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ORIGINAL ARTICLE

Tetrandrine achieved plasma concentrations capable of reversing MDR in vitro and had no apparent effect on doxorubicin pharmacokinetics in mice

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Abstract
Purpose Tetrandrine (Tet), a multidrug resistant (MDR) modulator, was a potential candidate for use in cancer therapy and exhibited potent biological activity in vitro and in vivo when combined with anticancer agents such as doxorubicin, paclitaxel. Our aims were to determine whether serum concentration of Tet, which was capable of blocking P-gp in vitro, could be safely achieved in mice and whether Tet induced pharmacokinetic alterations in serum doxorubicin disposition in mice.
Methods Tet of 30 mg/kg dose used to reverse MDR was administrated intraperitoneally in mice. Plasma Tet and serum doxorubicin concentration were analyzed by HPLC. CYP 3A4 activity was examined by HPLC with the substrate of nifedipine.
Results More than 1 μmol/L of Tet could at least tenfold reverse MDR in vitro. The plasma peak concentration of Tet was about 2 μmol/L and not less than 1 μmol/L until 18 h following Tet administration (i.p.) at 30 mg/kg. These suggested that the concentrations of Tet that were sufficient to inhibit P-gp might be achieved in mice receiving 30 mg/kg of Tet. Importantly, no significant difference was demonstrated between the doxorubicin pharmacokinetic parameters obtained in mice received doxorubicin only and doxorubicin plus Tet. This implied that Tet of 30 mg/kg did not alter the profiles of pharmacokinetics of doxorubicin including the clearance and AUC of doxorubicin. Furthermore, Tet did not significantly affect on CYP 3A4 activity in human liver microsomes until more than 25 μmol/L.
Conclusions 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.

Keywords Multidrug resistance · Pharmacokinetics · Tetrandrine · CYP 3A4 · P-glycoprotein · Doxorubicin

Abbreviations

Tet	Tetrandrine
MDR	Multidrug resistance
P-gp	P-glycoprotein
VCR	Vincristine
MTT	3-(4,5-Dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide
DPH	1, 6-Diphenyl-1,3,5-hexatriene
S.E.	Standard error
PBS	Phosphate-buffered saline
i.p.	Intraperitoneal injection

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Introduction

Multidrug resistance (MDR) is considered a major obstacle of providing successful chemotherapy to many patients. Multifactorial in etiology, classic MDR is associated with the overexpression of P-glycoprotein (P-gp), resulting in decreased cellular drug accumulation due to increase efflux of a broad variety of structurally and functionally unrelated natural products and anticancer drugs (epipodophyllotoxins, vinca alkaloids, and anthracyclines). Inhibiting P-gp as a strategy to reverse MDR in cancer patients has been studied extensively, but the results have generally been disappointing [1–4].

Several non-cytotoxic drugs can sensitize MDR cells to chemotherapeutic drug in vitro and in vivo [5–8]. Combined therapy with MDR-related cytotoxins and modulators could inhibit tumor growth and prolong the life span in animal models [9]. Unfortunately, the data regarding the clinical efficacy is not yet available in early clinical trials. Alternatively, the MDR reversal agent may expose the patient to unacceptable side effects or toxicity at doses required for effectiveness and/or affect on the pharmacokinetics of anticancer drug [10, 11]. These limitations have spurred efforts to search for new, more effective MDR modulators.

Viewing the course of research and development, there are three generations of MDR modulators [12, 13]. The first generation P-gp modulators are substrates of P-gp and thus worked by competing with the cytotoxic compounds for efflux by the P-gp pump; therefore, high serum concentrations of the modulators were necessary to reverse MDR in vivo and caused serious toxicity. Second-generation P-gp modulators have a better pharmacological profile than the first-generation compounds, such as the reversal of MDR, not only in vitro but also in vivo. But they also interact with cytochrome P450 isoenzyme 3A4 that significantly inhibits the metabolism and excretion of the cytotoxic agents, thus leading to unacceptable toxicity that has necessitated chemotherapy dose reductions in clinical trials. Therefore, the second-generation P-gp modulators limit their clinical usefulness. Third-generation MDR modulators should specifically and potently inhibit P-gp function, effect the reversal of MDR not only in vitro but also in vivo, and not affect cytochrome P450 3A4 (CYP 3A4) at relevant concentrations; thus not alter the plasma pharmacokinetics of coventional chemotherapeutic drugs. The third generation MDR modulators such as Tariquidar (XR9576) [14], Zosuquidar (LY335979) [15], Laniquidar (R102933) [16], ONT-093 (OC144-193) [17], GF120918 [18], and Biricodar (VX-710) [19] have showed promise in clinical trials. The continued development of these agents may establish the true therapeutic potential of P-gp-mediated MDR cancer patients.

Tetrandrine (Tet) is a bis-benzylisoquinoline alkaloid, isolated from the roots of Chinese medicinal herb *Radix stephania tetrandrae S. Moore*. It has been clinically used for the treatments of arrhythmia, hypertension, inflammation, silicosis, and occlusive cardiovascular disorders in China for decades [20–22]. It is relatively non-toxic to humans, even at the administration of 180 mg, intramuscularly (i.m.) three times daily (t.i.d.) [23, 24].

Fu et al. [25, 26] reported that tetrandrine potentially reversed MDR in vitro and Fu et al. [26, 27] confirmed the action in vivo. Importantly, the combination of Tet and Dox did not cause death amongst the animals and did not decrease their body weight. Therefore, there was no evidence of an increase in Dox-associated toxicity induced by Tet. Furthermore, Tet, as a potent inhibitor of the P-gp efflux pump, combined with daunorubicin, etoposide and cytarabine was used to treat the patients with refractory and relapsed acute myelogenous leukemia. Of 36 patients, 16 (44.4%) achieved complete remission, 9 (25%) achieved partial remission (PR), and not an increase of side effects was observed [28].

Though Tet is a promising MDR modulator, no reports are available to show whether serum concentrations of Tet which are capable of blocking P-gp in vitro can be safely achieved and whether Tet inhibit CYP 3A4 activity and alter the pharmacokinetic of anticancer drug. This study was conducted as a prelude to more thorough evaluation and refinement of chemomodulation strategies.

Materials and methods

Materials

Tetrandrine, ($C_{38}H_{42}O_6N_2$), a bisbenzylisoquinoline alkaloid isolated from the dried root of *Stephenia tetrandra S Moore* (Fig. 1). The Tet powder, with a purity of >98%, was obtained from Dr. Pan XP (Kuming Institute of Botany, Chinese Academy of Sciences).

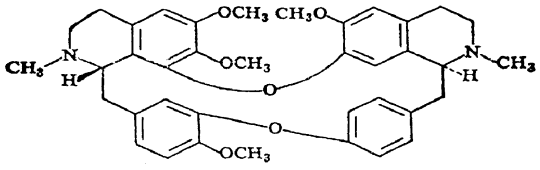

Fig. 1 The molecular structure of tetrandrine

Tet was freshly made to use. Vincristine (VCR), doxorubicin (Dox), paclitaxel (Taxol), dimethyl sulphoxide (DMSO) and 3-(4,5-dimethylthiazol-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. RPMI 1640 and DMEM were purchased from Gibco BRL.

Cell lines and cell culture

KB cells and KBv200 cells are human epidermoid carcinoma cell lines. KBv200 cells, a classical multidrug resistant human epidermoid carcinoma cell line with high level of P-gp expression which is the main cause for inducing MDR, was cloned from their parental drug-sensitive KB cells by stepwise exposure to increasing doses of vincristine and ethylmethane sulfonate (EMS) mutagenesis. The comparison with KB cells, KBv200 cells were approximately 100-fold resistant to VCR. KBv200 cells and parental sensitive KB cells were obtained from professor Liu XY (Chinese Academy of Medical Sciences, Beijng). KBv200 cells and KB cells were cultured with RPMI 1640 culture medium with 10% fetal bovine serum, benzylpenicillin (50 kU/L), and streptomycin (50 mg/L) at 37°C in a humidified atmosphere of 5% CO_2 + 95% air [26].

The MDR cell lines MCF-7/adr and the parental sensitive cell line MCF-7 were generously provided by Professor Liu XY (from the National Cancer Institute, USA). The MDR cell line MCF-7/adr and the parental cell line MCF-7 were grown as adherent monolayers in flasks in DMEM with 10% fetal bovine serum (FBS), benzylpenicillin (50 kU/L), and streptomycin (50 mg/L) at 37°C in a humidified atmosphere of 5% CO2 + 95% air. MCF-7/adr cells were approximately 100-fold resistant to Dox, and overexpression of P-gp was the main cause for the MDR [27].

Animals

NIH mice weighing 20 ± 3 g (8–10 weeks of age) were utilized in all experiments. The mice were obtained from the Center of Experimental Animals, Sun Yat-Sen University, and maintained and brought up in the Center. Animals received food and water as daily. Care and use of the animals followed the Guide for the Care and Use of Laboratory Animals of Center of Experimental Animals, Sun Yat-Sen University.

Cytotoxicity was examined by MTT assay

The cells were collected with trypsin and re-suspended to a final concentration of 1×10^5 cells/mL, and the cells of 0.18 ml aliquots were seeded in 96-well multiplates. The modulator of 10 μl and anticancer drug of 10 μl were added after 24 h incubation. After 68 h, MTT solution (10 mg/ml) of 10 μl was added to each well, and the plate was further incubated for 4 h, allowing viable cells to reduce the yellow MTT into dark-blue formazan crystals, which were dissolved in 150 μl of dimethyl sulphoxide (DMSO). The cell growth inhibition was evaluated by the MTT method on triplicate assays. IC_{50} values were calculated from cytotoxicity curves by Bliss's method. The degree of resistance was calculated by dividing the IC_{50} for MDR cells by that for parental sensitive cells. The fold-reversal of MDR was calculated by dividing the IC_{50} for cells to anticancer drug in the absence of modulator by that in the presence of modulator [26].

Pharmacokinetics of Tet in mice

Early studies showed Tet of 30 mg/kg could significantly reverse MDR in KBv200 and MCF-7/adr cell xenografts in nude mice [26, 27]. So the dose of 30 mg/kg was used to study the pharmacokinetics of Tet in mice. NIH mice were divided randomly into experimental group and control group according to weight. Each group included six mice with three female and three male. Mice in experimental group were treated by intraperitoneal injection (i.p.) of Tet of 30 mg/kg, while mice in control group received saline of same volume. Blood was collected from the retro-orbital plexus into cold heparin-coated glass tubes. To guarantee enough plasma samples for detection, two mice's blood samples (one female, one male) were mixed and the plasma sample was isolated. The blood samples were collected at 15 min, 40 min, 2 h, 3.5 h, 6 h, 10 h, 14 h, 18 h, 24 h, 48 h, 72 h, and 96 h after administration (i.p.) of Tet.

The plasma sample was extracted by ethanol and berbamine was used as the reference compound. These samples were analyzed by HPLC. The samples were chromatographed on a hypersil-C_{18} reversed-phase column. The mobile phase consists of SDS/CH_3CN/H_2O (0.062/70/30, W/V/V), pH 3.84, pumped at a flow rate of 0.7 ml/min with determination wavelength of 230 nm [29].

Pharmacokinetic studies of doxorubicin with or without Tet

To evaluate the effects of Tet on serum doxorubicin disposition, pharmacokinetic studies of doxorubicin were performed on mice treated with 10 mg/kg doxorubicin intravenously (i.v.) alone or combined with 30 mg/kg Tet (i.p.) every 24 h for 3 days prior to doxorubicin

dosing. Each group included six mice with three female and three male. Blood was collected from the retro-orbital plexus into cold heparin-coated glass tubes. To guarantee enough sample plasma for detection, two mice's blood samples (one female, one male) were mixed and the plasma sample was isolated. These samples were analyzed for doxorubicin and its metabolites by HPLC as Zhao et al. description [30]. Parameter assessment was calculated by pharmacokinetics statistics software 3P97. AUC, t1/2 and MRT were calculated using *T*-method. The ANOVA analysis was used to determine the statistical difference between $T_{1/2}\beta$, $AUC_{0\to 24h}$, $AUC_{0\to\infty}$, $MRT_{0\to 24h}$ and $MRT_{0\to\infty}$ of the two groups [31].

CYP 3A4 assays

Many MDR-class anticancer agents and P-gp modulators are metabolized by CYP 3A4, an isoform of the P450 system. Simultaneous administration of multiple CYP 3A4 substrates may overwhelm the system and result in decreased clearance of one or both compounds. Available literature, MDR modulators of the true therapeutic potential would not affect CYP 3A4 activity but still retained the potent activity toward the reversal of MDR. On the other hand, these MDR modulators which did not affect activity of CYP 3A4, theoretically would have not pharmacokinetic interaction with anticancer agents such as doxorubicin. The pharmacokinetic characteristics of doxorubicin in the presence or absence of Tet indicated a very minor overall effect of Tet on kinetic interaction with doxorubicin in mice. To deduce if Tet interfered with the pharmacokinetic of doxorubicin in human, CYP 3A4 activity was examined in the presence or absence of Tet in human liver microsomes.

Human livers were obtained from Cancer Center, Sun Yat-sen University under protocols approved by the Ethics Committee for the conduct of human research. Microsomes were prepared by differential centrifugation and the protein concentration of microsomes was determined by Bradford method with bovine serum albumin as the standard protein. Microsomal incubations with nifedipine, the CYP 3A substrate, and HPLC analysis were performed as Guengerich et al. [32] description with the following modification: the column temperature was 12°C instead of ambient temperature, and a Hypersil ODS C_{18} column (5 μm, 150 × 4.6 mm) instead of an octyldecylsilyl (C_{18}) reverse-phase HPLC column (3 μm, 800 × 6.2 mm). Incubations of the human livers microsomes with nifedipine (50 μM) were performed in the presence or absence of Tet and troleandomycin (TAO), one of the specific inhibitors of CYP 3A4, as the positive control at the concentration of 0.125, 0.25, 0.50, 1.0, 5.0, 25 μM, respectively.

Examination of P-gp expression

The cells were incubated at room temperature with the monoclonal anti-P-gp antibodies with UIC2 (Immunotech, Marseille, France) at a concentration of 12.5 μg/mL or with an isotype-matched mIgG2a control antibody (Sigma, St Louis, MO) at a concentration of 10 μg/mL for 15 min. Cell-bound antibody was detected by flow cytometry [33].

Results

The resistant characterization of KBv200 cells and MCF-7/adr cells compared with parental sensitive KB cells and MCF-7 cells

MCF-7 cells, MCF-7/adr cells, KB cells and KBv200 cells were cultured in the presence of full range various conventional chemotherapeutic drug concentration, such as doxorubicin (Dox), vincrinstine (VCR), or paclitaxel for 72 h, and cell death was determined by MTT assay. The comparison with parental sensitive KB cells and MCF-7 cells, KBv200 cells and MCF-7/adr cells were strong resistance to VCR, Dox and paclitaxel. In detail, the resistant-fold was showed in Table 1.

Table 1 Cytotoxic effect of doxorubicin, vincristine and paclitaxel on KB, KBv200, MCF-7 and MCF-7/adr cells

Drug	IC50 (μmol/L)		Fold of resistance	IC50 (μmol/L)		Fold of resistance
	MCF-7/adr	MCF-7		KBv200	KB	
Doxorubicin	14.69 ± 3.20	0.146 ± 0.032	100.8	1.50 ± 0.31	0.061 ± 0.021	24.6
Vincristine	2.49 ± 0.41	0.098 ± 0.021	25.4	0.82 ± 0.24	0.007 ± 0.002	113.2
Paclitaxel	13.88 ± 1.23	0.266 ± 0.052	52.2	1.68 ± 0.35	0.044 ± 0.012	38.5

The cells were cultured with a full range of concentrations of doxorubicin (Dox), paclitaxel or vincristine for 72 h. The concentrations required to inhibit growth by 50% (IC_{50} values) were calculated from the cytotoxicity curves (Bliss's software). Data represent means and standard errors of at least triplicate determinations. Fold of resistance was calculated by dividing the IC_{50} for the MDR cells by that for the parental sensitive cells

Effect of Tet on the reversal of MDR in vitro

Our earlier studies showed that Tet could reverse MDR in vitro and in vivo [26–28]. To further confirm that Tet still reversed MDR in low concentration that was able to achieve in vivo, the cytotoxicity of traditional anticancer drugs was detected in the presence or absence of Tet with low concentration and the fold-resistance was calculated as described in "Materials and methods". The results are shown in Fig. 2. As the results, Tet in the concentration of 0.125 μmol/L was still able to have about twice activity for reversing the resistance of traditional anticancer drugs such as Dox, VCR, paclitaxel in KBv200 cells. At 0.50 μmol/L, Tet had 5.4-, 8.1- and 10.6-fold reversing KBv200 cell resistant to Dox, VCR and paclitaxel, respectively, and had 7.8-, 2.3- and 4.2-fold reversing MCF-7/adr cell resistant to Dox, VCR and paclitaxel, respectively.

The pharmacokinetic of Tet in mice

Tet could reverse MDR in vitro and in vivo [26–28]. To further confirm whether Tet could achieve the plasma concentration required to reverse MDR in vitro, pharmacokinetic of Tet was studied in mice.

The NIH mice were selected as study model of pharmacokinetics. Tetrandrine of 30 mg/kg was injected (i.p.) in a volume of 10 ml/kg body weight. Six animals per time point were treated. Plasma samples were analyzed by HPLC. Single-compartment open model was used to evaluate the pharmacokinetics. The main pharmacokinetics parameters were as follows: $AUC_{0\rightarrow\infty}$: 45484.69 mg/L h, $AUC_{0\rightarrow 96h}$: 43658.29 mg/L h, $t_{1/2Ke}$:

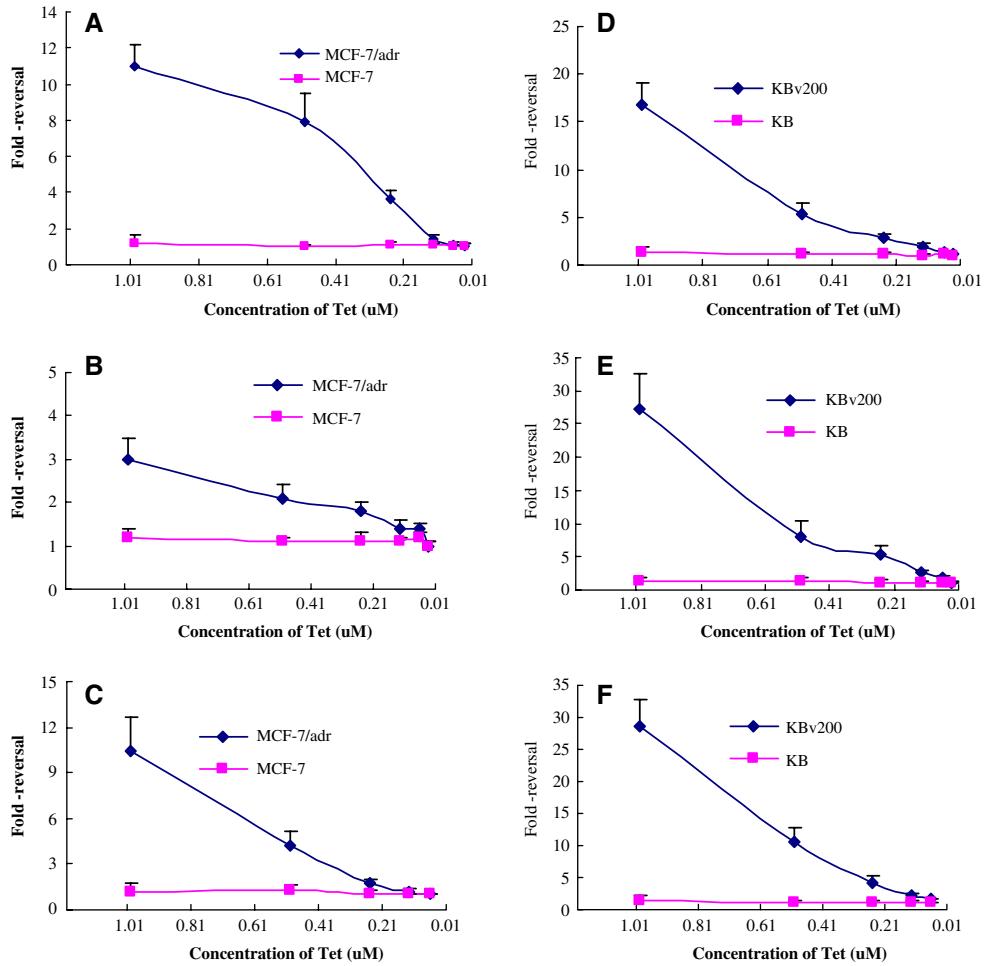

Fig. 2 Effect of tetrandrine (Tet) on enhancing the sensitivity of MCF-7 cells, MCF-7/adr cells, KB cells and KBv200 cells to the chemotherapeutic agent in vitro. Cytotoxicity was measured by MTT assay. The cells were cultured with a full range of concentrations of doxorubicin (Dox), paclitaxel or vincristine in the presence or absence of Tet for 72 h. The fold-reversal of MDR was calculated by dividing the IC_{50} for cells to the anticancer drug in the absence of Tet by that in the presence of Tet. Data represent means and standard errors of at least triplicate determinations: **a**, **d** for doxorubicin; **b**, **e** for vincristine; **c**, **f** for paclitaxel

25.6 h, Ke: 0.03/h, $t_{1/2Ka}$: 0.31 h, Ka: 2.27/h, V/F: 0.56 h, CL/F: 0.02 h. The equation was: Ct =1246 ($e^{-0.03t} - e^{-2.27t}$).

As shown in Fig. 3, the plasma peak concentration of Tet achieved 1.98 μmol/L and more than 1.0 μmol/L plasma concentration lasted until 18 h after adiministration (i.p.) of 30 mg/kg Tet. Furthermore, at the time of 48 h after adiministration of Tet, the concentration of Tet was still high (0.48 μmol/L) enough to reverse drug resistance, whereas at the same time point, chemotherapeutic drug such as doxorubicin was almost completely metabolized. These suggested that Tet could achieve the concentration that potently reversed MDR in vitro and lasted enough to meet the enhancement action of conventional chemotherapeutic drug under the dose used to treat KBv200 cell xenografts in nude mice by Fu et al. [27, 28].

Effect of Tet on pharmacokinetic of chemotherapeutic drug doxorubicin in mice

The pharmacokinetic (PK) profiles of doxorubicin in plasma of mice pretreated with or without of Tet were illustrated in Fig. 4. The Tet treatment group did not have higher plasma peak levels of doxorubicin. There were no significant differences in PK profiles in mice pretreated with or without Tet. This result suggested that Tet did not cause increases in doxorubicin concentrations in plasma.

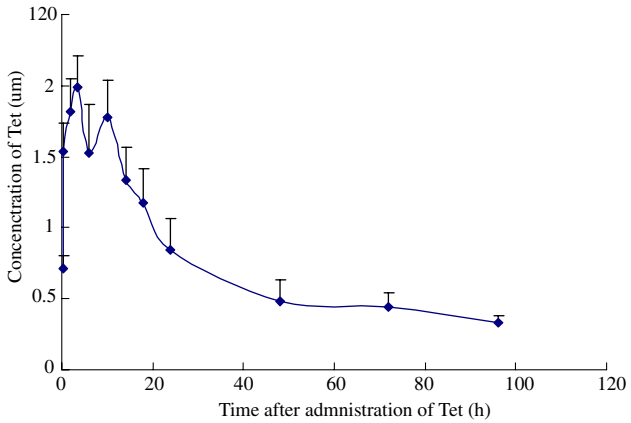

Fig. 3 The profile of pharmacokinetic of Tet in mice. 30 mg/kg of tetrandrine was injected (i.p.) in a volume of 10 ml/kg body weight. Six animals per time point were treated. While mice in the control group received non-tetrandrine solvent of same volume. Blood was collected from the retro-orbital plexus into cold heparin-coated glass tubes. To guarantee enough plasma samples for detection, two mice's blood samples (one female, one male) were mixed and the plasma sample was isolated. Plasma samples were analyzed by HPLC. The data represent mean and standard errors of three plasma samples. Tet could achieve the concentration that could potently reverse MDR in vitro and lasted enough to meet the enhancement action of conventional chemotherapeutic drug under the dose used to treat xenograft nude mice by Fu et al. [25–27]

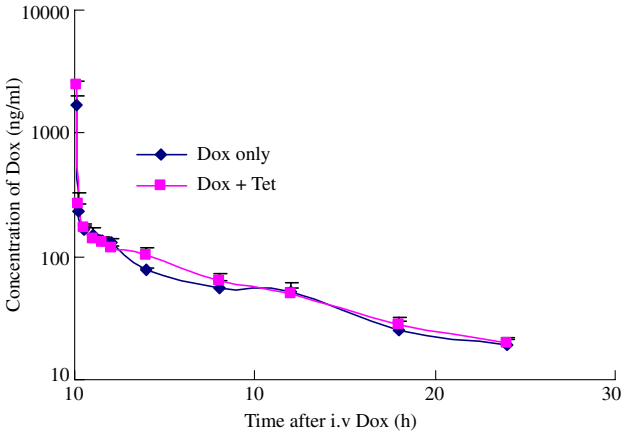

Fig. 4 Effect of tetrandrine on the profile of Dox pharmacokinetics in mice. The mice in experimental group were treated by i.p. Tet 30 mg/kg at d1 and d3, while mice in control group received non-Tet solvent of same volume. All mice were injected with doxorubicin 10 mg/kg (10 ml/kg) through caudal vein at d3 after i.p. Tet or non-Tet solvent. Blood was collected from the retro-orbital plexus into cold heparin-coated glass tubes. To guarantee enough plasma samples for detection, two mice's blood samples (one female, one male) were mixed and the plasma samples were analyzed for doxorubicin and its metabolites by HPLC. The data represent the mean and standard error of three independent samples. There were no significant differences in pharmacokinetics profiles in mice pretreated with or without Tet

Dox PK data were summarized in Table 2. The clearance and AUC of Dox and other pharmacokinetics indexes did not show marked difference between two group mice pretreated with or without Tet.

Effect of Tet on human CYP 3A4 activity

The distribution of P-gp and CYP 3A4 in tissues and their substrates appear to overlap [34, 35], therefore many of P-gp substrates interact with CYP 3A. As an inhibitor of MDR, Tet was determined whether it inhibited the activity of CYP 3A. TAO, a positive inhibitor of CYP 3A4, could significantly inhibit the activity of CYP 3A4 in a concentration dependent manner. But Tet did not obviously inhibit the activity of CYP 3A4 until the concetration of 25 μmol/L (Fig. 5).

Effect of Tet on expression of P-gp

No marked difference of the expression of P-gp was observed in KBv200 cells treated by Tet for 24 h (Fig. 6). These results provide further identification of the regulatory mechanisms that are not involved in the over-expression of P-gp in MDR cells.

Table 2 Dox pharmacokinetic parameters are summarized between two groups mice pretreated with or without Tet

Parameters	With Tet	Without Tet	*P*
$T_{1/2}$(h)β	12.320 ± 5.237	10.823 ± 2.576	>0.05
K10(1/h)	4.747 ± 1.371	4.013 ± 0.346	>0.05
Vc (L/kg)	0.000967 ± 0.000315	0.00126 ± 8.7E-05	>0.05
Cl(s)(L/h kg)	0.00431 ± 0.000295	0.00432 ± 0.00116	>0.05
$AUC_{0\text{-}24h}$(ng h/ml)	1848.707 ± 151.021	1622.453 ± 73.916	>0.05
$AUC_{0\text{-}\infty}$(ng h/ml)	2111.83 ± 149.153	1891.153 ± 46.343	>0.05
MRT0-24h(h)	6.0167 ± 0.329	6.485 ± 0.286	>0.05
MRT0-∞(h)	9.943 ± 1.185	10.9 ± 1.316	>0.05

The mice in experimental group were treated by i.p. Tet 30 mg/kg at d1 and d3, while mice in control group received non-Tet solvent of same volume. All mice were injected with doxorubicin 10 mg/kg (10 ml/kg) through caudal vein at d3 after i.p. Tet or non-Tet solvent. Blood was collected from the retro-orbital plexus into cold heparin-coated glass tubes. To guarantee enough sample plasma for detection, two mice's blood samples (one female, one male) were mixed and the plasma samples were analyzed for doxorubicin and its metabolites by HPLC. The Dox pharmacokinetic parameters were summarized. The data represent the means and standard error of three independent samples

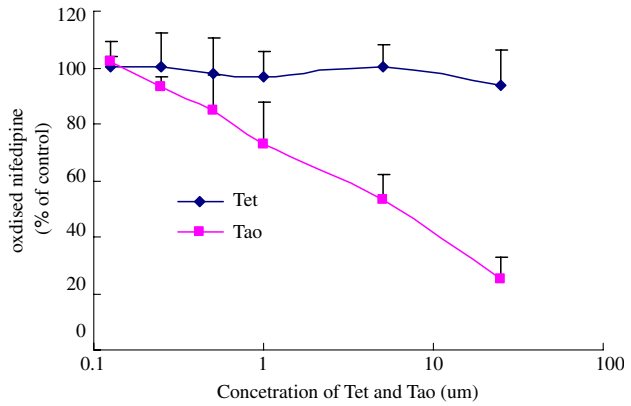

Fig. 5 Effect of tetrandrine and troleandomycin (TAO) on the activity of CYP 3A4. Human liver microsomes were prepared by differential centrifugation. Microsomal incubations with nifedipine, the CYP 3A substrate in the presence or absence of Tet and troleandomycin (TAO), one of the specific inhibitors of CYP 3A4, as the positive control, and HPLC analysis were performed as the description of "Experimental Design". The data represent the mean and standard error of three independent experiments. TAO could significantly inhibit the activity of CYP 3A4 in concentration dependent manner. But up to 25.0 μM, the Tet did not obviously inhibit the activity of CYP 3A4

Discussion

P-glycoprotein (P-gp) is well known because of its contribution to multidrug resistance during anticancer treatment. More recent work indicates that P-gp mediates the transcellular transport of many drugs in addition to anticancer compounds. Because of this reason, its potential role in clinically significant drug–drug interactions has just begun to be realized [6, 10].

Efforts to overcome multidrug resistance have primarily focused on attempts to inhibit P-gp. Since the discovery that verapamil and cyclosporin A were P-gp inhibitors and able to reverse P-gp mediated resistance, a considerable amount of research has been performed. The affinity of the first-generation MDR modulators was low for ABC transporters and necessitated the use of high doses, resulting in unacceptable high toxicity which limited their application. The second-generation MDR modulators had a better pharmacologic profile than the first-generation MDR modulators. But co-administration of an MDR modulator usually elevated the plasma concentrations of an anticancer drug by interfering with its clearance or inhibiting its metabolism and excretion, thus leading to unacceptable toxicity that necessitates chemotherapy dose reductions in clinical trials down to pharmacologically ineffective levels. In vivo, altered Dox tissue disposition had been reported in rodents when cyclosporine or PSC 833 was administered simultaneously with Dox [36, 37]. In human clinical studies, cyclosporine and tamoxifen had been shown to reduce doxorubicin clearance [38–41]. Therefore, the MDR modulators (the third generation) with a high serum concentrations capable of reversing MDR in vitro and the lack of pharmacokinetic interactions with anticancer drugs were needed to develop.

Tet could potently reverse P-gp-mediated MDR in vitro and in vivo [26, 27]. Tet of 1 μmol/L exhibited about tenfold reversal of MDR in KBv200 cells in vitro. Tet of 30 mg/kg could achieve sufficient plasma concentrations and keep enough time for meeting reversal of MDR in mice. Importantly, the profile of pharmacokinetics of doxorubicin was similar between with and without Tet. These suggested that Tet maybe belonged to the third generation MDR modulators.

Furthermore, many of the anticancer drugs are substrates both for ABC transporter proteins and for the cytochrome P450 isoenzyme 3A4 (CYP 3A4). Most of the second-generation MDR chemosensitizers are also

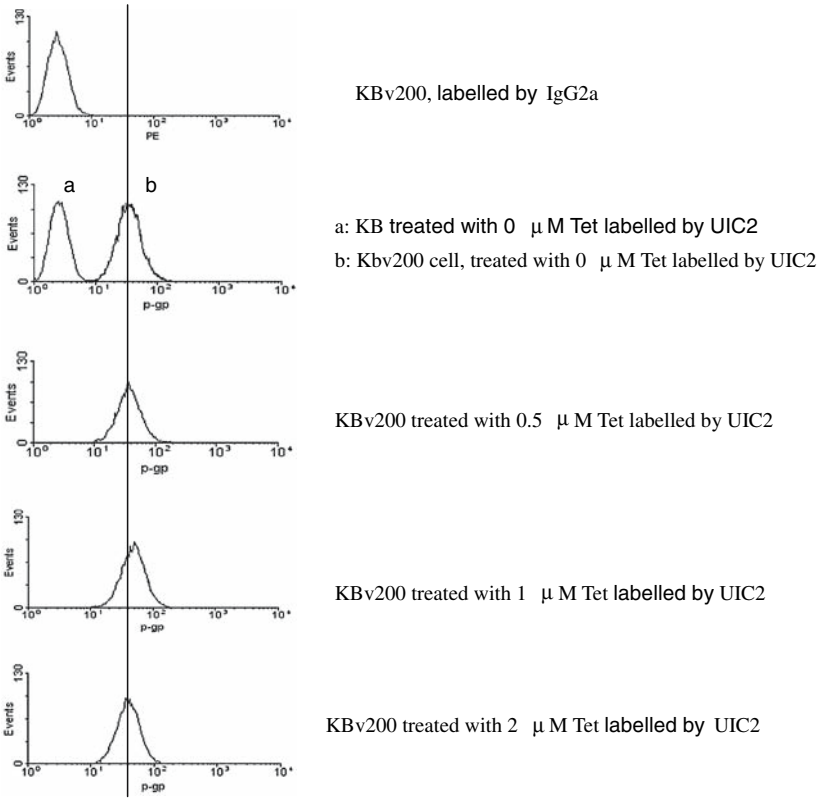

Fig. 6 Effect of Tet on the expression of P-gp. The cells were treated with Tet for 24 h. P-gp was examined as the description of "Materials and methods". **a** KBv200 cells were labelled by IgG2a; **b**, *a*: KB cells were labelled by UIC2, *b*: Kbv200 cells were labelled by UIC2; **c** KBv200 treated with 0.5 μM Tet and labelled by UIC2; **d** KBv200 treated with 1 μM Tet and labelled by UIC2; **e** KBv200 treated with 2 μM Tet and labelled by UIC2

substrates for CYP 3A4 and affect on CYP 3A4 activity which may result in unpredictable pharmacokinetic interactions. Co-administration of a MDR modulator may significantly elevate plasma concentrations of an anticancer drug by interfering with its clearance (e.g., via biliary elimination) or metabolism (e.g., via the cytochrome P450 system). This would increase the concentration of an anticancer drug leading to unacceptable side effects, necessitating dose reductions down to pharmacologically ineffective levels [42–44]. Because of these problems, MDR inhibitors have not improved the therapeutic efficiency of anticancer drugs unless such novel MDR modulators lack significant inhibition of CYP 3A4 activity. Tet did not significantly inhibit CYP 3A4 activity until 25 μmol/L concentration which was much higher than that capable of reversing MDR in vitro.

Our earlier work showed Tet could result in a significant inhibition of azidopine binding to P-gp in a dose-dependent manner [26, 27]. Furthermore, Tet did not regulate the expression of P-gp. These suggested Tet inhibited the function of P-gp by directly binding to P-gp but not involving in the regulation of p-gp expression.

In conclusion, Tet could achieve plasma concentrations capable of reversing MDR in vitro and also did not affect CYP 3A4 activity and the profile of pharmacokinetic of doxorubicin in mice. These implied that Tet, an old drug, was promising in the reversal of MDR for clinic.

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