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Effect of Cimetidine and Phenobarbital on Metabolite Kinetics of Omeprazole in Rats

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Omeprazole (OMP) is a proton pump inhibitor used as an oral treatment for acid-related gastrointestinal disorders. In the liver, it is primarily metabolized by cytochrome P-450 (CYP450) isoenzymes such as CYP2C19 and CYP3A4. 5-Hyroxyomeprazole (5-OHOMP) and omeprazole sulfone (OMP-SFN) are the two major metabolites of OMP in human. Cimetidine (CMT) inhibits the breakdown of drugs metabolized by CYP450 and reduces the clearance of coadministered drug resulted from both the CMT binding to CYP450 and the decreased hepatic blood flow due to CMT. Phenobarbital (PB) induces drug metabolism in laboratory animals and human. PB induction mainly involves mammalian CYP forms in gene families 2B and 3A. PB has been widely used as a prototype inducer for biochemical investigations of drug metabolism and the enzymes catalyzing this metabolism, as well as for genetic, pharmacological, and toxicological investigations. In order to investigate the influence of CMT and PB on the metabolite kinetics of OMP, we intravenously administered OMP (30 mg/kg) to rats intraperitoneally pretreated with normal saline (5 mL/kg), CMT (100 mg/kg) or PB (75 mg/kg) once a day for four days, and compared the pharmacokinetic parameters of OMP. The systemic clearance (CL_t) of OMP was significantly ($p<0.05$) decreased in CMT-pretreated rats and significantly ($p<0.05$) increased in PB-pretreated rats. These results indicate that CMT inhibits the OMP metabolism due to both decreased hepatic blood flow and inhibited enzyme activity of CYP2C19 and 3A4 and that PB increases the OMP metabolism due to stimulation of the liver blood flow and/or bile flow, due not to induction of the enzyme activity of CYP3A4.

Key words: Omeprazole, Phenobarbital, Cimetidine, Interaction, Pharmacokinetics

INTRODUCTION

Omeprazole (OMP, 5-methoxy-2-[[(4-methoxy-3,5-dimethyl-2-pyridinyl)methyl]sulphinyl]-1*H*-benzimidazole, Fig. 1) is a class referred to as proton pump inhibitor; it acts to regulate acid production in the stomach and is used to treat various acid-related gastrointestinal disorders (Howden, 1991; Wilde and McTavish, 1994). In the liver, it is metabolized to varying degrees by several CYP450 isoenzymes, which are further categorized into subfamilies of related polymorphic gene products (Petersen, 1994). The metabolism of OMP is to a large extent dependent on CYP3A4 and CYP2C19. OMP is metabolized to two major metabolites, 5-hydroxyomeprazole (5-OHOMP) and omeprazole sulfone (OMP-SFN), in humans (Fig. 1) (Regardh *et al.*, 1985; Andersson *et al.*, 1993, 1994).

Cimetidine (CMT, *N*-cyano-*N'*-methyl-*N''*-[2-[[(5-methyl-1*H*-imidazol-4-yl)methyl]thio]ethyl]guanidine) is a histamine H_2-receptor antagonist that is widely used to treat gastric and duodenal ulcers (Black, 1976). The most widely known effect of CMT is the inhibition of hepatic metabolism mediated by CYP450 isozymes such as CYP3A4, 2D6, 1A2, 2C9, and 2C19, the reduction in liver blood flow, and inhibition of the proximal tubular secretion of organic cations (Shiga *et al.*, 2000; Badyal and Dadhich, 2001; Yamano *et al.*, 2001; Szutowski *et al.*, 2002).

Phenobarbital (PB, 5-ethyl-5-phenyl-2,4,6(1*H*,3*H*,5*H*)-pyrimidinetrione) has been known to induce drug metabolism in laboratory animals and human (Carlie *et al.*, 1997). PB induction mainly involves mammalian CYP forms in gene families 2B and 3A, but the expression of other drug metabolising enzymes and genes of unknown function are also modulated (Sudjana-Sugiaman *et al.*, 1994).

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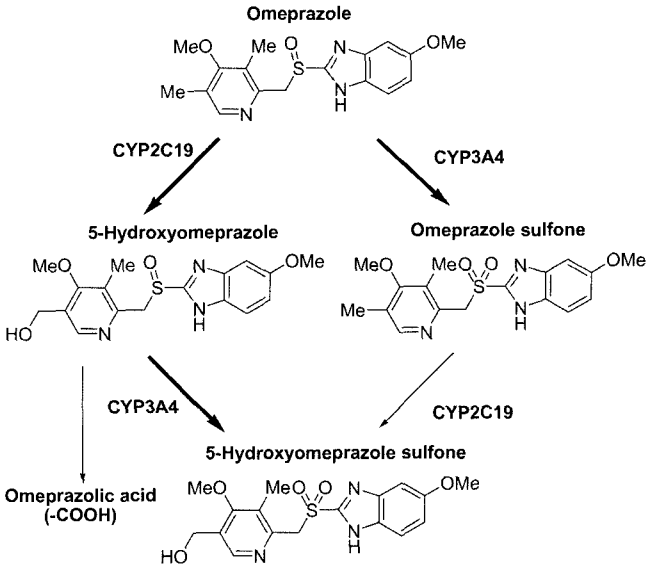

Fig. 1. Chemical structures of OMP and its metabolites, and the proposed major metabolic pathways of OMP

Since then, PB has been widely used as a prototype inducer for biochemical investigations of drug/steroid metabolism and the enzymes catalyzing this metabolism, as well as for genetic, pharmacological, and toxicological investigations (Kakizaki *et al.*, 2003). Fascination with complex mechanism of PB induction has been long attracting many scientists to this research field (Mino *et al.*, 1998; Marc *et al.*, 2000; Ejiri *et al.*, 2005).

In this study, the influence of CMT- (100 mg/kg) and PB-pretreatment (75 mg/kg) on the pharmacokinetic parameters of OMP was investigated in the rat. Thus, we performed the intravenous administration (30 mg/kg) of OMP to rats intraperitoneally pretreated with normal saline (5 mL/kg), CMT (100 mg/kg), and PB (75 mg/kg) once a day for four days, respectively.

And we also examined the liver weight, hematocrit, SGOT, and SGPT test for the pretreated rats and developed pharmacokinetic model of the OMP metabolism for further investigation.

MATERIALS AND METHODS

Chemicals and instruments

OMP, 5-OHOMP and OMP-SFN were kindly provided by AstraZeneca Pharmaceuticals Co. (Mölndal, Sweden). CMT was purchased from Choongwae Pharma Corp. (Seoul, South Korea). PB was purchased from Daewon Pharm. Co. Ltd. (Seoul, South Korea). Phenacetin and dichloromethane were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Acetonitrile and methanol were purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). AST14, ALT14, total protein, and bilirubin were purchased from Gilford Co. (Oberlin, OH, U.S.A.). Normal saline and heparin were obtained from common commercial sources. The water was purified using a Milli-Q system (Millipore Co., Milford, MA, U.S.A.). The HPLC system was from Shimadzu Corporation (Kyoto, Japan). It consisted of two pumps (Model LC-10AD*vp*), a degasser (Model DGU-12A) and a UV detector (Model SPD-10A*vp*) at 302 nm. Columns were Luna C_{18} reversed-phase 2 (250×4.6 mm, with a particle size of 5 μm, Phenomenex, Torrance, CA, U.S.A.).

Experimentals

Animals

Male Sprague-Dawley rats (220~250 g) were purchased from Jung-Ang Lab. Animal Co. (Seoul, South Korea). Animals were housed separately in a cage at a ventilated animal room with controlled temperature (19±1°C) and relative humidity (50±5%) and kept on 12 h light/dark cycle. During this period of time, animals had access to food and water ad libitum except when fasted overnight before administration of OMP. The animals were acclimated to the above conditions for at least 2 weeks before use in the experiments.

Animal treatment

Three groups of male Sprague-Dawley rats (n=6 per group), in a parallel design, received CMT (100 mg/kg, once a day), PB (75 mg/kg, once a day) and an equal volume of saline (as the control), respectively, by intraperitoneal injection for four consecutive days.

After the end of blood sampling, liver was perfused with isotonic saline solution via the portal vein, removed and weighed. SGOT, SGPT, total protein, and bilirubin value were measured by clinical chemistry analysis (SBA300, Gilford, Oberlin, OH, U.S.A.).

Blood was collected just before OMP administration and transferred to microcapillary tubes. Samples were centrifuged (2 min, 12,000 rpm) and the percent hematocrite determined (percent red blood cells in total blood volume) using a circular microcapillary tube reader (Superior®, 1.1~1.2×75 mm, Paul Marienfield KG, Bad Mergenthim, W-Germany) for each animal (Wintrobe, 1974).

Dosing and sample collection

Animals were anesthetized with diethyl ether, and left femoral vein and artery were catheterized with PE-50 polyethylene tubing (Intramedic®, Clay Adams Co., Parasippany, NJ, U.S.A.). Each rat received OMP (30 mg/kg/3 mL in 2% polyethylene glycol 400) *via* the left femoral vein. Blood (0.3 mL) was collected from the left femoral artery cannula at 2, 5, 10, 15, 30, 45, 60, 90, 120, and 240 min after OMP administration. Between each blood sample collection, 0.3 mL of normal saline was administered via

the left femoral vein cannula as fluid replacement. Blood samples were immediately centrifuged and the plasma (100 μL) was separated and immediately frozen at -70°C until analysis.

Assay of OMP and metabolites in rat plasma

Frozen plasma was allowed to thaw at room temperature. Internal standard (I.S., phenacetin, 100 μL of a 250 μg/mL stock solution in methanol), dichloromethane (5 mL), and 0.2 M borate buffer (400 μL, pH 9.0) were added to 100 μL of plasma in a glass tube. After shaking with a vortex mixer for 1 min and centrifugation at 2500 rpm for 10 min, the upper aqueous layer was aspirated and discarded. The remaining organic phase was transferred into a new glass tube and evaporated to dryness under a nitrogen stream in a centrifugal evaporator at 40°C (CVE-200D, Tokyo Rikakikai, Tokyo, Japan). The residue was finally reconstituted in 150 μL of mobile phase by vortex-mixing, of which 50 μL was then injected into the HPLC system (Kobayashi *et al.*, 1992; Cheng *et al.*, 2002).

Assay validation

Analyses were performed with the following HPLC system; Model LC-10AD*vp* pumps, a Model SPD-10A*vp* UV detector (Shimadzu Corp., Kyoto, Japan) and Luna C_{18} reversed-phase 2 (250×4.6 mm, 5 μm particle size, Phenomenex, Torrance, CA, U.S.A.). The mobile phase was acetonitrile-0.05 M phosphate buffer (pH 8.5) (30:70, v/v) at a flow rate of 0.5 mL/min. The eluate was monitored at UV wavelength of 302 nm. To examine the linearity of the assay, we prepared calibration curves for OMP, 5-OHOMP, and OMP-SFN at concentrations ranging from 50 to 2000 ng/mL in plasma. Standard samples were prepared by adding the analytes to drug-free plasma and were extracted and analyzed as described above. Peak-height ratios of each analyte to the I.S. were measured and the calibration curves were obtained from the least-squares linear regression. The regression line was used to calculate the concentrations of the respective analytes in the unknown samples.

To assess the absolute recoveries of the analytes extracted from plasma, we compared the peak heights of an extracted plasma sample containing a known amount of each of the analytes with the peak heights of an extracted water sample containing a known amount of each of the respective analytes. The final QC concentrations of OMP, OMP-SFN, and 5-OHOMP in plasma were 100, 500, and 1000 ng/mL.

The precision and accuracy of the assay were assessed by the intra- and inter-assay CVs and relative errors by determining each of the three analytes at 100, 500, and 1000 ng/mL in plasma.

To test the short- and long-term stability of the OMP, 5-OHOMP, and OMP-SFN, QC samples were stored under different conditions; at room temperature for 0, 4, and 24 h at -70°C for 1 month. And also, three freezing-thawing cycles (-70°C/room temperature) were applied to the QC samples on three consecutive days. The compounds were considered stable if the variation of assay (n=5) was less than 10% of initial time response.

Pharmacokinetic analysis

We tried to fit the OMP and its metabolites concentration-time profiles to various pharmacokinetic models with WinNonlin software program and selected a final model according to a previously reported model discrimination method (Gabrielsson and Weiner, 2000). The mechanistic pharmacokinetic model for OMP and its metabolite was finally developed on the basis of two-compartment model shown in Fig. 2. The whole model is described by the following equations.

$$V_1 \cdot \frac{dC_1}{dt} = In - Cl_d \cdot C_1 + Cl_d \cdot C_2 - Cl_m \cdot C_1 - Cl_e \cdot C_1$$

$$V_2 \cdot \frac{dC_2}{dt} = Cl_d \cdot C_1 - Cl_d \cdot C_2$$

$$V_{m1} \cdot \frac{dC_{m1}}{dt} = Cl_m \cdot C_1 + Cl_{md} \cdot C_{m2} - Cl_{em} \cdot C_{m1} - Cl_{me} \cdot C_{m1}$$

$$V_{m2} \cdot \frac{dC_{m2}}{dt} = Cl_{md} \cdot C_{m1} - Cl_{md} \cdot C_{m2}$$

where V is the volume of distribution, C is the concentration of OMP or 5-OHOMP, In is the input amount into compartment *1*, Cl is the clearance, the subscript *1, 2, m1* and *m2* are central and peripheral compartment for OMP and 5-OHOMP, respectively. And subscript *d* is the

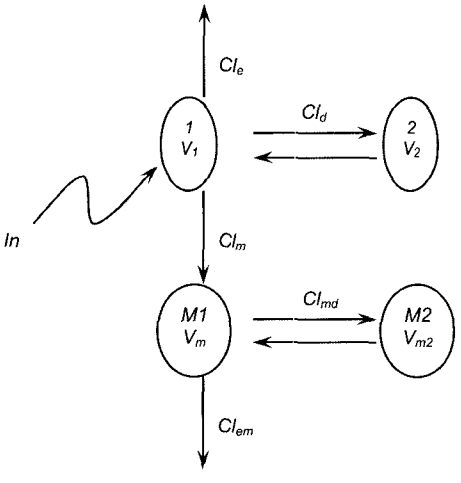

Fig. 2. The pharmacokinetic model for OMP and 5-OHOMP. *1* and *2* denote central and peripheral compartment of OMP, respectively. *M1* and *M2* denote central and peripheral compartment of 5-OHOMP, respectively.

distribution of OMP, *e* is the elimination of OMP, *md* is the distribution of 5-OHOMP, *em* is the elimination of 5-OHOMP.

Obtained pharmacokinetic parameters were compared among groups. The data were analyzed for statistical significance by Student's t-test ($P<0.05$). All calculated values were expressed as their mean±S.D.

RESULTS AND DISCUSSION

Clinical chemistry measurment

CMT showed no effect on the liver weight, hematocrit, SGOT, and SGPT in rats. However, PB has significantly ($P<0.05$) increased the liver weight, suggesting a liver enzyme induction (Waxman and Walsh, 1982).

Simultaneous determination of OMP, 5-OHOMP, and OMP-SFN

Typical HPLC chromatograms of OMP and its metabolites are shown in Fig. 3. The retention times for OMP, 5-OHOMP, OMP-SFN, and phenacetin (I.S.) were 12.0, 5.0, 11.0, and 10.5 min, respectively. No other interference peaks were observed in the chromatograms of blank plasma samples.

The mean recoveries of OMP, 5-OHOMP, and OMP-SFN from plasma were greater than 90%. The calibration curves for plasma samples were linear over the concentration range examined ($r^2>0.99$ for all the analytes). The responses were linear in the following ranges; 50~2000 ng/mL ($y = 0.00132x + 0.03627$, $r^2 = 0.9994$, n = 9), ($y = 0.0013049x - 0.02851$, $r^2 = 0.9986$, n = 9), and ($y = 0.00233x - 0.002345$, $r^2 = 0.9996$, n = 9) for OMP, 5-OHOMP, and OMP-SFN, respectively, where y is the ratio between the peak height of standard and the peak height of the I.S. and x is the standard content (ng/mL). The intra- and inter-assay CVs for the three analytes was less than 15% in plasma when signal-to-ratio was 10. The lower limit of quantitation was 50 ng/mL for all analytes in plasma. All analytes were shown to be stable in the frozen plasma at -70°C for at least three freeze-thaw cycles. Short-term stability showed that 5-OHOMP was stable in plasma at least 24 h at room temperature in the daylight, while OMP and OMP-SFN were not stable. Long-term stability showed all analytes were stable in plasma for at least one month when stored at -70°C.

On the other hand, all the concentrations of OMP-SFN in control, CMT- and PB-pretreated group were below the lower limit of quantitation at such a high dose of OMP (30 mg/kg). This result was due to a species difference between human and rat and coincided with other investigator (Cheng *et al.*, 2002).

Plasma concentration-time profiles of OMP and its metabolites

The plasma concentration-time profiles of the OMP and 5-OHOMP in the control, the CMT- and PB-pretreated rats after the intravenous administration of OMP (30 mg/kg) are shown in Fig. 4.

Table I lists the parameters of the final model obtained from WinNonlin program. The metabolite clearance (Cl_m) of OMP to 5-OHOMP was significantly ($P<0.05$) decreased in the CMT-pretreated group, but significantly ($P<0.05$) increased in the PB-pretreated group. These results mean that CMT inhibited the metabolism of OMP to 5-OHOMP and that PB induced the same metabolism. The elimination clearance (Cl_e) of OMP in control rats was negligible, but

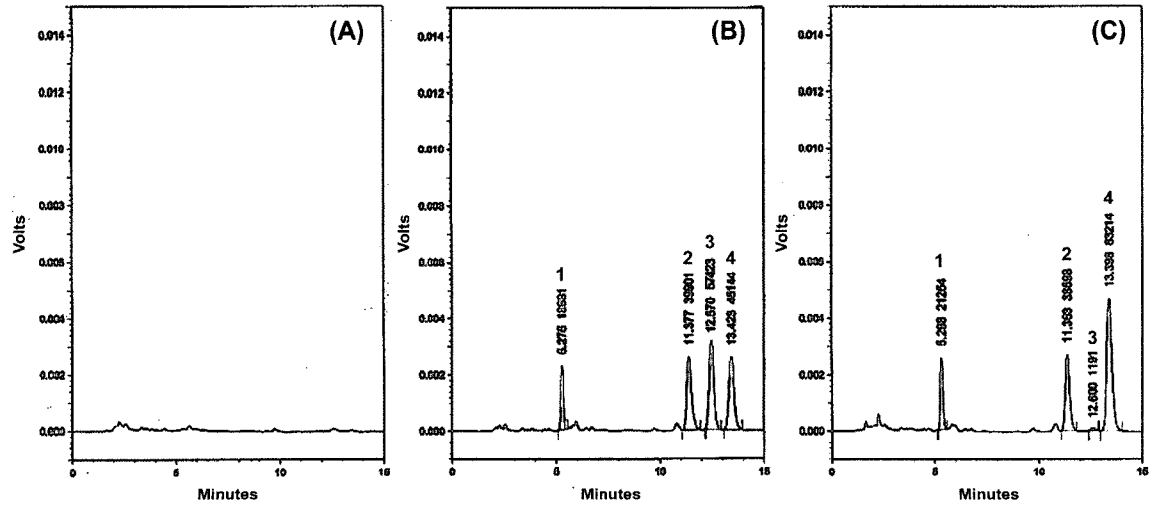

Fig. 3. Chromatograms of (A) blank rat plasma, (B) blank rat plasma spiked with OMP (1000 ng/mL), 5-OHOMP (1000 ng/mL), OMP-SFN (500 ng/mL), and internal standard (I.S., phenacetin 250 μg/mL) and (C) plasma sample (OMP: 1275.99 ng/mL, 5-OHOMP: 744.87 ng/mL) at 30 min after intravenous administration of OMP (30 mg/kg). Peaks: 1 = 5-OHOMP; 2 = I.S.; 3 = OMP-SFN; 4 = OMP.

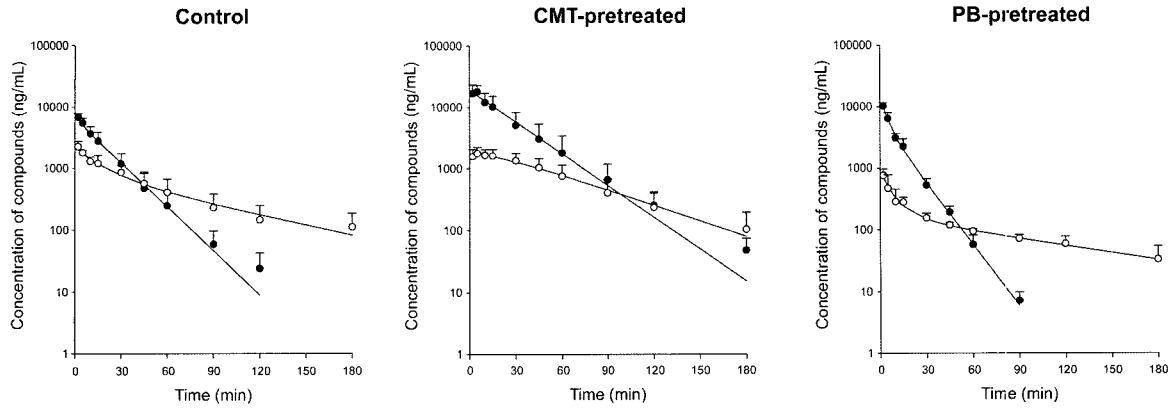

Fig. 4. Mean plasma concentration-time curves of OMP (-●-) and 5-OHOMP (-○-) after intravenous administration of OMP (30 mg/kg) to the control, the CMT- and PB-pretreated rats. Solid lines represent the model fitted values. Error bars mean the standard deviation of the mean.

the Cl_e in both PB- and CMT-pretreated rats was significantly (P<0.05) increased. These results mean that PB increased both the metabolism and elimination of OMP and 5-OHOMP and that CMT decreased the metabolism of OMP, but increased the elimination of OMP owing to the compensation mechanism of CMT, which compensated the decrease of metabolism of OMP to 5-OHOMP (Levy *et al.*, 2003).

Table II shows the pharmacokinetic parameters of OMP and 5-OHOMP on the basis of non-compartmental analysis. The plasma concentration of the OMP and 5-OHOMP was significantly (P<0.05) increased after the CMT pretreatment. The area under the curve ($AUC_{0-\infty}$) of OMP and 5-OHOMP was significantly (P<0.05) increased in CMT-pretreated rats than in control rats. Therefore, these results suggest that CMT inhibits CYP2C19 in rats, especially OMP to 5-OHOMP, not for OMP to OMP-SFN with CYP3A4. Whereas, the plasma concentrations of OMP and 5-OHOMP were significantly (P<0.05) decreased after PB treatment. The area under the curve ($AUC_{0-\infty}$) of

Table I. The parameters of pharmacokinetic model (Fig. 1) for OMP and 5-OHOMP obtained from WinNonlin program[#]

Parameters	Control	CMT-pretreated	PB-pretreated
Cl_d (mL/min/kg)	287.86 ± 80.12	520.15± 143.56*	96.76± 21.29*
Cl_m (mL/min/kg)	193.99 ± 56.50	47.74± 13.10*	258.79± 48.24*
Cl_e (mL/min/kg)	0.001± 0.0002	7.29± 1.39*	20.75± 5.21*
Cl_{em} (mL/min/kg)	281.05 ± 111.42	193.67± 67.78	1181.25± 691.43
Cl_{md} (mL/min/kg)	362.77 ± 132.41	271.26± 113.10	2606.10± 1618.96
V_1 (l/kg)	2397.42 ± 741.52	1317.87± 482.18	2176.70± 1006.58
V_2 (l/kg)	943.50 ± 311.35	73.80± 18.25*	688.27± 247.77
V_{m1} (l/kg)	417.02 ± 125.10	500.61± 140.17	1023.71± 440.19
V_{m2} (l/kg)	12393.11 ±4337.55	5523.76±1712.37	91729.36±37608.88*

[#]Mean±S.D. (n=5~6); *P<0.05 between control and the other groups.

Table II. Effect of the CMT- and PB-pretreatment on the pharmacokinetic parameters of OMP[#]

Parameters	Control		CMT-pretreated		PB-pretreated	
	OMP	5-OHOMP	OMP	5-OHOMP	OMP	5-OHOMP
$AUC_{0-\infty}$ (μg·min/mL)	154.64±16.67	106.74±9.36	545.25±102.88*	154.91±15.17*	107.32± 5.58*	25.40± 1.10*
$t_{1/2}$ (min)	11.93± 2.16	31.59±8.22	17.53± 6.28	21.56±10.00	7.10± 0.39	54.42±29.73
CL_t (mL/min/kg)	139.99±41.71	-	55.02± 17.22*	-	279.54±21.75*	-
MRT (min)	17.22± 2.11	45.58±8.85	25.29± 3.42*	31.11± 8.79	10.25± 0.69*	78.52±32.10
Vss (l/kg)	2.75± 0.23	-	1.39± 0.22*	-	2.86± 0.36	-

[#]Mean±S.D. (n=5~6); *P<0.05 between control and the other groups.

OMP and 5-OHOMP was significantly (P<0.05) decreased in PB-pretreated rats than in control rats. Therefore, these results suggest that PB increased the elimination of both OMP and 5-OHOMP due to increased hepatic blood flow and/or bile flow, due not to the induction of CYP3A4 enzyme (Branch *et al.*, 1974).

We came to a conclusion as follows. In CMT-pretreated rats, the $AUC_{0-\infty}$ of OMP was significantly (P<0.05) increased and CL_t was significantly (P<0.05) decreased as compared with control rats. On the other hand, in PB-pretreated rats, CL_t of OMP was significantly (P<0.05) increased and MRT of OMP was significantly (P<0.05) decreased as compared with the control rats. These results indicate that CMT inhibits the OMP metabolism due to both decreased hepatic blood flow and inhibited enzyme activity of CYP2C19 and 3A4 and that PB increases the OMP metabolism due to stimulation of the liver blood flow and/or bile flow, due not to induction of the enzyme activity of CYP3A4. But, we need further investigation to confirm these roles of enzymatic activity and hepatic blood flow.

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