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Effect of Clarithromycin on the Pharmacokinetics of Ambroxol in Rats

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ABSTRACT – This study investigated the effect of clarithromycin on the pharmacokinetics of ambroxol in rats. The pharmacokinetic parameters of ambroxol in rats were determined after the oral administration of ambroxol (12 mg/kg) in the presence or absence of clarithromycin (5 or 10 mg/kg). Compared with the control (given ambroxol alone), coadministration of clarithromycin significantly ($P<0.05$ at 5 mg/kg; $P<0.01$ at 10 mg/kg) increased the area under the plasma concentration-time curve (AUC), peak plasma concentrations (C_{max}) and absorption rate constant (K_a) of ambroxol. Clarithromycin increased the AUC of ambroxol in a dose dependent manner within the dose range of 5 to 10 mg/kg. The absolute bioavailability (AB%) of ambroxol in the presence of clarithromycin was significantly higher than that of the control ($P<0.05$ at 5 mg/kg; $P<0.01$ at 10 mg/kg), and the relative bioavailability (RB%) of ambroxol with clarithromycin was increased by 1.32- to 1.71-fold. However, there were no significant changes in time to reach peak concentration (T_{max}) and terminal half-life ($T_{1/2}$) of ambroxol in the presence of clarithromycin. Coadministration of clarithromycin enhanced the bioavailability of ambroxol, which may be due to the inhibition of intestinal and hepatic metabolism of ambroxol by CYP 3A4. Further studies for the potential drug interaction are necessary since ambroxol is often administrated concomitantly with clarithromycin in humans.

Key words – Ambroxol, Pharmacokinetics, Clarithromycin, Rats

Ambroxol, [trans-4-(2-amino-3,5-dibromobenzylamino)-cyclohexanole hydrochloride],[1] is used as a mucolytic drug that lowers sputum viscosity by normalizing secretion from bronchiolar glands, increases mucociliaryclearance,[2,3] and provides anti-oxidative[4] as well as anti-inflammatory capacity.[5-11] Ambroxol is well absorbed and excreted into urine as glucuronide form (40%), and the oxidized metabolites (20%), dibromoanthranilic acid (DBAA) and 6,8-dibromo-3-(trans-4-hydroxycyclohexyl)-1,2,3,4-tetrahydroquinazoline (DHTQ).[12] CYP3A4 predominantly involved in the metabolism of ambroxol to DBAA in humans.[13] CYP 3A4 inhibitor, ketoconazole has been reported to inhibit the production of DBAA (>80%) at 1 μM.

Clarithromycin, a macrolide antibacterial agent, is a substrate[14] and inhibitor of CYP3A4.[15,16] Coadministration of clarithromycin significantly increased the oral bioavailability of midazolam, a CYP 3A4 substrate.[17] Although clarithromycin is often coadministered with ambroxol in usual clinical practice. There is no report of pharmacokinetic interaction between clarithromycin and ambroxol. This study aimed to investigate the effects of clarithromycin on the pharmacokinetics of ambroxol in rats.

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Materials and Methods

Materials

Ambroxol hydrochloride, domperidone and clarithromycin were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, methanol, diethyl ether were obtained from Merck Co. (Darmstadt, Germany). All other chemicals were of reagent grade and all solvents were of HPLC grade.

Animal studies

Male Sprague-Dawley rats (270-300 g) were purchased from Dae Han Laboratory Animal Research Co. (Choongbuk, Korea), and a normal standard chow diet (No. 322-7-1, Superfeed Co, Gangwon, Korea) tap water was provided *ad libitum*. Throughout the experiment, the animals were housed three per cage in laminar flow cages, which was maintained at 22±2°C, 50-60% relative humidity, under a 12 h light-dark cycle. The animals were allowed to acclimatize for at least one week prior to the experiments. These experiments were performed 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 and approved by the animal care committee at our institution (Chosun University).

The rats were divided into four groups containing six each: the control group (12 mg/kg, oral), two experimental groups (12 mg/kg oral ambroxol coadministered with 5 or 10 mg/kg

of clarithromycin), and an IV group (intravenous administration of 4.0 mg/kg ambroxol). The rats were fasted for at least 24 h prior to the experiments but were given water *ad libitum*. Rat were anaesthetized with diethyl ether and the right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, NJ, USA) to allow blood sampling.

The drug were dissolved in 2 mL of distilled water and coadministered drugs were prepared by mixing ambroxol (12 mg/kg) and clarithromycin (5 or 10 mg/kg, respectively) for oral administration. Blood samples (0.5 mL) were collected at 0.1, 0.25, 0.5, 1, 2, 3, 4, 6, 8, and 12 hour postdose. The blood samples were centrifuged, and the plasma (0.2 mL) was taken and stored at −40°C until analyzed by HPLC.

HPLC assay

The plasma concentrations of ambroxol were determined by HPLC assay using a modification of the method reported by Botterblom *et al.*[18] 25 μL of domperidone (2 μg/mL) as the internal standard and 0.2 mL of plasma were added 0.2 mL of buffer PH 10 and 1.2 mL of diethyl ether. The mixture was then stirred for 2 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 200 μl of the mobile phase and centrifuged at 13,000 rpm for 5 min. 50 μl of the supernatant was then injected into the HPLC system. The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a UV-Vis detector (Model SPD-10A), a system controller (Model SCL-10A), a degasser (Model DGU-12A) and an autoinjector (SIL-10AD). The UV detector was set at 242 nm. The stationary phase was a μ-bondapack C_{18} column (3.9×300 mm, 10 μm, Waters Co., Ireland) and the mobile phase consisted of methanol: acetonitrile: 0.01 M phosphate buffer (pH 7.0): tetrahydrofuran (35:35:27.5:2.5, v/v/v/v %). The retention times at a flow rate of 1.5 mL/min were as follows: the internal standard, 3.9 min and ambroxol, 5.0 min (Figure 1). The calibration curve of ambroxol was linear within the range of 10-200 ng/mL. The intra-day (n=5) and inter-day (n=5) coefficients of variation were <5% for ambroxol.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed using the LAGRAN method computer program.[19] The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal method. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were obtained directly from the experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the terminal slope. The half-life ($T_{1/2}$) of the drug was obtained using the formula, 0.693/K_{el}. The absolute bioavailability (A.B.%) of ambroxol was calculated using the formula, $(AUC_{oral}/AUC_{iv}) \times (Dose_{i.v}/Dose_{oral}) \times 100$, and the relative bioavailability (R.B.%) of ambroxol was estimated using the formula, $(AUC_{ambroxol\ with\ clarithromycin} / AUC_{control}) \times 100$.

Statistical analysis

All the means are presented with their standard deviation. The pharmacokinetic parameters were compared with a one-way ANOVA, followed by a posteriori testing with the use of the Dunnett correction. A P value <0.05 was considered to be statistically significant.

Results and Discussion

Figure 2 shows the plasma concentration-time profiles of ambroxol after the oral administration of ambroxol with or without clarithromycin in the rats. Table I summarizes the pharmacokinetic parameters of ambroxol. The coadministration of clarithromycin (5 or 10 mg/kg, respectively) signifi-

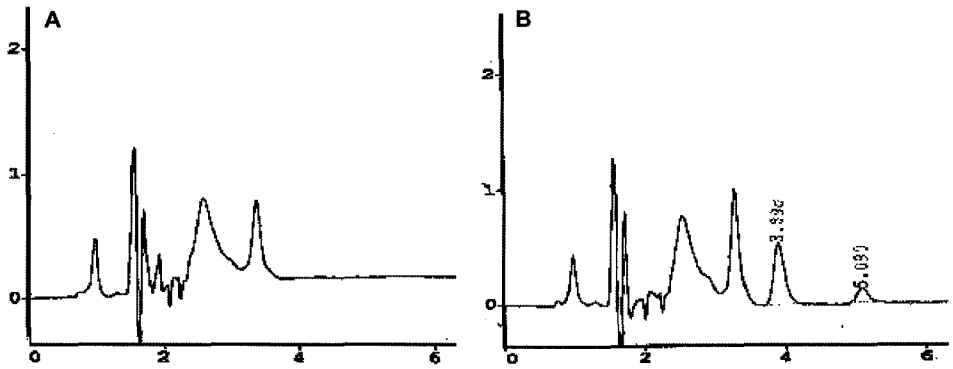

Figure 1–Chromatograms of blank plasma (A) and plasma (B) spiked with domperidone (3.9 min) and ambroxol (5.0 min).

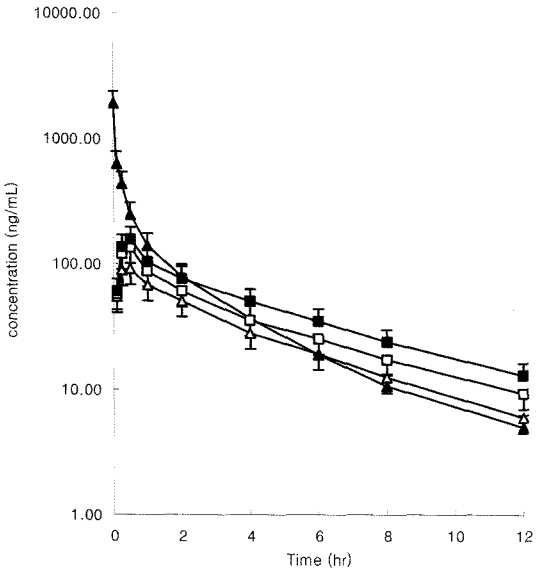

Figure 2–Mean plasma concentration-time profiles of ambroxol following an intravenous (4 mg/kg) or oral (12 mg/kg) administration of ambroxol in the presence or absence of clarithromycin to rats (Mean ± S.D., n = 6). (△) Control (ambroxol 12 mg/kg, oral), (□) Coadministered with 5 mg/kg clarithromycin, (■) Coadministered with 10 mg/kg clarithromycin, (▲) I.V. injection of ambroxol, 4 mg/kg. Bars represent the standard deviation.

cantly altered the pharmacokinetic parameters of ambroxol compared with the control group (given ambroxol alone). The absorption rate constant (K_a), peak plasma concentration (C_{max}) and the area under the plasma concentration-time curve (AUC) of ambroxol were significantly ($P<0.05$ at 5 mg/kg; $P<0.01$ at 10 mg/kg) increased with coadministration of clarithromycin. There were no significant changes in the time to reach peak plasma concentration (T_{max}) and the terminal plasma half-life ($T_{1/2}$) of ambroxol in the presence of clarithromycin. The absolute bioavailability (AB%) of ambroxol in the rats coadministered with clarithromycin ranged from 23.7% to 30.7%, which was significantly higher ($P<0.05$ at 5 mg/kg; $P<0.01$ at 10 mg/kg) than that in the control group (18.0%). The relative bioavailability (RB%) of ambroxol in the presence of clarithromycin was increased by 1.32 to 1.71 fold.

Ambroxol is used as a mucolytic drug that lowers sputum viscosity by normalizing secretion from bronchiolar glands, increases mucociliary clearance,[2,3] and provides anti-oxidative[4] as well as anti-inflammatory capacity.[5] Based on the broad spectrum of substrate specificities as well as colocalzation in the small intestine, the primary site of absorption for oral administration, CYP 3A4 has been recognized as major barrier for the drug absorption.[20,21] Therefore, inhibitors of CYP 3A4 should have a great impact on the bioavailability of many drugs which are CYP 3A4 substracts. Besides the extensive metabolism by CYP 3A4, suggesting that CYP 3A4 should act synergistically to linit the oral bioavalability of ambroxol in intestinal tract.[13]

Clarithromycin, a macrolide antibacterial agent, is a substrate[14] and inhibitor of CYP 3A4.[15,16] Coadministration of clarithromycin significantly increased the oral bioavailability of midazolam, a CYP 3A4 substrate.[17] In vitro studies have shown that potent inhibitors of CYP 3A (e.g. ketoconazole and ritonavir) alter the metabolism of clarithromycin.[22] In this studies, the coadministration of clarithromycin significantly enhanced the K_a of ambroxol, and the C_{max} and AUC of ambroxol were increased by 32 to 72%.

The coadministration of clarithromycin significantly enhanced the systemic bioavailability of ambroxol in rats. Taken all together, results from the present study were consistent with the previous reacts reported by Yeates *et al.*[23] that clarithromycin could dramatically decrease the clearance and increase the AUC of midazolam and triazolam. In addition, Nakatsuka

Table I–*Mean Pharmacokinetic Parameters of Ambroxol after an Intravenous (4 mg/kg) or Oral (12 mg/kg) Administration of Ambroxol in the Presence and Absence of Clarithromycin to Rats*

Parameters	Ambroxol (Control)	Clarithromycin Coadministration		I.V.
		5 mg/kg	10 mg/kg	
AUC (ng·hr/mL)	356±91.3	470±117.5 *	610±152.5**	662 ±165.5
C_{max} (ng/mL)	91.5±22.9	135.2±33.8*	157.0±39.3 **	-
T_{max} (hr)	0.5	0.5	0.5	-
K_a (hr^{-1})	5.9±1.5	9.1±2.3*	12.4±3.1**	-
$T_{1/2}$ (hr)	3.6±0.9	4.4±1.1	4.4±1.1	3.0 ± 0.75
A.B. (%)	18.0±4.5	23.7±5.9*	30.7±7.7**	-
R.B. (%)	100	132	171	-

Mean±S.D. (n=6), *$P<0.05$, **$P<0.01$, significant difference compared to the control (given ambroxol alone orally). AUC: area under the plasma concentration-time curve from 0 hr to infinity, C_{max}: peak plasma concentration, T_{max}: time to reach plasma peak concentration, K_a: absorption rate constant, $t_{1/2}$: terminal half-life, A.B. (%): absolute bioavailability, R.B. (%): relative bioavailability(AUC rate compared to $AUC_{control}$).

et al have reported that clarithromycin could increase the blood concentration of cabergoline in human.[24]

The coadministration of clarithromycin significantly increased the level of ambroxol, which also suggests that ambroxol is amajor substrate of CYP 3A4 in the small intestine.

Conclusion

The coadministration of clarithromycin significantly enhanced the systemic bioavailability of ambroxol in rats. The increased oral bioavailability of ambroxol in the presence of clarithromycin implying that clarithromycin could be effective to inhibit the metabolism of ambroxol in the intestine and liver.

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