INTRODUCTION

Antihistamines effectively inhibit histamine-mediated symptoms, such as sneezing and nasal discharge, because they have the ability to block histamine (H1) receptors (Tarnasky and Van Arsdel, 1990; Wang et al., 2001). Loratadine is a widely prescribed, non-sedating antihistamine with selective peripheral histamine H1-receptor antagonist activity that is not associated with performance impairment and has an excellent safety record (Bradley and Nicholson, 1987; Ramaekers et al., 1992; Kay et al., 1997; Philpot, 2000; Prenner et al., 2000). Loratadine is orally administered and is used to treat allergy symptoms, including sneezing, watery eyes, and runny nose. It is also used to treat skin hives and itching in people with chronic skin reactions. It has been widely used due to its efficacy in the treatment of allergic symptoms and lack of significant central and autonomic nervous side effects such as sedation and anticholinergic properties (Clissold et al., 1989).

Loratadine undergoes extensive first-pass metabolism in the liver (Hilbert et al., 1987) and CYP3A4 enzymes are responsible for the metabolism of loratadine (Yumibe et al., 1996). Loratadine is also a substrate of P-gp (Wang et al., 2001). Since P-gp is co-localized with CYP3A4 in the small intestine, P-gp and CYP3A4 may act synergistically in absorption and first-pass metabolism of drugs, respectively (Pichard et al., 1990; Wacher et al., 1998; Ito et al., 1999).

Curcumin is the major yellow pigment in turmeric, curry, and mustard and has been widely used in cosmetics and drugs (Govindarajan, 1980). Studies on the chemopreventive efficacy of curcumin have shown that it possesses both antiinitiating and antipromoting activities in several experimental systems (Huang et al., 1994; Deshpande et al., 1998). There are also reports that curcumin inhibits carcinogenesis in various tissues, including skin (Huang et al., 1997), colorectal (Rao et al., 1995), oral (Tanaka et al., 1994), forestomach (Singh et al., 1998) and mammary (Singletary et al., 1998) cancers. In
vitro and animal studies have suggested that curcumin may have antitumor (Aggarwal and Shishodia, 2006; Choi et al., 2006), antioxidant, antiarthritic, anti-amyloid, anti-ischemic (Shukla et al., 2008) and anti-inflammatory properties (Srivastava et al., 1995).

Appiah-Opong et al. (2008) reported that curcumin inhibits its human CYP3A4, C29 and D26, while Thapliyal and Maru, (2001) found that curcumin inhibits human CYP1A1, 1A2 and 2B1. Thus, the inhibitory effects of curcumin against human CYP enzymes remain somewhat controversial. Curcumin is an inhibitor of P-gp in the KB/MDR cell line (Effert et al., 2002), but the inhibitory effect of curcumin against P-gp is ambiguous elsewhere. Therefore, we re-evaluated the inhibition of CYP enzyme activity and P-gp activity by curcumin using CYP inhibition assays and rhodamine-123 retention assays in P-gp-overexpressing MCF-7/ADR cells.

Loratadine and curcumin interact with CYP enzymes and P-gp, and the tremendous increase in the use of health supplements may result in curcumin being taken concomitantly with loratadine to treat dermatological or allergic diseases as a combination therapy. It is important to assess the potential pharmacokinetic interactions after the concurrent use of loratadine and curcumin or curcumin-containing dietary supplements in order to assure the effectiveness and safety of drug therapy. However, the possible effects of curcumin on the bioavailability and pharmacokinetics of loratadine have not been reported. Since loratadine and curcumin share the same CY

MATERIALS AND METHODS

Chemicals and apparatus

Loratadine, curcumin and propranolol [an internal standard in high performance liquid chromatographic (HPLC) analysis of loratadine] were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Acetonitrile, methanol, diethyl ether were obtained from Merck Co. (Darmstadt, Germany). Other chemicals for this study were of reagent grade or HPLC grade.

The apparatus used in this study was a high-performance liquid chromatograph equipped with a Waters 1515 isocratic pump, a Waters 717 plus autosampler and a WatersTM 474 scanning detector (Waters Co., Milford, MA, USA). An HPLC column temperature controller (Phenomenex Inc., CA, USA), a Branson® ultrasonic cleaner (Branson Ultrasonic Co., Danbury, CT, USA), a vortex-mixer (Scientific Industries Co., NY, USA), and a high-speed microcentrifuge (Hitachi Co., Tokyo, Japan).

Animal experiments

Male Sprague-Dawley rats, 7-8 weeks old (270-300 g), were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Republic of Korea) and were given free access to commercial rat chow diet (No. 322-7-1, Superfeed Co., Gangwon, Republic of Korea) and tap water ad libitum. The animals were housed (four or five per cage) in laminar flow cages maintained at 22 ± 2°C and 50-60% relative humidity under a 12:12 h light-dark cycle. The experiments began after acclimation to these conditions for at least one week. The experiments were carried out in accordance with the “Guiding Principles in the Use of Animals in Toxicology” adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The Animal Care Committee of Chosun University (Gwangju, Republic of Korea) approved the design and the conduct of this study.

The rats were fasted for at least 24 h prior to beginning the experiments and had free access to tap water. Each animal was lightly anaesthetized with ether, and the femoral artery and vein were cannulated using polyethylene tubing (SP45, I.D. 0.58 mm, O.D. 0.96 mm; Natsume Seisakusho Co. LTD., Tokyo, Japan) for blood sampling and intravenous administration.

Oral and intravenous administration of loratadine

The rats were divided into seven groups (n=6, each): the oral group (4.0 mg · kg⁻¹ of loratadine dissolved in water, homogenized at 36°C for 30 min) without (control) or with 0.5 mg · kg⁻¹; 2.0 mg · kg⁻¹ or 8.0 mg · kg⁻¹ of oral curcumin; the intravenous group (1.0 mg · kg⁻¹ of loratadine, dissolved in 0.9% NaCl solution, homogenized at 36°C for 30 min) without (control) or with 2.0 mg · kg⁻¹ or 8.0 mg · kg⁻¹ of oral curcumin. Oral loratadine was administered intragastrically using a feeding tube, and curcumin was intragastrically administered 30 min before administration of loratadine. Loratadine for intravenous administration was injected through the femoral vein within 0.5 min.

Blood samples (0.35 ml) were collected into heparinized tubes via the femoral artery at 0 (as a control), 0.017 (at the end of infusion), 0.1, 0.25, 0.5, 1, 2, 4, 8, 12 and 24 h after intravenous infusion, and 0.25, 0.5, 1, 2, 3, 4, 8, 12 and 24 h after oral administration. Blood samples were immediately centrifuged for 3 min at 13,000 rpm and 0.2-ml aliquots of plasma were stored in a −40°C freezer until HPLC analysis of loratadine. Approximately 0.9 ml of whole blood collected from untreated rats was infused via the femoral artery at 0.5, 2, 4 and 8 h to replace the blood lost due to blood sampling.

HPLC assay

The plasma concentrations of loratadine were determined by the HPLC assay modified from the methods of Yin et al. (2003) and Amini and Ahmadziani (2004). Briefly, 50 μl of propranolol (0.21 μg · ml⁻¹, as the internal standard), 50 μl of 2 N sodium hydroxide solution and 1.1 ml of diethyl ether were added to 0.2 ml of the plasma samples. The mixture was then stirred for 3 min and centrifuged at 13,000 rpm for 10 min. 1.0 ml of the organic layer was transferred to a clean test tube and evaporated at 35°C under a stream of nitrogen. The residue was dissolved in 150 μl of the mobile phase and centrifuged at 13,000 rpm for 5 min. A 70-μl aliquot of the supernatant was injected into the HPLC system. Fluorescence detection was performed at excitation and emission wavelengths of 290 and 460 nm. The stationary phase was a Kromasil KR-100-5C, column (150×4.60 mm, 5 μm, EKA chemicals, Sweden) and the mobile phase was methanol/acetonitrile/0.05 M KH₂PO₄ (3:30:67, v/v/v, pH 2.0 adjusted with phosphoric acid). The retention time at a flow rate of 1.2 ml · min⁻¹ was as follows: internal standard at 4.49 min and loratadine at 11.86 min. The calibration curves of loratadine were linear within the range of 10-500 ng · ml⁻¹. The coefficients of variation were less than 13.3% for loratadine.
CYP 3A4 inhibition assay

The assay of inhibition of human CYP3A4 enzyme activity was performed in a multiwell plate using a CYP inhibition assay kit (GENTEST, Woburn, MA) as described previously (Crespi et al., 1997). Briefly, human CYP enzyme was obtained from baculovirus-infected insect cells. CYP substrate (7-BFC for CYP3A4) was incubated with or without test compounds in buffer containing the enzyme/substrate with 1 pmol of P450 enzyme and a NADPH-generating system (1.3 mM NADP, 3.54 mM glucose 6-phosphate, 0.4 U/ml glucose 6-phosphate dehydrogenase and 3.3 mM MgCl₂) in potassium phosphate buffer (pH 7.4). Reactions were terminated by adding stop solution after 45-min incubation. Metabolite concentrations were measured by spectrofluorometer (Molecular Device, Sunnyvale, CA) at an excitation wavelength of 409 nm and an emission wavelength of 530 nm. Positive control (1 μM ketoconazole for CYP3A4) was run on the same plate and produced 99% inhibition. All experiments were performed in duplicate, and the results are expressed as the percent of inhibition.

Rhodamine-123 retention assay

The procedures used for the Rho-123 retention assay were similar to a previously reported method (Han et al., 2008). MCF-7/ADR cells were seeded in 24-well plates. At 80% confluence, the cells were incubated in FBS-free DMEM for 18 h. The culture medium was then changed to Hanks' balanced salt solution and the cells were incubated at 37°C for 30 min. After incubation of the cells with 20 μM rhodamine-123 in the presence of curcumin (0, 1, 3 and 10 μM) for 90 min, the medium was completely removed. The cells were then washed three times with ice-cold phosphate buffer (pH 7.0) and lysed in lysis buffer. The rhodamine-123 fluorescence in the cell lysates was measured using excitation and emission wavelengths of 480 and 540 nm, respectively. Fluorescence values were normalized to the total protein content of each sample and presented as the ratio to control.

Pharmacokinetic analysis

The plasma concentration data were analyzed using a non-compartmental method on WinNonlin software version 4.1 (Pharsight Co., Mountain View, CA, USA). The elimination rate constant (Kₑ) was calculated by the log-linear regression of loratadine concentration data during the elimination phase, and the terminal half-life (t₁/₂) was calculated by 0.693/Kₑ. The peak concentration (Cmax) and time to reach the peak concentration (Tₚ₀) of loratadine in the plasma were obtained by visual inspection of the data in the concentration-time curve. The area under the plasma concentration-time curve (AUC₀-t) from time zero to the time of the last measured concentration (Cₗₐₜ) was calculated using the linear trapezoidal rule. The AUC zero to infinity (AUC₀-∞) was obtained by adding AUC₀-t, and the extrapolated area was determined by Cₗₐₜ/Kₑ. The total body clearance for the i.v. route (CL) was calculated from D/AUC, where D is the dose of loratadine. The mean residence time (MRT) was calculated by dividing the first moment of AUC (AUMC₀-∞) by AUC₀-∞. The apparent volume of distribution at steady state (Vdss) was estimated by the product of MRT and CL after i.v. dosing. The bioavailability (A.B.) of loratadine was calculated by AUCoral/AUCiv×Doseiv/Doseoral×100, and the

Fig. 1. Inhibitory effect of ketoconazole (A) and curcumin (B) on CYP3A4 activity. All experiments were performed in duplicate, and results are expressed as the percent of inhibition. The IC₅₀ value of curcumin on CYP3A4 activity is 2.71 μM.
relative bioavailability (R.B.) was calculated by \( \frac{\text{AUC}_{\text{with curcumin}}}{\text{AUC}_{\text{control}}} \times 100 \).

**Statistical analysis**

All the means are presented with their standard deviation. The pharmacokinetic parameters were compared using one-way analysis of variance (ANOVA), followed by a posteriori testing with the Dunnett correction. A p-value <0.05 was considered statistically significant.

**RESULTS**

**Inhibition of CYP3A4**

The inhibitory effect of curcumin on CYP3A4 activity is shown in Fig. 1. The IC\(_{50}\) of curcumin on CYP3A4 activity was 2.71 μM. Higher concentrations of curcumin inhibited CYP3A4 enzyme activity in a concentration-dependent manner.

**Rhodamine-123 retention assay**

As shown in Fig. 2, accumulation of rhodamine-123, a P-gp substrate, was reduced in MCF-7/ADR cells overexpressing P-gp compared to that in MCF-7 cells lacking P-gp. The concurrent use of curcumin enhanced the cellular uptake of rhodamine 123 in a concentration-dependent manner and showed statistically significant (\( p<0.01 \)) increase at the concentration range of 10 μM. This result suggests that curcumin significantly inhibits P-gp activity.

**Effects of curcumin on the plasma concentrations after oral administration**

The plasma concentration-time profiles of loratadine in the

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Loratadine (Control)</th>
<th>Loratadine+Curcumin</th>
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<tbody>
<tr>
<td></td>
<td>0.5 mg · kg(^{-1})</td>
<td>2.0 mg · kg(^{-1})</td>
</tr>
<tr>
<td>AUC(_{0-\infty}) (ng·h·ml(^{-1}))</td>
<td>926 ± 167</td>
<td>1,079 ± 205</td>
</tr>
<tr>
<td>C(_{\text{max}}) (ng·ml(^{-1}))</td>
<td>84.2 ± 15.2</td>
<td>97.0 ± 18.4</td>
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<tr>
<td>T(_{\text{max}}) (h)</td>
<td>0.83 ± 0.26</td>
<td>0.92 ± 0.21</td>
</tr>
<tr>
<td>t(_{1/2}) (h)</td>
<td>10.5 ± 1.9</td>
<td>10.8 ± 2.1</td>
</tr>
<tr>
<td>A.B. (%)</td>
<td>6.5 ± 1.2</td>
<td>7.6 ± 1.4</td>
</tr>
<tr>
<td>R.B. (%)</td>
<td>100</td>
<td>117</td>
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(Mean ± S.D., n = 6). *\( p<0.05 \), **\( p<0.01 \), significant difference compared to the control (given loratadine alone) group. AUC\(_{0-\infty}\): area under the plasma concentration-time curve from 0 h to infinity, C\(_{\text{max}}\): peak plasma concentration, T\(_{\text{max}}\): time to reach peak plasma concentration, t\(_{1/2}\): terminal half-life, A.B. (%): absolute bioavailability, R.B. (%): relative bioavailability.
presence and absence of curcumin were characterized in rats and illustrated in Fig. 3. The mean pharmacokinetic parameters of loratadine are summarized in Table 1. As shown in Table 1, the presence of curcumin (2.0 or 8.0 mg·kg⁻¹) significantly altered the pharmacokinetic parameters of loratadine compared to those in the control group given loratadine alone. Curcumin significantly (2.0 mg·kg⁻¹, p<0.05; 8.0 mg·kg⁻¹, p<0.01) increased the AUC₀-∞ of loratadine by 39.4-66.7%. The C_max was significantly (2.0 mg·kg⁻¹, p<0.05; 8.0 mg·kg⁻¹, p<0.01) increased by 34.2-61.5% in the presence of curcumin. The A.B. of loratadine was significantly increased 40.0-66.1% (2.0 mg·kg⁻¹, p<0.05; 8.0 mg·kg⁻¹, p<0.01) compared to that in the oral control group. Consequently, the R.B. of loratadine was increased by 1.39- to 1.67-fold. However, there were no significant changes in the T_max and the t₁/₂ of loratadine in the presence of curcumin.

**Effects of curcumin on the plasma concentrations after i.v. administration**

The pharmacokinetic profiles of loratadine were also evaluated after its intravenous administration (1.0 mg·kg⁻¹) in the presence or absence of curcumin (2.0 or 8.0 mg·kg⁻¹) and illustrated in Fig. 4. As summarized in Table 2, curcumin had no effect on the pharmacokinetic parameters of intravenous loratadine although it had a significant effect on the bioavailability of loratadine given orally, suggesting that curcumin may be mainly due to increased intestinal absorption caused via P-gp and CYP3A4 inhibition rather than to reduced renal and hepatic elimination of loratadine.

**DISCUSSION**

With the great interest in herbal components as alternative medicines, much effort is currently being expended to identify natural compounds of plant origin that modulate P-gp and metabolic enzymes, however, there is far less information on the pharmacokinetic interactions between herbal components and medicines. More preclinical and clinical investigations on the herbal constituents-drug interaction should be performed to prevent potential adverse reactions or to utilize those interactions for a therapeutic benefit. Therefore, the present study evaluated the effect of curcumin, a naturally occurring flavonoid, on the pharmacokinetics of loratadine in rats to examine a potential drug interaction between curcumin and loratadine via the dual inhibition of CYP3A4 and P-gp.

Loratadine has low bioavailability because of poor solubility and first-pass metabolism in the liver and epithelial cells of the small intestine. Yumibe et al. (1996) reported that loratadine is metabolized by CYP 3A4 in both liver and small intestine. Since loratadine is also a substrate for the efflux pump, P-gp (Wang et al., 2001), the oral bioavailability of loratadine might be affected by this transporter. P-gp co-localized with CYP3A4 may function synergistically in regulating the bioavailability of many orally administered compounds (Pichard et al., 1990; Wacher et al., 1998; Ito et al., 1999). Based on their broad overlap in substrate specificities as well as their co-localization in the small intestine, which is the primary site of absorption for orally administered drugs, CYP3A4 and P-gp are recognized as a concerted barrier to drug absorption (Wolozin et al., 2000; Cummins et al., 2002). CYPs contribute significantly to first-pass metabolism and oral bioavailability of many drugs. The first-pass metabolism of compounds in the intestine limits the absorption of toxic xenobiotics and may ameliorate side effects. Moreover, induction or inhibition of intestinal CYPs may be responsible for significant drug-drug interactions when one agent decreases or increases the bioavailability and absorption rate constant of a concurrently administered drug (Kaminsky and Fasco, 1991).

As shown in Figs. 1 and 2, curcumin inhibited CYP3A4 and P-gp. In this study, the presence of curcumin increased the AUC and C_max of oral loratadine. Since curcumin acts as an inhibitor of efflux transporters, i.e., P-gp, located in intestinal cells, the presence of curcumin might decrease the efflux of loratadine by this transporter in the intestine. The enhanced oral bioavailability of loratadine contributed to the competitive inhibition of loratadine metabolism by CYP 3A4 both in the liver and intestine. This result was consistent with previous studies showing that a single oral administration of cimeti-

![Fig. 4. Mean plasma concentration-time profiles of loratadine after i.v. administration of loratadine (1.0 mg·kg⁻¹) without (●) or with 2.0 mg·kg⁻¹ (○) or 8.0 mg·kg⁻¹ (▼) of curcumin to rats. Bars represent the standard deviations (n=6).](image-url)
Curcumin on the Pharmacokinetics of Loratadine

Corresponding Author

Curcumin Effect on the Pharmacokinetics of Loratadine

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