P-gp Induction by Curcumin: An Effective Antidotal Pathway

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Abstract

**Aim:** We had reported that low dose curcumin could reduce the absorption of talinolol. The investigation in this study provided more information about inductive effect of high dose curcumin and further elucidated the mechanism between curcumin and drug interactions mediated by intestinal P-gp.

**Methods:** The pharmacokinetics of talinolol after high dose curcumin was studied in 12 healthy volunteers using HPLC-MS/ESI method. The function, expression and mRNA levels of p-gp were determined in p-gp over-expressing caco-2 cells by flowcytometry or real-time quantitative polymerase chain reaction.

**Results:** High dose curcumin decreased the AUC0-∞ and Cmax of talinolol by 42% and 29%, respectively. CL/F was significantly increased by approximately 77% versus control. *In vitro* studies, curcumin and its metabolite tetrahydrocurcumin significantly upregulated function, expression and MDR1 mRNA levels of p-gp in a concentration- and time-dependent manner, with significant results observed as soon as 1 h after incubation. The remarkable increases in p-gp expression were not accompanied by proportional increases in p-gp transport activity.

**Conclusions:** Simultaneous administration of curcumin might alter the pharmacokinetics of co-administered drugs by p-gp induction, by which curcumin may confer protection from endogenous and exogenous toxins.

Keywords: Curcumin; p-gp; Absorption; Caco-2 cells; Antidotal pathway

Introduction

P-glycoprotein (p-gp) is a multidrug transporter belonging to the adenosine 5′-triphosphate (ATP)-binding cassette transporter family. In humans, it is the product of multi-drug resistance gene MDR1. P-gp is expressed in the apical membrane of many pharmacologically important epithelial barriers, such as intestinal epithelial cells, and influences net drug absorption by facilitating secretion of a variety of structurally and pharmacologically unrelated hydrophobic compounds from the blood into the lumen of the gastrointestinal tract [1]. P-gp has been viewed as a therapeutic target for specific inhibition to overcome the well-known problems of drug resistance in anticancer therapy. On the other hand, its polarized expression is consistent with the proposed role of p-gp as a secretory protective system, contributing to the gastrointestinal epithelial barrier in limiting bioavailability of its substrates. Thus, by using its efflux properties, a possible antidotal pathway against the damage induced by xenobiotics that are substrates of this transporter could be proposed [2]. A lot of drugs have been found to induce p-gp, including dexamethasone, rifampicin and chemotherapeutic agents, namely doxorubicin, daunorubicin and vinblastine [3]. However, due to dose-limiting toxicity of these synthetic p-gp-inducing agents, much effort is currently being expended toward identifying natural and dietary compounds that are less toxic to animals, plentiful and inexpensive [4].

The present study focused on curcumin, which is natural polyphenol and the major constituent of turmeric powder, a spice and colouring agent extracted from the root of *Curcuma longa* and widely used in Asian food because of its wide range of biological and pharmacological activities, including anti-inflammatory, anti-carcinogenic, antioxidant, anti-allergic, antidepressant, antibacterial, hypcholesterolemic, hypoglycemic and antitumor activities [5]. The safety of curcumin and its derivatives has been studied in various clinical trials, and it is clear that curcumin is not toxic, even at a high dose of 8 g/day for 3 months [6]. Curcumin has been recognized as an effective inducer of intestinal p-gp by effectively decreasing the absorption of p-gp substrate talinolol in healthy volunteers [7]. However, *in vitro* experimentation is required to elucidate the mechanism of p-gp induction by curcumin. Most studies examined the mechanism of the reduced bioavailability using inducers of intestinal p-gp on drug transport. Such studies are mainly performed *in vitro* with polarized caco-2 cells [8,9]. However, there are very few studies examining directly the relationship between p-gp induction and drug transport through changes in expression and function of p-gp in intestinal cells, following curcumin treatment *in vitro*. Therefore, we have undertaken characterization of these parameters in caco-2 cells, which will provide a greater understanding of the underlying changes in p-gp expression and function underpinning the molecular changes associated with the decreased talinolol absorption across the intestine. Two clinically important p-gp substrates, namely talinolol and the fluorescent Rhodamine 123 (Rh-123), the known p-gp inducer rifampicin and inhibitor cyclosporin A (CsA), the first immunosupressor that has been shown to modulate p-gp activity in laboratory models and entered very early into clinical trials for reversal of MDR, were used in this study [10].

Materials and Methods

Chemicals and reagents

Talinolol tablet formulation (Cordanum®-50mgtablet) and reference
standard were generously provided by Cordenau, Arzneimittelwerk Dresden GmbH (Dresden, Germany); Rh-123, rifampicin, CsA, propranolol, phosphates buffer salt (PBS) and diphenyltetrazolium bromide (MTT) were purchased from SIGMA (Steinheim, Germany); curcumin soft capsule (ZhiKePing®-50mg, containing curcumin, demethoxycurcumin and bisdemethoxycurcumin), the reference standard of curcumin and tetrahydrocurcumin (Figure 1) were obtained from ShenWei pharmaceutical com. Ltd. (Shijiazhuang, P.R. China), its adjuvant (corn oil) has no drug-drug interactions with talinolol in vitro investigation.

Caco-2 cells were obtained at passage 18 from Shanghai cell bank (Shanghai, P.R. China). Dulbecco’s modified Eagle’s medium (DMEM), non-essential amino acid (NEAA), antibiotic-antimycotic mixed stock solution, glucose, PBS buffer (0.01M, pH 7.25), L-glutamine, 1 mM ethylenediaminetetraacetic acid (EDTA), 0.25% trypsin, Hanks’ balanced salt solution (HBSS) and human transferrin (4 mg/mL) were obtained from Invitrogen (Carlsbad, CA). Fetal bovine serum (FBS) was from Hyclone Laboratories (Logan, UT). Mouse monoclonal antibody P170/p-gp/MDR Ab-2 (mAb) was from CHEMICON International Inc, USA, and fluorescein isothiocyanate (FITC)-labeled affinity-purified Goat Anti-Mouse IgG(H+L) as a secondary antibody was from Kirkegaard Perry Laboratories, KPL, USA. Mouse IgG2a-FITC was purchased from ImmunoTools GmbH (Friesoythe, Germany). Transwell polycarbonate cell culture inserts (24 mm diameter, 0.4 μm pore size) were from Costar Corp. (Bedford, MA, USA). Dilution of curcumin, tetrahydrocurcumin, Rh-123 and CsA were prepared in dimethyl sulfoxide (DMSO) and stored at -20°C and were diluted with the culture medium to make the indicated concentrations. All other reagents or solvents used were either analytical or high-performance liquid chromatography grade, and were purchased from Merck (Germany).

Subjects and study design

The study cohort consisted of 12 healthy, nonsmoking Chinese men ranging in age from 23 to 26 years and weighing between 56 and 73 kg (mean ± SD: 68 ± 8 kg). All subjects were in good health as determined by standard physical examinations, a 12-lead electrocardiogram (ECC), and the results of routine biochemical and hematological tests. None of the subjects had a previous history of drug allergy, alcohol or drug abuse, cardiovascular diseases (systolic blood pressure>140 mmHg; diastolic blood pressure>95 mmHg), tachyarrhythmia, gastrointestinal disorder or stenoses, diabetes mellitus or chronic diseases. The results of renal and liver function tests were normal. No concomitant drugs were allowed 2 months before and during the study period, including over-the-counter drugs. The subjects were also asked not to consume alcoholic or caffeine-containing foods and beverages 12 h prior to and throughout the successive 3-week study periods. The protocol has been approved from the Ethics Committee of the second Xiangya Hospital of Central South University (Changsha, China). Prior to screening examination, all subjects provided written informed consent. The study was conducted in a self-controlled two-period study in a randomized, open-labeled design in 12 healthy volunteers, with a wash-out period of 2 weeks between the administration of a single oral dose of 50 mg talinolol (period 1) and the consecutive 6-day administration of 600 mg curcumin (200 mg, tid, po), concomitantly with a single oral dose of 50 mg talinolol (period 2) on the seventh day. This dosage regimen (600 mg/day) was selected to maintain a higher curcumin concentration in the intestinal segment during the major absorption process of talinolol. Curcumin and talinolol were given with 200 mL mineral water. The subjects were asked to remain in a seated position for 4 h after taking talinolol, after which they were allowed to perform usual daily activities in the clinical trial room; however, strenuous activity and exercise were prohibited. All subjects received a standardized meal 1.5, 4 and 10 h after drug administration. Serial blood samples were collected from an in-dwelling venous catheter (anticoagulated with sodium heparin) at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48 and 60 h after talinolol administration. Blood samples were collected in plastic containers, and immediately centrifuged. The separated plasma samples were immediately frozen at -80°C until assayed.

Pharmacokinetics and statistical analysis of talinolol

The specific and sensitive reverse phase high performance liquid chromatography-electrospray ionization mass spectrometry (HPLC-MS/ESI) method to analyze the plasma concentrations of unchanged talinolol has been described elsewhere [7]. Noncompartmental analysis was used to characterize talinolol plasma concentration-time profiles. The pharmacokinetic calculations were performed using DAS (ver.2.0, China) software and SPSS 11.0 statistical software package (ver. 11.0, SPSS Inc., Chicago, IL). All data are expressed as mean values ± standard deviation (means ± SD). Comparisons of mean values were analyzed by the least significant difference t-test, if homogeneity of variance test failed, by Wilcoxon signed-rank test. Differences were considered to be significant at p<0.05 (two-sided test). Inter-individual variability was determined using coefficients of variation (C.V.).

Caco-2 cell culture

Caco-2 cells (passage 18) were used as the authentic standard

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**Figure 1:** Chemical structures of talinolol (A), propanolol (B), curcumin (C), demethoxycurcumin (D), bisdemethoxycurcumin (E) and tetrahydrocurcumin (F).
in each run of the assay, since they were demonstrated to express sufficient levels of MDR1 mRNA. Caco-2 cells (passage 25-36) were grown in complete medium, consisting of DMEM supplemented with 10% heat-inactivated FBS, 0.1 mM NEAA, 25 mM glucose, 2 mM L-glutamine, 100 U/ml penicillin, and 100 g/ml streptomycin, 0.25 µM amphotericin and 6 µg/ml transferrin in an atmosphere of 95% air and 5% CO2 at 37°C. When the cells reached 80% confluence, they were subcultured using 1 mM EDTA and 0.25% trypsin with medium exchange every 2-3 days. Drug solutions were prepared in HBSS containing 10 mM Hepes, 4.2 mM NaHCO3, and 0.5% DMSO. Cell monolayers were used for transport studies at 16-20 days after seeding with the monolayers transepithelial electrical resistance (TEER) of 800-1000 Ω·cm-2 measured using a Millicell-ERS before and after transport experiments. P-gp expression and function in this system was validated by flow cytometry.

**Cytotoxicity assays**

For the *in vitro* evaluation of the cytotoxicity, the MTT assay that measures mitochondrial activity was performed to find noncytotoxic dosage of drugs in caco-2 cells, and only when the survival rates of cells above 90%, the doses were considered as the non-cytotoxic dosage. The cells were seeded onto 48-well plates at a density of 5×104 cells/cm² to obtain confluent monolayers at the day of the experiment. On the day of the experiment, the cells were washed twice with PBS buffer and exposed to curcumin, tetrahydrocurcumin, CsA or rifampicin, respectively, in fresh cell culture medium for 6, 12, 24, 48 and 72h. For the MTT assay, at each selected time point, the cell culture medium was removed, and the cells were washed twice with PBS, followed by the addition of fresh cell culture medium containing 0.5 mg/L MTT and incubation at 37°C in a humidified, 5% CO2-95% air atmosphere for 4 h. After this incubation period, the cell culture medium was removed, and the formed formazan crystals dissolved in 100% DMSO. The absorbance was measured at 490 nm in a multi-well plate reader (Wellscan MK2, Thermo Electron, USA). The percent viability relative to that of the control cells was used as the cytotoxicity measure.

**In vitro evaluation of p-gp expression, function and MDR1 mRNA levels**

For the *in vitro* evaluation of p-gp expression, function and MDR1 mRNA levels, the cells were seeded onto 24-well plates at a density of 5×104 cells/cm² to obtain confluent monolayers at the day of the experiment. Given the cytotoxicity data obtained, the maximum non-cytotoxic dosage for curcumin, tetrahydrocurcumin, CsA or rifampicin were 1, 5, 10 and 0 µmol/L, respectively. On the day of the experiment, the cells were washed twice with PBS buffer and exposed to curcumin (0.1, 0.5, 1.0 µmol/L) or tetrahydrocurcumin (0.5, 1.0, 5.0 µmol/L), respectively, in fresh cell culture medium for 1, 6, 12, 24, 48 and 72 h, respectively, using CsA (5 µmol/L) as a negative control and rifampicin (10 µmol/L) as a positive control. The blank control group was treated with PBS buffer.

For the evaluation of p-gp expression, the cells were washed twice with PBS and trypsinized with 0.25% trypsin/1 mM EDTA to obtain a cell suspension. The cells were then centrifuged (300 g/10 min) and suspended in PBS buffer containing 10% FBS and p-gp antibody P170/p-gp/MDR Ab-2. The antibody dilution in this experiment was applied according to the manufacturer's instructions for flow cytometry. The cells were then incubated for 24 h at 4°C in the dark. After this incubation period, the cells were washed twice with PBS buffer containing 10% FBS and p-gp antibody conjugated with FITC, suspended in ice-cold PBS and kept on ice until analysis. Mouse IgG2a-FITC was used as an isotype-matched negative control to estimate nonspecific binding of the FITC-labeled anti-p-gp antibody. Fluorescence measurements of isolated cells were taken with a flow cytometer. The fluorescence of FITC-labeled antibody was measured by a 530 ± 15 nm band-pass filter (FL1). Acquisition of data for 10,000 cells was gated to include viable cells based on their forward and side light scatter and the propidium iodide (4 µg/mL) incorporation (propidium iodide interfaces with a nucleic acid helix with a resultant increase in fluorescence intensity emission at 615 nm). The mean values of fluorescence intensity for 10,000 cells were the parameter used for comparison. Nonlabeled cells were analyzed in each experiment by a 530 ± 15 nm band-pass filter (FL1), in order to detect a possible contribution from cells autofluorescence to the analyzed fluorescence signals.

For the p-gp transport activity assay, the cells were washed twice with PBS buffer and trypsinized with 0.25% trypsin/1 mM EDTA to obtain a cell suspension. The cells were then centrifuged (300 g/10 min) and suspended in PBS buffer containing 500 µmol/L Rif-123 and incubated at 37°C. After the accumulation of the fluorescent substrate, the cells were washed twice with ice-cold PBS with 10% FBS and centrifuged (300 g/10 min) at 4°C. The obtained cell pellet was then suspended in DMEM containing curcumin (0.1, 0.5, 1.0 µmol/L), curcumin (0.5, 1.0, 5.0 µmol/L), or the plasma samples (at peak concentrations) from volunteers, respectively. The cells were incubated for 1 h at 37°C. After this period, the cells were washed twice with ice-cold PBS with 10% FBS, suspended in ice-cold PBS and immediately analyzed, as described above for the p-gp activity assay. The intracellular fluorescence of Rh-123 was measured by a 530 ± 15 nm band-pass filter (FL1).

For the evaluation of MDR1 mRNA levels of p-gp, total RNA was extracted from confluent monolayers of caco-2 cells using an RNeasy mini-kit and an RNase-free DNase set. The reverse transcription (RT) was conducted in 20 µL of two-step RT reaction mix containing 4 µL of the extracted total RNA (2 µg/ml), 1×TaQMan RT buffer, 5.5 mM MgCl2, 500 µM dATP, 500 µM dGTP, 500 µM dCTP, 500 µM dUTP, 2.5 µM random hexamer, 0.4 U/µL of RNase inhibitor and 1.25 U/µL MultiScrie reverse transcriptase. The mixture was incubated at 25°C for 10 min and subsequently at 48°C for 30 min. RT reaction was terminated by heating at 95°C for 5 min, followed by cooling at 4°C for 5 min, giving the RT product. Primer pairs and TaqMan probes for MDR1 mRNA were designed using the Primer Express 1.0 program (Applied Biosystems). The real-time quantitative polymerase chain reaction (PCR) was performed as follows: the 25 µL of reaction mixtures contained 1×TaqMan buffer A, 5.5 mM MgCl2, 400 µM dUTP, 200 µM dATP, 200 µM dCTP, 200 µM dGTP, 0.02 U/µL AmpliTaq Gold DNA polymerase, 200 nM each forward and reverse primer, 100 nM TaqMan probe and 1 µL of RT product. The reaction was performed in quintuplicate for each RT product. β-actin was used as the internal standard. During the extension phase of PCR, consisting of an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, and 60°C for 1 min, the nucleolytic DNA polymerase cleaved the hybridization probe, and the resulting relative increase in the reporter fluorescent dye emission was monitored in real time using a sequence detector. The fluorescent dye emission was a function of cycle number and was determined using the sequence detector software, giving the threshold cycle number (Ct) at which PCR amplification reached a significant threshold. The value of the Ct was linearly correlated with logarithmic value of genomic DNA quantity. The PCR products obtained by this real-time quantitative PCR procedure were confirmed to be the expected
products by electrophoresis through 3.0% agarose gels in the presence of ethidium bromide with visualization under UV illumination. The PCR products for the target proteins were undetectable in the real-time PCR procedure without reverse transcription. The mRNA levels of MDR1 are expressed as concentrations relative to β-actin mRNA.

Data analysis

All values are given as the means ± SD. Statistical comparisons were performed by one-way analysis of variance followed by Student’s t-test. P values of less than 0.05 (two-tailed) were considered statistically significant.

Results

In vivo studies

No clinically significant effects on blood pressure and heart rates were observed after administration of talinolol alone or after curcumin.

The main pharmacokinetic data of talinolol in healthy volunteers (n=12) were presented in Table 1. As we expected, coadministration of high dose curcumin decreased the absorption of talinolol significantly in comparison with control, the AUC∞ and Cmax were reduced significantly by approximately 42% and 29%, respectively, CL/F was significantly increased by approximately 77%, tmax and t1/2 values of talinolol were not significantly affected by curcumin. The interindividual variability in AUC and Cmax of talinolol was comparable in each period; the C.V. of AUC∞ and Cmax were 21% and 32% after high dose curcumin, and 23% and 37% after talinolol alone, respectively. The mean plasma concentrations versus time profiles before and after high dose curcumin were presented on Figure 2.

In vitro studies

Curcumin and its metabolite tetrahydrocurcumin significantly upregulated the expression, function and MDR1 mRNA levels of p-gp in a concentration- and time-dependent manner, with significant results observed as soon as 1 h after incubation.

P-gp expression levels were increased by 3.0-, 3.4-, 3.6-fold, and 2.7-, 3.1-, 3.4-fold higher after 6 h incubation with curcumin (0.1, 0.5, 1.0 μmol/L) and tetrahydrocurcumin (0.5, 1.0, 5.0 μmol/L), respectively, and the relative MDR1 mRNA levels were significantly increased by 51.9%, 53.8%, 55.8%, and 26.9%, 29.8%, 30.8%, respectively, with significant results observed as soon as 6 h after incubation. However, the remarkable increases in p-gp expression were not accompanied by proportional increases in p-gp transport activity, the corresponding increases in p-gp activity were only of 6.2%, 22.3%, 33.8%, and 13.3%, 25%, 41.3%, respectively, with significant results observed as soon as 1 h after incubation. The plasma samples at peak concentrations significantly accelerated the efflux of Rh-123 by 61.5%. Rifampicin, a well-described p-gp activator, significantly increased the function, expression and mRNA of p-gp by 69.8%, 4.17-fold and 67.3%, respectively, while the inhibitor CsA decreased them by 51.4%, 32% and 36.5%, respectively (Table 2). The p-gp in PBS buffer treated cells remained unchanged over 7 days.

Discussion

Induction of intestinal p-gp by several agents was reported to reduce bioavailability of orally administered drugs [11]. The present study focused on curcuminoids. A commercial grade curcuminoids (Sigma cat # C1386) containing curcumin, demethoxycurcumin and bisdemethoxycurcumin, which is commonly known as curcumin, has been reported to induce p-gp and reduce the absorption of its substrate talinolol [7]. The investigation in the present study proved that high dose curcumin seemed more effective in p-gp induction than the previous low dose curcumin, according to the AUC decrease of the substrate (42% versus 32%), since there was no significant difference in pharmacokinetic parameters between the controls of the two studies. The transport of substrate by p-gp is coupled to ATP hydrolysis, and there is evidence for stimulation of adenosine triphosphatase (ATPase) activity of p-gp by drug substrates or modulators from diverse systems [12]. A large number of compounds that interact with p-gp ATPase have been identified [13]. Modulators such as verapamil stimulate the ATPase, resulting in induction of p-gp and decreased bioavailability of its substrate talinolol [10,14]. Curcumin has also been reported to be able to stimulate the p-gp ATPase activity at low concentrations (0.5-1 μM) [15]. Interestingly, the concentrations used in the present in vitro studies were 0.1-1 μM for curcumin, which were extremely consistent with the concentrations above mentioned, thus we guess the decreased bioavailability of talinolol, and the activated function of p-gp in the present studies, were due to stimulation of the p-gp ATPase activity by curcumin. The induction of p-gp by plasma samples was much stronger than by curcumin or tetrahydrocurcumin alone (Table 2), which demonstrated the mixture of curcumin and tetrahydrocurcumin, and maybe other metabolites of curcuminoids in the plasma samples.

Table 1: Pharmacokinetic parameters of talinolol in healthy volunteers administered as a single oral dose of 50 mg alone (control) or concomitantly with curcumin (means ± SD, n=12).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>Curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC∞ (mg·h·mL⁻¹)</td>
<td>1712.1 ± 284.7</td>
<td>982.3 ± 188.0*</td>
</tr>
<tr>
<td>Cmax (μg·mL⁻¹)</td>
<td>1810.2 ± 293.2</td>
<td>1051.0 ± 231.5*</td>
</tr>
<tr>
<td>tmax (h)</td>
<td>2.0 ± 0.7</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>t1/2 (h)</td>
<td>12.2 ± 2.2</td>
<td>13.3 ± 6.1</td>
</tr>
<tr>
<td>CL/F (L·h⁻¹)</td>
<td>28.3 ± 4.8</td>
<td>50.1 ± 12.9*</td>
</tr>
</tbody>
</table>

AUC∞: Area Under Plasma Concentration–time curve from 0 to 60 hours; Cmax: Highest observed plasma concentration; tmax: Time to reach Cmax; t1/2: Elimination half-life; CL/F: Total clearance

Figure 2: The mean plasma concentration versus time profiles in healthy volunteers administered as a single oral dose of 50 mg alone (control) or concomitantly with curcumin (means ± SD, n=12).
resulted in a synergistic induction of p-gp functions. To the best of our knowledge, it has not yet been investigated whether these other metabolites are effective in modulating p-gp-related drug efflux in humans. Further studies are required to identify which of the metabolites are active in inducing a variety of beneficial physiological functions in animals and humans.

We further elucidated the mechanism by quantifying p-gp expression and function underpinning the molecular changes associated with the decreased talinolol absorption across the intestine using caco-2 cell lines. Induction of p-gp in our in vitro studies occurred with rifampicin treatment. We have compared the effect of rifampicin against vehicle control on p-gp expression and substrate accumulation function over time. The induction of p-gp by rifampicin was found to be time-dependent in caco-2 cells. Changes in p-gp induction corresponded to changes in p-gp function, as evidenced by reduced accumulation of the p-gp substrate Rh-123 (Table 2). It was shown previously that rifampicin could significantly increase mRNA and protein expression of pregnane X receptor (PXR), and is a well-known potent inducer of PXR [16]. Like p-gp, PXR is multispecific, recognizing a number of endogenous metabolites and xenobiotics as ligands, such as components of herbal remedies, dietary constituents [17,18]. Herbal medicines, such as St. John’s wort, have been shown to induce PXR significantly [18]; Traditional Chinese Medicines, Wu Wei Zi (Schisandra chinensis Baill) and Gan Cao (Glycyrrhiza uralensis Fisch) induced PXR and Increased warfarin clearance by recognizing a number of endogenous metabolites and xenobiotics as ligands, such as components of herbal remedies, dietary constituents [17,18]. Herbal medicines, such as St. John’s wort, have been shown to induce PXR significantly [18]; Traditional Chinese Medicines, Wu Wei Zi (Schisandra chinensis Baill) and Gan Cao (Glycyrrhiza uralensis Fisch) induced PXR and increased warfarin clearance by recognizing a number of endogenous metabolites and xenobiotics as ligands, such as components of herbal remedies, dietary constituents [17,18].

### Table 2:
The concentration-dependent induction of p-gp function, expression and relative MDR1 mRNAs levels by curcumin and tetrahydrocurcumin. The blank control group was treated with PBS buffer (means ± SD, n=5).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Con. (μmol/L)</th>
<th>Function¹</th>
<th>Expression²</th>
<th>MDR1 mRNA levels³</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank control</td>
<td>—</td>
<td>648.82 ± 9.23</td>
<td>28.75 ± 6.25</td>
<td>1.04 ± 0.25</td>
</tr>
<tr>
<td>Curcumin</td>
<td>0.1</td>
<td>608.52 ± 7.52</td>
<td>114.55 ± 5.27</td>
<td>1.58 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>504.24 ± 7.13</td>
<td>126.01 ± 4.53</td>
<td>1.60 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>429.31 ± 8.30</td>
<td>133.13 ± 5.58</td>
<td>1.62 ± 0.05</td>
</tr>
<tr>
<td>Tetrahydrocurcumin</td>
<td>0.5</td>
<td>562.52 ± 6.54</td>
<td>106.32 ± 5.10</td>
<td>1.32 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>486.72 ± 8.93</td>
<td>117.88 ± 6.38</td>
<td>1.35 ± 0.06</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>380.74 ± 7.85</td>
<td>124.93 ± 3.87</td>
<td>1.36 ± 0.04</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>10</td>
<td>195.82 ± 5.98</td>
<td>148.75 ± 4.99</td>
<td>1.74 ± 0.27</td>
</tr>
<tr>
<td>Plasma samples</td>
<td>—</td>
<td>250.02 ± 10.16</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>CsA</td>
<td>5</td>
<td>982.28 ± 10.25</td>
<td>19.59 ± 3.11</td>
<td>0.66 ± 0.07</td>
</tr>
</tbody>
</table>

¹The intracellular fluorescence of Rh-123; ²Relative MDR1 mRNA levels of p-gp (ratio of MDR1 mRNA levels and mRNA levels of the internal reference β-actin); ³P<0.05 versus blank control group; ⁴P<0.05 versus curcumin and tetrahydrocurcumin groups

In a word, simultaneous administration of curcumin might alter the pharmacokinetics of co-administered drugs by upregulation of function, expression and MDR1 mRNA of p-gp; the current results have also shed light on the cellular protective effects of curcumin. Both curcumin and tetrahydrocurcumin are known to have cellular protective and general detoxifying functions, according to traditional Chinese medicine and published biomedical literature. Our results suggest that the activation of PXR and/or ATPase and consequent decrease in lung damage, and a significant enhancement in survival time [22]. These results prompted the screening and selection of potent and safe p-gp inducers using caco-2 cell line. Our present study clearly indicated that curcumin was effective in increasing p-gp expression and activity (Table 2), and the absorption of the substrate talinolol was significantly decreased by approximately 42% (Table 1). In fact, when in the presence of this known p-gp inducer, p-gp expression increased in a concentration- and time-dependent manner, with significant results observed as soon as 6 h after incubation. This rapid increase in p-gp upregulation was also reported by Ehret et al. [23], who showed that venlafaxine increases the expression of MDR1 and MRP genes in caco-2 cells during an acute (1.5, 3 and 6 h) treatment period. Similar results were observed for another known p-gp inducer, rifampicin, and for several nonsteroidal anti-inflammatory drugs (NSAIDs), including diclofenac, fenbufen and indomethacin [24]. The present remarkable increases in p-gp expression levels induced by curcumin and tetrahydrocurcumin were not accompanied by proportional increases in p-gp transport activity. For example, the exposure of caco-2 cells to curcumin (0.5μM) for 6 h increased the protein and MDR1 mRNA expression levels to approximately 3.4-fold and 58.3% of control values, respectively, although p-gp transport activity increased only by 22.3%. These data indicate that although p-gp is being highly expressed and incorporated into the cell membrane upon exposure to the tested inducer, the magnitude of the expected protective effect against a xenobiotic did not increase in a similar trend. Noteworthy, our data suggest that, for the screening of p-gp inducers, both p-gp expression and activity should be investigated, since an increase in the first may not be reflected in an increase in the second parameter. Similarly, Takara et al. [24] noted that p-gp transport function remained unchanged in caco-2 cells exposed to several NSAIDs, in spite of the observed increase in MDR1 mRNA. One possible explanation for the differences noted between p-gp expression and activity levels in these cells is that caco-2 full differentiation into enterocytes could be needed to obtain fully functional p-gp.
induction of the drug-effluxing/detoxifying transporter may be the mechanism by which these traditional Chinese medicines confer protection from endogenous and exogenous toxins. Further studies are needed to clarify the mechanism of curcumin on the cellular efflux of deleterious xenobiotics.

The major limitation of the present study is that talinolol is a substrate of not only p-gp, but also of OATP2B1, which is expressed in the intestinal epithelial cells and has been claimed to be involved in talinolol absorption [25], therefore absorption kinetics of talinolol can be more complex. Further studies are required to identify the interaction of curcumin with OATP2B1, as well as its effect on p-gp.

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