Multi-Drug Resistance

USING CHINESE BOTANICALS AND BOTANICAL ISOLATES IN CONJUNCTION WITH CHEMOTHERAPY TO OVERCOME MDR IN CANCER TREATMENT

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Introduction

WHAT IS MDR?

MDR

- MDR is defined as insensitivity of cancer cells to cytotoxic and cytostatic actions of a number of structurally and functionally unrelated drugs. Cancer cells are intrinsically resistant to anti-cancer agents because of genetic and epigenetic heterogeneity.
- Also, there are some host factors which include poor absorption, rapid metabolism and excretion that can result in low serum drug levels. The mechanisms include alteration in the expression of proteins involved in the apoptotic signalling such as p53, Bcl2 family of proteins.
- Drug efflux proteins (P-gp, MRPs) and metabolising enzyme (CYP450) are major factors in drug interactions. Overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic profiles of multidrug regimens.(Pal & Mitra, 2006).

MDR

- Multidrug-resistance (MDR) is the chief limitation to the success of chemotherapy. According to the National Cancer Institute, multidrug-resistance is a phenomenon where cancer cells adopt to anti-tumour drugs in such a way that makes the drugs less effective. Studies have shown that 40% of all human cancers develop MDR.
- Deaths due to cancer occur in most of the cases when the tumour metastasises. Chemotherapy is the only choice of treatment in metastatic cancer, and MDR limits that option.
- Cancer defends itself against chemotherapeutic regimes by several mechanisms including MDR. Therefore, a detailed understanding of ABC-(ATP-binding cassette) transporters mediated drug resistance would help to formulate strategies to overcome this problem.

MDR

- Chemotherapy kills drug-sensitive cells, but leaves behind a higher proportion of drug-resistant cells. As the tumour begins to grow again, chemotherapy may fail because the remaining tumour cells are now resistant.
- That cells have mechanisms to transport a variety of molecules out of the cytoplasm has been known for decades. For example, organic cation transporters were some of the earliest such mechanisms identified, and the kidney's secretory capability in this regard was first demonstrated in 1947.
- The presence of ATP-binding cassette, ABC-transport proteins in tumour cells circumvents an intracellular accumulation of chemotherapeutic drugs.

MDR prevention

- Recent investigations have shown that preventing the emergence of MDR at the onset of chemotherapy treatment, rather than reversing MDR once it has developed, may assist in overcoming drug resistance.
- Recent studies have demonstrated that several small-molecule inhibitors, including Pgp inhibitors, are capable at preventing the development of MDR when co-treated with cytotoxic drugs in different *in vitro* and *in vivo* model systems. Preventing or delaying the emergence of drug resistance is likely to enhance the effectiveness of chemotherapy and improve clinic outcomes for patients with cancer.

- A prevention strategy for circumventing drug resistance in cancer chemotherapy. Curr Pharm Des. 2001 Nov; 7(16):1595-614.
- Novel strategies to prevent the development of multidrug resistance (MDR) in cancer. Oncotarget. 2017 Oct 13; 8(48): 84559–84571. doi: 10.18632/oncotarget.19187

Gut Microbiome & Chemotherapy Metabolism

- The significance of the gut microbiota as a determinant of drug pharmacokinetics and accordingly therapeutic response is of increasing importance with the advent of modern medicines characterised by low solubility and/or permeability, or modified-release.
- These physicochemical properties and release kinetics prolong drug residence times within the gastrointestinal tract, wherein biotr
- Modulating the microbiome, either through exogenous replacement (probiotics) or curtailing interventions, such as antibiotics or specific inhibitors, affords exciting opportunities to improve healthcare outcomes and advance personalised medicine. Transformation by commensal microbes can occur.
- The Impact of the Gut Microbiota on Drug Metabolism and Clinical. Yale J Biol Med. 2016 Sep; 89(3): 375–382.

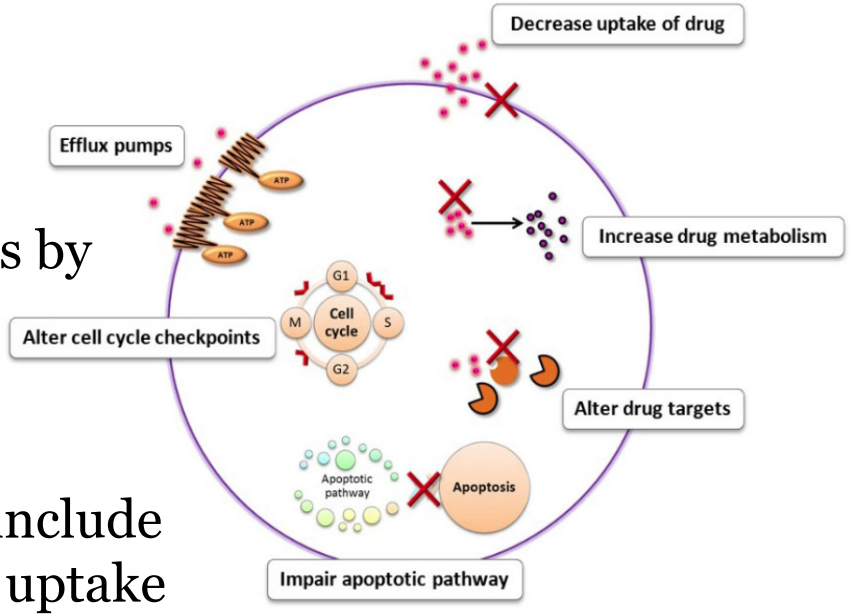
Chemotherapy-driven Dysbiosis

- Montassier et al., (2015) found that faecal samples collected after chemotherapy exhibited significant decreases in abundances of Firmicutes and Actinobacteria and significant increases in abundances of Proteobacteria compared to samples collected before chemotherapy.
- Following chemotherapy, patients had reduced capacity for nucleotide metabolism, energy metabolism, metabolism of co-factors and vitamins, and increased capacity for glycan metabolism, signal transduction and xenobiotics biodegradation.
- Chemotherapy-driven dysbiosis in the intestinal microbiome. AP&T. Volume42, Issue5.September 2015 Pages 515-528 https://doi.org/10.1111/apt.13302

Mechanisms of MDR towards cancer chemo-therapeutic drugs

The MDR cancer cells may subsequently develop cross-resistance to several unexposed and structurally unrelated chemotherapeutic agents

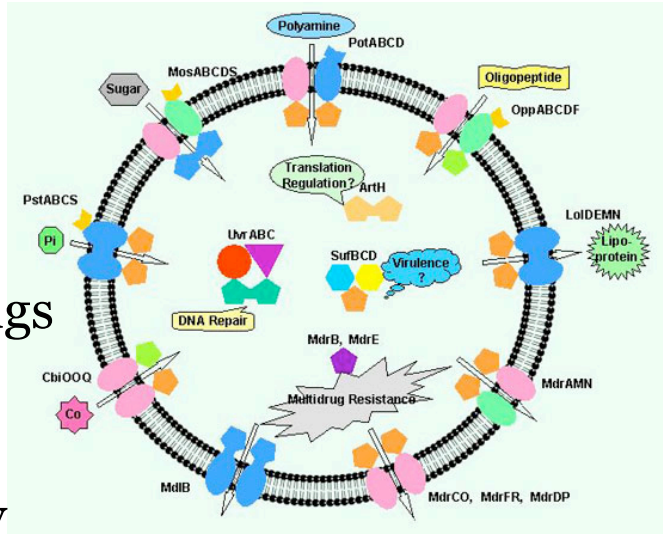
- Cancer cells can develop resistance to multiple drugs by various mechanisms as depicted.



- Mechanisms include (a) decreased uptake of drug, (b) reduced intracellular drug concentration by efflux pumps, (c) altered cell cycle checkpoints, (d) altered drug targets, (e) increased metabolism of drug and (f) induced emergency response genes to impair apoptotic pathway.

ATP-Binding Cassette (ABC)

ABC transporters are transmembrane proteins that utilise the energy of adenosine triphosphate (ATP) hydrolysis to carry out certain biological processes including translocation of various substrates across membranes and non-transport-related processes such as translation of RNA and DNA repair.



- When the ABC transporter proteins are overexpressed in cancer cells they can export anticancer drugs and render tumours resistant
- Nine ABCC subfamily members, the so-called Multidrug Resistance Proteins (MRPs) 1-9, have been implicated in mediating multidrug resistance in tumour cells.

MDR

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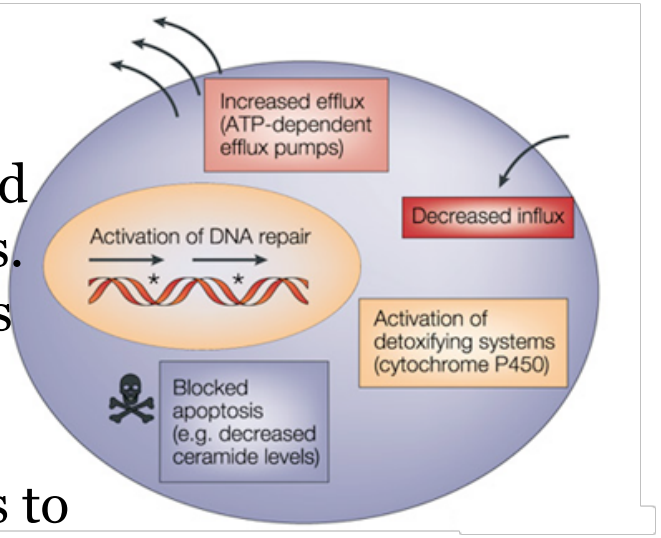
Studies have shown that 40% of all human cancers develop MDR.

- Tumour cells adopt several mechanisms to evade death induced by anti-tumour agents. These include changes in apoptotic pathways and activation of cell-cycle check points to increase DNA repair.



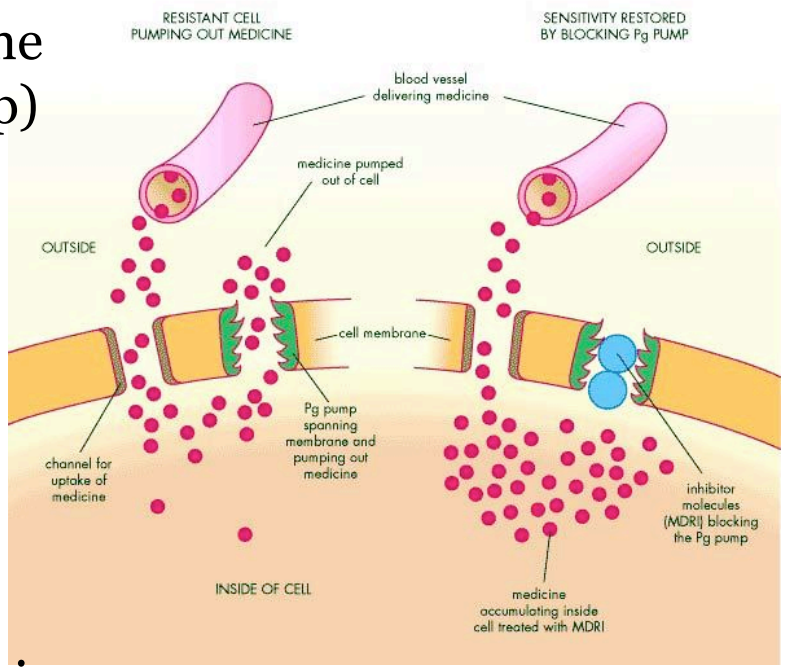
- Cancer cells develop resistance by increased expression of multidrug-resistant proteins and altered anti-tumour drug transport mechanisms.

MDR1
(Pgp)

P-gp plays an important role in altering the pharmacokinetics of a wide variety of drugs.

Tumours with detectable levels of P-gp are 3-4 fold more susceptible to chemotherapeutic failure than P-gp negative tumours. Therefore the role of P-gp in the development of MDR is very significant.

- Plasma membrane glycoprotein (Pgp) was the first ABC-transporter detected in various cancers exerting resistance to a variety of chemically unrelated cytotoxic agents including anti-tumour drugs such as doxorubicin, vinblastine, ritonavir, indinavir and paclitaxel. It works as an energy-dependent efflux pump and can recognise a wide range of substrates.

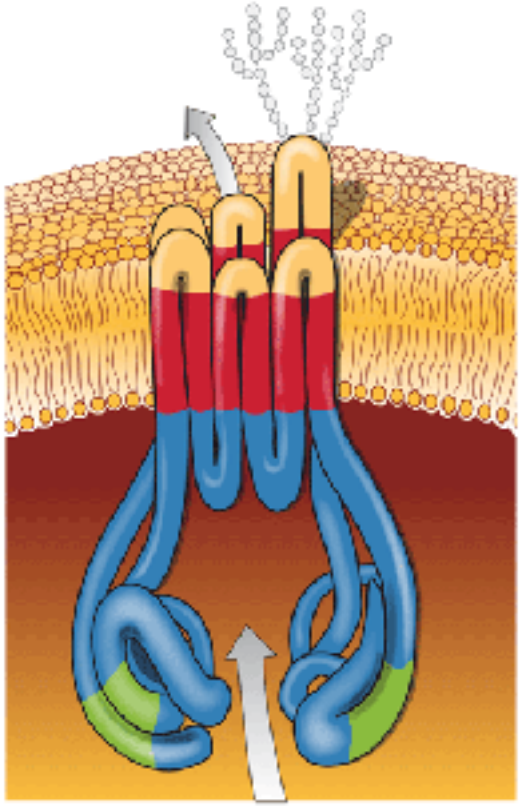


Pgp Efflux

The Pgp is a 170 kDa intrinsic membrane protein that effluxes a wide range of drugs from the cell.

The membrane lipid bilayer plays and important role in Pgp function and may regulate both binding and transport of drugs.

- Pgp expression has been linked to the efflux of chemotherapeutic drugs in human cancers leading to multidrug resistance.
- Pgp activity can also result in low oral absorption and poor brain penetration.
- Interaction of drugs with the Pgp may also cause an increase in toxicity of co-administered compounds.



Multidrug Resistance Protein 1 (MRP1)

Like Pgp, MRP1 is also overexpressed in tumour cells and represents a major obstacle to drug delivery.

- High levels of expression of MRP1-7 proteins were observed in non-small-cell lung cancer. In breast cancer, there is also a significant expression of this protein which may increase the chance of treatment failure.
- Studies have also shown that over expression of MRP causes resistance to methotrexate (MTX), also known as BCRP, ABCP and ABCG2 and anti-foliates such as ZD1694 in colorectal cancer.

Breast Cancer Resistance Protein (BCRP)

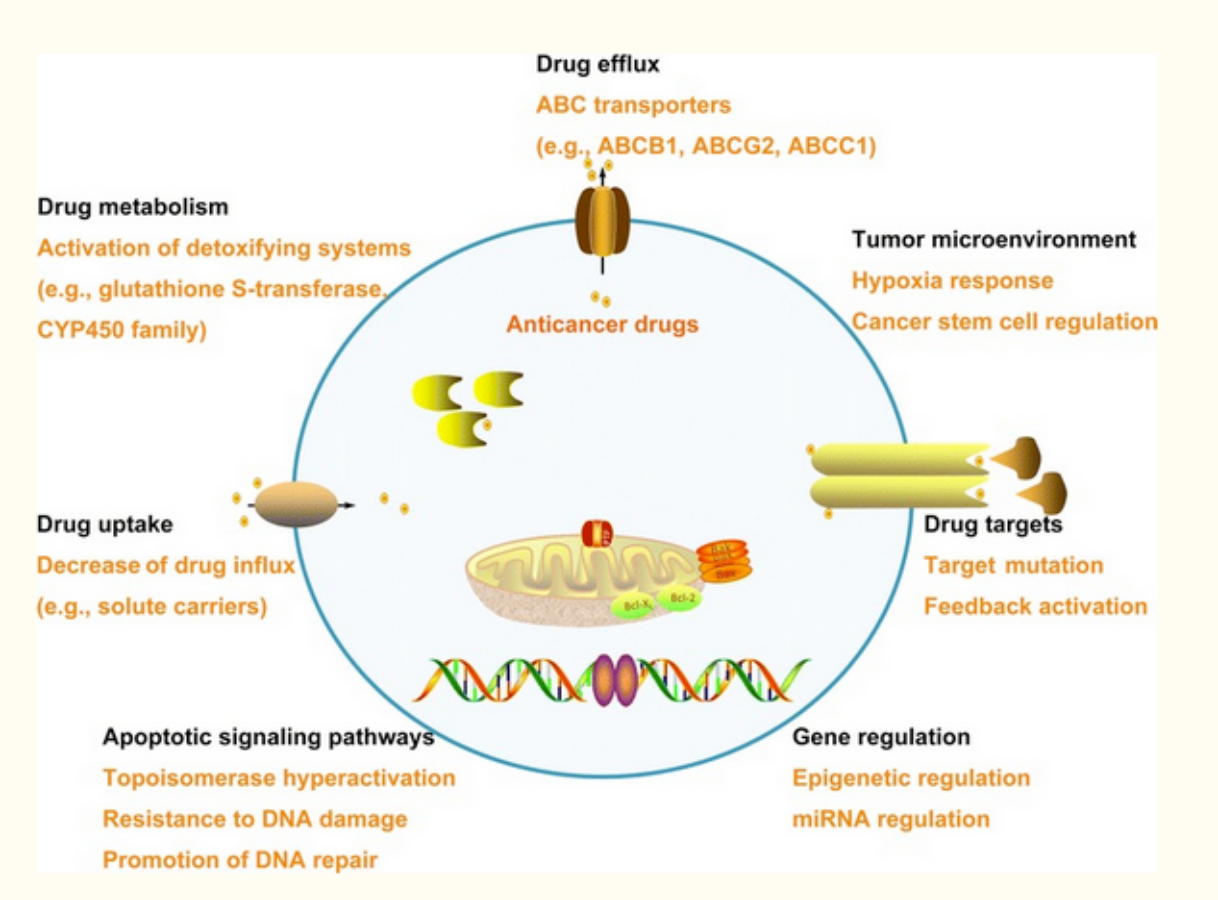
Overexpression of BCRP was reported in the plasma membrane of drug-resistant ovarian, breast, colon, gastric cancer, and fibrosarcoma cell lines.

- Since BCRP is expressed in the gastrointestinal tract, it is thought that this protein may affect the bioavailability of the drugs. Its overexpression in several types of cancer makes it a relevant target of strategies aimed at defeating multidrug-resistance.
- BCRP belongs to a novel branch of the ABC-transporter family. The members of this subfamily are about half the size of the full-length ABC transporters, thus known as half-transporters.

The main mechanism of MDR is overexpressing ATP-binding cassette (ABC) transporters to increase drug efflux, resulting in a decrease in intracellular drug concentration.

Other mechanisms of MDR are reducing drug uptake by influx transporters, boosting drug metabolism, blocking apoptotic signalling pathways, elevating adaptability by epigenetic regulation and microRNA regulation, mutation in drug targets or feedback activation of other targets and signalling pathways, and change of tumour microenvironment.

ABCB1, ATP-binding cassette subfamily B member 1; ABCG2, ATP-binding cassette subfamily G member 2; ABCC1, ATP-binding cassette subfamily C member 1; CYP450, cytochrome P450



Li et al., 2017

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Botanicals & MDR

HERBS AND ISOLATES IN MDR

Chinese Botanicals and Isolates

- Chinese botanicals and isolates (CBIs) consist of a large number of components and have been safely used in humans for thousands of years, they could serve as a natural chemical pool for screening of MDR modulators. A considerable portion of anti-cancer agents currently used in the clinic were isolated from medicinal plants.
- Certain CBIs not only have anticancer properties, but may also alter the expression or function of drug transporters. The combined use of CBIs with conventional chemotherapeutic drugs may increase the efficacy and reduce the side effects (Wang Zj et al, 2010).

Chinese Botanicals and Isolates

- P-gp is vulnerable to inhibition, activation or induction by many compounds as well as CBIs (Breier et al, 2005).
- Modulation of P-gp activity with selective inhibitors could also be a useful strategy to increase the oral bioavailability of P-gp substrate drugs, in particular, to develop oral formulations of anticancer drugs transported by P-gp .

Multi-Drug Resistance (MDR)

- Ginsenosides
  - Rg3 reversed multi-drug resistance and restored the sensitivity of resistant KBV20 cell line to various anticancer drugs. Increased animal life span in an in vivo MDR model in a dose-independent manner (Kim SH et al, 2003; Yue et al, 2006).
  - Combination of purified saponins containing Rb1, Rb2, Rc, Rd, Re and Rg1 reversed MDR (Liu et al, 2008; Si & Tien, 2005)
- Quercetin/Kaempferol
  - Quercetin is less potent than kaempferol but more effective than genistein and daidzein in reversing MDR (Kioka et al, 1992).
  - Quercetin reverses MDR through action on mitochondrial membrane potential and the induction of apoptosis (Kothan et al, 2004)
- Puerarin
  - Down-regulates MDR1 expression via nuclear factor κ-B and CRE (cAMP response element) transcriptional activity (Hien et al, 2010)

Plasma Membrane Glycoprotein (Pgp)

- Curcumin
  - Enhances sensitivity to vincristine by the inhibition of P-gp in SGC7901/VCR cell line (Chang et al, 2006).
  - Curcumin derivatives reversed MDR by inhibiting P-gp efflux (Tang et al, 2005)
  - Bisdemethoxycurcumin modified from curcumin resulted in greater inhibition of P-gp expression (Um et al, 2008).
  - Curcumin derivatives reversed MDR by inhibiting P-gp efflux (Limtrakul et al, 2004).
- Honokiol
  - Down-regulates expression of P-glycoprotein at mRNA and protein levels in MCF-7/ADR, a human breast MDR cancer cell line (Xu et al, 2006)
- Quercetin
  - Inhibits overexpression of P-gp mediated by arsenite (Kioka et al, 1992).

P-glycoprotein

- Pheophorbide a (Pa) from Scutellaria barbata could significantly inhibit the growth of R-HepG2 cells with an IC50 value at 25.0 microM after 48 hours treatment (Tang et al, 2007).
- Scutellaria barbata and low dose 5-FU can significantly inhibit the tumour growth both in vitro and in vivo (Xu et al, 2013).
- Scutellaria barbata (SB) elevated expression of Bax, p53, Akt, and JNK by up-regulating the apoptotic pathway and down-regulating the survival pathway in prostate cancer cells (Wong et al, 2009)

P-glycoprotein

- Steroidal saponin from *Trillium tschonoskii* (TTS) could reverse the MDR in HCC cells and significantly enhance chemosensitisation. TTS inhibited HepG2 and R-HepG2 cells survival in a dose-dependent manner by 75% and 76%, respectively ($p<0.01$), as well as colony formation 77% and 81% ($p<0.01$).
- TTS also repressed expression of many other MDR genes, such as MRP1, MRP2, MRP3, MRP5, MVP and GST-π. In vivo, TTS dose-dependently reduced R-HepG2 cells xenografts tumour formation by inhibiting tumour cells proliferation (Wang et al, 2013)

P-glycoprotein

- Kampo extract medicines, Senkyu-cha-cho-san (SCCS) and Sokei-kakketsu-to (SKKT), effected intestinal absorption of P-glycoprotein (P-gp) in vivo.
- Concomitant administration of each Kampo extract medicine unexpectedly showed the tendency to decrease Cmax and AUC of talinolol. Decreased intestinal absorption of talinolol might be caused, not by the inhibition of P-gp, but by the inhibition of organic anion transporting peptides by both Kampo extract medicines (Iwanaga et al, 2012).

Formulas

- SCCS: Chuan Xiong Cha Tiao San
  - Menthae Haplocalycis (bo he)
  - Ligusticum chuanxiong (chuan xiong)
  - Angelicae Dahuricae (bai zhi)
  - Notopterygium incisum (qiang huo)
  - Asarum heterothropoides (xi xin)
  - Cyperus Rotundi carbonized (chao xiang fu)
  - Schizonepeta tenuifolia (jing jie)
  - Ledebouriellae Divaricatae (fang feng)
  - Glycyrrhizae Uralensis Preparata (zhi gan cao)
  - Cameliae (lu cha)
- SKKT: Shu Jing Huo Xue Tang
  - Paeoniae Alba (bai shao)
  - Angelicae Sinensis (dang gui)
  - Ligusticum chuanxiong (chuan xiong)
  - Rehmanniae Glutinosae (sheng di)
  - Pruni Persicae (tao ren)
  - Atractylodes lancea (cang zhu)
  - Poriae Cocos (fu ling)
  - Achyranthis Bidentatae (niu xi)
  - Clematis chinensis (wei ling xian)
  - Stephaniae Tetrandrae (han fang ji)
  - Notopterygium incisum (qiang huo)
  - Ledebouriellae Divaricatae (fang feng)
  - Gentianae Longdancao (long dan cao)
  - Angelicae Dahuricae (bai zhi)
  - Citri Reticulatae (chen pi)
  - Glycyrrhizae Uralensis (gan cao)
  - Zingiberis Recens (sheng jiang)

Diosgenin

- Diosgenin is a naturally occurring steroidal sapogenin and is one of the major bioactive compounds found in Trigonella foenum-graecum seeds. In addition to being a lactation aid, diosgenin has been shown to be hypocholesterolaemic, gastro-and hepato-protective, anti-oxidant, anti-inflammatory, anti-diabetic, and anti-cancer. Diosgenin has a unique structural similarity to oestrogen.
- Diosgenin has also been reported to reverse multi-drug resistance in cancer cells and sensitise cancer cells to standard chemotherapy. (Sethi, Shanmugam, Warrier et al., 2018

Diosgenin

- The anticancer mode of action of diosgenin has been demonstrated via modulation of multiple cell signalling events involving critical molecular candidates associated with growth, differentiation, apoptosis, and oncogenesis.
- Altogether, these preclinical and mechanistic findings strongly implicate the use of diosgenin as a novel, multitarget-based chemo-preventive or therapeutic agent against several cancer types. (Raju & Mehta 2009)

Diosgenin

- Akt signalling has gained recognition for its functional role in more aggressive, therapy-resistant malignancies and is frequently constitutively active in cancer cells. Diosgenin inhibits pAkt expression another functional downstream target of Akt, was inhibited by Diosgenin in ER(+) but not in ER(-) BCa cells.
- Additionally, we found that diosgenin caused G1 cell cycle arrest by downregulating cyclin D1, cdk-2 and cdk-4 expression in both ER(+) and ER(-) BCa cells resulting in the inhibition of cell proliferation and induction of apoptosis. and XIAP. The Raf/MEK/ERK pathway.
- Studies indicate diosgenin significantly inhibits tumour growth in both MCF-7 and MDA-231 xenografts in nude mice. (Srinivasan, Koduru, Kumar et al., 2009)

Breast Cancer Resistance Protein (BCRP)

- Flavonoids, a major class of natural compounds widely present in foods and herbal products, have been shown to be human BCRP inhibitors (Zhang S et al, 2005).
- The flavones Retusin (Origanum vulgare) and Ayanin (Croton schiedeanus) were found to be highly potent inhibitors of BCRP (Pick et al, 2011)
- Chrysin (Passiflora caerulea) and biochanin A (Trifolium pratense) were the most potent BCRP inhibitors, producing significant increases in mitoxantrone accumulation at concentrations of 0.5 or 1.0μM and in mitoxantrone cytotoxicity at a concentration of 2.5μM (Zhang S et al, 2004; Wang & Morris, 2007).

Apoptosis pathways in cancer

All: Cancer Letters. Volume 332, Issue 2 May 2013

- Emodin and Rhein from *Rheum* (Dahuang) and *Polygonum cuspidatum* (Huzhang) produce ROS and ROS-induced apoptosis.
- Artemisinin from *Artemisia annua* (Qinghao) been shown to kill tumour cells through a ROS-dependent mechanism.
- Berberine from *Coptis chinensis* (Huanglian) induces ROS-mediated apoptosis.
- The flavones Chrysin and Apigenin from *Scutellaria Baicalensis* (Huangqin) potentiate the cytotoxicity of anti-cancer drugs by depleting cellular GSH.
- Wogonin from *Scutellaria Baicalensis* (Huangqin) can sensitise TNFα-resistant tumour cells to undergo TNFα-induced apoptosis.

Apoptosis pathways in cancer

- Evodiamine, a constituent from Chinese herb Evodiae fructus (Wuzhuyu) promoted the phosphorylations of Raf-1 kinase and Bcl-2 and was found to be superior to that of paclitaxel (Carcinogenesis 2005; 26:968-75).
- Kanglaite (KLT) injection is an anti-tumour new drug which extracts from Chinese medicine-*coix seed* and could inhibit some anti-apoptotic gene and activate some pro-apoptotic genes. KLT may induce the apoptosis of tumour cell by way of up-regulate the expression of p53 genes (Lu et al, 2008).
- Several studies show that phytochemicals, such as curcumin, EGCG, genistein, quercetin, and reseveratrol, can reverse chemo-resistance to cisplatin and/or paclitaxel by modulating NF-κB or/and MDR-transporter proteins both in vitro and in vivo (Surh, 2003; Aggarwal et al, 2004; Weir et al, 2007).

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Chinese Botanical Studies

HERBS, ISOLATES AND COMPOUNDS

Chinese Medicinals and MDR

- Efferth et al. (2002) investigated the activity of 22 drugs from traditional Chinese medicine toward sensitive and MDR1- or MRP1-overexpressing multidrug-resistant human CCRF-CEM leukaemia cells.
- These herbal drugs included artesunate, artemisinin, baicalein, baicalin, berberine, bufalin, cantharidin, cephalotaxine, curcumin, daidzein, daidzin, diallyl disulfide, ginsenoside Rh2, glycyrrhizic acid, isonardosinon, homoharringtonine, nardosinon, nardofuran, puerarin, quercetin, tannic acid, and tetrahydronardosinon.
- TCM-derived compounds can modulate multidrug resistance. Artesunate and bufalin revealed both high anti-leukaemic activity if applied alone as well as modulation effects in combination with daunorubicin. Homoharringtonine, artesunate, and bufalin have potent anticancer activity. The latter two also increased the accumulation of daunorubicin in the multidrug resistant cells.

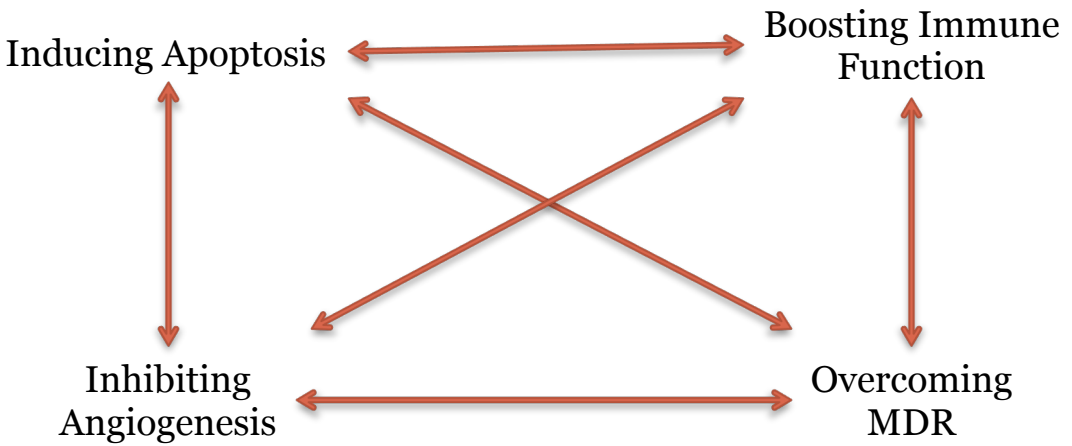
Chinese Medicinals and MDR

- Fractions from the PB group (herbs with the ability to promote blood circulation and remove blood stasis) showed more significant effects than fractions from the CH group (herbs with the ability to clear away heat and toxic materials). Fractions from dichloromethane (CH_2Cl_2) extracts were more effective than fractions from ethyl acetate (EtOAc) extracts (Yang L et al, 2011).
- Aqueous extracts of 12 Chinese medicinal herbs, Anemarrhena asphodeloides, Artemisia argyi, Commiphora myrrha, Duchesnea indica, Gleditsia sinensis, Ligustrum lucidum, Rheum palmatum, Rubia cordifolia, Salvia chinensis, Scutellaria barbata, Uncaria rhychophylla and Vaccaria segetalis demonstrated growth inhibitory activity on some or all of the cancer cell lines, but only two showed activity against the normal mammary epithelial cells (Shoemaker et al, 2005).

Traditional Chinese Medicine in Cancer Therapy

There is a molecular basis for the reported synergies of TCM herbs when administered alongside so-called 'conventional therapies' in tumour cell regulation, which help in bringing about homeostasis (Parekh et al, 2009).

- By identifying potent bio-actives derived from TCM, and tailoring formulations that encapsulate/ incorporate them into cutting-edge drug delivery systems for parenteral administration, one can envision overcoming the shortfalls that have prevented TCM being accepted by the West as a real adjunct/alternative to conventional cancer therapies.
- With a library of over 250,000 individual therapeutic compounds at our disposal, many of which have yet to be successfully isolated and tested for both safety and efficacy, there are certainly challenges that lay ahead.



Sheng Mai Injection

- 'Shengmai Injection' (SMI), consisting of *Panax ginseng* (renshen) and *Ophiopogon japonicus* (maidong), down-regulated P-gp expression in peripheral blood lymphocyte membrane. When used together with oxaliplatin, 5-fluorouracil or folinic acid, the injection prolonged the survival rate of colon cancer patients (Kaye, 1999).
- The injection also enhanced the efficacy of tamoxifen and nifedipine in combination therapy (Wang & Yang, 2001).
- SMI might improve human immune function to facilitate the chemotherapy of patients with stomach cancer (Lin et al, 1995).
- SMI injection would not influence the efficacy of chemotherapy on advanced NSCLC patients, while it could improve the quality of life, increase the body weight of patients, alleviate adverse reactions of chemotherapy as myelosuppression so as to improve the tolerance of organism to chemotherapy (Cao et al, 2008).

Kanglaite Injection

- Kanglaite (KLT) was effective in reversing MDR of cells and increasing the sensitivity of cancer cells to chemotherapeutic agents by a quite small effective dosage (2-8μl/ml) which was far below its IC50 for K562/vcr cells (Yang H et al, n.d.).
- KLT has been proven to play its anticancer role through inhibition of the mitosis of tumour cells during G2/M phases, induction of apoptosis and inhibition of the formation of newly generated blood vessels (Li, 2001).
- Standard treatment course for KLT is 200 ml (2 bottles) per day via intravenous drip x 42 days (84 bottles). Clinical experiences in China and Russia suggest 2 treatment courses (Ruan et al, 2006).

Artemisinin, Artesunate and Di-hydroartemisinin

- A, A & D-h increased cytotoxicity of pirarubicin and doxorubicin in P-glycoprotein-overexpressing K562/adr cells, and in MRP1-overexpressing GLC4/adr cells (Reungpatthanaphong & Mankhetkorn , 2002).
- Artesunate all showed blockade of rhodamine (Rho)-123 transport and decreased basal-to-apical (B-A) P-gp-mediated DIG transport at concentrations of 100 μM and 1 mM (Oga et al, 2012).
- Artesunate induced apoptosis and reactive oxygen species in neuroblastoma cells that over-express P-glycoprotein (Michaelis et al, 2009). Downregulates expression of Survivin mRNA (Wang, 2010).
- More than 70 cell lines from different tumour types have been reported to be inhibited by artesunate and its related compound artemisinin (Efferth et al, 2001; Efferth et al, 2003).

Schisandra lignans extract (SLE)

- 10μM verapamil (a known P-gp inhibitor) and SLE (0.5, 2.0, and 10.0μg/ml) were observed to significantly enhance the uptake and inhibit the efflux ratio of P-gp substrates in Caco-2 and L-MDR1 cells.
- A single-dose SLE at 500mg/kg could increase the area under the plasma concentration time curve of digoxin and vincrisine significantly without affecting terminal elimination half-time.
- Long-term treatment with SLE for continuous 10 days could also increase the absorption of P-gp substrates with greatly down regulation of P-gp expression in rat intestinal and brain tissues (Liang et al, 2013).

Species (pin yin name)	Family	Active Components	In Vitro and In Vivo Activity	Refs
Fructus Schizandrae (wu wei zi)	Magnoliaceae	schizandrin A schizandrin B schizandrin C schizandrol A schizandrol B	25 μg/ml (KBv200, MCF-7/Dox, vincristine)	Huang M et al, 2008
Silybum marianum	Asteraceae	silymarin	Inhibited OATP1B1- and BCRP-mediated rosuvastatin (BCRP) transport	Deng et al, 2008
Isolate & Source	Grapevines, pine, soy and legumes	Resveratrol	30 μM (MCF7, mitoxantrone, bodipy-FL-prazosin)	Cooray et al, 2004
	Soy	Daidzein (8-hydroxydaidzein)	Decreased mRNA expression levels of MDR1 and MRP 1	Lo, 2012
	Citrus fruits	Hesperetin & Naringin	Significant inhibition of OATP2B1	Shirasaka et al, 2013
	Soy	Genistein	3 μM-10 μM (K562/BCRP, SN-38 and mitoxantrone)	Imai et al, 2004

Species (pin yin name)	Family	Active Components	In Vitro and In Vivo Activity	Refs
Curcuma wenyujin (yu jin)	Zingiberaceae	Sesquiterpenes Diterpenoids	Markedly increased doxorubicin accumulation in MCF-7/ADR cells.	Yang L et al (1), 2011
Chrysanthemum indicum (ye huang ju)	Asteraceae	Pyrethrins	Sensitised resistant cancer cells at a non-toxic concentration (10 μg mL^{-1})	
Salvia chinensis (dan shen)	Lamiaceae	Sesquiterpenes	Enhanced apoptosis induced by doxorubicin in MCF-7/ADR cells, and restore the effect of docetaxel on the induction of G2/M arrest in A549/Taxol cells	Yang L et al (2), 2011
Ligusticum chuanxiong (chuan xiong)	Apiaceae	Ligustrazine Tetramethylpyrazine		
Cassia tora (jue ming zi)	Fabaceae	Cinnamaldehyde	Salvia reduced microvessel density (MVD)	Liu F et al, 2012
Glycyrrhiza glabra (gan cao)	Leguminosae	Glycyrrhetinic acid	100 μM(KB/MRP, doxorubicin) Targets both the proteasome and peroxisome proliferator-activated receptors (PPARs)	Nabekura et al, 2008; Lallemand et al, 2011

Source	Isolate	In Vitro and In Vivo Activity	Refs
Citrus , tomato	Naringenin	3 μM-10 μM (K562/BCRP, SN-38 and mitoxantrone)	Imai et al, 2004
Citrus	Tangeretin	Inhibited P-glycoprotein and increased doxorubicin accumulation	Wesołowska et al, 2012
Scutellaria polyodon, Cirsium rhinoceros	Acacetin	1 -3 μM (K562/BCRP, SN-38 and mitoxantrone)	Imai et al, 2004; Shim et al, 2007
Ginger	Kaempferol (Zingiber zerumbet)	2 -3 μM (K562/BCRP, SN-38 and mitoxantrone).	Imai et al, 2004
Ginger	Kaempferol (Zingiber zerumbet)	Showed a potent P-gp inhibitory effect	Chung et al, 2007
Grapes, onions, tea	Myricetin	25 μM (MDCKII-MRP1, vincristine) Inhibited MRP1 by perturbation of the lipid phase of membranes.	van Zanden et al, 2005; Zhang Sz et al, 2004; Wesołowska et al, 2009

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Botanicals and Chemotherapy

ADVANTAGES, LIMITS AND CONCERNS

Complementary Oncology

- Scientifically-based therapies of complementary medicine cannot replace the well studied conventional cancer-destructive therapies such as surgery, chemo-, radio- or hormone therapy. Accordingly, they are by no means "alternative therapies".
- Complementary approaches in oncology that are recommended as additional to standard cancer destructive therapies claim to optimise this therapy. A great body of data emerging from scientifically sound clinical trials prove that defined complementary procedures are beneficial for the patients (Beuth & Schierholz, 2007).

Drug/Herb Interaction

- P-glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4) together constitute a highly efficient barrier for many orally absorbed drugs. Available literature and clinical reports indicate that many drugs and herbal active constituents are substrates for both P-gp and CYP3A4.
- Exposure with pure herbal agents such as hypericin, kaempferol and quercetin or extract of SJW resulted in higher uptake or influx of ritonavir and erythromycin. Hypericin, kaempferol and quercetin also caused a remarkable inhibition of cortisol metabolism. (Pal & Mitra, 2006).
- Multiple doses of baicalin decreased the expression of hepatic CYP3A2 by approximately 58% (Tian et al, 2013)

Drug/Herb Interactions

- Inhibition or induction of P-gp by co-administered drugs or food as well as herbal constituents may result in pharmacokinetic interactions leading to unexpected toxicities or under-treatment. On the other hand, modulation of P-gp expression and/or activity may be a useful strategy to improve the pharmacological profile of anticancer P-gp substrate drugs.
- Several variants in the ABCB1 coding regions result in amino acid change and potentially affect P-gp expression and activity. Hoffmeyer et al (2000) reported an association among a SNP in exon 26 (C3435T) of ABCB1, reduction in duodenal P-gp levels, and higher peak plasma concentrations of the P-gp substrate digoxin in healthy volunteers.
- Also, genetic variability in the MDR1 gene affects absorption and tissue distribution of P-gp substrate drugs (Marchetti et al, 2007).

Drug/Herb Interactions

- The prevalence of CAM use among cancer patients receiving conventional therapy is 54%– 77%, and that about 72% of patients do not inform their treating physician. CAM use significantly increases the risk for interactions with anticancer drugs, especially because of the small therapeutic range and steep dose–toxicity curve of these drugs (Lee et al, 2012).
- CAM use substantially increases the risk for interactions with anticancer drugs, especially because of the narrow therapeutic window of these compounds. However, for most CAMs, it is unknown whether they affect metabolising enzymes and/or drug transporter activity (Marchetti et al, 2007).
- It is important to note that various methods have been used to conduct in vivo and in vitro assessments of herb-drug interactions (Zhou et al, 2003). Yet, many of these studies were conducted in animals but not in humans (Lee et al, 2012).

CYP 3A4

- P-glycoprotein (P-gp), multiple drug resistance associated proteins (MRPs), and cytochrome P450 3A4 together constitute a highly efficient barrier for many orally absorbed drugs.
- Drug efflux proteins (P-gp, MRPs) and metabolising enzyme (CYP450) are major factors in drug interactions. Overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic profiles of multidrug regimens (Pal & Mitra, 2006).

COP & BER

- Coptis extract (COP) and its major constituent, Berberine (BER) increase ROS production, reduce MDR, and enhance the inhibitory effects of chemotherapeutic agents on A549 cell growth.
- Combinations of COP or BER with chemotherapeutic agents (5-FU, CPT, and TAX) exhibited a stronger inhibitory effect on A549 cell growth (He et al, 2012).
- Repeated administration of Berberine (300 mg, t.i.d., p.o.) decreased CYP2D6, 2C9, and CYP3A4 activities (Guo et al, 2011). CYP2D6 was inhibited by tetrahydropalmatine (THP) from Corydalis genus or Stephania rotunda and BER (Zhao Y et al, 2012).

COP & BER

- BER caused CYP3A4 and P-glycoprotein inhibition in the liver and gut wall, respectively, and because of an increase in gastric-emptying time causing increased cyclosporine A bioavailability and reduced metabolism (Cicero & Ertek, 2009).
- Cyclosporin A, a circumventor of MDR, markedly decreases glucosylceramide levels (Lavie et al, 1997). Glucosylceramide has clinical significance for the early identification of drug-resistant tumours (Lucci et al, 1998).
- Levels of glucosylceramide synthase mRNA, glucosylceramide synthase protein, and P-glycoprotein (P-gp) increased in parallel (Gouazé et al, 2004).

Tetrandrine

- Tetrandrine (TET) reversed MDR in vitro and modulated Pgp-mediated drug efflux (Tian & Pan, 1997; Choi et al, 1998). A combination of tetrandrine with doxorubicin or vincristine in vitro demonstrated synergistic anticancer effects (Sun et al, 1999). Tetrandrine reduced P-gp expression (Li et al, 2006).
- TET at the tested dose of combination treatment could achieve plasma concentrations that reversed MDR in experimental models and it had no apparent effect on doxorubicin pharmacokinetics in mice and CYP 3A4 activity in human liver microsomes (Dai et al, 2007).

Tetrandrine

- Co-administration of TET restores the sensitivity of MDR cancer cells to doxorubicin, paclitaxel, docetaxel, and vincristine (Fu LW et al, 2002; Zhu X et al, 2005) through the inhibition of P-gp.
- In mice with MDR MCF-7/adr or KBv200 cell xenografts, co-administration of TET increases the anticancer activity of doxorubicin and vincristine without a significant increase in toxicity (Fu L et al, 2004).
- **Cytotoxicity:** Dogs administered tetrandrine at low dose (3 mg/kg) and medium dose (10 mg/kg) levels showed no toxic changes. Administration of the drug at a high dose level (40 mg/kg) for 2 months may induce focal necrosis of liver cells, abnormal liver function, and acceleration of erythrocyte sedimentation rate. After the dogs had received tetrandrine for 6 months continuously, necrosis of liver tissue appeared (Tainlin et al, 1982).

Herb	Anti-Cancer Effect	Reference	CYP 450 Enzyme	Reference
Salvia miltiorrhiza (dan shen)	Diminution of cell proliferation. Neo-tanshinlactone is 10-fold more potent than tamoxifen citrate	Lee CY et al, 2008 Wang et al, 2004.	Inhibits CYP 1A2, 2C9, 2D6 and 3A4.	Chan, 2001; Qui et al, 2008
Panax ginseng (ren shen)	Ginsenoside Rp1 inhibits insulin-like growth factor 1 receptor (IGF-1R)/Akt pathway and cancer cell proliferation.	Kang et al, 2011	Inhibits CYP3A4, 2C9, 2C19, 2D6, 2E1	Henderson et al, 1999; Foster et al, 2002
Angelica sinensis (dang gui)	Acetone extract could induce G1/S arrest and activate the mechanism of apoptosis in human cancer cells	Cheng YL et al, 2004	Inhibits CYP 1A2, 2C9, 2D6, 2E1 and 3A	Lo et al , 1995; Sevior et al, 2010
Rheum officinale (da huang)	Emodin reverses multi-drug resistance in MCF-7/Adr cells and down-regulates ERCC1 protein expression. Suppressed the tumour growth derived from Side population (SP) cells through down-regulating ABCG2 expression. Down-regulates MRP1	Fu JM et al, 2012; Li XX et al, 2013	Inhibits CYP1A1, CYP1B1, CYP2E1	Sun et al, 2000; Shimpo et al, 2003; Mahadevan et al, 2007

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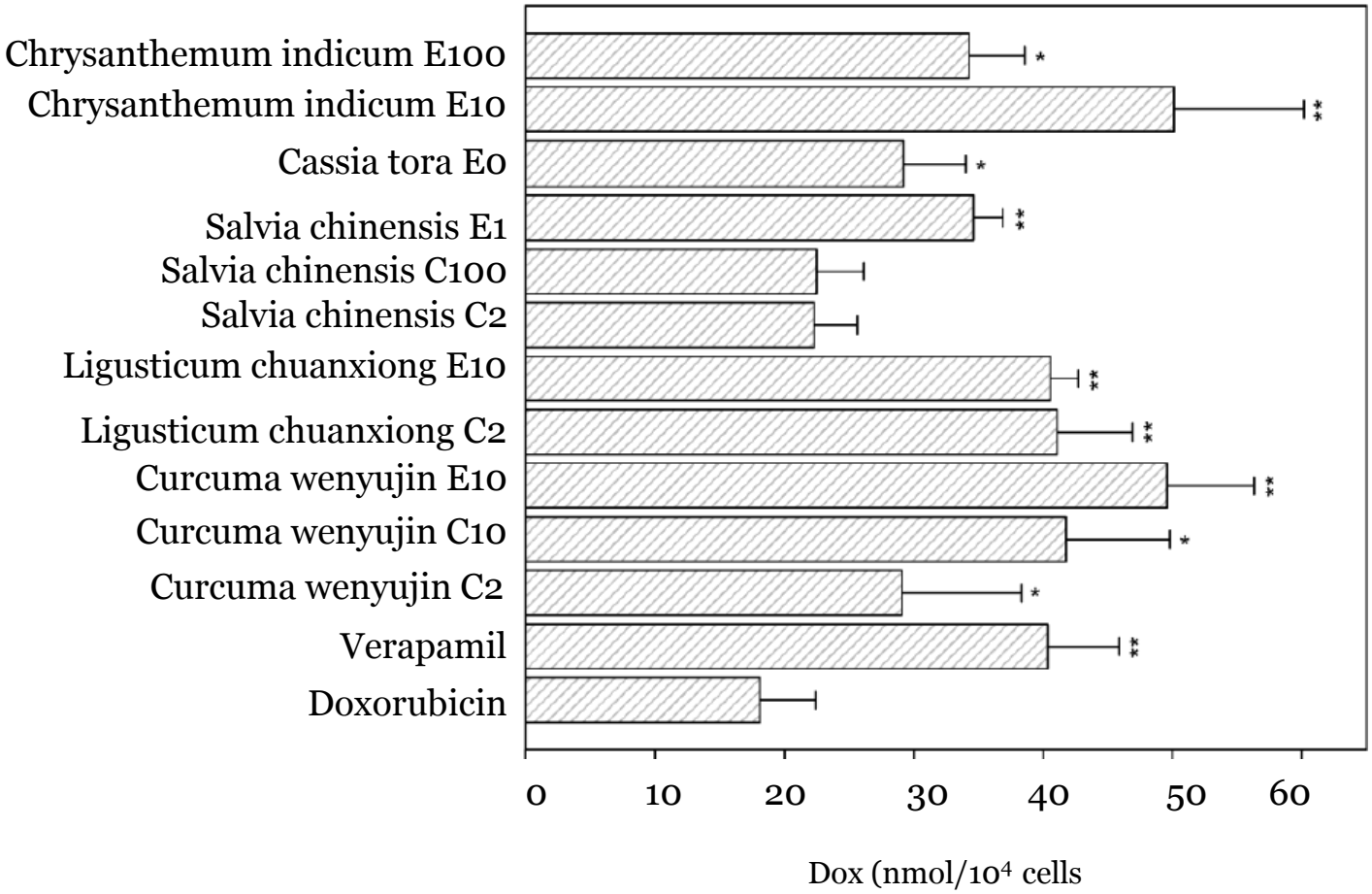
Additional Studies

PROTOCOLS

Effects of fractions on doxorubicin (Dox) accumulation in MCF-7/ADR cells.



Notes: Bars represent means ± standard deviations (SD) of triplicate determinations; ** and * represent $p < 0.01$ and 0.05, respectively, compared with the control group (doxorubicin) in the MCF-7/ADR cells.

Notes

- The dried and powdered herbal materials (100 g) were extracted with 95% EtOH (500 mL) for 3 h thrice at 80°C. The EtOH extracts were concentrated under reduced pressure, suspended in 200 mL water, and then partitioned exhaustively with equal volume of CH2Cl2 and EtOAc successively.
- The CH2Cl2 extracts were chromatographed respectively over a silica gel column with gradient elution by MeOH/CH2Cl2 (0:100, 1:99, 2:98, 5:95, 10:90, 20:80, 100:0, each four column volume) to yield 7 fractions (C0, C1, C2, C5, C10, C20, C100); the EtOAc extracts were followed in the same treatment to obtain another 7 fractions (E0, E1, E2, E5, E10, E20, E100). Each herb yielded 14 fractions, denoted by species name combined with fraction number.
- All fractions were dried and dissolved in DMSO to a final concentration of 40 mg mL–1.

Notes

- Cells were incubated with fractions for 48 h. IC50 and IC10 values (μg mL–1) in MTT test experiment were expressed as means ± standard deviations of three experiments.
- Reversing fold in MDR reversal activity experiment is the IC50 ratio of doxorubicin alone to doxorubicin with sample in MCF-7/ADR cells. (The IC50 of doxorubicin alone for MCF-7/ADR cells and MCF-7 cells used here were 1.14 and 0.13 μg mL–1, respectively.
- The IC50 of doxorubicin in the presence of 5 μg mL-1 verapamil for the MCF-7/ADR cells and the MCF-7 cells were 0.14 and 0.18 μg mL–1, respectively.)

Source: Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, People's Republic of China; School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, People's Republic of China (2010)

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Protocols

- Fu LW, et al (2001) MTT cell proliferation assay in combination with other in vitro drug evaluation assays to screen potential MDR modifiers from a series of naturally occurring bis-benzylisoquinoline alkaloids (BBIs) that were isolated from natural plants. These include berbamine, oxyacanthine, tetrandrine, guattegaumerine and constitute a series of almost 400 phenylalanine-derived metabolites with a rich and varied chemistry and pharmacology.
- In Vitro screening showed potent activities to restore sensitivity of resistant tumour cells, such as MCF-7/adr and KBv200 cells, to many antitumour drugs including doxorubicin and vincristine.
- Measurement of radioactive [3H]-Vincristine indicated that these BBIs increased intracellular drug accumulation in MDR cells, but had little effect on drug-sensitive cells.

Protocols

- The mechanism of these compounds to reverse MDR was associated with the increase in the intracellular drug accumulation through inhibiting the activity of P-gp. Another important feature is that the in vitro cytotoxic effect of these naturally occurring BBIs themselves on tumour cells was very low.
- Tetrandrine potentiated the cytotoxicity of Dox; a 20.4-fold reversal of resistance was achieved in the presence of 2.5 μmol/l of TET. Accumulation and efflux studies with the P-gp substrates, Dox and Fura-2, demonstrated that TET inhibited the P-gp-mediated drug efflux. In addition, TET lowered cell membrane fluidity in a concentration-dependent manner (Fu LW et al, 2002).
- In vitro studies showed that co-administration of TET at 2.5 μM, which has little cytotoxicity alone, reversed the sensitivity of KBv200 cells to paclitaxel and docetaxel around 10-fold (Zhu XM et al, 2005).

Protocols

- Zuo Jin Wan (ZJW), comprised of Rhizoma Coptidis and Fructus Evodiae in the ratio of 6 : 1, has been identified to have anticancer activity. ZJW could increase the concentration of chemotherapeutic drugs in HCT116/L-OHP cells in a dose-dependent manner. ZJW could also reverse drug resistance of colorectal cancer cells by decreasing P-gp level in vitro and in vivo.
- ZJW reversed MDR via increasing the sensitivity of MDR cells to chemotherapeutic agents. Second, ZJW reversed MDR through down-regulation of P-gp in vitro and in vivo. And third, combination of chemotherapy with ZJW prolonged the overall survival time of xenograft model and reduced the tumour volume (Sui et al, 2013).
- Recent findings have found that berberine and coptisine, which are the major active constituents of Coptis, were found to reverse ABCB1-mediated MDR in human MDR cancer cells (Min et al, 2006; He et al, 2012).

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