaling pathway.[105]

Quercetin inhibits noradrenaline-promoted invasion of human breast cancer cells by blocking β-adrenergic signaling.

Endogenous catecholamines such as adrenaline (A) and noradrenaline (NA) are released from the adrenal gland and sympathetic nervous system during exposure to stress. The adrenergic system plays a central role in stress signaling, and excessive stress was found to be associated with increased production of reactive

oxygen species (ROS). Overproduction of ROS induces oxidative damage in tissues and causes the development of diseases such as cancer. In this study, we investigated the effects of quercetin-3-O-glucuronide (Q3G), a circulating metabolite of quercetin, which is a type of natural flavonoid, on the catecholamine-induced β2-adrenergic receptor (β2-AR)-mediated response in MDA-MB-231 human breast cancer cells expressing β2-AR. Treatment with A or NA at concentrations above 1μM generated significant levels of ROS, and NA treatment induced the gene expression of heme oxygenase-1 (HMOX1), and matrix metalloproteinase-2 (MMP-2) and -9 (MMP9). Inhibitors of p38 MAP kinase (SB203580), cAMP-dependent protein kinase (PKA) (H-89), activator protein-1 (AP-1) transcription factor (SR11302), and NF-κB and AP-1 (Tanshinone IIA) decreased MMP2 and MMP9 gene expression. NA also enhanced cAMP induction, RAS activation and phosphorylation of ERK1/2. These results suggested that the cAMP-PKA, MAPK, and ROS-NF-κB pathways are involved in β2-AR signaling. Treatment with 0.1μM Q3G suppressed ROS generation, cAMP and RAS activation, phosphorylation of ERK1/2 and the expression of HMOX1, MMP2, and MMP9 genes. Furthermore, Q3G (0.1μM) suppressed invasion of MDA-MB-231 breast cancer cells and MMP-9 induction, and inhibited the binding of [(3)H]-NA to β2-AR. These results suggest that Q3G may function to suppress invasion of breast cancer cells by controlling β2-adrenergic signaling, and may be a dietary chemopreventive factor for stress-related breast cancer.[126]

Inhibits AR expression and down-regulation of c-Jun

Cell extracts treated with quercetin or without treatment were used for checking protein expression levels of c-Jun and cAMP response element binding protein (CREB)-binding protein (CBP). Quercetin dramatically induced the protein expression of c-Jun which in turn inhibited the AR expression. [75] Another mechanism whereby quercetin suppressed PC was through the modulation of AP-1. [76]

Anticarcinogenic action of quercetin by downregulation of phosphatidylinositol 3-kinase (PI3K) and protein kinase C (PKC) via induction of p53 in hepatocellular carcinoma (HepG2) cell line.

Protein kinase C (PKC) is a key regulator of cell growth and differentiation in mammalian cells and hyperactivation of PKC is believed to play an important role in tumor progression. PKC is downstream to signaling protein of phosphatidylinositol 3-Kinase (PI3K), a known up-regulator of cell proliferation and survival. Accumulation of reactive oxygen species (ROS) triggers oxidative stress in the tumor microenvironment, leading to the hyperactivation of various oxidative stress-stimulated signaling molecules. Quercetin (QUE) is reported to show antitumor activity both in vitro and in vivo; however, the molecular mechanism is yet to be thoroughly explored. HepG2 cells display cellular functions similar to the normal hepatocytes with high degree of morphological and functional differentiation, therefore HepG2 cell line is chosen as the suitable model for drug targeting. Present study is aimed to establish the signaling pathway involved in the anticarcinogenic action of QUE in HepG2 cell line. HepG2 cells were treated with different doses of QUE. Protein level and gene expression were analysed by Western blotting and RT-PCR, respectively. PKC activity was measured by non-radioactive-tagged phosphorylation. Results showed downregulation of expression of PI3K, PKC, COX-2 and ROS caused by QUE. Additionally, QUE enhanced the expression of p53 and BAX in HepG2 cells. Overall, results of the current study suggested that QUE elicited anticarcinogenic action by upregulation of p53 and BAX in HepG2 cells via downregulation of ROS, PKC, PI3K and COX-2, confirming our earlier report on the animal model.[129]

Protective against Idarubin toxicity

Idarubicin is a synthetic anthracycline anticancer drug widely used in the treatment of some hematological malignancies. The studies in our laboratory have clearly demonstrated that idarubicin can undergo reductive bioactivation by NADPH-cytochrome P450 reductase to free radicals with resulting formation of DNA strand breaks, which can potentially contribute to its genotoxic effects [Celik, H., Arinç, E., Bioreduction of idarubicin and formation of ROS responsible for DNA cleavage by NADPH-cytochrome P450 reductase and its potential role in the antitumor effect. J Pharm Pharm Sci, 11(4):68-82, 2008]. In the current study, our aim was to investigate the possible protective effects of several phenolic antioxidants, quercetin, rutin, naringenin, resveratrol and trolox, against the DNA-damaging effect of idarubicin originating from its P450 reductase-catalyzed bioactivation.

METHODS: DNA damage was measured by detecting single-strand breaks in plasmid pBR322 DNA using a cell-free agarose gel method.

RESULTS: Our results indicated that, among the compounds tested, quercetin was the most potent antioxidant in preventing DNA damage. Quercetin significantly decreased the extent of DNA strand breaks in a dose-dependent manner; 100 microM of quercetin almost completely inhibited the DNA strand breakage. Unlike quercetin, its glycosidated conjugate rutin, failed to provide any significant protection against idarubicin-induced DNA strand breaks except at the highest concentration tested (2 mM). The protective effects of other antioxidants were significantly less than that of quercetin even at high concentrations. Quercetin was found to be also an effective protector against DNA damage induced by mitomycin C.

CONCLUSION: We conclude that quercetin, one of the most abundant flavonoids in the human diet, is highly effective in reducing the DNA damage caused by the antitumor agents, idarubicin and mitomycin C, following bioactivation by P450 reductase.[93]

Induced apoptosis in association with death receptors and fludarabine in cells isolated from CLL patients

Quercetin is a flavonoid naturally present in food and beverages belonging to the large class of phytochemicals with potential anti-cancer properties. Here, we investigated the ability of quercetin to sensitise primary cells from chronic lymphocytic leukaemia (CLL) to death receptor (DR) agonists, recombinant TNF-related-apoptosis-inducing ligand (rTRAIL) and anti-CD95, and to fludarabine, a widely used chemotherapeutic drug against CLL.

Peripheral white blood cells were isolated from patients and incubated with medium containing 50 ng ml anti-CD95 agonist antibody; 10 ng ml recombinant TRAIL; 10-25 microM quercetin and 3.5-14 microM fludarabine. Neutral Red assay was used to measure cell viability, where as apoptosis was assessed by determining caspase-3 activity, exposure to Annexin V and PARP fragmentation.

Quercetin significantly enhanced anti-CD95- and rTRAIL-induced cell death as shown by decreased cell viability, increased caspase-3 and -9 activities, and positivity to Annexin V. In addition, association of quercetin with fludarabine increases the apoptotic response in CLL cells of about two-fold compared with quercetin monotreatment.

This work shows that resistance to DR- and fludarabine-induced cell death in leukaemic cells isolated from CLL patients can be ameliorated or bypassed by the combined treatment with quercetin. Considering the low toxicity of the molecule, our study results are in favour of a potential use of quercetin in adjuvant chemotherapy in combination with other drugs.[102]

Inhibits Angiogenesis Mediated Human Prostate Tumor Growth by Targeting VEGFR- 2 Regulated AKT/mTOR/P70S6K Signaling Pathways.

Angiogenesis is a crucial step in the growth and metastasis of cancers, since it enables the growing tumor to receive oxygen and nutrients. Cancer prevention using natural products has become an integral part of cancer control. We studied the antiangiogenic activity of quercetin using ex vivo, in vivo and in vitro models. Rat aortic ring assay showed that quercetin at non-toxic concentrations significantly inhibited microvessel sprouting and exhibited a significant inhibition in the proliferation, migration, invasion and tube formation of endothelial cells, which are key events in the process of angiogenesis. Most importantly, quercetin treatment inhibited ex vivo angiogenesis as revealed by chicken egg chorioallantoic membrane assay (CAM) and matrigel plug assay. Western blot analysis showed that quercetin suppressed VEGF induced phosphorylation of VEGF receptor 2 and their downstream protein kinases AKT, mTOR, and ribosomal protein S6 kinase in HUVECs. Quercetin (20 mg/kg/d) significantly reduced the volume and the weight of solid tumors in prostate xenograft mouse model, indicating that quercetin inhibited tumorigenesis by targeting angiogenesis. Furthermore, quercetin reduced the cell viability and induced apoptosis in prostate cancer cells, which were correlated with the downregulation of AKT, mTOR and P70S6K expressions. Collectively the findings in the present study suggest that quercetin inhibits tumor growth and angiogenesis by targeting VEGF-R2 regulated AKT/mTOR/P70S6K signaling pathway, and could be used as a potential drug candidate for cancer therapy.[106]

Quercetin reduces cisplatin nephrotoxicity in rats without compromising its anti-tumor activity.

Nephrotoxicity is the major limitation for the clinical use of cisplatin as an anti-tumoural drug. Our aim was to investigate the protective effect of quercetin on cisplatin nephrotoxicity in a rat tumour model in vivo and to examine the mechanisms of renal protection.

Breast adenocarcinoma (13762 Mat B-III) cells were inoculated subcutaneously in male Fischer rats and 7 days later, the rats were administered daily with quercetin [50 mg/kg/day, intraperitoneally (i.p.)] or vehicle. Four days after that, the rats were given a single dose of cisplatin (4 mg/kg, i.p.) or vehicle. Tumour growth and renal function were monitored throughout the experiment. Two or 6 days after cisplatin administration, the rats were killed and the kidneys and tumours were removed to examine renal function and toxicity markers in both tissues.

In the kidney, cisplatin treatment induced: (i) a decrease in renal blood flow and glomerular filtration rate, (ii) tubular necrosis/apoptosis, (iii) increased lipid peroxidation and decreased endogenous antioxidant systems, (iv) increased expression of inflammation markers and (v) increased activity of the apoptosis executioner caspase-3. Cisplatin effectively reduced tumour size and weight.

Co-treatment with quercetin partially prevented all the renal effects of cisplatin, whereas it did not impair its anti-tumour activity. In conclusion, in a model of tumour-bearing rats, quercetin prevents the nephrotoxic effect of cisplatin without affecting its anti-tumour activity.[100]

Impact of quercetin on the expression of heparanase in cervical cancer cells

To detect the expression of heparanase (HPA) in cervical cancer cells and investigate the impact of quercetin on the expression of HPA, and the molecular mechanism that quercetin inhibits the growth of cervical cancer cells.

The experimental groups included cervical cancer cell lines (HeLa and Caski) exposed to different concentrations of quercetin (20, 40 and 80 μmol/L) in the culture medium. The control groups included a negative control group, which was cultured with RPMI 1640 only, and a positive control group, in which cervical cancer cells were transfected with HPA small interference RNA (siRNA) to silence HPA expression. The cellular expression levels of HPA were detected with fluorescence quantitative real-time PCR and western blot analysis at 24, 48 and 72 hours after treatment.

(1) HPA was significantly expressed in both cervical cancer cell lines (HeLa and Caski), and it exists both nucleus and cytoplasm. (2) The real-time PCR shows as follows: as the quercetin concentration increased (20, 40 and 80 μmol/L), the mRNA expression level of HPA decreased ($P < 0.01$), in which the inhibition of HPA expression was concentration dependent. In addition, the inhibition of HPA expression was also time dependent. As time growth, the expression level of HPA mRNA (24, 48 and 72 hours) in HeLa and Caski cells decreased ($P < 0.01$). Compared with negative control group, the expression level of HPA mRNA decreased in different concentrations of quercetin (40 and 80 μmol/L) in both HeLa and Caski cells (all $P < 0.05$); Compared with positive control group, the expression level of HPA mRNA expressed no obvious difference in quercetin (80 μmol/L) group ($P > 0.05$) in HeLa cells, while it was opposite in Caski cells ($P < 0.01$). (3) The result of western blot shown that, as the quercetin concentration increased (20, 40 and 80 μmol/L) and time growth (24, 48 and 72 hours), the expression level of HPA protein decreased ($P < 0.01$), and the inhibition of HPA protein expression was concentration and time dependent. Compared with negative control group, the expression level of HPA protein decreased in different concentrations of quercetin (40 and 80 μmol/L) in both HeLa and Caski cells (all $P < 0.05$); Compared with positive control group, the expression level of HPA protein expressed no obvious difference in quercetin (80 μmol/L) group (> 0.05) in both HeLa cells and Caski cells (all $P > 0.05$). Quercetin could inhibits the expression of HPA in cervical carcinoma cell lines, which inhibition is concentration and time dependent.[110]

Quercetin Suppresses Cyclooxygenase-2 Expression and Angiogenesis through Inactivation of P300 Signaling.

Quercetin, a polyphenolic bioflavonoid, possesses multiple pharmacological actions including anti-inflammatory and antitumor properties. However, the precise action mechanisms of quercetin remain unclear. Here, we reported the regulatory actions of quercetin on cyclooxygenase-2 (COX-2), an important mediator in inflammation and tumor promotion, and revealed the underlying mechanisms. Quercetin significantly suppressed COX-2 mRNA and protein expression and prostaglandin (PG) E(2) production, as well as COX-2 promoter activation in breast cancer cells. Quercetin also significantly inhibited COX-2-mediated angiogenesis in human endothelial cells in a dose-dependent manner. The in vitro streptavidin-agarose pulldown assay and in vivo chromatin immunoprecipitation assay showed that quercetin considerably inhibited the binding of the transactivators CREB2, C-Jun, C/EBPβ and NF-κB and blocked the recruitment of the coactivator p300 to COX-2 promoter. Moreover, quercetin effectively inhibited p300 histone acetyltransferase (HAT) activity, thereby attenuating the p300-mediated acetylation of NF-κB.

Treatment of cells with p300 HAT inhibitor roscovitine was as effective as quercetin at inhibiting p300 HAT activity. Addition of quercetin to roscovitine-treated cells did not change the roscovitine-induced inhibition of p300 HAT activity. Conversely, gene delivery of constitutively active p300 significantly reversed the quercetin-mediated inhibition of endogenous HAT activity. These results indicate that quercetin suppresses COX-2 expression by inhibiting the p300 signaling and blocking the binding of multiple transactivators to COX-2 promoter. Our findings therefore reveal a novel mechanism of action of quercetin and suggest a potential use for quercetin in the treatment of COX-2-mediated diseases such as breast cancers.[99]

Inhibits Benzo(a)pyrene toxicity induced PC

Benzo(a)pyrene (BaP)-mediated toxicity is prostate cancer and the chemopreventative potential of quercetin was studued. Quercetin inhibited both BaP-mediated effects on peroxiredoxin (Prx) I and II, in 22Rv1 human prostate cancer cells. In Prostate cancer cells, quercetin inhibited BaP-mediated upregulation of Prx I and had tendency to neutralize BaP-mediated downregulation of Prx II. Quercetin also inhibited BaP-induced concentrations of reactive oxygen species as well. These results suggest that Prx I and II may be involved in BaP-mediated toxicity and the potential chemopreventative mechanisms of quercetin. [77]

Liposomal quercetin: antitumor efficacy *in vivo* and *in vitro*.

Quercetin was encapsulated in polyethylene glycol 4000 liposomes. Biodistribution of liposomal quercetin i.v. at 50 mg/kg in tumor-bearing mice was detected by high-performance liquid chromatography. Induction of apoptosis by liposomal quercetin *in vitro* was tested. The antitumor activity of liposomal quercetin was evaluated in the immunocompetent C57BL/6N mice bearing LL/2 Lewis lung cancer and in BALB/c mice bearing CT26 colon adenocarcinoma and H22 hepatoma. Tumor volume and survival time were observed. The mechanisms underlying the antitumor effect of quercetin *in vivo* was investigated by detecting the microvessel density, apoptosis, and heat shock protein 70 expression in tumor tissues.

Results: Liposomal quercetin could be dissolved in i.v. injection and effectively accumulate in tumor tissues. The half-time of liposomal quercetin was 2 hours in plasma. The liposomal quercetin induced apoptosis *in vitro* and significantly inhibited tumor growth *in vivo* in a dose-dependent manner. The optimal dose of liposomal quercetin resulted in a 40-day survival rate of 40%. Quantitative real-time PCR showed that liposomal quercetin down-regulated the expression of heat shock protein 70 in tumor tissues. Immunohistochemistry analysis showed that liposomal quercetin inhibited tumor angiogenesis as assessed by CD31 and induced tumor cell apoptosis.
Conclusions: Our data indicated that pegylated liposomal quercetin can significantly improve the solubility and bioavailability of quercetin and can be a potential application in the treatment of tumor. [63]

Induces Human DNA Topoisomerase II Inhibition, Cell Cycle Arrest and Apoptosis in Hepatocellular Carcinoma Cells.

Dietary flavonoids have been associated with reduced risk of cancer including hepatocellular carcinoma (HCC). Quercetin-3-O-glucoside (Q3G) has been shown to possess anti-proliferative and antioxidant activities. The objectives of this study were to assess the anti-proliferative properties of Q3G in human liver cancer cells (HepG2); assess the cytotoxicity on normal primary cells; and elucidate its possible mechanism of action(s).

MATERIALS AND METHODS:
Using a dose- and time-dependent study, we evaluated the antiproliferative properties of Q3G in HepG2 cells using MTS cell viability assay and lactate dehydrogenase release assay. To elucidate the mechanism of action, we performed cell-cycle analysis using flow cytometry. Cell death via apoptosis was analyzed by DNA fragmentation assay, caspase-3 induction assay and fluorescence microscopy. DNA topoisomerase II drug screening assay was performed to assess the effect of Q3G on DNA topoisomerase II.

RESULTS:
Q3G treatment inhibited cell proliferation in a dose- and time-dependent manner in HepG2 cells with the blockade of the cell cycle in the S-phase. Additionally, Q3G exhibited a strong ability to inhibit DNA topoisomerase II. Furthermore, DNA fragmentation and fluorescence microscopy analysis suggested that Q3G induced apoptosis in HepG2 cells with the activation of caspase-3. Interestingly, Q3G exhibited

significantly lower toxicity to normal cells (primary human and rat hepatocytes and primary lung cells) than sorafenib ($p<0.05$), a chemotherapy drug for hepatocellular carcinoma. The results suggest that Q3G is a potential antitumor agent against liver cancer with a possible mechanism of action via cell-cycle arrest and apoptosis. Further research should be performed to confirm these results in vivo.[117]

Quercetin and EGCG exhibit chemopreventive effects in cholangiocarcinoma cells via suppression of JAK/STAT signaling pathway.

Quercetin and epigallocatechin-3-gallate (EGCG) are dietary phytochemicals with antiinflammatory and antitumor effects. In the present study, we examined the effects of these two compounds on Janus-like kinase (JAK)/signal transduction and transcription (STAT) pathway of cholangiocarcinoma (CCA) cells, because CCA is one of the aggressive cancers with very poor prognosis and JAK/STAT pathway is critically important in inflammation and carcinogenesis. The results showed that the JAK/STAT pathway activation by proinflammatory cytokine interleukin-6 and interferon-γ in CCA cells was suppressed by pretreatment with quercetin and EGCG, evidently by a decrease of the elevated phosphorylated-STAT1 and STAT3 proteins in a dose-dependent manner. The cytokine-mediated up-regulation of inducible nitric oxide synthase (iNOS) and intercellular adhesion molecule-1 (ICAM-1) via JAK/STAT cascade was abolished by both quercetin and EGCG pretreatment. Moreover, these flavonoids also could inhibit growth and cytokine-induced migration of CCA cells. Pretreatment with specific JAK inhibitors, AG490 and piceatannol, abolished cytokine-induced iNOS and ICAM-1 expression. These results demonstrate beneficial effects of quercetin and EGCG in the suppression of JAK/STAT cascade of CCA cells. Quercetin and EGCG would be potentially useful as cancer chemopreventive agents against CCA.[124]

Quercetin: Direct binding of Bcl-2 family proteins by triggers its pro-apoptotic activity.

Bcl-2 family proteins are important regulators of apoptosis and its anti-apoptotic members, which are overexpressed in many types of cancer, are of high prognostic significance, establishing them as attractive therapeutic targets. Quercetin, a natural flavonoid, has drawn much attention because it exerts anticancer effects, while sparing normal cells. A multidisciplinary approach has been employed herein, in an effort to reveal its mode of action including dose-response antiproliferative activity and induced apoptosis effect, biochemical and physicochemical assays and computational calculations. It may be concluded that, quercetin binds directly to the BH3 domain of Bcl-2 and Bcl-xL proteins, thereby inhibiting their activity and promoting cancer cell apoptosis.[122]

Quercetin suppresses insulin receptor signaling through inhibition of the insulin ligand-receptor binding and therefore impairs cancer cell proliferation.

Although the flavonoid quercetin is known to inhibit activation of insulin receptor signaling, the inhibitory mechanism is largely unknown. In this study, we demonstrate that quercetin suppresses insulin induced dimerization of the insulin receptor (IR) through interfering with ligand-receptor interactions, which reduces the phosphorylation of IR and Akt. This inhibitory effect further inhibits insulin stimulated glucose uptake due to decreased cell membrane translocation of glucose transporter 4 (GLUT4), resulting in impaired cancer cell proliferation. The effect of quercetin in inhibiting tumor growth was also evident in an in vivo model, indicating a potential future application for quercetin in the treatment of cancers.[123]

Dietary quercetin inhibits bone loss

In the present study, we demonstrate for the first time the effects of dietary quercetin on bone loss and uterine weight loss by ovariectomy in vivo. Female mice were ovariectomized (OVX) and were randomly allocated to 3 groups: a control diet or a diet with 0.25% (LQ) or 2.5% quercetin (HQ). After 4 weeks, dietary quercetin had no effects on uterine weight in OVX mice, but bone mineral density of the lumbar spine L4 and femur measured by peripheral quantitative computed tomography (pQCT) was higher in both the sham and the HQ groups than in the OVX group. Histomorphometric analysis showed that the HQ group restored bone volume (BV/TV) completely in distal femoral cancellous bone, but did not reduce the osteoclast surface area and osteoclast number when compared with the OVX group. In in-vitro experiments using mouse monocyte/macrophage cell line RAW264.7 cells, however, quercetin and its conjugate, quercetin-3-O-beta-D: -glucuronide dose-dependently inhibited the receptor activator of nuclear factor-kappa B ligand (RANKL)-induced osteoclast differentiation, and the RANKL-stimulated expression of

osteoclast related genes was also inhibited by quercetin. The luciferase reporter assay showed that quercetin did not appear to have estrogenic activity through estrogen receptors. These results suggest that dietary quercetin inhibits bone loss without effect on the uterus in OVX mice and does not act as a potent inhibitor of osteoclastogenesis or as a selective estrogen receptor modulator in vivo. [87]

Improves mitochondrial biogenesis and enhances exercise performance

We examined the effects of 7 days of quercetin feedings in mice on markers of mitochondrial biogenesis in skeletal muscle and brain, and on endurance exercise tolerance. Mice were randomly assigned to one of the following three treatment groups: placebo, 12.5 mg/kg quercetin, or 25 mg/kg quercetin. Mice underwent a treadmill performance run to fatigue or were placed in voluntary activity wheel cages, and their voluntary activity (distance, time, and peak speed) was recorded. Quercetin increased mRNA expression of PGC-1alpha and SIRT1 ($P < 0.05$), mtDNA ($P < 0.05$) and cytochrome c concentration ($P < 0.05$). These changes in markers of mitochondrial biogenesis were associated with an increase in both maximal endurance capacity ($P < 0.05$) and voluntary wheel-running activity ($P < 0.05$). These benefits of querectin on fitness without exercise training may have important implications for enhancement of athletic and military performance and may also extend to prevention and/or treatment of chronic diseases. [88]

Cognitive-Enhancing Effect of Quercetin in Parkinson's Disease Induced by 6-Hydroxydopamine

Oxidative stress has been reported to induce cognitive impairment in Parkinson's disease. This paper aimed to determine the effect of quercetin, a substance possessing antioxidant activity, on the cognitive function in a rat model of Parkinson's disease. Male Wistar rats, weighing 200–250 g, were orally given quercetin at doses of 100, 200, 300mg/kg BWonce daily for a period of 14 days before and 14 days after the unilateral lesion of right substantia nigra induced by 6-hydroxydopamine (6-OHDA). Their spatial memory was assessed at 7 and 14 days of treatment and neuron density was determined,malondialdehyde (MDA) level, the activity of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were evaluated at the end of the experiment. In addition, the activity of acetylcholinesterase (AChE) was also measured. It was found that all doses of quercetin enhanced spatial memory. Therefore, it is suggested that the cognitive-enhancing effect of quercetin occurs partly because of decreased oxidative damage resulting in increased neuron density.[101]

Anti-fibrotic effect of liposomal quercetin and inflammatory cytokines in pulmonary fibrosis.

It is widely accepted that inflammatory cells and cytokines play vital roles in the process of pulmonary fibrosis. The aim of this study was to evaluate the preventative effects of liposomal quercetin against bleomycin-induced pulmonary fibrosis in vivo. The underlying molecular mechanisms were also investigated. Bleomycin was injected intratracheally at a single dose of 5U/kg for pulmonary fibrosis induction. Liposomal quercetin was intravenously injected 1 day prior to bleomycin administration and continued to the end of the study (for 4weeks). Our results showed that liposomal quercetin diminished the increase of total cell counts and macrophage counts in bronchoalveolar lavage fluid. The neutrophil and lymphocyte counts were also significantly decreased both on day 7 and 14 after liposomal quercetin injection ($P<0.05$). The levels of TNF-alpha, IL-1beta, and IL-6 in bronchoalveolar lavage fluid at day 7 were strikingly reduced in liposomal quercetin treated group compared with bleomycin-induced group (TNF-alpha: 56.21+/-3.16pg/ml vs.79.85+/-6.91pg/ml; IL-1beta: 37.64+/-2.10pg/ml vs. 73.29+/-5.78pg/ml; IL-6: 88.52+/-5.96pg/ml vs. 128.56+/-8.72pg/ml; $P<0.05$). Moreover, the treatment with liposomal quercetin exerted approximately 35.8% reduction of the hydroxyproline content in contrast to the bleomycin-induced group ($P<0.05$). Histopathological assessment revealed that treatment with liposomal quercetin apparently lessened the lung fibrosis areas and collagen deposition accompanied with decreased expression of TGF-beta1. Thus, our results suggested that liposomal quercetin could attenuate the bleomycin-induced pulmonary fibrosis in vivo by the suppression of inflammatory cytokines.[91]

Reduces blood pressure and lipids

Regular consumption of flavonoids may reduce the risk for CVD. However, the effects of individual flavonoids, for example, quercetin, remain unclear. The present study was undertaken to examine the effects of quercetin supplementation on blood pressure, lipid metabolism, markers of oxidative stress, inflammation, and body composition in an at-risk population of ninety-three overweight or obese subjects aged 25-65 years with metabolic syndrome traits. Subjects were randomized to receive 150 mg quercetin/d

in a double-blinded, placebo-controlled cross-over trial with 6-week treatment periods separated by a 5-week washout period. Mean fasting plasma quercetin concentrations increased from 71 to 269 nmol/l ($P < 0.001$) during quercetin treatment. In contrast to placebo, quercetin decreased systolic blood pressure (SBP) by 2.6 mmHg ($P < 0.01$) in the entire study group, by 2.9 mmHg ($P < 0.01$) in the subgroup of hypertensive subjects and by 3.7 mmHg ($P < 0.001$) in the subgroup of younger adults aged 25-50 years. Quercetin decreased serum HDL-cholesterol concentrations ($P < 0.001$), while total cholesterol, TAG and the LDL:HDL-cholesterol and TAG:HDL-cholesterol ratios were unaltered. Quercetin significantly decreased plasma concentrations of atherogenic oxidised LDL, but did not affect TNF-alpha and C-reactive protein when compared with placebo. Quercetin supplementation had no effects on nutritional status. Blood parameters of liver and kidney function, haematology and serum electrolytes did not reveal any adverse effects of quercetin. In conclusion, quercetin reduced SBP and plasma oxidised LDL concentrations in overweight subjects with a high-CVD risk phenotype. Our findings provide further evidence that quercetin may provide protection against CVD.[92]

Quercetin protects against high glucose-induced damage in bone marrow-derived endothelial progenitor cells.

Endothelial progenitor cells (EPCs), a group of bone marrow-derived pro-angiogenic cells, contribute to vascular repair after damage. EPC dysfunction exists in diabetes and results in poor wound healing in diabetic patients with trauma or surgery. The aim of the present study was to determine the effect of quercetin, a natural flavonoid on high glucose-induced damage in EPCs. Treatment with high glucose (40 mM) decreased cell viability and migration, and increased oxidant stress, as was evidenced by the elevated levels of reactive oxygen species (ROS), malondialdehyde (MDA) and superoxide dismutase in bone marrow-derived EPCs. Moreover, high glucose reduced the levels of endothelial nitric oxide synthase (eNOS) phosphorylation, nitric oxide (NO) production and intracellular cyclic guanosine monophosphate (cGMP). Quercetin supplement protected against high glucose-induced impairment in cell viability, migration, oxidant stress, eNOS phosphorylation, NO production and cGMP levels. Quercetin also increased Sirt1 expression in EPCs. Inhibition of Sirt1 by a chemical antagonist sirtinol abolished the protective effect of quercetin on eNOS phosphorylation, NO production and cGMP levels following high glucose stress. To the best of our knowledge, the results provide the first evidence that quercetin protects against high glucose-induced damage by inducing Sirt1-dependent eNOS upregulation in EPCs, and suggest that quercetin is a promising therapeutic agent for diabetic patients undergoing surgery or other invasive procedures.[125]

Effective against high altitude cerebral edema.

The present study was undertaken to elucidate the intervention of quercetin against high altitude cerebral edema (HACE) using male Sprague Dawley rats as an animal model. This study was also programmed to compare and correlate the effect of both quercetin (flavonoid) and dexamethasone (steroid) against HACE. Six groups of animals were designed for this experiment, (I) normoxia, (II) hypoxia (25,000 ft, 24 h), (III) normoxia+quercetin (50 mg/kg body wt), (IV) normoxia+dexamethasone (4 mg/kg body wt), (V) hypoxia+quercetin (50 mg/kg body wt), (VI) hypoxia+dexamethasone (4 mg/kg body wt). Quercetin at 50 mg/kg body wt, orally 1h prior to hypoxia exposure, was considered as the optimum dose, due to a significant reduction in the level of brain water content and cerebral transvascular leakage ($P < 0.001$), as compared to control (24 h hypoxia). Dexamethasone was administered at 4 mg/kg body wt, orally, 1h prior to hypoxia exposure. Both drugs (quercetin and dexamethasone) could efficiently reduce the hypoxia-induced hematological changes. Quercetin was observed to be a more potent antioxidative and anti-inflammatory agent. It blocks nuclear factor kappa-beta (NFκB) more significantly ($P < 0.05$) than the dexamethasone-administered hypoxia-exposed rats. Histopathological findings demonstrate the absence of an edema and inflammation in the brain sections of quercetin-administered hypoxia-exposed rats. The present study reveals quercetin to be a potent drug against HACE, as it efficiently attenuates inflammation as well as cerebral edema formation without any side effects of steroid therapy (dexamethasone).[107]

Anti-fibrotic effect of liposomal quercetin and inflammatory cytokines in pulmonary fibrosis

The present study reports the possible role of oxidative stress and inflammation (role of nuclear factor, NFkB) in hypoxia-induced transvascular leakage in brain of rats. The rats were exposed to a simulated altitude of 25,000 ft for different durations: 0, 3, 6, 12, 24, and 48h. Brain water content, transvascular leakage, oxidative stress, and proinflammatory parameters were studied at different durations of hypoxic exposure. The results revealed that maximum increase in transvascular leakage in brain of rats was observed at 24h of hypoxic exposure (240.16 ± 1.95 relative fluorescence units (r.f.u)/g tissue) compared with control (100.58 ± 1.79 r.f.u/g tissue). There was a significant increase in reactive oxygen species (ROS) and lipid peroxidation (MDA), with concomitant reduction in antioxidants. Hypoxic exposure resulted in a significant increase in NFκB protein expression levels and in the DNA binding activity in the 24-h hypoxic exposure ($p<0.001$) compared with control. There was a significant increase in proinflammatory cytokines, with concomitant upregulation of cell adhesion molecules. Simultaneously, to rule out the fact that inflammation causes cerebral edema, the rats were pretreated with curcumin (100 mg/kg body weight) 1h prior to 24-h hypoxia. Curcumin pretreatment significantly attenuated the hypoxia-induced cerebral transvascular leakage ($p<0.05$), with concomitant downregulation in the expression of brain NFκB levels ($p<0.001$). The present study therefore reveals that inflammation (NFκB) plays a significant role in hypoxia-induced cerebral edema.[108]

Targeting oxidative stress attenuates trinitrobenzene sulphonic acid induced inflammatory bowel disease like symptoms in rats: Role of quercetin.

This study was aimed to investigate the beneficial effects of quercetin (QCT) against trinitrobenzene sulfonic acid (TNBS) induced clinical, morphological, and biochemical alterations in rats.

MATERIALS AND METHODS:
Colitis in rats was induced by administration of TNBS (25 mg dissolved in 0.25 ml of 30% ethanol) 8 cm into the rectum of the rat using a catheter. The animals were divided into six experimental groups (n = 6); naive (saline only without TNBS administration), control (saline + TNBS), standard (sulfasalazine 25 mg/kg + TNBS), QCT (25) (QCT 25 mg/kg + TNBS), QCT (50) (QCT 50 mg/kg + TNBS), QCT (100) (QCT 100 mg/kg + TNBS). Sulfasalazine (25 mg/kg) and QCT (25, 50 and 100 mg/kg) were administered per oral for 11 days and the colonic damage was evaluated in terms of macroscopical (body weight, stool consistency, rectal bleeding, and ulcer index) and biochemical parameters (myeloperoxidase activity, lipid peroxidation, nitrite, and glutathione).

RESULTS:
Treatment with QCT (50, 100 mg/kg) for 10 days following TNBS administration significantly attenuated the clinical, morphological, and biochemical alterations induced by TNBS, whereas it was found to be not effective at its lower dose (25 mg/kg) throughout the experimental protocol.

CONCLUSION:
QCT attenuates the clinical, morphological and biochemical alterations induced by TNBS possibly via its antioxidant mechanism.[119]

Long-term quercetin dietary enrichment decreases muscle injury in mdx mice.

BACKGROUND & AIMS:
Duchenne muscular dystrophy results from a mutation in the dystrophin gene, which leads to a dystrophin-deficiency. Dystrophic muscle is marked by progressive muscle injury and loss of muscle fibers. Activation of the PGC-1α pathway has been previously shown to decrease disease-related muscle damage. Oral administration of the flavonol, quercetin, appears to be an effective and safe method to activate the PGC-1α pathway. The aim of this investigation was to determine the extent to which long term dietary quercetin enrichment would decrease muscle injury in dystrophic skeletal muscle. We hypothesized that a quercetin enriched diet would rescue dystrophic muscle from further decline and increase utrophin abundance.

METHODS:
Beginning at three-months of age and continuing to nine-months of age mdx mice (n = 10/group) were assigned to either to mdx-control receiving standard chow or to mdx-quercetin receiving a 0.2% quercetin-enriched diet. At nine-months of age mice were sacrificed and costal diaphragms collected. One hemidiaphragm was used for histological analysis and the second hemidiaphragm was used to determine

gene expression via RT-qPCR.

RESULTS:
The diaphragm from the mdx-quercetin group had 24% ($p \leq 0.05$) more muscle fibers/area and 34% ($p \leq 0.05$) fewer centrally nucleated fibers compared to the mdx-control group. Further, there were 44% ($p \leq 0.05$) fewer infiltrating immune cells/area, a corresponding 31% ($p \leq 0.05$) reduction in TNF gene expression, and a near 50% reduction in fibrosis. The quercetin-enriched diet increased expression of genes associated with oxidative metabolism but did not increase utrophin protein abundance.

CONCLUSIONS:
Long-term quercetin supplementation decreased disease-related muscle injury in dystrophic skeletal muscle; however the role of PGC-1α pathway activation as a mediator of this response is unclear.[120]

Dosage

There are lots of quercetin products of the market, but they won't do much good if the quercetin is not activated for use by the body. We sell Source Naturals because it combines its quercetin with bromelain and a non-acidic form of vitamin C. Bromelain is an enzyme derived from pineapple that is known to increase the body's ability to absorb various substances. Studies suggest that vitamin C has a synergistic relationship with Quercetin, which improves quercetin's use by the body.

Taking quercetin with bromelain on an empty stomach thirty minutes before a meal results in a high degree of bioavailability. Also, taking it sublingually for acute allergy symptoms such as sinusitis and/or asthma. Because the bioavailability of quercetin is about 30% through the stomach wall, taking it sublingually is far-superior, and therefore far more effective results, especially in acute allergy symptoms, such as hay fever. [73, 74]

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