Reversal effects of traditional Chinese herbs on multidrug resistance in cancer cells

Multidrug resistance (MDR) continues to be a major obstacle for successful anticancer therapy. In this work, fractions from 17 clinically used antitumour traditional Chinese medicinal herbs were tested for their potential to restore the sensitivity of MCF-7/ADR and A549/Taxol cells to a known antineoplastic agent. The effects of these fractions were evaluated by MTT method and an assay of the cellular accumulation of doxorubicin. 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 CH2Cl2 extracts were more effective than fractions from EtOAc extracts. Five herbs (Curcuma wenyujin, Chrysanthemum indicum, Salvia chinensis, Ligusticum chuanxiong Hort. and Cassia tora L.) could sensitise these resistant cancer cells at a non-toxic concentration (10 µg mL−1), and markedly increased doxorubicin accumulation in MCF-7/ADR cells, which necessitates further investigations on the active ingredients of these herbs and their underlying mechanisms.
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**Keywords**
- traditional Chinese medicine (TCM),
- multidrug resistance (MDR),
- P-glycoprotein (P-gp),
- modulator,
- MCF-7/ADR cells,
- A549/Taxol cells

1. **Introduction**

Currently chemotherapy treatment for cancer patients is severely limited because of the tumour cells’ resistance to a wide variety of drugs, a phenomenon called multidrug resistance (MDR; Ambudkar et al., 1999; Gottesman, 2002). One of the major causes of MDR is the overexpression of a membrane-bound protein, P-glycoprotein (P-gp) in tumour cells. P-gp is a member of the ATP binding cassette (ABC) superfamily of energy-dependent efflux protein pumps, which operates by translocating a substrate from the intracellular to the extracellular compartment, resulting in reduced intracellular concentration, tumour cell survival and resistance to antineoplastic drugs (Ling, 1997). It is important to note that cancer cells can exhibit an MDR phenotype by mechanisms other than those involving P-gp. Other proteins such as the multidrug resistance proteins (MRPs; Deeley & Cole, 1997), lung cancer resistance-related protein (LRP; Scheper et al., 1993) and breast cancer-resistance protein (BCRP; Ross et al., 1999) were identified and shown to act in the same way. Now P-gp and other ABC transporters are viewed as targets in the treatment of multidrug resistant cancers (Sarkadi & Muller, 1997).

It has long been recognised that membrane transport of anticancer drugs can be blocked, with consequential reversal of MDR, by the use of pharmacological agents termed modulators, chemosensitisers or MDR-reversing agents. Examples are verapamil, trifluoperazine, chloroquine, cyclosporine A and rapamycin (Fardel, Lecureur, & Guillouzo, 1996). These compounds work successfully in some patients, however most results of clinical trials are disappointing and they may expose the patients to unacceptable side-effects or toxicity at the doses required for effectiveness or affect the pharmacokinetics of the anticancer drug (Kerb, Hoffmeyer, & Brinkmann, 2001).

Natural sources are a fertile ground to find novel drugs with activity against MDR cancer cells. In some countries, especially China, traditional herbal medicines are often used together with main-stream chemotherapeutic agents. The clinically used traditional Chinese herbs
for the treatment of tumour can be classified into four categories based on the theory of Traditional Chinese Medicine (TCM): drugs (CH group) for ‘Clearing away Heat and Toxins’, drugs (PB group) for ‘Promoting Blood Flow to Remove Stasis’, drugs for ‘Invigoration’ and toxic drugs. Drugs for ‘Invigoration’ have indirect antineoplastic action by enhancing organism's immunity and been used clinically to minimise radiotherapy and chemotherapy-induced toxicity (Fu & Chen, 2008). Toxic drugs are excluded in our study because cancer is primarily a disease of old age, less toxic therapy is a major priority. For these reasons, we selected herbs from the first two groups. In the present study, we report the evaluation of the effects of these herbs on tumour cell MDR.

2. Results and discussion
Each herb investigated (Supplementary Table S1 – online only) was first extracted with 95% EtOH, suspended in water and then partitioned with CH2C12 and EtOAc. Extracts of CH2C12 and EtOAc were chromatographed, respectively, over a silica gel column to yield 14 fractions of C0-C100 and E0-E100.

We first examined the reversal effects of these fractions (from 17 Chinese medicinal herbs) on MDR in MCF-7/ADR cells, and then validated the effects of selected fractions in A549/Taxol cells. The multidrug-resistant MCF-7/ADR and A549/Taxol cells used here were 8.77- and 3768-fold more resistant to doxorubicin and docetaxel, respectively, compared to their drug-sensitive parental cell lines (Supplementary Tables S2 and S3 – online only).

In TCM, the first seven plants in Supplementary Figure S1(a) (online only), Sparganium stoloniferum, Ligusticum chuanxiong Hort., Curcuma wenyujin, Polygonum orientale, Caesalpinia sappan, Prunus Persica and Salvia chinensis, belong to the PB group; the other 10 plants, Cassia tora L., Pteris multifida Poir., Euonymous alatus, Portulaca oleracea, Chrysanthemum indicum, Sedum sarmentosum, Lobelia chinensis, Semiaquilegia adoxoides, Houttuynia cordata and Hedyotis diffusa, to the CH group. Supplementary Figure S1(b) (online only) shows the relationship between the reversing fold (RF) and percentage of MeOH in the mobile phase conditioned on herb category and extract solvent. The colour lines in graphs are LOESS fits corresponding to each category or extract solvent. The RF values of fractions from plants in PB group (the blue line) are higher than those in CH group (the purple line). Fractions from CH2Cl2 extracts (the red line) have higher RF values than those from EtOAc extracts (the green line). The RF values are highest in fractions where the percentage of MeOH in the mobile phase is in the 2–10% range. Samples eluted with 0% and 1% MeOH in CH2Cl2 abound with wax, fatty acids, chlorophyll, etc., were often regarded as inactive
constituents. Therefore, the general trend is that the less polar fractions are expected to contain active compounds than the more polar ones, which can be explained by the fact that the drug binding sites of P-gp and other pumps are likely accessible from the lipid bilayer, rather than the aqueous phase, and the partitioning of the transport substrate into the membrane plays an important role in modulating both drug binding to the protein (Romsicki & Sharom, 1999) and the rate of drug transport (Lu, Liu, & Sharom, 2001).

Eleven fractions of different polarities with RF value over 30 were selected for our further investigations. These fractions at 10 µg mL⁻¹ (lower than their IC10 values) are non-toxic with over 90% survival of MCF-7/ADR and MCF-7 cells in our experiments (Supplementary Table S2 – online only). A549/Taxol cell, a kind of P-gp overexpressed cell line established by in vitro continuous stepwise exposure to drugs, was then used to validate the reversal effects of these selected fractions (Zhang et al., 2010). As shown in Supplementary Table S3 (online only), all fractions at 10 µg mL⁻¹ (also non-toxic to A549/Taxol and A549 cells at this concentration) had the ability to synergetically potentiate the inhibitory effect of docetaxel on the growth of A549/Taxol cells, which further suggested the role of the fractions played in the inhibition of P-gp function. Curcuma wenyujin called ‘E zhu’ in Chinese has been clinically used for suppression of tumours, from which several constituents, e.g. β-elemene, with potential antitumour and tumour MDR reversal activities have been characterised. Not surprisingly, Curcuma wenyujin C2 showed exceptionally strong effect. Curcuma wenyujin C10 and E10 were relatively less effective on their reversal ability in A549/Taxol cells than MCF-7/ADR cells. Ligusticum chuanxiong C2 lowered IC50 of docetaxel more than Ligusticum chuanxiong E10 did. The fractions in plant Salvia chinensis exhibited effective reversal activity, while they had poor function in cell accumulation of doxorubicin in MCF-7/ADR cells (Supplementary Figure S2 – online only). These data suggested that Salvia chinensis might have mechanisms other than the effect shown on P-gp function.

The decrease of cellular drug accumulation induced by P-gp is thought to be one of the main causes of MDR. Supplementary Figure S2 (online only) illustrates the effects of fractions on the accumulation of doxorubicin in MCF-7/ADR cells. Doxorubicin accumulation in the positive group (verapamil) was 2.23-fold higher than that in the control group. Curcuma wenyujin C10 (2.31-fold), Curcuma wenyujin E10 (2.74-fold) and Chrysanthemum indicum E10 (2.77-fold) had stronger
effects on doxorubicin accumulation than verapamil, which suggested that these three fractions were most suitable for further investigations on active ingredients.

3. Conclusions
In conclusion, our results showed that fractions from PB group were more effective than those from CH group and that fractions from CH2Cl2 extracts had more potential than those from EtOAc extracts to sensitisise the tumour MDR cells to antineoplastic agents. Five herbs could effectively reverse tumour cells’ MDR and significantly increase cellular accumulation of doxorubicin, which suggested that medicinal plants were good natural screening libraries to discover new chemosensitisers. Further studies are promised to identify from these fractions the compounds responsible for these activities and to clarify their underlying mechanisms.

References


