CHIRALITY 19:485–490 (2007)

Stereoselective Regulation of MDR1 Expression in Caco-2 Cells by Cetirizine Enantiomers

SHUIJIE SHEN, YING HE, AND SU ZENG*

Department of Pharmaceutical Analysis and Drug Metabolism, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China

ABSTRACT MDR1-encoded P-glycoprotein (P-gp) is a drug efflux transporter mainly expressed in liver, kidney, intestine, brain (at the level of the blood-brain barrier), and placenta. It thus plays important roles in drug absorption, distribution, and excretion. Cetirizine is a second-generation nonsedating antihistamine used to treat allergic disease of respiratory system, skin and eyes. To evaluate P-gp expression and function in Caco-2 cells pretreated with cetirizine enantiomers, we assessed the sensitivity of Caco-2 cells to paclitaxel using the MTT assay and the polarized transport of rhodamine-123 and doxorubicin across Caco-2 monolayers. RT-PCR and flow cytometry were used to assay MDR1 mRNA and P-gp protein respectively. The sensitivity of Caco-2 cells to paclitaxel decreased significantly after cells were pretreated with 100 μM *R*-cetirizine but increased upon treatment with *S*-cetirizine. The efflux of rhodamine-123 and doxorubicin was enhanced significantly after Caco-2 monolayers were pretreated with 100 μM *R*-cetirizine but was reduced by *S*-cetirizine. The MDR1 mRNA and P-gp levels in Caco-2 cells were increased by 100 μM *R*-cetirizine and decreased by 100 μM *S*-cetirizine. These results suggest that *R*-cetirizine up-regulates MDR1 expression while *S*-cetirizine down-regulates MDR1 expression. *Chirality 19:485–490, 2007.*

KEY WORDS: MDR1; P-glycoprotein; Caco-2 cells; cetirizine; stereoselective; drug–drug interaction

INTRODUCTION

MDR1 or P-glycoprotein (P-gp) is a multidrug efflux transporter, which plays an important role in drug absorption, distribution, and excretion. It has been reported that P-gp can be induced or suppressed by a variety of drugs or xenobiotics.[1–3] The regulation of P-gp could influence the pharmacokinetic behavior of other drugs in vivo and may lead to drug–drug interactions by altering intestinal absorption, brain distribution, proximal renal-tubular excretion, or biliary excretion. Therefore, these regulatory effects should be estimated when two or more drugs are coadministrated.

Caco-2 cells originate from human colorectal carcinoma and used as a model for intestinal epithelial cells. It is well known that Caco-2 cells express P-gp and other ATP-binding cassette proteins such as multidrug resistant-associated proteins (MRPs).[4]

Cetirizine is a highly efficacious and long-acting second-generation nonsedating antihistamine for the treatment of allergic disorders of the respiratory tract, skin, and eyes.[5] *R*-cetirizine (levocetirizine) has high affinity to H_1 receptor but *S*-cetirizine has no antihistaminic efficacy.[6] Unlike first-generation antihistamines, cetirizine is nonsedating. Since it is transported by P-gp located at the brain-blood barrier, only a small amount of the drug reaches the central nervous system (CNS).[7] Cetirizine is a substrate of P-gp but it is still unclear whether it has any effect on MDR1 expression.

Enantiomers often have different pharmacological, toxicological, pharmacokinetic (PK), and pharmacodynamic (PD) characteristics[8] including their transport by P-gp,[9] but do enantiomers behave differently with regard to MDR1 expression? In this study, the toxicity of paclitaxel to Caco-2 cells was compared with and without pretreatment with cetirizine enantiomers; polarized transport of rhodamine-123 and doxorubicin across Caco-2 monolayers were also estimated. Reverse transcriptase-PCR (RT-PCR) and flow cytometry techniques were used to assay MDR1 mRNA and protein expression. We found that cetirizine enantiomers can regulate MDR1 expression in Caco-2 cells and that these regulatory effects were totally different.

MATERIALS AND METHODS

Chemicals

Enantiomers of cetirizine hydrochloride were provided by Minsheng Pharmaceutical Industry Group (Hangzhou, China). Enantiomers of cetirizine hydrochloride were

*Correspondence to: Prof. Su Zeng, Department of Drug metabolism and Pharmaceutical Analysis, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, 310058, China. E-mail: zengsu@zju.edu.cn
Received for publication 5 September 2006; Accepted 8 February 2006
DOI: 10.1002/chir.20400
Published online 12 April 2007 in Wiley InterScience (www.interscience.wiley.com).

prepared as 20.0 mM DMSO stock solutions. These two stock solutions were diluted with HBSS (137 mM NaCl, 5.4 mM KCl, 1.3 mM $CaCl_2$, 0.4 mM $MgSO_4$, 0.5 mM $MgCl_2$, 0.3 mM Na_2HPO_4, 0.4 mM KH_2PO_4, 4.2 mM $NaHCO_3$, 5.6 mM glucose, and 25 mM HEPES) before use. DMSO concentration did not exceed 0.5% in culture medium or incubation buffer. Paclitaxel was a product of Sichuan Taiji Pharmaceutical (Chengdu, China). Camptothecin was obtained from Sichuan Jiangyuan Natural Products (Chengdu, China). Lucifer yellow, rhodamine-123 and doxorubicin were purchased from Sigma (St Louis, Mo). All other chemicals were obtained commercially and were of analytical grade requiring no further purification.

Cell culture

Caco-2 cells (51–55 passages) obtained from the Chinese Academy of Medical Sciences (CAMS, Beijing, China) were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM, Gibco, NY) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, NY), 1% nonessential amino acid (Gibco), 100 U/ml penicillin G and 100 μg/ml streptomycin sulfate. Cells were grown in a humidified atmosphere of 5% CO_2 at 37°C.

Paclitaxel Resistance of Caco-2 Cells

Caco-2 cells were digested and seeded in 25 cm^2 flask at a density of 8×10^5 cells/cm^2. The next day, Caco-2 cells were pretreated with series of concentrations of *R*- or *S*-cetirizine (10.0, 20.0, 50.0, 100.0, 200.0.0 μM) in culture medium. Blank control was set up by adding the same volume of DMSO. After cultured for another 48 h, cells were harvested and resuspended in complete medium without cetirizine after rinsed with HBSS twice. Cells were seeded at 96-well plate at a density of 7.5×10^4 cells/ml, 100 μl per well. After cultured for 24 h, culture medium was replaced with 200.0 μl fresh complete medium containing 0.005, 0.02, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, 100.0 μg/ml of paclitaxel, three triplicates for each concentration. Blank control was set up by adding the same volume of DMSO.

After incubation of Caco-2 cells with paclitaxel for 48 h at 37°C, 20 ml MTT solution (5 mg/ml in PBS) was added to each well. Culture medium was removed carefully after further incubation for 4 h at 37°C and 150 ml DMSO was added to each well. The plate was shaken for 15 min using an orbital mixer. The absorbance at 570 nm was measured using a plate reader (SPECTRAmax M2, Molecular Devices). Growth inhibition rate was calculated according to eq. 1. The IC_{50} values of paclitaxel in Caco-2 cells with or without cetirizine pretreatment were derived from the "four parameter logistic equation" (also called "Hill Equation," eq. 2) using the SoftMax Pro 4.7.1 software (Molecular Devices).

$$\%\text{Inhibition} = \left[1 - \frac{\overline{OD}_{\text{treated}}}{\overline{OD}_{\text{nontreated}}}\right] \times 100 \qquad (1)$$

$$\%\text{Inhibition} = \left[\frac{A - D}{1 + (\frac{C}{IC_{50}})^B} + D\right] \times 100 \qquad (2)$$

In eq. 1, where $\overline{OD}_{\text{treated}}$ = mean OD value of wells treated by paclitaxel and $\overline{OD}_{\text{nontreated}}$ = mean OD value of wells nontreated by paclitaxel. In eq. 2, where A = maximum inhibition rate of paclitaxel; B = Hill coefficient or called Hillslope; C = paclitaxel concentration; D = inhibition rate of blank control.

Rhodamine-123 and Doxorubicin Transport Across Caco-2 Monolayers

Caco-2 cells were harvested with 0.25% trypsin-0.02% EDTA solution when reaching 80% confluence and seeded at Transwell inserts (12 mm i.d., 1.13 cm^2 in bottom area, Corning Costar) in 12-well plates at a density of 1.0×10^5 cells/cm^2. Culture medium was replaced every other day for the first 14 days and daily for next 7 days until the monolayers expressed differentiated properties that closely resemble morphologic and functional characteristics of normal enterocytes. The integrity of the monolayer was checked by measuring the transepithelial electrical resistance (TEER) value across the monolayer using a Millicell-ERS Voltohmmeter (Millipore) and monitoring the permeability of the paracellular leakage marker lucifer yellow across the monolayer. The cell monolayers were considered adequate enough for transport experiments when the P_{app} for lucifer yellow was $\leq 0.2 \times 10^6$ cm/s and TEER value $\geq 550\ \Omega \cdot cm^2$.

Caco-2 cell monolayers were washed twice by HBSS after the basolateral and apical side culture media were removed. The cell monolayers were pretreated with *R*- or *S*-cetirizine. Briefly, HBSS containing 10.0, 20.0, 50.0, 100.0, 200.0 μM of *R*- or *S*-cetirizine was added to both sides of the Transwell inserts. A blank control was run on the same plate by adding HBSS. The incubation medium was removed after incubation for 1 h at 37°C, and cells were washed carefully three times with HBSS. The Caco-2 monolayers were then ready for rhodamine-123 and doxorubicin transport experiments.

In the uptake experiments, rhodamine-123 and doxorubicin stock solutions were diluted to 5.0 and 30.0 μM respectively in HBSS and 0.5 ml substrate solution was added to the apical side and 1.5 ml HBSS to the basolateral side. In the efflux experiments, donor solutions were added to the basolateral side of the monolayer and HBSS was added to the apical side. This transport device was shaken for 2 h at 37°C using an orbital shaker. After incubation, receiving solution was collected to a 96-well clear flat bottom black plate (FLUOTRAC™ 200, Greiner Microlon) and fluorescence intensity was determined using a plate reader (SPECTRA max M2, Molecular Devices). For rhodamine-123, λ_{ex} was set at 500 nm, λ_{em} 540 nm, and for doxorubicin, λ_{ex} was set at 480 nm, λ_{em} 590 nm. Apparent permeation coefficient (P_{app}) and efflux ratio (ER) values were calculated using eqs. 3 and 4.

$$P_{\text{app}} = \frac{dQ}{dt \cdot A \cdot C_0} \qquad (3)$$

$$\text{Efflux Ratio} = \frac{P_{\text{app(BL–AP)}}}{P_{\text{app(AP–BL)}}} \qquad (4)$$

TABLE 1. The IC_{50} values of paclitaxel in Caco-2 cells pretreated with cetirizine enantiomers

Concentration (μmol/M)	IC_{50} (μmol/M)		Relative resistance[a]	
	R-cetirizine	*S*-cetirizine	*R*-cetirizine	*S*-cetirizine
Non-treated	9.847 ± 0.854		–	–
10.0	9.137 ± 0.876	10.094 ± 0.746	0.928	1.025
20.0	9.917 ± 0.973	9.864 ± 0.594	1.007	1.002
50.0	10.718 ± 0.879	8.842 ± 1.164	1.088	0.898
100.0	13.222 ± 0.631*	7.713 ± 0.967*	1.343	0.783
200.0	16.300 ± 0.659*	6.085 0.901*	1.655	0.618

The values are the mean ± SD of three independent experiments.
[a]The relative resistance = IC_{50} values in the *R*-cetirizine (*S*-cetirizine) treated Caco-2 cells/IC_{50} values in nontreated Caco-2 cells.
*The IC_{50} was statistically different from that of nontreated Caco-2 cells, $P < 0.05$.

In eq. 3, C_0 is the initial concentration of the substrate on the donor side; dQ/dt represents the substrate transport rate. A is cell monolayer area, here $A = 1.13$ cm^2. In eq. 4, $P_{app(BL\text{-}AP)}$ represents the P_{app} value from the basolateral side to the apical side and $P_{app(AP\text{-}BL)}$ represents the P_{app} value from the apical to basolateral side.

Reverse Transcriptase-PCR Analysis of MDR1 mRNA in Caco-2 Cells

Caco-2 cells were seeded in 25 cm^2 cell flasks at a density of 2×10^5 cells/cm^2. Cells were cultured for 10 days at 37°C, 5% CO_2, and culture medium was changed every 2 days. Then, the culture medium was replaced by fresh medium containing 100.0 μM *R*- or *S*-cetirizine. A blank control was set up by adding the same volume of solvent to culture medium. After incubation for 24 h, MDR1 mRNA was evaluated by RT-PCR. Total RNA was isolated using TRizol reagent (Bio Basic Inc., Canada) and dissolved in 50 μl RNase-free water. MDR1 and β-globin cDNA were synthesized using M-MuLV reverse transcriptase (Fermentas, Lithuania) with Oligo$(dT)_{18}$ random primer from 0.1 μg total RNA. Primer MDR1-F (5′ CCC ATC ATT GCA TAT GCA GG) and MDR1-R (5′ GTT CAA ACT TCT ACT CCT GA) were used to amplify MDR1,[10] product size 157, annealing temperature 58°C. Primer globin-F (5′ CAA CTT CAT CCA CGT TCA CC) and globin-R (5′ GAA GAG CCA AGG ACA GGT AC) were used to amplify human β-globin, product size 268, annealing temperature 58°C. β-globin was used as a loading control. PCR reaction was performed in a 25 μl system with Taq DNA polymerase (Sangon, Shanghai) in a thermal cycler (Mastercycler gradient, Eppendorf, Germany), 35 cycles for each gene. PCR products were separated on 2% agarose gel containing 0.5 μg/ml ethidium bromide and photographed with a gel analysis system (Rio-Rad, Italy). Band volume data were acquired using Rio-Rad Quantity One software.

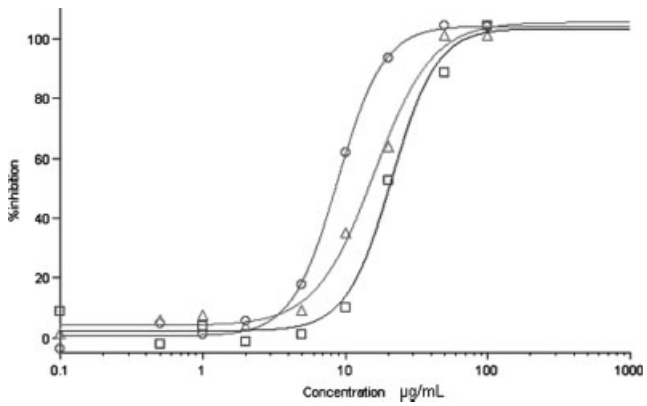

Fig. 1. The growth inhibitory curves of paclitaxel in Caco-2 cells pretreated with or without cetirizine enantiomers. Cells were pretreated with or without 100.0 μmol/M cetirizine enantiomers for 48 h at 37°C, and then the cytotoxicity of paclitaxel was evaluated. △: nontreated cells, □: cells treated with *R*-cetirizine, ○: cells treated with *S*-cetirizine.

Flow Cytometry Analysis of MDR1 Protein Expression in Caco-2 Cells

Caco-2 cells were seeded in cells flasks and pretreated with 100.0 μM *R*- or *S*-cetirizine for 72 h with the same method as described above. After pretreatment, cells were digested and resuspended in phosphate buffered saline (PBS; 137 mM NaCl, 2.7 mM KCl, 1.5 mM KH_2PO_4, and 8.1 mM Na_2HPO_4) at a density of 2×10^6 cell/ml. Five microliter of rhodamine B isothiocyanate (RBITC) labeled P-gp antibody (Biosynthesis Biotechnology, Beijing) was added to 50 μl cell suspension, mixed gently, and incubated for 30 min at 4°C. The cell suspension was centrifuged for 5 min at 1000 rpm. Cells were resuspended in 400 μl PBS, and then the fluorescence intensity was measured with a flow cytometer (FACScan, Becton-Dickinson, San Jose, CA). Fluorescence signal was collected through a 530 ± 30 nm band-pass filter. CellQuest Pro software from BD was used for acquisition and analysis of data.

Data Statistical Analysis

Data were expressed as mean ± S.D. Statistical analyses were performed using SPSS (version 12.0) Software. Analysis of variance (ANOVA) was used to compare differences between *R*- and *S*-cetirizine pretreatment. Differences were considered as statistically significant at $P \leq 0.05$.

RESULTS

Paclitaxel Resistance of Caco-2 Cells

The IC_{50} value of paclitaxel in Caco-2 cells increased after *R*-cetirizine pretreatment, but decreased after *S*-cetirizine pretreatment. Table 1 presents the effects of pre-

TABLE 2. Effects of cetirizine enantiomers on the transport of rhodamine-123 and doxorubicin

Cetirizine (μmol/l)		Rhodamine-123 (5.0 μmol/l)			Doxorubicin (30.0 μmol/l)		
		*P*app ($\times 10^{-6}$ cm/s)			*P*app ($\times 10^{-6}$ cm/s)		
		AP→BL	BL→AP	ER	AP→BL	BL→AP	ER
Non-treated		0.712 ± 0.040	6.850 ± 0.309	9.62	0.203 ± 0.021	1.322 ± 0.029	6.51
10.0	R	0.625 ± 0.058	7.106 ± 0.556	11.4	0.226 ± 0.029	1.374 ± 0.098	6.08
	S	0.763 ± 0.082	7.965 ± 0.215	10.4	0.189 ± 0.020	1.201 ± 0.117	6.35
20.0	R	0.720 ± 0.104	7.666 ± 0.681	10.6	0.216 ± 0.039	1.340 ± 0.158	6.20
	S	0.738 ± 0.068	7.933 ± 0.917	10.7	0.276 ± 0.015	1.750 ± 0.098	6.34
50.0	R	0.837 ± 0.081	7.702 ± 0.745	9.20	0.248 ± 0.019	1.466 ± 0.207	5.91
	S	0.892 ± 0.062	8.878 ± 0.635	9.95	0.236 ± 0.026	1.291 ± 0.136	5.47
100.0	R	0.578 ± 0.041	8.638 ± 0.460	14.9	0.231 ± 0.020	2.011 ± 0.090	8.70
	S	0.954 ± 0.068	7.002 ± 0.621	7.34	0.400 ± 0.059	1.820 ± 0.143	4.55
200.0	R	0.550 ± 0.347	8.715 ± 0.109	15.8	0.154 ± 0.010	1.396 ± 0.114	9.06
	S	1.318 ± 0.116	6.506 ± 0.244	4.94	0.259 ± 0.031	0.870 ± 0.082	3.36

The permeability of rhodamine-123 and doxorubicin were determined in Caco-2 cell monolayers pretreated with cetirizine enantiomers. Each value represents the mean ± S.D. for three independent monolayers.

treatment with cetirizine enantiomers on the IC_{50} value of paclitaxel to Caco-2 cells. Low concentrations of cetirizine enantiomers had no significant effects on IC_{50} value until concentration reached 100.0 μM. Compared with controls, the IC_{50} value of paclitaxel in Caco-2 cells pretreated with 100.0 and 200.0 μM of *R*-cetirizine was increased by 34 and 65% and decreased by 21 and 38% upon pretreatment with 100.0 and 200.0 μM of *S*-cetirizine, respectively. Figure 1 shows the growth inhibition curves by paclitaxel in Caco-2 cells pretreated with 100.0 μM of cetirizine enantiomers. The growth inhibition curve shifted in a concentration-dependent manner after the cells were pretreated with cetirizine enantiomers.

Polarized Transport of Rhodamine-123 and Doxorubicin Across Caco-2 Monolayers

Rhodamine-123 and doxorubicin are typical P-gp substrates and were selected as probes of P-gp expression and function in Caco-2 monolayers.[11] Table 2 summarizes the polarized transport of rhodamine-123 and doxorubicin across Caco-2 monolayers.

The ER values indicate the extent of efflux of the substrates. In the control group, rhodamine-123 and doxorubicin exhibited clear efflux, which indicated that Caco-2 monolayers expressed fully functional P-gp. The ER values of rhodamine-123 and doxorubicin increased significantly in a concentration-dependent manner after the monolayers were pretreated with 100.0 or 200.0 μM of *R*-cetirizine. On the contrary, pretreatments with 100.0 or 200.0 μM of *S*-cetirizine gave a decrease in ER. There were no obvious effects on rhodamine-123 and doxorubicin transport after the monolayers were pretreated with low concentration of cetirizine enantiomers.

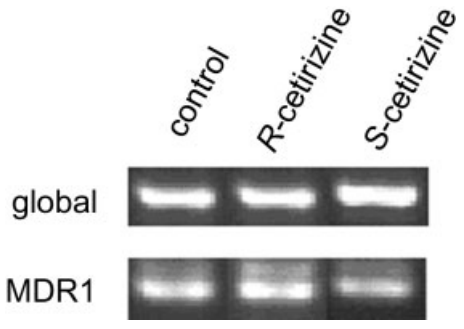

Fig. 2. Reverse transcriptase-PCR analysis of MDR1 mRNA in Caco-2 cells without or with 100 .0 μmol/l cetirizine enantiomers. globin is shown as a loading control.

MDR1 mRNA Level in Caco-2 Cells

The RT-PCR agarose gel is shown in Figure 2. The MDR1 mRNA level in Caco-2 cells increased by 47% after the cells were pretreated with 100.0 μM *R*-cetirizine for 24 h, while it decreased by 28% after the cells were pretreated with 100.0 μM *S*-cetirizine for 24 h (Table 3).

MDR1 Protein Level in Caco-2 Cells

After the cells were pretreated with 100.0 μM of *R*-cetirizine for 72 h, the mean fluorescence intensity increased by 35% compared with nontreated cells, which indicated that P-gp expression increased. Pretreatment with 100.0 μM of *S*-cetirizine for 72 h mean fluorescence intensity of P-gp expression decreased by 22%, which indicated a decrease (Fig. 3).

TABLE 3. The levels of MDR1 mRNA in Caco-2 cells treated with cetirizine enantiomers

Relative volume			Regulated	
Control	*R*-cetirizine	*S*-cetirizine	R/ Control	S/ Control
0.828 ± 0.162	1.220 ± 0.306	0.593 ± 0.159	1.47	0.72

Rio-Rad Quantity One software was used to acquire the band volume data.-Data were obtained from three independent experiments.
Relative Volume = $Volume_{(MDR1)}/Volume_{(globin)}$.

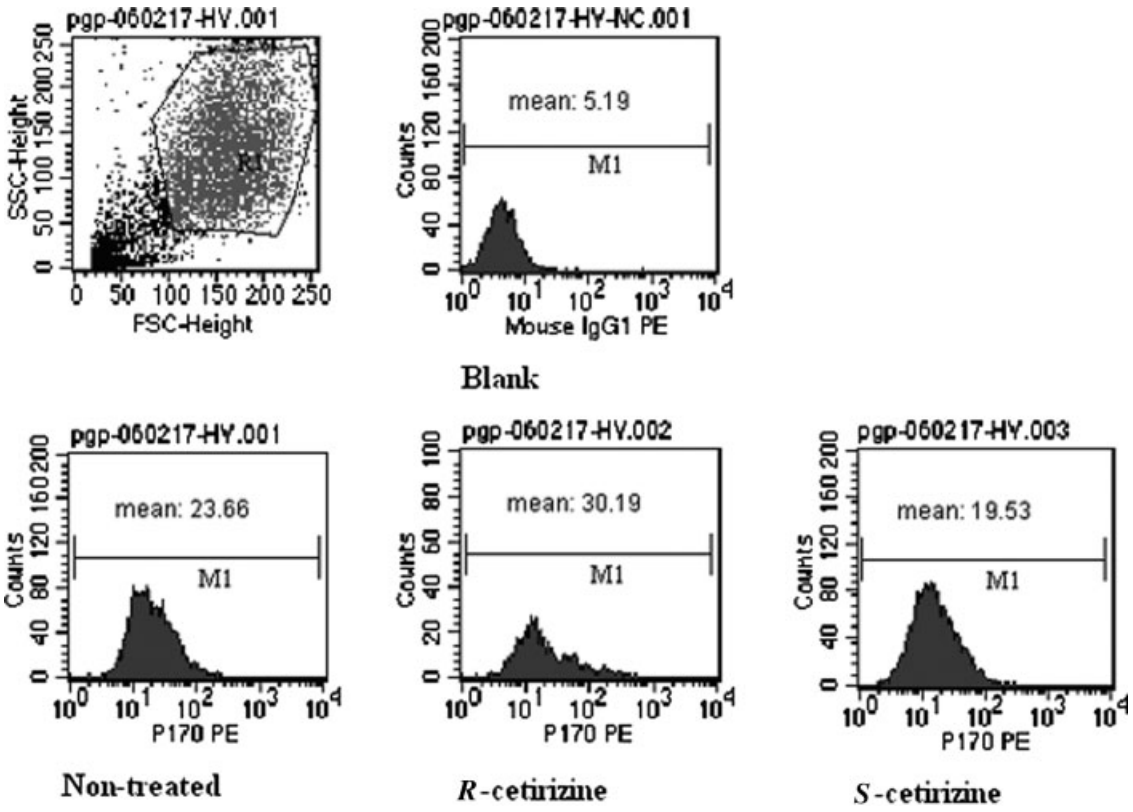

Fig. 3. Flow cytometry analysis of P-gp in Caco-2 cells pretreated with or without cetirizine enantiomers (100.0 μM). The mean values in the figures are fluorescent intensity. Fluorescence signal was collected through a 530 ± 30 nm band-pass filter. CellQuest Pro software from BD was used for acquisition and analysis of data.

DISCUSSION

It is well known that a large number of drugs are transported by P-gp, so alteration of P-gp expression may change the absorption, distribution, and excretion of these drugs. It has been recognized that MDR1 expression can be induced or inhibited by various types of drugs and herb drugs, food ingredients etc.[1–3,12–15] It seems that there is no structural or pharmacological relationship between them. Although induction or inhibition may not be perceptible in vivo, we must keep it in mind that it may take effect in certain circumstances. P-gp mediated drug–drug interactions occur when one drug is a substrate of P-gp and another drug can induce or inhibit P-gp expression or function significantly. Drug–drug interactions produced by P-gp induction or inhibition would cause drug toxicity or loss of pharmacological efficacy. For example, the serum concentration of digoxin, a substrate of P-gp, decreases when coadministrated with rifampin because rifampin induces P-gp expression.[16]

Fortunately, more attention has been paid to drug–drug interactions caused by P-gp induction or inhibition recently.[17–20] To avoid this kind of unwanted drug–drug interactions, the effect of a new drug on P-gp regulation should be estimated during preclinical drug development.

Our results showed that *R*-cetirizine up-regulated MDR1 expression while *S*-cetirizine down-regulated MDR1 expression in a concentration-dependent manner. Low concentrations of cetirizine enantiomers had no effect. The regulation of MDR1 could occur either at transcriptional or translational level. It seems that cetirizine acted at transcriptional level since the P-gp protein level varied with the mRNA level. P-gp protein level and mRNA level could be inferred from flow cytometry and reverse transcriptase-PCR data, and the increase or decrease ratio of MDR1 mRNA and protein level in Caco-2 cells treated with 100.0 μM of *R/S*-cetirizine were similar (about 30%, Table 3, Fig. 2).

It has been reported that MDR1 expression and function is regulated by many mechanisms and is related to many signal transduction pathways.[21–24] Alteration of gene transcription, mRNA stability and processing, protein stability, membrane incorporation, structure are involved in MDR1 regulation. This study is the first report of enantiomers having different regulatory effects on MDR1 expression and further research on the special regulation effects of cetirizine enantiomers would be helpful to understanding the mechanism of MDR1 regulation.

In addition, we must be aware that Caco-2 cells not only expresses P-gp but also MRPs and it is quite possible that MRPs were also involved in this experiment. Camptothecin, which is a substrate of P-gp and MRPs but more sensitive to MRPs than paclitaxel,[25] was selected to evaluate its cytotoxicity to Caco-2 cells with or without cetirizine enantiomers pretreatment. The results showed that the growth inhibition curve of camptothecin shifted similarly with paclitaxel both in direction and extent. Therefore, MRPs

may also be regulated like P-gp. Further experiments are required to validate this hypothesis.

LITERATURE CITED

1. Tabe Y, Konopleva M, Contractor R, Munsell M, Schober WD, Jin L, Tsutsumi-Ishii Y, Nagaoka I, Igari J, Andreeff M. Up-regulation of MDR1 and induction of doxorubicin resistance by histone deacetylase inhibitor depsipeptide (FK228) and ATRA in acute promyelocytic leukemia cells. Blood 2006;107:1546–1554.
2. Han Y, Tan TM, Lim LY. Effects of capsaicin on P-gp function and expression in Caco-2 cells. Biochem Pharmacol 2006;71:1727–1734.
3. Chung SY, Sung MK, Kim NH, Jang JO, Go EJ, Lee HJ. Inhibition of P-glycoprotein by natural products in human breast cancer cells. Arch Pharm Res 2005;28:823–828.
4. Hidalgo IJ, Li J. Carrier-mediated transport and efflux mechanisms in Caco-2 cells. Adv Drug Deliver Res 1996;22:53–66.
5. Polli JW, Baughman TM, Humphreys JE, Jordan KH, Mote AL, Salisbury JA, Tippin TK, Serabjit-Singh CJ. P-glycoprotein influences the brain concentrations of cetirizine (Zyrtec), a second-generation nonsedating antihistamine. J Pharm Sci 2003;92:2082–2089.
6. Devalia JL, De Vos C, Hanotte F, Baltes E. A randomized, double-blind, crossover comparison among cetirizine, levocetirizine, and ucb 28557 on histamine-induced cutaneous responses in healthy adult volunteers. Allergy 2001;56:50–57.
7. Chen C, Hanson E, Watson JW, Lee JS. P-glycoprotein limits the brain penetration of nonsedating but not sedating H1-antagonists. Drug Metab Dispos 2003;31:312–318.
8. Zeng S. Chiral drug and chiral pharmacology. Hangzhou: Zhejiang University Publishing House; 2002.
9. He Y, Zeng S. Determination of the stereoselectivity of chiral drug transport across Caco-2 cell monolayers. Chirality 2006;18:64–69.
10. Takara K, Takagi K, Tsujimoto M, Ohnishi N, Yokoyama T. Digoxin up-regulates multidrug resistance transporter (MDR1) mRNA and simultaneously down-regulates steroid xenobiotic receptor mRNA. Biochem Biophys Res Commun 2003;306:116–120.
11. van der Sandt IC, Blom-Roosemalen MC, de Boer AG, Breimer DD. Specificity of doxorubicin versus rhodamine-123 in assessing P-glycoprotein functionality in the LLC-PK1, LLC-PK1:MDR1 and Caco-2 cell lines. Eur J Pharm Sci 2000;11:207–214.
12. Kubota H, Ishihara H, Langmann T, Schmitz G, Stieger B, Wieser HG, Yonekawa Y, Frei K. Distribution and functional activity of P-glycoprotein and multidrug resistance-associated proteins in human brain microvascular endothelial cells in hippocampal sclerosis. Epilepsy Res 2006;68:213–228.
13. Gurley BJ, Barone GW, Williams DK, Carrier J, Breen P, Yates CR, Song PF, Hubbard MA, Tong Y, Cheboyina S. Effect of milk thistle (*Silybum marianum*) and black cohosh (*Cimicifuga racemosa*) supplementation on digoxin pharmacokinetics in humans. Drug Metab Dispos 2006;34:69–74.
14. Wang EJ, Barecki-Roach M, Johnson WW. Quantitative characterization of direct P-glycoprotein inhibition by St John's wort constituents hypericin and hyperforin. J Pharm Pharmacol 2004;56:123–128.
15. Zhou S, Lim LY, Chowbay B. Herbal modulation of P-glycoprotein. Drug Metab Rev 2004;36:57–104.
16. Greiner B, Eichelbaum M, Fritz P, Kreichgauer HP, von Richter O, Zundler J, Kroemer HK. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. J Clin Invest 1999;104:147–153.
17. Lin JH. Drug-drug interaction mediated by inhibition and induction of P-glycoprotein. Adv Drug Deliv Rev 2003;55:53–81.
18. DuBuske LM. The role of P-glycoprotein and organic anion-transporting polypeptides in drug interactions. Drug Saf 2005;28:789–801.
19. Wang JS, DeVane CL, Gibson BB, Donovan JL, Markowitz JS, Zhu HJ. Population pharmacokinetic analysis of drug-drug interactions among risperidone, bupropion, and sertraline in CF1 mice. Psychopharmacology (Berl) 2006;183:490–499.
20. Ejsing TB, Pedersen AD, Linnet K. P-glycoprotein interaction with risperidone and 9-OH-risperidone studied in vitro, in knock-out mice and in drug-drug interaction experiments. Hum Psychopharmacol 2005;20:493–500.
21. Hu Z, Jin S, Scotto KW. Transcriptional activation of the MDR1 gene by UV irradiation. Role of NF-Y and Sp1. J Biol Chem 2000;275:2979–2985.
22. Ohga T, Uchiumi T, Makino Y, Koike K, Wada M, Kuwano M, Kohno K. Direct involvement of the Y-box binding protein YB-1 in genotoxic stress-induced activation of the human multidrug resistance 1 gene. J Biol Chem 1998;273:5997–6000.
23. Rohlff C, Glazer RI. Regulation of the MDR1 promoter by cyclic AMP-dependent protein kinase and transcription factor Sp1. Int J Oncol 1998;12:383–386.
24. Raggers RJ, Vogels I, van Meer G. Upregulation of the expression of endogenous Mdr1 P-glycoprotein enhances lipid translocation in MDCK cells transfected with human MRP2. Histochem Cell Biol 2002;117:181–185.
25. Lalloo AK, Luo FR, Guo A, Paranjpe PV, Lee SH, Vyas V, Rubin E, Sinko PJ. Membrane transport of camptothecin: facilitation by human P-glycoprotein (ABCB1) and multidrug resistance protein 2 (ABCC2). BMC Med 2004;2:16.