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Curcuma drugs and curcumin regulate the expression and function of P-gp in Caco-2 cells in completely opposite ways

Xiao-Long Hou[a,1], Kyoko Takahashi[b], Ken Tanaka[c], Katsuhiko Tougou[a], Feng Qiu[d], Katsuko Komatsu[c], Koichi Takahashi[e], Junichi Azuma[a,*]

[a] *Department of Clinical Pharmacology and Pharmacogenomics, Graduate School of Pharmaceutical Science, Osaka University, 1-6 Yamadaoka, Suita City, Osaka, Japan*
[b] *Department of Medicinal Resources, Graduate School of Pharmaceutical Science, Osaka University, 1-6 Yamadaoka, Suita City, Osaka, Japan*
[c] *Division of Pharmacognosy, Department of Medicinal Resources, Institute of Natural Medicine, University of Toyama, 2630 Sugitani, Toyama, Japan*
[d] *Department of Natural Product Chemistry, Shenyang Pharmaceutical University, 103 Road Wenhua, Shenyang, China*
[e] *Department of Pharmaceutics, School of Pharmaceutical Sciences, Mukogawa Women's University, 9-11-68 koushien, Hyogo, Japan*

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ABSTRACT

Curcumin is a phenolic compound isolated from rhizomes of *C. longa*, *C. aromatica* and other *Curcumas* except *C. zedoaria*. Recently, both curcumin and *Curcumas* have become prevalent as supplement. P-gp has been reported as an important determinant for drug absorption in small intestine. In this study, Caco-2 cell monolayers were treated with methanol extracts of *Curcumas* (0.1 mg/ml) or curcumin (30 μM) for 72 h to investigate the relationship between the potential affects of *Curcumas* and curcumin on P-gp. [^{3}H]-digoxin and rhodamine 123 were used to evaluate P-gp activity. All *Curcumas* significantly increased the activity of P-gp by up-regulating the expressions of P-gp protein and *MDR1* mRNA levels. Interestingly, contrary to *Curcumas*, curcumin treatment inhibited the activity of P-gp with a decrease in P-gp protein and *MDR1* mRNA expression levels. *Curcumas* might alter the pharmacokinetics of co-administrated drugs by up-regulating the function and expression levels of intestinal P-gp. However, curcumin has no relationship with the inductive effect of *Curcumas* since curcumin showed an opposite effects. Caution should be exercised when *Curcumas* or curcumin are to be consumed with drugs that are P-gp substrates because *Curcumas* and curcumin might regulate the function of P-gp in completely opposite ways.

1. Introduction

In East Asia, *Curcuma* species (*C. longa*, *C. aromatica, C. zedoaria*) have been used as traditional medicines because of their various pharmacological activities including enhancing wound healing, promoting digestion, anti-cancer, anti-oxidant, hepatoprotective etc. (Araujo and Leon, 2001; Matsuda et al., 1998). Recent pharmacological studies have demonstrated that the rhizomes of *C. longa* expressed antiallergic (Ram et al., 2003) and antidepressant activities (Yu et al., 2002). Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) is the major yellow pigment from rhizomes of *C. longa*, *C. aromatica* and other *Curcumas* except *C. zedoaria*. Curcumin possesses wide range of pharmacological activities (Maheshwari et al., 2006). Especially, its effectivity as cancer chemopreventive agent has been affirmed by in vitro experiments and clinical trials (Thangapazham et al., 2006; Garcea et al., 2005). However, although both *Curcuma* drugs and curcumin have become prevalent as supplements in the world, little is known about the correct application of them because it is always thought that *Curcuma* drugs and curcumin share the same effects since curcumin is the main component from *Curcuma* drugs.

As herbal medicinal products have become prevalent throughout the world, the fatalness of herb–drug interactions stands out (Mills et al., 2005; Fugh-Berman and Ernst, 2001). Clinical trials have showed that many herbal supplements or over-the-counter remedies may modulate the activity of drug metabolism enzymes and/or drug transporters and further influence the bioavailability of co-administrated drugs (Gurley et al., 2004; Saruwatari et al., 2003; Anderson et al., 2003). In another of our report, we have indicated that *C. longa* and *C. zedoaria* might inhibit the activity and protein expression of cytochrome P450 3A4, which is the major phase I metabolizing enzyme and responsible for the metabolism of about 60% of drugs in current clinical use (Hou et al., 2007). On the other hand, Naganuma et al. (2006) reported that *C. longa* and curcumin inhibited the function of both sulfotransferase (SULT) and UDP-glucuronosyl transferase (UGT) activity in Caco-2 cells.

P-glycoprotein (P-gp) is a drug transporter belonging to the ATP-binding cassette transporter family. In humans, it is the product of

* Corresponding author. Tel.: +81 6 6879 8163; fax: +81 6 6879 8253.
E-mail address: Azuma@phs.osaka-u.ac.jp (J. Azuma).
[1] Present address: Department of Pharmaceutics, School of Pharmaceutical Science, Mukogawa Women's University, Hyogo, Japan.

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multi-drug resistance gene *MDR1*. P-gp is expressed in the apical membrane of many pharmacologically important epithelial barriers, including the intestinal epithelium, hepatocytes and renal tubular cells. In intestinal epithelial cells, P-gp is expressed on the apical surface and excretes drugs and xenobiotic compounds to the apical side. Thus P-gp plays an important role in regulating the intestinal absorption of xenobiotic compounds (Chan et al., 2004). The inhibition/induction of intestinal P-gp has been reported as a significant reason for herb–drug interaction (Jodoin et al., 2002; Perloff et al., 2001; Han et al., 2006).

Caco-2 cell model is a well-established in vitro model to study drug absorption and related mechanism in small intestine (Hilgers et al., 1990; Neuhaus et al., 2006). Since Caco-2 cell expresses P-gp as much as normal intestinal epithelial cells do, it has become a well-used model to evaluate the affect of drugs on intestinal P-gp. In this study, Caco-2 cells were utilized to detect the *Curcuma*–drug interaction mediated by intestinal P-gp. The major goals of this study are as following: (1) To show some information for the correct application of *Curcuma* drugs. (2) To investigate the relationship between the application of curcumin and *Curcuma* drugs.

2. Materials and methods

2.1. Materials and chemicals

Caco-2 cells (HTB37) were obtained at passage 18 from American Type Culture Collection (Manassas, VA). *C. longa* (Okinawa, Japan), *C. zedoaria* (Okinawa, Japan) and *C. aromatica* (Okinawa, Japan) used in this study were correctly identified by the molecular biological method previously reported (Cao et al., 2001). All drugs are stored in the Museum of Material Medica, Institute of Natural Medicine, Toyama University, Japan. Dulbelcco's modified Eagle's medium (DMEM), non-essential amino acid (NEAA), antibiotic–antimycotic mixed stock solution, glucose, ethlenediaminertetracetic acid (EDTA), trypsin, rhodamine 123, liquid scintillation cocktail were obtained from Nacalai Tesque (Kyoto, Japan). Fetal bovine serum (FBS) was from ICN Biomedicals, Inc. (Aurora, Ohio). [^{3}H]-digoxin (specific radio activity of 21.8 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA, USA). Verapamil was from Sigma Chemical Co. (St. Louis, MO, USA). Transwell polycarbonate cell culture inserts (24 mm diameter, 0.4 μm pore size) were from Costar Corp. (Bedford, MA, USA). Millicell ERS device was obtained from Millipore (Bedford, MA, USA). Stock solution of *Curcuma* extracts and curcumin were prepared at 50 mg/ml and 100 mM in dimethyl sulfoxide (DMSO) and stored in −20 °C.

2.2. Cell culture condition

Caco-2 cells at passage 35–44 were used for all experiments. Cell cultures were maintained in a humidified 37 °C incubator with a 5% carbon dioxide in air atmosphere. Caco-2 cells were grown in plastic tissue culture dishes in a medium consisting of DMEM containing 25 mM glucose, 4 mM L-glutamine, 0.1 mM nonessential amino acids, 100 units/ml penicillin, 100 units/ml streptomycin, 250 nM amphotericin and supplemented with 10% heat-inactivated FBS. When the cells reached 80% confluence, they were removed using 0.2% trypsin/EDTA, diluted with 1:4 and reseeded onto fresh tissue culture dishes. Medium was changed at 2–3 days.

2.3. Preparation of Curcuma extracts

The methanol extracts of *Curcuma* rhizomes were prepared and analyzed as previously described (Sasaki et al., 2003). In briefly, powdered drugs were dipped in methanol (1 l × 2) for 12 h at room temperature, the combined supernatants were evaporated to obtain the methanol extracts. The methanol extracts were freeze-dried into resultant powders. Samples were analyzed using a HPLC apparatus consisting of pumps (Shimadzu LC-10AT, Japan), a degasser (Shimadzu DGU-12A, Japan) an autosampler (Shimadzu SIL-10A, Japan), and a UV–vis detector (Shimadzu SPD-10A, Japan). The reversed-phase separation was performed in Waters Symmetry C_{18} column (4.6 mm × 150 mm, 5 μm particles). The mobile phase was composed of 0.25% acetic acid (A) and acetonitrile (B). The gradient was as follows: 0 min: 60% A, 40% B; 15 min, 40% A, 60% B; 35 min, 0% A, 100% B; 40 min, 60% A, 40% B. The elution was performed at a rate of 1 ml/min. Curcumin was detected at 410 nm and identified according to the retention time. A methanol stock solution of curcumin was prepared at 100 μM. Concentrations were obtained by extrapolation of peak area from a standard curve.

2.4. Rhodamine 123 efflux and accumulation

Caco-2 cells were seeded at 5×10^5 cells/cm^2 onto Transwell insert. *Curcuma* drugs (0.1 mg/ml) or curcumin (30 μM) were supplied to the apical compartment on day 18. Control groups were treated with 0.2% DMSO. After 72 h, *Curcuma* drugs/curcumin were removed and transport experiments were conducted on monolayers with transepithelial electrical resistance (TEER) of 800–1000 Ω cm^2. Monolayer TEER was measured before and after transport experiments.

To start transport experiment, Transwell inserts were washed with warmed PBS twice, and equilibrated with transport medium (HBSS buffer with 10 mM HEPES and adjusted to pH 7.4 with 1 N NaOH) for 30 min. Transport buffer (2.6 ml) with 5 μM rhodamine 123 was then added to the basolateral compartment and transport buffer (1.5 ml) was added to the apical compartment. Transwell plate was incubated at 37 °C. At 15, 30, 60, 90 min, 50 μl aliquots were withdrawn from the apical compartment for analysis. Positive control groups were pretreated with verapamil (100 μM) for 30 min and during the experiments. Then the cells were thoroughly washed three times with ice-cold PBS. Cells were then solubilized with 1% triton X for analysis of rhodamine 123 fluorescence and P-gp total protein.

2.5. Bi-directional [^{3}H]-digoxin transport study

Experimental protocol for bi-directional [^{3}H]-digoxin transport was similar to that for the rhodamine 123 efflux except that [^{3}H]-digoxin was added to the apical (for apical to basolateral transport: A–B) or basolateral (for basolateral to apical transport: B–A) compartments. At 15, 30, 60, 90 min, 50 μl aliquots were removed from the receiver compartments and mixed with 3 ml of scintillation cocktail and analyzed in a liquid scintillation counter. Apparent permeability coefficient (P_{app}) was calculated as $P_{app} = (dQ/dt)/(AC_0)$ (cm/s) where dQ/dt (nmol/s) was the flux rate, A (cm^2) was the effective surface area of the cell monolayer, and C_0 (nmol/ml) was the initial drug concentration in the donor chamber. Net efflux was expressed as the quotient of P_{app} (B–A) to P_{app} (A–B).

2.6. Rhodamine 123 fluorometric analysis

Rhodamine 123 was quantified with fluorometric analysis, 485 nm (exitation) and 530 nm (emission), using a PerkinElmer LS50B luminescence spectrophotometer. Rhodamine 123 in samples was determined based on calibration curves constructed from a series of standards.

2.7. P-gp and MDR1 expression

Caco-2 cells were cultured and treated with *Curcuma* drugs or curcumin as described in the previous section. Then, cell monolayers were washed with PBS for 3 times. Total cellular protein or total RNA was extracted. Total cellular protein was extracted by lysis buffer containing 150 mM NaCl, 10 mM Tris (pH 7.4), 1 mM EDTA, plus 1% Triton X-100, 1% deoxycholic acid and protease inhibitor mixture, followed by centrifugation at 1500 *g* for 10 min. Protein extraction was conducted on ice to minimize any potential protein degradation. Total RNA was extracted from the treated cells using TRIzol reagent.

2.8. Western blot analysis of P-gp

Protein concentration was determined by the BCA protein assay reagent kit (Pierce), and bovine serum albumin was used as a standard. Proteins (20 μg) from the total cell lysate were analyzed by SDS-PAGE (7.5% gel). After blotting, the Immobilon-P membrane (Millipore) was blocked with 5% skim milk in PBS with 0.5% Tween 20 at room temperature for 1 h. Immunoblots were incubated at room temperature for 1 h with the primary monoclonal antibody, C219 (1:1000). After further washing, the membranes were incubated for 1 h with anti-mouse IgG horseradish peroxidase conjugate (1:3000). The protein was visualized by exposing the membrane to a Kodak film for 1–5 min(s) in a dark room. Blots were reprobed with antibody to glyceraldehydes-3-phosphate dehydrogenase (GAPDH) as a loading control. Quantitative analysis of immunoblotted bands was performed by computer program (NIH Image, version 1.63).

2.9. RNA extraction and SYBR® GREEN I real time reverse transcriptase-polymerase chain reaction (RT-PCR) for MDR1

First strand cDNA was generated from 1 μg total RNA by using the oligo(dT) first strand primer. Real time PCR was performed with 50,000 fold diluted SYBR® GREEN I dye (Invitrogen). For *MDR1*, the forward primer sequence used was 5′-CTGTATTGTTTGCCACCACGA-3′ and the reverse primer sequence used was 5′-AGGGTGTCAAATTTATGAGGCAGT-3′; for *β-actin,* the forward primer sequence used was 5′-GGTCATCACCATTGGCAATGA-3′ and the reverse primer sequence used was 5′-GTAGTTTCGTGGATGCCACAGG-3′. Aliquots of the reverse–transcription reaction mixture (1 μl) were amplified and detected using a ABI PRISM 7700 sequence Detector System (Applied Biosystems) with the following profile: 1 cycle of 95 °C for 5 min, and 45 cycles each of 95 °C for 15 s and 60 °C for 1 min. The *MDR1* mRNA levels were normalized relative to *β-actin* mRNA level in each sample.

2.10. Reversibility of cellular P-gp expression and function

Caco-2 cells cultured on Transwell inserts were exposed to *Curcuma* drugs (0.1 mg/ml) or curcumin (30 μM) on day 18. After 72 h, the medium was reverted to medium without *Curcuma* drugs/curcumin. The cells were cultured for another 72 h. P-gp function was measured by rhodamine 123 efflux, P-gp expression level was determined by Western blot. P-gp function and expression were compared to those of control.

2.11. Statistical analysis

Statistical evaluation of data was performed by one-way analysis of variance followed by Bonferroni/Dunn multiple range test. Significant differences from untreated control cells are indicated as $^{*}P < 0.01$.

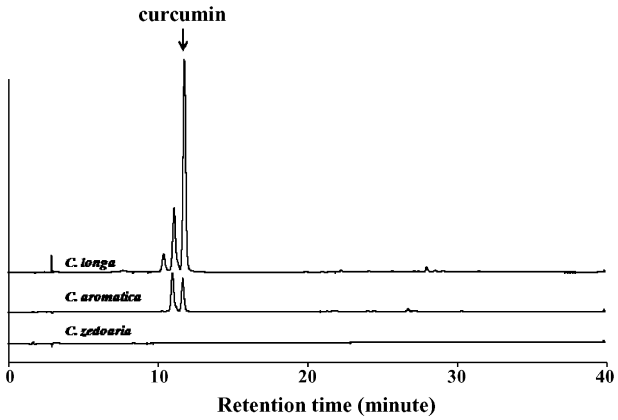

Chart 1. HPLC chromatographs of *C. longa*, *C. aromatica* and *C. zedoaria* methanol extracts. Black arrows pointed to the peaks of curcumin. The peaks were detected at 410 nm.

3. Results

3.1. Effects of Curcuma drugs and curcumin on rhodamine 123 accumulation and efflux

The methanol extracts of *C. longa, C. zedoaria* and *C. aromatica* were analyzed by HPLC. In both *C. longa* and *C. aromatica* extracts, the peaks of curcumin were detected (Chart 1). The curcumin contents in extracts of *C. longa* and *C. aromatica* were 3.9% and 1.2%, respectively. On the other hand, there was no curcumin was detected in the extract of *C. zedoaria*. In the following studies, 0.1 mg/ml of *Curcuma* extracts and 30 μM of curcumin were used because they were the largest dose without cytotoxicity (Hou et al., 2007).

The affect of *Curcuma* drugs/curcumin on P-gp was screened by rhodamine 123 accumulation and efflux. Verapamil (100 μM), a well-documented P-gp inhibitor, significantly increased rhodamine 123 accumulation in Caco-2 cells by acute exposure. After the cells were treated with *Curcuma* drugs (0.1 mg/ml), rhodamine 123 accumulation was decreased by about 30–50%. However, curcumin treatment raised rhodamine 123 accmulation by about 2 fold (Fig. 1). After Caco-2 cells were treated for 72 h with *Curcuma* drugs or curcumin, all *Curcuma* drugs treatment significantly increased the efflux of rhodamine 123, whereas curcumin inhibited the efflux of rhodamine 123 by about 30% (Fig. 2). Furthermore, our results

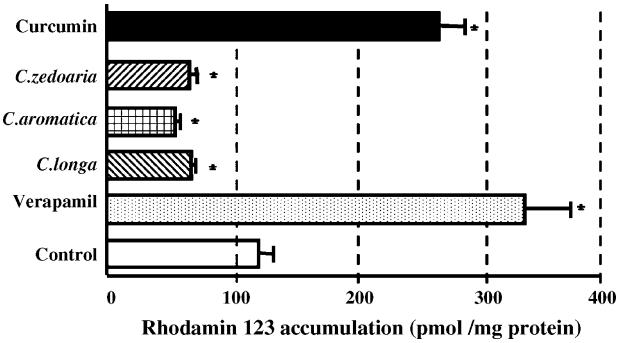

Fig. 1. Effects of *Curucuma* drugs on rhodamine 123 accumulation in Caco-2 cells. Caco-2 cells were treated with *Curcuma* drugs (0.1 mg/ml) or curcumin (30 μM) for 72 h. The accumulation of rhodamine 123 was measured after incubating the Caco-2 cells at 37 °C for 90 min without treatment of *Curcuma* drugs/curcumin. Positive Control groups were pretreated with verapamil (100 μM) for 30 min and during the accumulation experiment. Results are means ± S.E.M. from triplicate experiments. $^{*}P < 0.01$ compared with control.

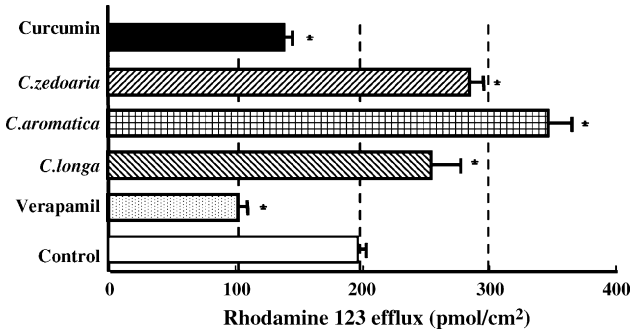

Fig. 2. Effects of *Curcuma* drugs/curcumin on rhodamine 123 efflux. Caco-2 cells were treated with *Curcuma* drugs (0.1 mg/ml) or curcumin (30 μM) for 72 h. The efflux of rhodamine 123 (10 μM) was measured after incubating the Caco-2 cells at 37 °C for 90 min without treatment of *Curcuma* drugs/curcumin. Positive Control groups were pretreated with verapamil (100 μM) for 30 min and during the experiment. Results are means ± S.E.M. from triplicate experiments. $^{*}P < 0.01$ compared with control.

indicated that all *Curcuma* drugs increased the efflux of rhodamine 123 in a dose-dependent way when the concentration was below 0.1 mg/ml (data not shown).

3.2. Effects of Curcuma drugs and curcumin on [^{3}H]-digoxin transport

The affects of *Curcuma* drugs and curcumin on P-gp function were further evaluated by measuring transepithelial transport of [^{3}H]-digoxin across Caco-2 cell monolayers. TEERs measured before and after transport studies showed no significantly differences (data not shown). Transepithelial [^{3}H]-digoxin transport across Caco-2 monolayers showed a marked asymmetry, with B–A permeability exceeding A–B permeability by a ratio of 3.8 (Table 1). The polarized permeability is characteristic of an efflux system that facilitates the transfer of intracellular [^{3}H]-digoxin back to apical compartments. Verapamil (100 μM) significantly reduced the asymmetry of [^{3}H]-digoxin transport by increasing A–B permeability and reducing B–A permeability and the efflux ratio was 2.0. Curcumin (30 μM) treatment for 72 h enhanced the A–B permeability from 2.1 to 3.1 and inhibited the B–A permeability from 8.0 to 6.3, as a result, the net efflux was decreased to 2. However, all *Curcuma* drugs (0.1 mg/ml) treatment enhanced the net efflux of [^{3}H]-digoxin by modestly reducing the A–B permeability and significantly induced the B–A permeability. Especially, *C. aromatica* induced the B–A permeability to 18.8 and resulted a net efflux as 15.7 (Table 1).

Table 1
Apparent permeability coefficient ($P_{app} \times 10^{-6}$ cm/s) and net efflux ratio of bi-directional [^{3}H]-digoxin (50 nM) transport across Caco-2 cell monolayers

Sample	P_{app} ($\times 10^{-6}$ cm/s)		Net efflux
	A–B	B–A	
Control	2.1 ± 0.4	8.0 ± 0.2	3.8
Verapamil	2.7 ± 0.3*	5.5 ± 0.5*	2.0
C. longa	1.4 ± 0.3*	13.4 ± 1.8*	9.6
C. aromatica	1.2 ± 0.4*	18.8 ± 2.4*	15.7
C. zedoaria	1.3 ± 0.8*	16.6 ± 2.5*	12.8
curcumin	3.1 ± 0.6*	6.3 ± 0.5*	2.0

Caco-2 cells were treated with *Curcuma* drugs (0.1 mg/ml) or curcumin (30 μM) for 72 h. The transport experiment was performed in the absence of *Curcuma* drugs/curcumin. Positive Control groups were pretreated with verapamil (100 μM) for 30 min and during the experiment. Results are means ± S.E.M. from triplicate experiments.

* $P < 0.01$ compared with control.

3.3. Effects of Curcuma drugs and curcumin on P-gp and MDR-1 expression

To investigate the affects of *Curcuma* drugs (0.1 mg/ml) and curcumin (30 μM) on P-gp and *MDR-1* expression, after Caco-2 cell monolyers were treated with *Curcuma* drugs and curcumin for 72 h, the lysates and total mRNA were recovered for western blot and

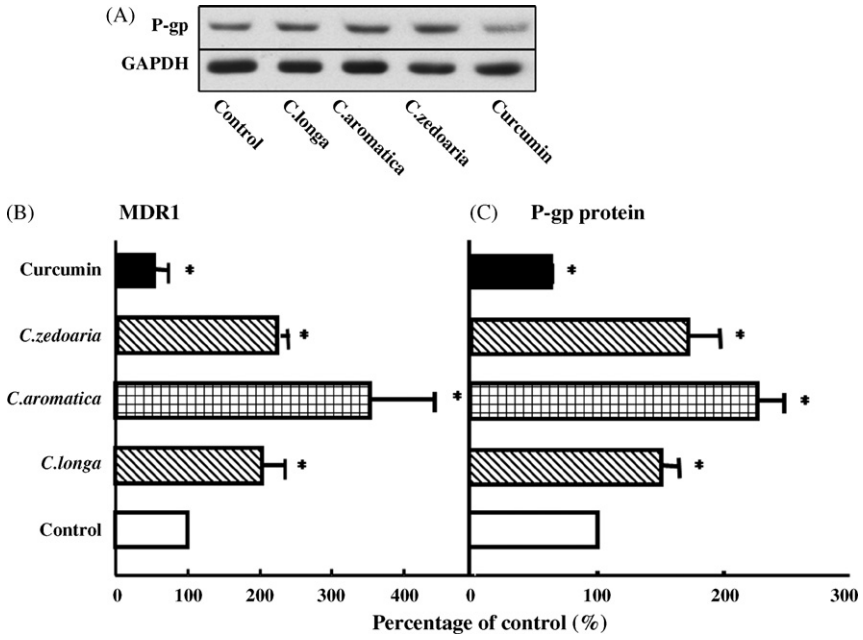

Fig. 3. Effects of *Curcuma* drugs/curcumin on *MDR1* and P-gp protein expression levels. Caco-2 cells were treated with *Curcuma* drugs (0.1 mg/ml) or curcumin (30 μM) for 72 h. After removing *Curcuma* drugs/curcumin, total mRNA or cell lysates were prepared for real-time RT-PCR or Western blot. (A) Representative Western blot for P-gp. (B) Quantitative analysis of *MDR1*. The results were normalized with that of *β-actin*. Results are means ± S.E.M. from triplicate experiments. $^{*}P < 0.01$ compared with control. (B) Quantitative analysis of P-gp immunoactive protein. The band intensities were normalized with that of GAPDH. Results are means ± S.E.M. from triplicate experiments. $^{*}P < 0.01$ compared with control.

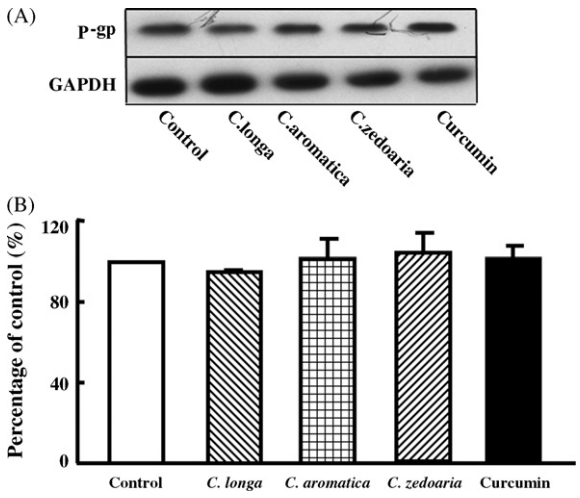

Fig. 4. Reversibility of P-gp protein up/down-regulation by *Curcuma* drugs/curcumin. Caco-2 cells treated with *Curcuma* drugs/curcumin for 72 h were cultured for another 72 h under standard conditions without *Curcuma* drugs/curcumin. Then, cell lysates were prepared for Western blot. (A) Representative Western blot for P-gp. (B) Quantitative analysis of P-gp immunoactive protein. The band intensities were normalized with that of GAPDH. Results are means ± S.E.M. from triplicate experiments.

SYBR® GREEN I real time RT-PCR, respectively. As shown in Fig. 3, curcumin significantly decreased the expression levels of P-gp and *MDR1* by 48% and 35%. On the contrary, all *Curcuma* drugs up-regulated the expression levels of P-gp and *MDR1*. Especially, *C. aromatica* exhibited significant inductive effects on P-gp and *MDR1* expression levels by 2.3 and 3.5 fold, respectively.

3.4. Reversibility of cellular P-gp expression and function

To study the reversibility of cellular P-gp expression and function, Caco-2 monolayers were treated with *Curcuma* drugs (0.1 mg/ml) or curcumin (30 μM) for 72 h, then the monolayer was cultured in medium free of *Curcuma* drugs/curcumin for 72 h. P-gp function was evaluated by rhodamine 123 efflux and P-gp expression was determined by Western blot. As shown in Figs. 4 and 5, after 72 h' washout, the P-gp function and expression level of both *Curcuma* drugs treated monolayers and curcumin treated monolayers have reverted to the baseline level as control.

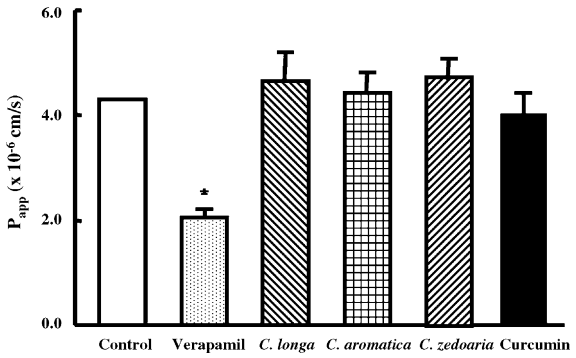

Fig. 5. Reversibility of P-gp activity up/down-regulation by *Curcuma* drugs/curcumin. Caco-2 cells treated with *Curcuma* drugs/curcumin for 72 h were cultured for another 72 h under standard conditions without *Curcuma* drugs/curcumin. The efflux of rhodamine 123 (10 μM) was measured. Positive Control groups were pretreated with verapamil (100 μM) for 30 min and during the experiment. Results are means ± S.E.M. from triplicate experiments. $^{*}P < 0.01$ compared with control.

4. Discussion

Intestinal P-gp plays an important role in the absorption and pre-systemic elimination of many peroral xenobiotics, including drugs. In this study, we evaluated the affects of *Curcuma* drugs and curcumin on the expression and function of intestinal P-gp. Using Caco-2 monolayers as a model of small intestine, we found that *Curcuma* drugs might influence drug disposition by up-regulating the expression and function of intestinal P-gp. Interestingly, our results showed that the main pigment from *Curcuma* drugs, curcumin, exhibited an opposite effect to *Curcuma* drugs by inhibiting the expression and function of P-gp. Furthermore, it was proved that these affects were reversible after 72 h' washout. To our knowledge, this is the first report about the effects of *Curcuma* drugs on expression and function of P-gp, this is also the first report about the relationship between the effects of *Curcuma* drugs and curcumin on P-gp. Moreover, *Curcuma* drugs identified by molecular biological method were used in this study.

In 1998, Romiti et al. (1998) demonstrated that curcumin (25 μM) treatment for 72 h would inhibit the function and expression of P-gp in primary cultures of rat hepatocytes. Anuchapreeda et al. (2006) further verified that curcumin can decrease the expression of *MDR1* in leukemic cells recovered from patients. Moreover, Zhang et al. (2007) showed that oral administration of curcumin to male Sprague-Dawley rat decreased the expression of intestine P-gp, and further modulated the pharmacokinetics of peroral celiprolol, which is a substrate of P-gp. However, attention should be paid to the reports that little curcumin is detected in blood after curcumin administration with 4–8 g/day because curcumin is rapidly metabolized to its metabolites (Lin and Pan Lin-Shiau, 2000; Ireson et al., 2002). It makes no sense to treat hepatocytes or leukemic cells directly with curcumin. On the other hand, species differences have been observed between human and rat P-gp when prototypical inducers were added to induce the activity of P-gp in human and rat hepatocytes (Nishimura et al., 2006). Thus, it is quite necessary to confirm the affects of curcumin and *Curcuma* drugs on intestinal P-gp using human-derived materials. Using Caco-2 cells, our study confirmed the inhibitory effects of curcumin on the function and expression of P-gp. Although the *MDR1* down-regulation mechanism keeps unclear from the present study, it is of interest to recall that both AP-1 transcriptional factor and nuclear factor kappa B (NF-kB) can regulate the expression of *MDR1* (Chen et al., 2005; Daschner et al., 1999; Kuo et al., 2002). Curcumin has been reported to be a potent inhibitor of both AP-1 transcriptional factor and NF-kB (Foryst-Ludwig et al., 2004; Divya and Pillai, 2006). On the other hand, Madan et al. (2001) has reported that *C. longa* can activate NF-kB.

Chearwae et al. has previously demonstrated that when the concentration was below 30 μM, curcumin exhibited dose-dependent inhibitory activity on the activity and expression of P-gp in multidrug-resistant KB cells (Anuchapreeda et al., 2002; Chearwae et al., 2004). Curcumin content in *C. longa* and *C. aromatica* extracts occupied only 3.9% (10.6 μM) and 1.2% (3.3 μM), respectively. Moreover, there was no curcumin content was detected in *C. zedoaria* extract. Therefore, we speculated that there must be at least one compound owing P-gp inductive activity in *Curcuma* drugs. *Curcuma* rhizomes are multi-component systems. Except for curcumin, some essential oils, such as β-bisabolene, β-sesquiplellendrene and ar-curcumene which belong to bisabolane type and curcumenol, curcumol, which belong to guaiane type, have been isolated from *Curcuma* rhizomes. Reports showed that these essential oils exhibit multiple biological activities (Lai et al., 2004; Syu et al., 1998). The inductive mechanism of *Curcuma* drugs on P-gp must be very complicated because it might be a combined effect of some compound(s) with curcumin.

In this study, we reported that both *Curcuma* drugs and curcumin modulated the function of P-gp at the concentration of 0.1 mg/ml and 30 μM. Human clinical trials indicated no dose-limiting toxicity when curcumin was orally administrated up to 10 g/day (Cheng et al., 2001). Sharma et al. (2001) reported that *Curcuma* extract (curcuminoid:essential oil = 10:1) could be administrated safely at doses of up to 2.2 g/day. Taking the gastric fluid volume to be 1–3 l for an adult human with the body weight of 60 kg, the concentrations of *Curcuma* drugs and curcumin in the gastric fluid would be 0.7–2.2 mg/ml and 8.7–26.0 mM, respectively. It is possible for administrated *Curcuma* drugs and curcumin to affect the pharmacokinetics of co-administrated drugs by interfering with the intestinal P-gp function. Furthermore, it is also reasonable to speculate that *Curcuma* drugs co-administration might decrease the effect of cancer drugs, which are used to gastric cancer since the P-gp inhibitory activity has been reported as a mechanism of anti-cancer action.

In summary, the results of this study confirmed that 72 h' treatment of curcumin inhibited the expression and function of intestinal P-gp. Interestingly, we found that all *Curcuma* drugs' treatment significantly induced the expression and function of P-gp. These results suggested that *Curcuma* drugs and curcumin might alter the pharmacokinetics of drugs in completely different direction and caution should be exercised when *Curcuma* drugs or curcumin are to be consumed with drugs that are P-gp substrates. Further studies are needed to clarify the affects of *Curcuma* drugs and curcumin on P-gp in the clinic.

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