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Pharmacokinetics of Verapamil and Its Major Metabolite, Norverapamil from Oral Administration of Verapamil in Rabbits with Hepatic Failure Induced by Carbon Tetrachloride

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The aim of this study was to investigate the pharmacokinetic changes of verapamil and its major metabolite, norverapamil, after oral administration of verapamil (10 mg/kg) in rabbits with slight, moderate and severe hepatic failure induced by carbon tetrachloride. The plasma verapamil concentrations in all groups of hepatic failure were significantly higher ($p<0.01$) than the control. However, the plasma norverapamil concentrations in severe hepatic failure were significantly higher ($p<0.05$) than the control. The peak concentrations (C_{max}) and the areas under the plasma concentration-time curve (AUC) of verapamil in the rabbits were significantly ($p<0.01$) higher than the control. The absolute bioavailability ($F_{A,B}$) and the relative bioavailability ($F_{R,B}$) of verapamil in the rabbits with hepatic failure were significantly higher (13.6-22.2% and 150-244%, respectively) than the control (9.1% and 100%, respectively). Although the AUC and C_{max} of its major metabolite, norverapamil, in slight, moderate hepatic failure were not significantly lower than the control, the metabolite-parent AUC ratio in all groups of hepatic failure was decreased significantly ($p<0.05$, in slight group; $p<0.01$, in moderate and severe group) than the control. This could be due to decrease in metabolism of verapamil in the liver because of suppressed hepatic function in the hepatic failure groups because verapamil is mainly metabolized in the liver. From our data, it would seem appropriate that in patients with liver disease, doses of verapamil should be decreased by degree of hepatic failure.

Key words: Verapamil, Norverapamil, Pharmacokinetics, Bioavailability, Hepatic failure

INTRODUCTION

Verapamil is a calcium channel blocking drug that is widely used as an antiarrhythmic agent to control supraventricular tachyarrhythmias. The potent vasodilating and negative inotropic properties of verapamil also make it useful for treating hypertension, ischemic heart disease, and hypertrophic cardiomyopathy (Fleckenstein *et al.*, 1977; Gould *et al.*, 1982; Lewis *et al.*, 1978). Verapamil is rapidly absorbed after being administered orally and is widely distributed throughout the body. Orally administered verapamil is subject to an extensive first-pass hepatic metabolism (extraction fraction = 0.79) from the portal circulation, resulting in a low systemic bioavailability (10 to 20%) (Schomerus *et al.*, 1976). The primary metabolic pathways of verapamil include *N*-demethylation and *N*-dealkylation. Cytochrome P-450(CYP) 3A4 is mainly responsible for the *N*-demethylation of verapamil, while the *N*-dealkylated metabolite is formed by CYP1A2. Norverapamil is a *N*-demethylated matabolites, that is found after the oral verapamil administration and appears to have approximately 20% of the coronary vasodilator activity of the parent compound in dogs (Eichelbaum *et al.*, 1979, 1984).

Since verapamil is eliminated by the liver and undergoes significant first-pass metabolism, one would anticipate that altered hepatic function might result in changes in the drug's intrinsic and systemic clearances, as well as systemic bioavailability. Various diseases can alter the pharmacokinetics characteristics of other drugs which undergo extensive first-pass metabolism and have systemic clearances approaching hepatic blood flow (Freedman *et al.*, 1981; Schwartz *et al., 1985)*, well-controlled studies of the effects of hepatic disorders on the disposition of

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verapamil are unavailable. The aim of this study was to investigate the effect of hepatic failure induced by carbon tetrachloride on the bioavailability of verapamil and its major metabolite, norverapamil, after orally administering verapamil to rabbits.

MATERIALS AND METHODS

Materials

Verapamil, norverapamil, and the internal standard, propranolol, were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Acetonitrile, triehylamine and diethylether were purchased from Merck Co. (Darmstadt, Germany). Phosphoric acid was purchased from the Junsei Co. (Tokyo, Japan). The other chemicals were of reagent grade and were used without further purification. The apparatuses used HPLC (Model LC-10A, Shimadzu Co., Kyoto, Japan), a syringe pump (Model 341B, Sage Co., Kyoto, Japan), a vortex mixer (Scientific Industries, Seoul, Korea) and a centrifuge (Abbot Co., TM, USA).

Animal experiments and drug administration

White male New Zealand rabbits weighing 2.0-2.4 kg were fasted for at least 36 h prior to the experiment and were given water ad libitum. The rabbits were placed under 25% urethane (4 mL/kg) anesthesia and the right femoral artery was cannulated with polyethylene tubing (PE-50, Intramedic, Clay Adams, USA) for blood sampling at room temperature. The animals were kept in these facilities 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" that were adopted by the Society of Toxicology (USA) in July 1989 and revised in March 1999. The animal care committee of our institution (Chosun University) approved this study.

Slight, moderate and severe hepatic failure rabbits induced with carbon tetrachloride (CCl_4:olive oil = 10 : 90, v/v) 0.5 mL/kg, 1.0 mL/kg and 2.0 mL/kg subcutaneous injection, respectively. Verapamil 10 mg/kg (20 mg, dissolved in 10 mL distilled water) was administered orally to the rabbits.

Blood samples (1.5 mL) were drawn from the femoral artery at 0, 0.1, 0.25, 0.5, 0.75, 1, 2, 4, 8, 12, and 24 h after administering the drug. The plasma samples were centrifuged at 3,000 rpm for 10 min. The plasmas (0.5 mL) were stored at -70°C until analyzed by HPLC.

HPLC assay

The plasma verapamil and norverapamil concentrations were determined by a HPLC assay using a modification of the method repored by Krecic-Shepard *et al.*. Briefly, 0.1 mL of propranolol HCl (400 ng/mL), as the internal standard, 50 μL of a 2 N sodium hydroxide solution and 6 mL of diethylether were added to 0.5 mL of the plasma samples. The mixture was then stirred for 3 min and centrifuged at 5,000 rpm for 10 min. Five milliliters of the organic layer were transferred to a clean test tube and evaporated at 35°C under a stream of nitrogen. The residue was then dissolved in 250 μL of the mobile phase, which was centrifuged at 5,000 rpm for 5 min, and 50 μL of the solution was injected into the HPLC system.

The HPLC system consisted of two solvent delivery pumps (Model LC-10AD, Shimadzu Co., Japan), a fluorescence detector (Model RF-10A), a system controller (Model SCL-10A), degasser (Model DGU-12A) and a autoinjector (SIL-10AD). The fluorescence detector was set at an excitation wavelength of 280 nm and an emission wavelength of 310 nm. The stationary phase used was a Kromasil KR 100-5C8 column (5 μm, 4.6×250 mm, EKA chemicals, Sweden). The mobile phase consisted of acetonitrile:0.05 M KH_2PO_4 with 0.05% triethylamine (30:70, v/v). The pH of the buffer was adjusted to 4.0 with 20% phosphoric acid. The mobile phase was filtered by passing through a 0.45 μm pore size membrane filter.

The retention times at a flow rate of 1.5 mL/min were as follows: internal standard, 4.5 min, norverapamil, 12.2 min, and verapamil, 13.4 min. Linear regression analysis using a least-square fit was performed. The calibration curve was obtained from the standard samples at the following concentration: 2, 10, 20, 50, 100, 200, and 400 ng/mL. The following regression equations were obtained: y=0.023x-0.088 (r=0.999) for verapamil, y=0.0348x -0.149 (r=0.999) for norverapamil.

Pharmacokinetic analysis

Non-compartmental pharmacokinetic analysis was performed by the LAGRAN method using the LARGAN computer program (Rocci *et al.*, 1983). The area under the plasma concentration-time curve from time zero to infinity (AUC) was computed using the LAGRAN method in order to reduce the errors associated with using the trapezoidal rule. The peak plasma concentration (C_{max}) and the time to reach the peak plasma concentration (T_{max}) were determined by a visual inspection of the experimental data. The elimination rate constant (K_{el}) was estimated by regression analysis from the slope of the line of best fit, and the half-life ($t_{1/2}$) of the drug was obtained by $0.693/K_{el}$. The absolute bioavailability ($F_{A.B}$%) of verapamil after being administered orally to the AUC of verapamil administered intravenously was calculated by $AUC_{oral}/AUC_{iv} \times Dose_{i.v}/Dose_{oral} \times 100\%$ and the relative bioavailability ($F_{R.B}$%) of verapamil was estimated by $AUC_{hepatic\ failure}/AUC_{control} \times 100\%$. The metabolite-parent AUC ratio (M.R) was estimated by $AUC_{norverapamil}/AUC_{verapamil}$.

Statistical analysis

All the means are presented with their standard deviation. The analysis of variance (ANOVA) with Scheffe's test was used to determine any significance difference between the control groups and groups co-administered or pre-treated with quercetin. A p value < 0.05 was considered significant.

RESULTS AND DISCUSSION

Clinical laboratory data in carbon tetrachloride-induced hepatic failure rabbits were shown in table. sGOT, sGPT and ALP in all carbon tetrachloride-induced hepatic failure rabbits increased significantly ($p < 0.01$, respectively) to the normal rabbits. Bilirubin in moderate and severe carbon tetrachloride-induced hepatic failure rabbits increased significantly ($p < 0.05$, respectively) to the normal rabbits. The mean plasma concentration-time profiles of verapamil and its main metabolite, norverapamil, after oral administering verapamil orally to rabbits with hepatic failure are shown in Fig. 1 and Fig. 2. The mean pharmacokinetic parameters of verapamil and norverapamil after the oral administration of verapamil to rabbits with hepatic failure are shown in Table I and Table II.

The plasma verapamil concentrations in all groups of hepatic failure were significantly higher ($p<0.01$) than the control. However, the plasma norverapamil concentrations in severe hepatic failure were significantly higher ($p<0.05$) than the control. The peak concentrations (C_{max}) and the areas under the plasma concentration-time curve (AUC) of verapamil in the rabbits were significantly ($p<0.01$) higher than the control. The T_{max} was significantly ($p<0.01$) longer than the control and $t_{1/2}$ in moderate and severe hepatic failure rabbits was significantly ($p<0.05$) longer than the control. The absolute bioavailability ($F_{A.B}$) and the relative bioavailability ($F_{R.B}$) of verapamil in the rabbits with hepatic failure were significantly higher (13.6-22.2% and 150-244%, respectively) than the control (9.1% and 100%, respectively). Although the AUC and C_{max} of its major metabolite, norverapamil, in slight, moderate hepatic failure were not significantly lower than the control, the metabolite-parent AUC ratio in all groups of hepatic failure was decreased significantly ($p<0.05$, in slight group; $p<0.01$, in moderate and severe group) than the control.

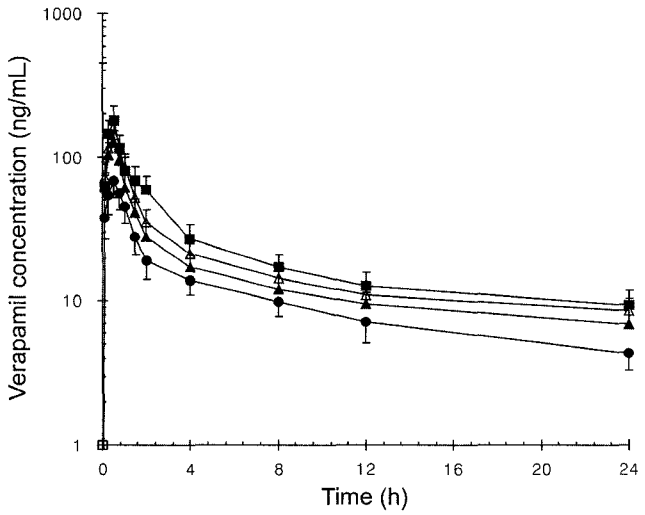

Fig. 1. Mean plasma concentration of verapamil after oral administration of verapamil (10 mg/kg) in control (●) and rabbits with slight (△), moderate (▲), and severe (■), hepatic failure induced by carbon tetrachloride. The bars represent the standard deviation (n=6).

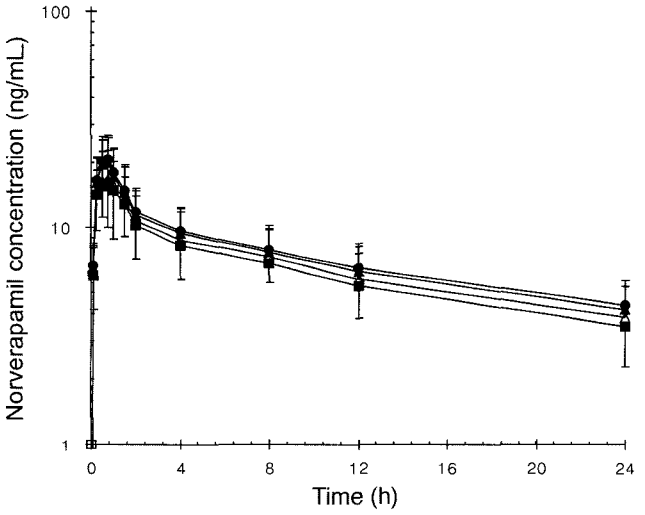

Fig. 2. Mean plasma concentration of its main metabolite, norverapamil, after oral administration of verapamil (10 mg/kg) in control (●) and rabbits with slight (△), moderate (▲), and severe (■), hepatic failure induced by carbon tetrachloride. The bars represent the standard deviation (n=6).

Table I. Laboratory data in control rabbits and rabbits with slight, moderate, and severe hepatic failure induced by carbon tetrachloride

Parameters	Control	Hepatic failure		
		Slight	Moderate	Severe
sGOT (U/L)	42.0 ± 16.1	119 ± 49.0**	581 ± 161**	1476 ± 422**
sGPT (U/L)	48.0 ± 20.2	154 ± 53.1**	259 ± 66.0**	552 ± 99.8**
ALP (U/L)	95.0 ± 34.0	151 ± 48.0**	218 ± 72.0**	361 ± 92.3**
Bilirubin (mg/dL)	0.30 ± 0.081	0.40 ± 0.160	0.50 ± 0.181*	0.50 ± 0.192*

Mean±S.D. (n = 6), *p<0.05, **p<0.01, significant difference compared to control.

Table II. Pharmacokinetic parameters of verapamil after oral administration of verapamil (10 mg/kg) in control rabbits and rabbits with slight, moderate and severe hepatic failure induced by carbon tetrachloride

Parameters	Control	Hepatic failure			I.V 2 mg/kg
		Slight	Moderate	Severe	
C_{max} (ng/mL)	68 ± 16.4	124 ± 29.8**	142 ± 35.3**	181 ± 44.1**	-
T_{max} (min)	30 ± 9.5	60 ± 9.1**	90 ± 16.1**	120 ± 26.1**	-
AUC (ng/mL·h)	319 ± 68	478 ± 94**	622 ± 129**	778 ± 151**	701 ± 149
$t_{\frac{1}{2}}$ (h)	11.6 ± 3.41	16.2 ± 4.82	17.7 ± 3.98*	18.9 ± 4.83*	9.9 ± 2.4
$F_{A.B}$ (%)	9.1 ± 2.12	13.6 ± 2.54**	17.7 ± 3.67**	22.2 ± 4.52**	100
$F_{R.B}$ (%)	100	150 ± 28.1**	195 ± 32.8**	244 ± 38.3**	-

Mean±S.D. (n = 6), *p<0.05, **p<0.01, significant difference compared to control

C_{max}: peak concentration; T_{max}: time to reach peak concentration; AUC: area under the plasma concentration-time curve from time zero to infinity; $F_{A.B}$: absolute bioavailability compared to AUC of I.V. administration; $F_{R.B}$: relative bioavailability compared to AUC of control.

Table III. Pharmacokinetic parameters of of its main metabolite, norverapamil, after oral administration of verapamil (10 mg/kg) in control rabbits and rabbits with slight, moderate and severe hepatic failure induced by carbon tetrachloride

Parameters	Control	Hepatic failure		
		Slight	Moderate	Severe
C_{max} (ng/mL)	20.6 ± 2.48	19.9 ± 5.48	16.8 ± 3.94	16.0 ± 3.27*
T_{max} (min)	45 ± 6.25	45 ± 3.55	45 ± 6.25	45 ± 9.11
AUC (ng/mL·h)	309 ± 47.1	294 ± 54.9	279 ± 48.7	258 ± 31.1*
$t_{\frac{1}{2}}$ (h)	19.9 ± 4.12	19.3 ± 3.99	19.8 ± 4.98	19.5 ± 29.4
$F_{R.B}$ (%)	100	95.1 ± 22.5	90.3 ± 27.4	83.4 ± 19.6
M.R	0.97 ± 0.27	0.62 ± 0.18*	0.45 ± 0.12**	0.33 ± 0.07**

Mean±S.D. (n = 6), * p < 0.05, ** p < 0.01, significant difference compared to control

C_{max}: peak concentration; T_{max}: time to reach peak concentration; AUC: area under the plasma concentration-time curve from time zero to infinity; $F_{R.B}$: relative bioavailability compared to AUC of control ; M.R: metabolite-parent AUC ratio.

As drugs with extensive hepatic first-pass metabolism show pronounced changes in their pharmacokinetic profile in hepatic failure, it would be expected that hepatic failure would decrease total systemic clearance and increase the oral bioavailability of verapamil. Wooodcock *et al.* reported the pharmacokinetics of verapamil after a single intravenous does were compared in normal subjects and in patients with biopsy-confirmed liver disease. Following a 5 mg intravenous dose, the subjects with liver dysfunction showed a 3-fold decrease in systemic clearance and a 1.7-fold decrease in apparent volume of distribution, with a corresponding increase in the plasna half-life. Somogyi *et al.* evaluated the disposition and bioavailability of verapamil, studying patients with histological proven hepatic cirrhosis. All of the pharmacokinetic parameters evaluated were altered. The volume of distribution was increased by 44%, oral and systemic clearance were reduced 5- and 2-fold. An increase in the terminal plasma half-life from 3.7 to 14.2 h was also observed. Following oral administration, subjects with liver disease showed higher peak drug concentrations, shorter times to achieve maximum drug plasma concentrations, and a significant increase in systemic bioavailability (22.0% *vs* 52.3%). The influence of mesocaval shunt surgery was evaluated in a patient with hepatic failure (Eichelbaum *et al.*, 1980), using simultaneous administration of intravenous and oral trideuterated verapamil. The only parameter significantly altered by surgery was systemic bioavailability. Changes in hepatic blood flow (indocyanine green clearance 447 mL/min before *vs* 165 mL/min after) appeared to have little effect on the systemic clearance of verapamil (391 mL/min before *vs* 383 mL/min after).

These observations support the conclusion that metabolism of verapamil in the liver could be due to decrease because of suppressed hepatic function in the hepatic failure groups because verapamil is mainly metabolized in the liver. From our data, it would seem appropriate that in patients with liver disease, doses of verapamil should be decreased by degree of hepatic failure.

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