# Stereoselective and Multiple Carrier-Mediated Transport of Cetirizine Across Caco-2 Cell Monolayers with Potential Drug Interaction

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*ABSTRACT* The aim of this study was to explore potential transport mechanisms of cetirizine enantiomers across Caco-2 cells. Cetirizine displayed polarized transport at concentrations ranging from 4.0 to 80.0 μM, with the permeability in the secretory direction being 1.4- to 4.0-fold higher than that in the absorptive direction. Cetirizine enantiomers were transported distinctively different from each other. In the presence of inhibitors of P-glycoprotein (P-gp) and multidrug resistance-associated protein (MRP), the absorptive transport was enhanced and secretory efflux was diminished. When verapamil, indomethacin, or probenecid were present, the difference in the absorptive permeability of R-cetirizine and S-cetirizine substantially intensified, whereas quinidine could eliminate. R-cetirizine significantly increased the efflux ratio of rhodamine-123 and doxorubicin in a fashion indicative of the upregulation of P-gp and MRP activities. However, S-cetirizine played a role of an inhibitor for P-gp and MRP. Ranitidine modified the absorption of cetirizine enantiomers, suggesting that the potential drug–drug interaction would significantly change the cetirizine pharmacokinetics. In conclusion, the results indicated that there are several efflux transporters including P-gp and MRP participating the absorption and efflux of cetirizine, which showed enantioselectivity in the transmembrane process. In addition, both P-gp and MRP functions could be modulated by cetirizine in chiral discriminative ways. *Chirality 22:684–692, 2010.* 

*KEY WORDS:* P-gp; MRP; Caco-2 cells; cetirizine; enantioselective; drug interaction

## INTRODUCTION

Cetirizine (Zyrtec®), the nonsedating antihistamine, is commonly used as the first-line agent for the treatment of seasonal and perennial allergic rhinitis and chronic idiopathic urticaria. It is one of the second generation H1-histamine receptor antagonists that shows some significant advantages beyond the first generation compounds.[1] It has demonstrated excellent efficacy and a favorable safety profile related to its low metabolism and absence of cardiac effects.

Cetirizine is a racemic mixture of R-cetirizine (now available under the trademark Xyzal®) and S-cetirizine. In most medicinal racemates, one of the enantiomers is more active than the other, and a number of studies have shown that cetirizine and its enantiomers follow this rule.[2] Optically pure cetirizines show some stereoselective pharmacological effects. S-cetirizine possesses potent activity in treating seasonal and perennial allergic rhinitis, the symptoms of allergic asthma, chronic idiopathic urticaria, some types of physical urticaria, and other disorders including those that would benefit from an inhibitory action on eosinophil function, S-cetirizine, which is not presently commercially available is more useful for the treatment of urticaria. It provides this effective treatment while avoiding the concomitant liability of adverse effects associated with the administration of racemic cetirizine by providing an amount which is insufficient to cause adverse effects associated with the administration of racemic. R-cetirizine is preferred in the treatment of allergic disorders, and it avoids the adverse effects including, but not limited to, sedation and somnolence, headache, gastrointestinal disturbance, dizziness, nausea, cardiac arrhythmias, and other cardiovascular effects. These results are due to the highly sensitive nature of antihistamine activity upon the precise stereochemistry between the drug and histamine receptor.[3] R-cetirzine has demonstrated a twofold higher affinity for the human H1-receptor compared with cetirizine, and ~30-fold higher affinity than S-cetirizine did.[4] Thus, the anti-H1 activity of cetirizine is primarily attributed



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to R-cetirizine, the eutomer, whereas S-cetirizine can be considered as the distomer.

The therapeutic difference of cetirizine enantioners may have relation with the absorption, distrubution, metabolism, and excretion (ADME) profile of each enantiomer. Absorption within the gastrointestinal tract is the first step governing the entry of drug in bloodstream and eventually its tissue distribution. Therefore, intestinal absorption and secretion is of special importance and should be investigated to fully understand the mechanism which is responsible for the enantioselective pharmacokinetic properties of cetirizine. The pharmacokinetic properties of cetirizine enantiomers have been studied in vivo, but no work has been reported on the comparative absorptive behaviors of the two enantiomers.[5] In a previous in vitro studies, we investigated that R-cetirizine upregulates MDR1 expression, whereas S-cetirizine downregulates MDR1 expression.[6] In this study, we investigated the intestinal absorptive differences between the two enantiomers in the Caco-2 cell model system.

Caco-2 cells originate from a human colorectal carcinoma and spontaneously differentiate on microporous filter membranes into polarized monolayers. They acquire many features of absorptive intestinal cells during culture such as microvillons structure, hydrolysis enzymes, and carrier-mediated transport systems for sugar, amino acids, and several drugs.[7] Caco-2 cells also express several efflux transport proteins that may hamper drug's absorption. These properties make the system particularly useful as an in vitro model for determining a drug's absorptive characteristics, studying the transport mechanism of drugs, and elucidating their metabolism.[8,9]

Therefore, to evaluate the intestinal absorption and efflux mechanisms of cetirizine enantiomers, this study was designed to study the transport characteristics of the two enantiomers using Caco-2 cell monolayers as a model of human intestinal epithelium.

## MATERIALS AND METHOD

### *Materials*

R-cetirzine and S-cetirizine were provided by Hangzhou Minsheng Pharmaceutical Group Co., (Zhejiang, People's Republic China). Sodium dodecyl sulfate (SDS), Lucifer yellow, cyclosporin A (CsA), sodium azide, verapamil, cimetidine, quinidine, indomethacin, diclofenac, ranitidine, cefradine, doxorubicin, and rhodamine-123 were purchased from Sigma (St. Louis, MO). Probenecid was kindly provided by Shanghai Jicheng Pharmaceutical Plant (Shanghai, People's Republic China). All solvents used were of HPLC grade and all chemical were of analytical grade.

### *Cell Culture*

Caco-2 cells obtained from Chinese Academy of Medical Sciences (CAMS, Beijing, People's Republic China) were cultured in high-glucose Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10% fetal bovine serum (Gibco), 1% nonessential amino acid (Gibco), and 100 U/mL antibiotic–antimycolic solution. Cells were grown in a humidified atmosphere of 5%$CO_2$ at 37°C.

After reaching 80% confluency, Caco-2 cells were harvested with 0.25% trypsin-EDTA solution and seeded in 12 mm i.d. Transwell® inserts (catalog number 3460, Corning Coster Corp.) in 12-well plates at a density of $1.0 \times 10^5$ cells/cm$^2$. Culture medium was replaced every other day for the first 14 days and daily thereafter for next 7 days until the monolayers expressed differentiated properities that closely resemble morphologic and functional characteristics of normal enterocytes.

### *Transport Experiments*

Caco-2 cells in Transwells at passage 60–70 were used for transport experiments. The integrity of the monolayer was checked by measuring transepithelial electrical resistance (TEER) value across the monolayer using Millicell-ERS voltohmmeter (Millipore) and by monitoring the permeability of the paracellular leakage marker Lucifer yellow across the monolayer.[10] The cell monolayers were considered tight enough for transport experiments when the *P*app for Lucifer yellow was less than $0.2 \times 10^{-6}$ cm/s and TEER value $>450\ \Omega\ \text{cm}^2$.

All transport studies were conducted at 37°C unless specified otherwise. Before the experiment, the inserts were washed twice and preincubated for 30 min with warm transport medium, Hank's Balanced Salt Solution containing 25 mM of HEPES, pH 7.4. Cetirizine enantiomers were dissolved in transport buffer into the desired final concentration ranging from 4.0 to 80.0 μM. The concentration interval for cetirizine was chosen to cover the range of luminal concentrations expected at the absorption site in the human jejunum. The solutions were sterile filtered and added on either the apical (AP, 0.5 ml) or the basolateral (BL, 1.5 ml) side of the inserts, whereas the receiving compartment contained the corresponding volume of transport medium. Transport studies were conducted in the absorptive direction (AP→BL) and the secretory direction (BL→AP), separately. After 1 h incubation, 0.5 ml samples were collected from the receiving sides of the cell monolayers for HPLC analysis.

### *Evaluation of Energy Depletion on the Transport of Cetirizine Enantiomers*

To determine energy dependency of cetirizine transport, transport medium depleted in glucose was used in both sides of the cell monolayers. ATP inhibitor, Sodium azide, was added to both AP and BL side and the monolayers were incubated for 1 h at 37°C.[11] Transport experiments also performed at 4°C to investigate the effect of temperature on the transport of cetirizine enantiomers.

### *Evaluation of Carrier-Mediated Transport*

To detect any significant carrier-mediated transport of cetirizine and to evaluate the possible stereoselectivity caused by transport protein, the bi-directional transport rates of R-cetirizine and S-cetirizine were determined in the presence of several inhibitors. P-gp inhibitors (CsA and verapamil), the nonspecific inhibitors of MRPs (indomethacin and probenecid), the organic cation transporter

(OCT) inhibitors (cimetidine), and the mixed inhibitor of P-gp, MRP, and OCT (quinidine) were added to AP and BL compartments to investigate the inhibition of P-gp-, MRP-, and OCT-mediated transport of cetirizine enantiomers across Caco-2 cell momolayers.

### *The Influence of Cetirizine Enantiomers on the Transport of Typical P-gp and MRP Substrates*

To investigate whether cetirizine enantiomes affect the function of P-gp and MRP, the transport characteristics of P-gp substrate rhodamine-123 (5.0 μM) and MRP substrate doxorubcin (30.0 μM) were studied in the presence of R-cetirizine or S-cetirizine (100.0 μM). Rhodamine-123 was quantified using SPECTRA max M2 (Molecular Devices) operating at excitation wavelength of 500 nm and emission wavelength of 540 nm. Similarly, doxorubcin was quantified at excitation wavelength of 480 nm and emission wavelength of 590 nm.

### *Investigate the Transport of Cetirizine Enantiomers in the Presence of Potential Clinicial Coadministered Drugs*

To explore the potential drug–drug interactions concerning cetirizine enantiomers, we examined the transport characteristics of R-cetirizine and S-cetirizine in the presence of diclofenac potassium (0.37 mM), ranitidine (2.85 mM), or cefradine (7.16 mM). Diclofenac potassium is a potent analgesic, nonsteroidal, anti-inflammatory drugs (NSAIDs). Ranitidine is a histamine H2-receptor antagonist, which is widely used to treat and prevent ulcers in the stomach and intestines. Cefradine, which belongs to a group of cephalosporins, is an antibiotic medicine used to treat bacterial infections. It is highly possible that these commonly used drugs might be simultaneously administered with cetirizine. Therefore, we dissolved these drugs in the transport medium into the assumed drug concentrations in the gastrointestinal tract to determine the possible interactions concerning the absorption of cetirizine enantiomers.

### *Ion-Pairing RP-HPLC Analysis of Cetirizine Enantiomers*

R-cetirizine and S-cetirizine in samples were quantified by ion-pairing RP-HPLC. To 0.5 ml of samples obtained from transport study in Caco-2 cell monolayers, 20 μl of diazepam was added as the internal standard. SDS was used as ion-pairing reagent. The HPLC conditions were as follows: Shimadzu LC-2010C HPLC system with UV detection at 230 nm, an Aglient Zorbax SB-C18 column (5 μm, 150 × 4.6 mm I.D.) with an ODS guard column (10 μm, 10 × 5 mm I.D.). The mobile phase consisted of a mixture of acetonitrile/0.02 M phosphate buffer solution containing 10 mM SDS and 1.5% TEA (pH 3.13) (54:46, v/v) at a flow rate of 0.75 ml/min. The injection volume was 100 μl.

### *Calculations and Statistics*

Transport rate of each enantiomer was obtained according to eq. 1.

Permeability of each enantiomer was estimated by calculating *P*app according to eq. 2.

The extent of the polarized transport was measured by efflux ration shown as eq. 3

$$V = \frac{dQ}{dt \cdot A} \tag{1}$$

$$P\text{app} = \frac{dQ}{dt \cdot A \cdot C_0} \tag{2}$$

$$\text{Efflux Ratio} = \frac{P\text{app}_{(\text{BL-AP})}}{P\text{app}_{(\text{AP-BL})}} \tag{3}$$

where $C_0$ is the initial concentration in the donor compartment and $A$ is the surface area of the monolayer. $dQ/dt$ is the rate of appearance of cetirizine enantiomers on the receiving side $P\text{app}_{(\text{BL-AP})}$ and $P\text{app}_{(\text{AP-BL})}$ are the BL→AP and AP→BL permeability of each enantiomer.

Results are given as mean ± SD. The differences among the different groups were evaluated by a one- or two-way analysis of variance (ANOVA) with a post-hoc test (Dunnett's multiple comparion test). The differences in *P*app of enantiomers were evaluated using paired *t*-test. $P < 0.05$ was considered to be statistically significant.

## RESULTS

### *Validation of HPLC Method*

This work presented simple and reliable HPLC method for the determination of cetirizine enantiomers in the transport medium. Representative chromatograms for cetirizine with internal standard in HBSS are shown in Figure 1. Under the chromatographic conditions described earlier, the total running time was within 15 min with acceptable separation of the compounds of interest. There were no interferences from the matrix components and from the presence of drugs such as CsA, verapamil, cimetidine, quinidine, indomethacin, diclofenac, ranitidine, and cefradine.

Calibration curves were constructed by performing a regression linear analysis of the peak area ratios of the enantiomers to the internal standard versus the enantiomer concentrations. The calibration curves of each enantiomer were linear over the concentration ranges from 0.10 to 2.00 μM, i.e., R-cetirizine, $Y = 2.500X - 0.0995$, $r = 1.000$; S-cetirizine, $Y = 2.541X - 0.0537$, $r = 0.9995$.

The intraday and interday precision and accuracy were analyzed at concentrations of 0.10, 0.50, 2.00 μM in five replicates within 1 day and on 5 consecutive days, respectively. Validation data indicated this method was sensitive and reliable with acceptable accuracy and precision (Table 1). The limit of detection, defined as the lowest T1 concentration of cetirizine which can be detected (signal-to-noise ratio 3) for each enantiomer, was 0.01 μM. The limit of quantification, defined as the lowest concentration of cetirizine which can be quantitatively determined with suitable precision and accuracy (signal-to-noise ratio

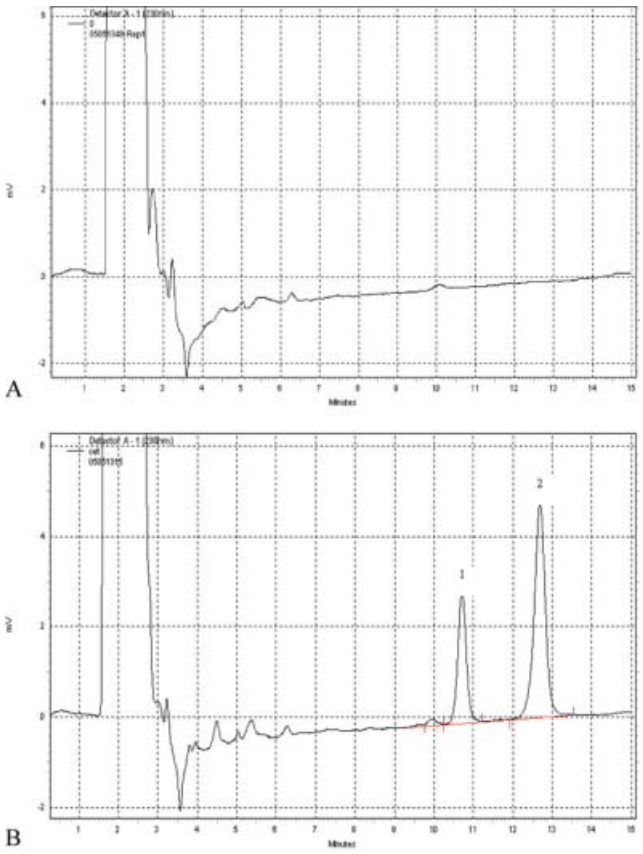

**Fig. 1.** Representative chromatogram of blank transport medium (**A**) and the sample spiked with I.S. and cetirizine (**B**). 1: Diazepam (I.S.); 2: R- or S-cetirizine. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

> 10) for each enantiomer, was 0.05 μM (RSD < 8.5%, $n$ = 5).

### *Polarized and Enantioselective Transport of Cetirizine*

Transport of cetirizine enantiomers across Caco-2 cell monolayers occurred in both AP→BL and BL→AP directions. Cetirizine displayed polarized transport at concentrations ranging from 4.0 to 80.0 μM, with the secretory *P*app being 1.4- to 4.0-fold higher than the absorptive *P*app. The *P*app $_{(BL-AP)}$ was independent on the concentration applied, whereas *P*app $_{(AP-BL)}$ decreased with increasing concentration, suggesting saturation of certain transporters. The efflux ratio increased as the concentration increased.

As shown in Figure 2, the bi-directional transport of R-cetirizine and S-cetirizine were in different manner. In the absorptive direction, the transepithelial transport rate of R-cetirizine was higher than S-cetirizine. Meanwhile, the secretory transport rate of cetirizine enantiomers did not show any significant difference in the concentration range 4.0 μM to 20.0 μM. With increasing concentration (40.0–80.0 μM), the distinctive transport difference between cetirizine enantiomers was observed.

Several models to describe the kinetics of the absorptive and secretory transport of cetirizine enantiomers (Michaelis-Menten model and Non-Michaelis-Menten model, single and two binding sites, substrate inhibition, and the sigmoid models) were fitted and compared using Sigmaplot 9.0 (SPSS, Chicago, IL). The choice of model was confirmed by *F*-test and Akaike's information criterion.[12] It was found that in the absorptive transport, a

**TABLE 1. Precision and accuracy date for cetirizine enantiomers**

| Concentration added (μmol/l) | Concentration found (μmol/l) | | | |
|---|---|---|---|---|
| | R-cetirizine | RSD (%) | S-cetirizine | RSD (%) |
| Intra-day ($n$ = 5) | | | | |
| 0.100 | 0.100 ± 0.004 | 4.5 | 0.098 ± 0.005 | 4.9 |
| 0.500 | 0.500 ± 0.009 | 1.8 | 0.497 ± 0.011 | 2.3 |
| 2.000 | 2.001 ± 0.010 | 0.5 | 1.989 ± 0.016 | 0.8 |
| Inter-day ($n$ = 5) | | | | |
| 0.100 | 0.099 ± 0.006 | 6.5 | 0.094 ± 0.007 | 7.7 |
| 0.500 | 0.501 ± 0.015 | 3.0 | 0.494 ± 0.016 | 3.2 |
| 2.000 | 2.004 ± 0.030 | 1.5 | 1.982 ± 0.028 | 1.4 |

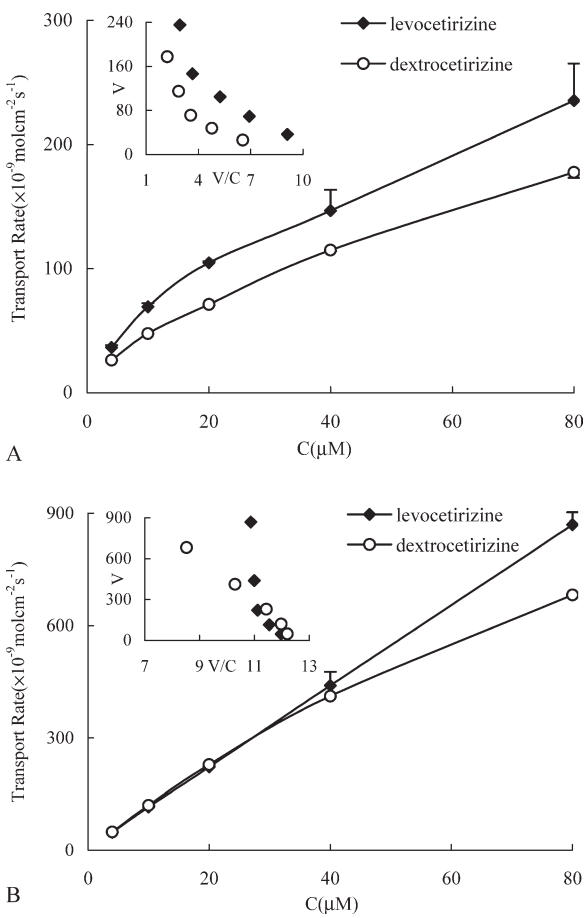


**Fig. 2.** Transport rate of cetirizine enantiomers across Caco-2 cell monlayers in the absorptive (**A**) and secretory (**B**) direction. The insets represented the Eadie-Hofstee transformation of the data for the bi-directional transport. Each data point represents the mean ± SD for three independent monolayers.

dual-transporter Michaelis-Menten model (eq. 4) was the best fit for both enantiomers. In the secretory direction, R-cetirizine and S-cetirizine followed the classic Michaelis-Menten model of one-binding site kinetics (eq. 5).

$$V = \frac{V_{\max 1}[S]}{Km_1 + [S]} + \frac{V_{\max 2}[S]}{Km_2 + [S]} \quad (4)$$

$$V = \frac{V_{\max}[S]}{Km + [S]} + Kd \cdot [S] \quad (5)$$

Where $V$ is the transport rate, $[S]$ is the initial concentration. $V_{\max}$ is the maximum transport rate; $V_{\max 1}$ and $V_{\max 2}$ are the high- and low-affinity maximum transport rate, respectively. $Km$ is the Michaelis-Menten constant; $K\mathrm{m}_1$ and $K\mathrm{m}_2$ are the high- and low-affinity Michaelis-Menten constants, respectively. $Kd$ is the coefficient of nonsaturable transport.[13]

The transport kinetic parameters were shown in Table 1. In the absorptive direction, $Km_2$ was more than 10 times higher than $K\mathrm{m}_1$ for each enantiomer. The in vitro clearance (CLint= $V_{\max}/Km$, using only kinetic parameters for the high-affinity component) demonstrated distinctive differences between enantiomers in the bi-directional transport. The clearance of S-cetirizine was higher than that of R-cetirizine, which was consistent with the in vivo data.[2]

### *Effect of Temperature and ATP Inhibitor on the Transepithelial Transport of Cetirizine Enantiomers*

Bi-directional transport of cetirizine enantiomers across Caco-2 cell monolayers was investigated at both 37°C and 4°C. The permeability of both enantiomers at 4°C was significantly lower than at 37°C. The polarized transport that can be obviously observed at 37°C was almost disappeared when the temperature dropped to 4°C. The lower temperature also diminished the permeability difference between R-cetirizine and S-cetirizine. Transport experiment in the presence of ATP inhibitor demonstrated that BL→AP transport rate of each enantiomer was decreased by almost 36%. Furthermore, AP→BL transport of cetirizine did not show any significant enantioselectivity (Fig. 3).

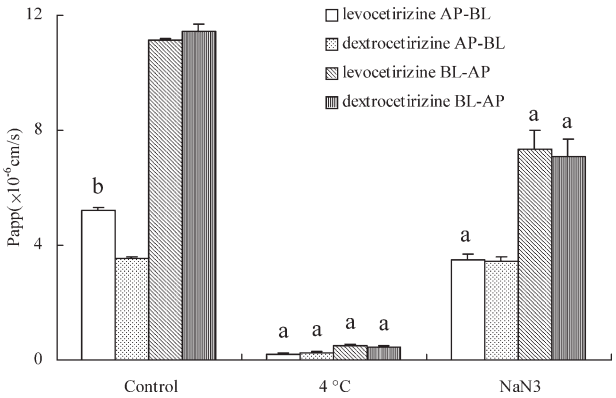


**Fig. 3.** Effect of temperature and ATP inhibitor on the transport of cetirizine enantiomers across Caco-2 cell monolayers. The experiments were performed at concentration of 20.0 μM levocetirizine or dextrocetirizine. Each value represents the mean ± SD for three independent monolayers. "a" indicates *P*app was significantly lower than the corresponding control value, $P < 0.05$. "b" indicates *P*app of levocetirizine was statistically different from dextrocetirizine, $P < 0.05$.

### *Effect of Potential Inhibitors on the Transepithelial Transport of Cetirizine Enantiomers*

The polarized transport of cetirizine suggested that it was likely to be mediated by some efflux transporters. To determine which carriers may be involved in the efflux of cetirizine, a variety of inhibitors were used. The first group was CsA (10 μM) and verapamil (100 μM), typical inhibitors of P-gp. Inclusion of verapamil or CsA abolished the profound polarized transport of cetirizine, with the $P\mathrm{app}_{(\mathrm{AP\text{-}BL})}$ for R-cetirizine and S-cetirizine increasing slightly and the $P\mathrm{app}_{(\mathrm{BL\text{-}AP})}$ decreasing to a great extent. The second group was indomethacin (100 μM) and probenecid (2 mM), the nonspecific inhibitors of MRPs. The results showed that the transport rate in the secretory direction appeared to decrease by about 18%, whereas the absorptive transport displayed distinct enhancement, which was different from the pattern of CsA and verapamil. The third group was cimetidine (50 μM), the inhibitor of OCT. Cimetidine did not influence the transport of each enantiomer. Finally, quinidine (50 μM), the mixed inhibitor of P-gp, MRP, and OCT,[14] sharply raised the AP→BL transport rate demonstrating predominantly absorptive rather than secretory transport.

As demonstrated in Figure 4, verapamil, indomethacin, and probenecid substantially intensified the difference in the absorptive permeability of R-cetirizine and S-cetirizine. Yet, the difference between R-cetirizine and S-cetirizine was no longer manifested in the presence of quinidine.

### *Modulation of P-gp and MRPs Activity by Cetirizine Enantiomers*

Since P-gp and MRPs might participate in the transport of cetirizine, the interplay of these important efflux proteins with cetirizine enantiomers was necessary to be examined. Rhodamine-123 is selectively transported in Caco-2 cell monolayers by P-gp and is often used to study the function of P-gp. As for dororubicin, it has been reported as a MRP substrate.[15] In this study, rhodamine-123 and doxorubicin were used as probes to study the cetirizine enantiomers on the P-gp and MRPs activity by determining the bi-directional transport of doxorubicin in the presence of cetirizine enantiomers.

The results are shown in Figure 5. The control experiments demonstrated that this cell model system expressed adequate amounts of P-gp and MRP thus leading to a sufficiently adequent efflux ratio for rhodamine-123 and doxorubicin. In the presence of R-cetirizine, the efflux ratios of rhodamine-123 and doxorubicin were enhanced by 48 and 67%, respectively. On the other hand, the efflux ratios of these two substrates were reduced to 47 and 87% of the original when S-cetirizne was presented. It was interesting to note that P-gp and MRP were vulnerable to inhibition or activation by cetirizine in enantioselective way. R-cetirizine

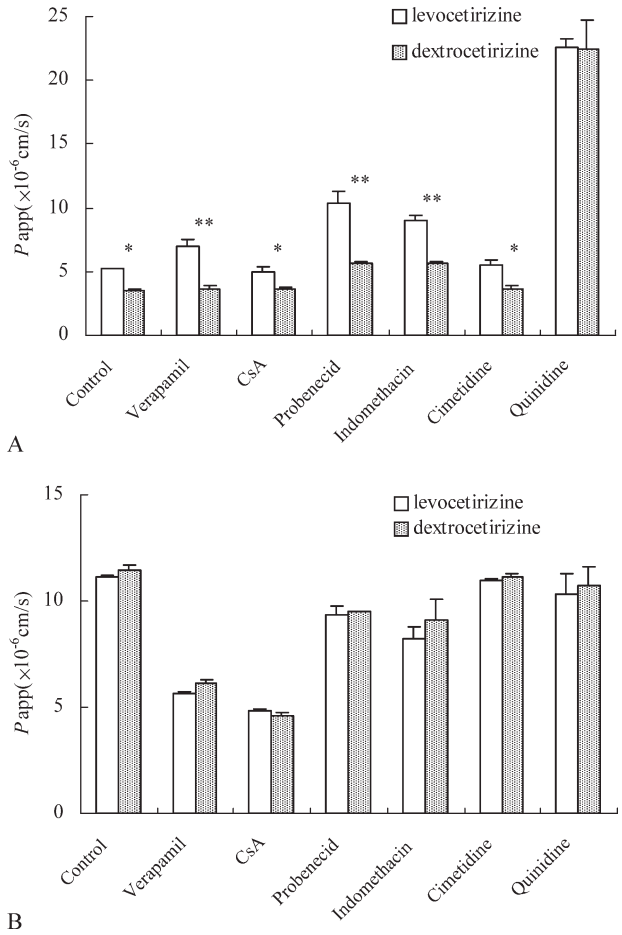


**Fig. 4.** Effect of inhibitors on transport of cetirizine enantiomers across Caco-2 cell monlayers Caco-2 cell monolayers were incubated at 37°C for 1 h with levocetirizine or dextrocetirizine added either to the apical side (**A**) or basolateral side (**B**) of the cell monolayers. CsA (10 μM), verapamil (100 μM), indomethacin (100 μM), probenecid (2 mM), cimetidine (50 μM), or quinidine (50 μM) was added to both the apical and basolateral side. Each value represents the mean ± SD for three independent monolayers. Significantly different between levocetirizine and dextrocetirizine by paired *t*-test, * $P < 0.05$, ** $P < 0.01$.

played as an inducer/activator of P-gp and MRP, whereas S-cetirizine mannered as an inhibitor of these efflux transporters.

### *Potential Clinicial Coadministered Drugs Influence the Transport of Cetirizine Enantiomers*

The interplay and modulation of efflux transporters with cetirizine enantiomers represent an important mechanism for many clinically important drug–drug interactions. Diclofenac potassium, ranitidine, and cefradine were chose to investigate whether they would alter the absorption of cetirizine enantiomers from the viewpoint of drug–drug and drug–protein interactions. The results in Figure 6 show that diclofenac potassium and cefradine did not affect the transport characteristics of cetirizine enantiomers. The polarized and enantioselective permeability of cetirizine did not change significantly compared with the control experiment. In the presence of ranitidine, the absorptive transport rate of R-cetirizine and S-cetirizine boosted up by 15.7- and 19.5-fold. Meanwhile, the seretory transport rate of R-cetirizine and S-cetirizine increased by 2.0-fold and 1.3-fold. The absorptive permeability was predominantly higher than the secretory direction and the enantioselectivity was enlarged. The results indicated that the coadministration of cetirizine with ranitidine could modify the absorption and the systemic toxicity of cetirizine.

## DISCUSSION

This study demonstrated that cetirizine was predominantly transported in the BL→AP direction in Caco-2 cell monolayers. The polarized transport of cetirizine was shown to be concentration-dependent and saturable. The biphasic and concave hyperbolic Eadie-Hofstee plots (Fig. 2, insets) indicated that multiple carriers participated in the transport process.[11,16,17] The transport might occur in the presence of high-affinity, low-capacity and low-affinity, high-capacity carrier systems.[18] The model fitting results indicated that there might be two carriers participated in the absorptive transport and one in the secretory transport.

Experiments performed at 4°C or in the presence of ATP inhibitor indicate that the transport of cetirizine was energy-dependent. Temperature changes may affect the drug diffusivity, membrane fluidity, transporter activity, intracellular trafficking, and other factors that may contribute to the change in permeability. Furthermore, the lower

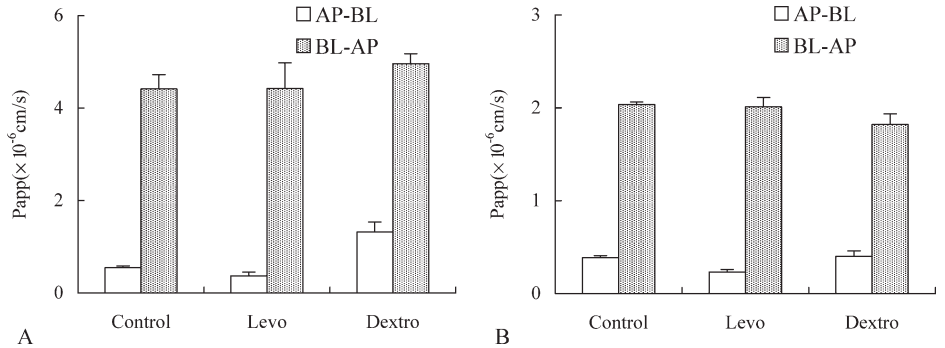


**Fig. 5.** Effect of levocetirizine and dextrocetirizine on the transport of rhodamine-123 (**A**) and doxorubicin (**B**) across Caco-2 cell monolayers. Rhodamine-123 (5.0 μM) and doxorubcin (30.0 μM) were added in the presence of levocetirizine or dextrocetirizine (100.0 μM). Each value represents the mean ± SD for three independent monolayers.

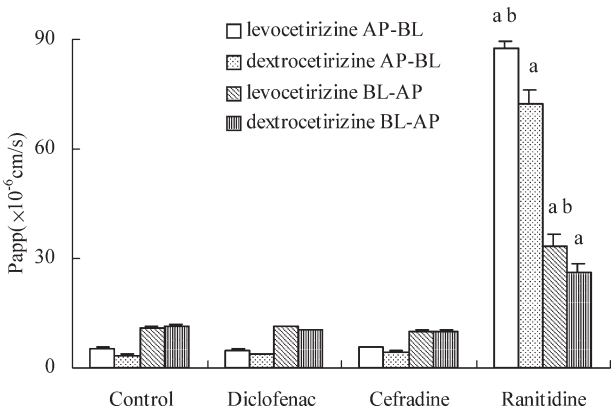


**Fig. 6.** Effect of clinicial coadministered drugs on the transport of cetirizine enantiomers. The experiments were performed at concentration of 20.0 μM levocetirizine or dextrocetirizine in the presence of diclofenac potassium (0.37 mM), ranitidine (2.85 mM), or cefradine (7.16 mM). Each value represents the mean ± SD for three independent monolayers. "a" indicates *P*app was significantlyhigher than the corresponding control value, $P < 0.05$. "b" indicates *P*app of levocetirizine was statistically higher than dextrocetirizine, $P < 0.05$.

temperature will reduce the contribution of energy-dependent mechanisms such as P-gp efflux system to the drug transport process.[19] It is important to note that reduced permeability with the presence of ATP inhibitor can be attributed to inhibition of carrier-mediated transport mechanism. Therefore, these results are in agreement with inhibition of ATP-dependent efflux pumps.

Since several efflux carriers work cooperatively in the transport process, we try to elucidate the possible efflux transporter(s) responsible for restricting the permeation of cetirizine. Previous studies reported that cetirizine was the substrate of P-gp.[20] Its affinity for P-gp at Blood-Brain Barrier (BBB) may explain the lack of central nervous system side effects of this modern H1-antagonist. Therefore, the efflux transport of cetirizine in Caco-2 cell monolayers was partly mediated by P-gp. Moreover, this study suggested MRP besides P-gp might be implicated in transepithelial transport of cetirizine. P-gp and MRP are two membrane proteins implicated in the active efflux of drugs and xenobiotics. They belong to the ATP-binding cassette (ABC) superfamily of transport proteins and function as energy-dependent efflux pumps, decreasing free cellular concentrations of drugs and xenobiotics. P-gp as well as MRP2 is expressed at the apical side in Caco-2 cells, whereas MRP1 and MRP3 would be present at the basolateral side.[21] The basolateral location of MPR1 and MPR3 makes the interpretation of efflux transport data intricate since MRP1 or MRP3 is an absorptive efflux transporter, whereas P-gp and MRP2 are secretory efflux transporters.

In this study, we particularly investigated the stereoselectivity in the transport of cetirizine across Caco-2 cell monolayers. Living organisms are based on a plethora of chiral molecules and often display different biological responses to drug enantiomers. It is not uncommon for one enantiomer to be active, whereas the other is toxic in biological systems. Thus, each enantiomer is necessary to undertake pharmacokinetic, metabolic, physiological, and toxicological evaluation in the search for drugs with greater therapeutic benefits and low toxicity. Biomembrane permeability and cellular uptake of chiral drugs may exist enantioselectivity if the intracellular micromolecules interact with the enantiomers in chiral discriminative ways. Caco-2 cell model system is very suited for this study since the purpose of this study is to evaluate the kinetic characteristics of transepithelial transport of each enantiomer of cetirizine.

The results showed that R-cetirizine and S-cetirizine were transported in different manners (Fig. 2, Table 2). It was likely that certain enantioselective transporter(s) might participate in the transport process. Therefore, we considered that MRPs and/or P-gp might be responsible for the enantioselective transport of cetirizine. In the presence of verapamil, indomethacin, and probenecid, the difference of the absorptive permeability between R-cetirizine and S-cetirizine substantially intensified in comparison with control experiments. One possible explanation for the phenomena is that cetirizine enantiomers might have different binding sites on MRP with different affinities. Verapamil is a substrate for both P-gp and MRP-1 and indomethacin and probenecid are the nonspecific MRP inhibitors. They might competitively inhibit the binding effect of one enantiomer on the MRP(s), and thus caused the transport difference between R-cetirizine and S-cetirizine much more profound. A previous study reported there was no difference in the BBB transport properties of cetirizine's two enantiomers in vivo, indicating that P-gp transport was not different between the two.[22] However, we could not absolutely exclude P-gp's effort in the enantioselective transport of cetirizine because in the presence of quinidine, the enantioselective transport was disappeared. Quinidine combined the inhibition effect of P-gp, MRP, and OCT. We considered that cetirizine enantiomers could no longer bind to these efflux transporters when the big molecular structure of quinidine blocked the binding sites on these proteins. Hence, the efflux transport of cetirizine

**TABLE 2. Transport kinetic parameters of cetirizine enantiomers across Caco-2 cell monolayers**

| AP→BL | R-cetirizine | S-cetirizine | BL→AP | R-cetirizine | S-cetirizine |
|---|---|---|---|---|---|
| $V_{max1}$ (μM cms$^{-1}$) | 35.0 | 22.1 | $V_{max}$ (μM cms$^{-1}$) | 10.1 | 2106.1 |
| $Km_1$ (μM) | 3.3 | 1.6 | $Km$ (μM) | 3.9 | 166.8 |
| $V_{max2}$ (μM cms$^{-1}$) | 448.3 | 506.8 | $Kd$ (cms$^{-1}$) | 10.8 | 0 |
| $Km_2$ (μM) | 97.6 | 179.4 | | | |

was reversed and the difference between the two enantiomers was eliminated. To point out the exact mechanism for the stereoselectivity was difficult because it is highly possible that more than one carrier mediated the transport of cetirizine in chiral discriminative ways. Therefore, efforts are needed to establish cell lines transfected with a human gene that lead to a higher, purer expression of a transporter gene in future studies.

P-gp, first discovered in 1976 in tumor cells, is resistant to multiple anticancer drugs. It has been demonstrated that the continuous exposure of tumor cells to some anticancer drugs induces P-gp in vitro and in vivo. It was reported that aspirin enhanced P-gp expression in human T lymphoma cells.[23] Many studies also have demonstrated that MRP is vulnerable to inhibition, activation, or induction.[24] This suggested that drugs other than anticancer drugs might also modulate P-gp and MRP activity. In this study, an interesting modulation effect by R-cetirizine and S-cetirizine was observed. R-cetirizine played as an inducer/activator of P-gp and MRP, whereas S-cetirizine mannered as an inhibitor of the efflux transporters. It is possible that P-gp or MRP might interact with cetirizine enantiomers in entirely different manners. This interpretation could also illuminate that MPR and/or P-gp might account for the enantioselective transport of cetirizine. P-gp and MRP modulation may be useful in clinical therapeatics by improving the pharmacokinetic and pharmacodynamic properties of substrates for which efficacy would be limited by the efflux proteins.

The activity of P-gp and MRP significantly affect cetirizine pharmacokinetics from the viewpoints of drug–drug and drug–protein interactions. The enantioselectivity observed in absorption might influence the pharmacological activity and pharmacokinetics of cetirizine. In this study, we found ranitidine, a P-gp substrate, would modify the transport characteristics of cetirizine enantiomers. Based on the broad substrate specificity and tissue distribution of P-gp, the distribution of P-gp substrates may substantially be altered either intentionally or unintentionally. A number of clinically important drug–drug interactions, the mechanisms of which previously were unexplained or attributed solely to inhibition of cytochrome P450 (CYP), are mediated by P-gp or concomitantly through P-gp and CYP modulation.[25] The coadministration of cetirizine with other P-gp substrates or inhibitors could modify the ADME profile of cetirizine, especially the enantioselective pharmacokinetics.

Cetirizine is a zwitterion at physiological pH and is well absorbed when given orally according to the biopharmaceutical classification system (BCS). Previously, in vivo experiments compared the plasma and urinary pharmacokinetic parameters of R- and S-cetirizine.[5] The results showed that R-cetirizine had a higher plasma AUC, a higher $C_{max}$, a longer terminal half-life ($t_{1/2}$), a lower nonrenal clearance, and a smaller volume of distribution (Vd). Our findings agreed with the enantioselective pharmacokinetics of cetirizine, indicating that R-cetirizine was the eutomer owning better bioavailability than S-cetirizine. Furthermore, the different absorptive characteristics between cetirizine enantiomers observed in this study might account in part for the stereoselective ADME profile of this chiral drug.

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