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Pharmacokinetics of omeprazole after intravenous and oral administration to rats with liver cirrhosis induced by dimethylnitrosamine

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Abstract

The aim of this study is to report the pharmacokinetics of omeprazole after intravenous (20 mg/kg) and oral (40 mg/kg) administration to rats with liver cirrhosis induced by dimethylnitrosamine (cirrhotic rats) with respect to CYP isozyme changes. The expressions of CYP1A2 and 3A1 decreased in cirrhotic rats and omeprazole is reported to be mainly metabolized via CYP1A1/2, 2D1, and 3A1/2 in male Sprague–Dawley rats. Hence, the pharmacokinetics of omeprazole could be changed in cirrhotic rats. After intravenous administration to cirrhotic rats, the AUC (1180 μg min/ml versus 474 μg min/ml) and CL_{NR} (17.4 ml/min/kg versus 42.3 ml/min/kg) of omeprazole were significantly greater and slower, respectively, than the controls. This could be due to decrease in the expressions of CYP1A2 and 3A1 in cirrhotic rats. The significantly slower CL_{NR} could be supported by significantly slower *in vitro* CL_{int} for the disappearance of omeprazole from hepatic microsomal study (0.102 ml/min/mg protein versus 0.144 ml/min/mg protein) and slower hepatic blood flow rate in cirrhotic rats. After oral administration to cirrhotic rats, the AUC difference was considerably greater (451% versus 149%) than that after intravenous administration, possibly due to decrease in intestinal first-pass effect of omeprazole in addition to decrease in hepatic metabolism of omeprazole in cirrhotic rats.

Keywords: Omeprazole; Pharmacokinetics; Liver cirrhosis; Dimethylnitrosamine; CYP1A2 and 3A1; Rats

1. Introduction

Omeprazole, 5-methoxy-2-[{(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl}sulphoxide]-1*H*-benzimidazole, is a proton pump inhibitor in gastric parietal cells. The drug has greater antisecretory activity than histamine H_2-receptor antagonists and has been widely used in the treatment of peptic ulcer, efflux oesophagitis, and Zollinger–Ellison syndrome (Berglindh and Sachs, 1985; Im et al., 1985). Recently, it was reported (Lee et al., 2006) that omeprazole was mainly metabolized via hepatic microsomal cytochrome P450 (CYP) 1A1/2, 2D1, and 3A1/2 (not via CYP2B1/2, 2E1, and 2C11) in male Sprague–Dawley rats. For example, in rats pretreated with 3-methylcholanthrene and dexamethasone (main inducers of CYP1A1/2 and 3A1/2 in rats, respectively), the time-averaged nonrenal clearance (CL_{NR}) of intravenous omeprazole (the CL_{NR} of omeprazole could represent the metabolic clearance of omeprazole in rats) was significantly faster (43.8 and 33.2% increase, respectively) than the respective controls. On the other hand, in rats pretreated with quinine and troleandomycin (main inhibitors of CYP2D1 and 3A1/2 in rats, respectively), the CL_{NR} of intravenous omeprazole was significantly slower (12.9 and 20.9% decrease, respectively) than the respective controls. However, the CL_{NR} of intravenous omeprazole was not significantly changed in rats pretreated with orphenadrine, isoniazid, and sulphaphenazole (main inducers of CYP2B1/2 and 2E1, and a main inhibitor of CYP2C11 in rats, respectively) compared with the respective controls.

Abbreviations: AUC, total area under the plasma concentration–time curve from time zero to time infinity; CL, time-averaged total body clearance; CL_R, time-averaged renal clearance; CL_{NR}, time-averaged nonrenal clearance; V_{ss}, apparent volume of distribution at steady state; MRT, mean residence time; V_{max}, maximum velocity; K_m, Michaelis–Menten constant; CL_{int}, intrinsic clearance; $Ae_{0-24\,h}$, percentage of dose excreted in 24-h urine; $GI_{24\,h}$, percentage of dose recovered from the gastrointestinal tract (including its content and feces) at 24 h; C_{max}, peak plasma concentration; T_{max}, time to reach a C_{max}; F, extent of absolute oral bioavailability

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Recently, it was obtained from our laboratories that in male Sprague–Dawley rats with liver cirrhosis induced by dimethylnitrosamine (cirrhotic rats), the expressions of CYP2B1/2, 2C11, and 2E1 increased, but those of CYP1A2 and 3A1 decreased compared with the controls. Hence, it could be expected that the pharmacokinetics of omeprazole could be changed in cirrhotic rats compared with the controls.

Several studies reported that peptic ulcer occurs with increased frequency among patients with liver cirrhosis (Kirk et al., 1980; Rabinovitz et al., 1989, 1990; Ichiyanagui et al., 1995; Siringo et al., 1995) and *Helicobacter pylori* infection is implicated in the pathogenesis of peptic ulcer in liver cirrhotic patients (Zullo et al., 1999). Omeprazole is frequently used in patients with liver cirrhosis to treat peptic ulcer disease, and is also used for the healing of mucosal lesions after endoscopic sclerotherapy of esophageal varices in liver cirrhosis and extrahepatic portal vein obstruction, EHPVO (Kumar et al., 2003). Although it was reported that the total area under the plasma concentration–time curve from time zero to time infinity (AUC) and extent of absolute oral bioavailability (F) of omeprazole were significantly greater, and metabolism of omeprazole were significantly slower in patients with liver cirrhosis (Rinetti et al., 1991; Sauvet and Schouler, 1992; Pique et al., 2002), the pharmacokinetic changes of omeprazole in cirrhotic patients with respect to CYP isozyme changes seems not to be reported yet. Hence, omeprazole was chosen in this study using liver cirrhotic rats as an animal model. The purpose of this study is to report the significantly greater AUC of omeprazole after intravenous and oral administration of the drug, and significantly slower CL_{NR} of omeprazole after intravenous administration of the drug to cirrhotic rats with respect to CYP isozyme changes.

2. Materials and methods

2.1. Chemicals

Omeprazole and torasemide (an internal standard of high-performance liquid chromatographic, HPLC, analysis of omeprazole) were donated from Yungjin Pharmaceutical Company (Seoul, South Korea) and Roche Pharmaceutical Company (Mannheim, Germany), respectively. Dimethylnitrosamine, reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), tris(hydroxymethyl) aminomethane (Tris®)-buffer, and ethylenediamine tetraacetic acid (EDTA) were purchased from Sigma–Aldrich Corporation (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade.

2.2. Animals

Male Sprague–Dawley rats (weighing 175–235 g) were purchased from Charles River Company Korea (Orient, Seoul, South Korea). The rats were randomly divided into two groups: the control and cirrhotic rats. All rats were maintained in a light-controlled room (light: 07:00–19:00 h, dark: 19:00–07:00 h) kept at a temperature of $22 \pm 2\,^{\circ}C$ and a relative humidity of $55 \pm 5\%$ (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University, Seoul, South Korea). Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under the supply of filtered pathogen-free air and with water and food (Sam Yang Company, Seoul, South Korea) ad libitum. The Animal Care and Use Committee of the College of Pharmacy, Seoul National University, approved this animal study protocol.

2.3. Induction of liver cirrhosis induced by dimethylnitrosamine

Dimethylnitrosamine (dissolved in 0.9% NaCl-injectable solution to produce a 0.01 mg/ml) at a dose of 0.01 mg/kg was repeatedly injected intraperitoneally on three consecutive days a week for 4 weeks (Ohara and Kusano, 2002). For control rats, the same volume of 0.9% NaCl-injectable solution was injected. During the pretreatment, rats had free access to food and water. Ten days after the last dimethylnitrosamine injection (or 0.9% NaCl-injectable solution), the experiment was performed. Laboratory cirrhotic rats produced by dimethylnitrosamine administration simulate the clinical features of human liver cirrhosis such as mortality, hepatic parenchymal cell destruction, connective tissue formation, and nodular regeneration (Kang et al., 2002). Liver cirrhosis in rats induced by dimethylnitrosamine was evident based on the liver microscopy; there was extensive micronodular cirrhosis with regenerative hepatocellular changes and bile ductular proliferation (Bae et al., 2004). It was reported (Jenkins et al., 1985; Jezequel et al., 1987) that dimethylnitrosamine was used as a reproducible animal model of hepatic cirrhosis.

2.4. Preliminary study

The following preliminary study was performed in control ($n = 5$) and cirrhotic ($n = 4$) rats to measure the liver and kidney functions. The 24-h urine was collected for the measurement of creatinine level. Plasma was collected for the measurement of total proteins, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), total bilirubin, alkaline phosphatase (ALP), and creatinine levels (analyzed by Green Cross Reference Lab., Seoul, South Korea), and plasma protein binding of omeprazole. The whole kidney and liver of each rat were excised, rinsed with 0.9% NaCl-injectable solution, blotted dry with tissue paper, and weighed. Small portions of each organ were fixed in a 10% neutral phosphate-buffered formalin and then processed for routine histological examination with hematoxylin–eosin staining.

2.5. Measurement of V_{max}, K_m, and CL_{int} for the disappearance of omeprazole in hepatic microsomes

The procedures are similar to the reported methods (Bae et al., 2004). The livers of control ($n = 5$) and cirrhotic ($n = 4$) rats were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) in an ice-cold buffer of 0.154 M KCl/50 mM Tris–HCl in 1 mM EDTA, pH 7.4. The homogenate was centrifuge at $10{,}000 \times g$ for 30 min and the supernatant fraction was further centrifuged at $100{,}000 \times g$ for

90 min. Protein content was measured using the reported method (Bradford, 1976). The V_{max} (the maximum velocity) and K_m (the Michaelis–Menten constant; the concentration at which the rate is one-half of V_{max}) for the disappearance of omeprazole were determined after incubating the above microsomal fractions (equivalent to 0.5 mg protein), a 5-μl aliquot of omeprazole (dissolved in 0.1 M carbonate buffer of pH 9.8 having substrate concentrations of 1, 2.5, 5, 10, and 20 μM), and a 50-μl (1 mM) aliquot of NADPH (dissolved in Tris–HCl buffer, pH 7.4) in a final volume of 0.5 ml by adding 0.1 M phosphate buffer, pH 7.4, in a water-bath shaker kept at 37 °C and at a rate of 500 oscillations per min (opm). All of the above microsomal incubation conditions were linear. The reaction was terminated by the addition of 1 ml of diethyl ether after a 5-min incubation. Omeprazole was measured by the reported HPLC method (Kang et al., 1999). The kinetic constants (K_m and V_{max}) for the disappearance of omeprazole were calculated using the nonlinear regression method (Duggleby, 1995). The intrinsic clearance (CL_{int}) for the disappearance of omeprazole was calculated by dividing the respective V_{max} by the respective K_m.

2.6. Measurement of plasma protein binding of omeprazole using the equilibrium dialysis technique

Protein binding of omeprazole to plasma of control and cirrhotic rats was determined by the reported method using the equilibrium dialysis technique (Shim et al., 2000). One milliliter of plasma was dialyzed for 8 h at 37 °C against 1 ml of isotonic Sørensen phosphate buffer of pH 7.4 containing 3% (w/v) dextran in a 1-ml dialysis cell (Spectrum Medical Industries, Los Angeles, CA) using a Spectra/Por 4 membrane (mol. wt. cutoff of 12,000–14,000 Da; Spectrum Medical Industries). Omeprazole (dissolved in 0.1 M carbonate buffer of pH 9.8) was spiked into the plasma side at an omeprazole concentration of 10 μg/ml. After 8-h incubation, two 100-μl aliquots were collected from each compartment and stored in a −70 °C freezer (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until HPLC analysis of omeprazole (Kang et al., 1999). In the preliminary study, it took approximately 6 h to reach an equilibrium of omeprazole between plasma and buffer sides and binding of omeprazole to 4% human serum albumin was constant, 91.7 ± 0.785%, at omeprazole concentrations ranging from 1 to 200 μg/ml. Therefore, the 8-h incubation time and an omeprazole concentration of 10 μg/ml were arbitrarily chosen in this plasma protein binding study.

2.7. Intravenous and oral administration of omeprazole to rats

The procedures for the pretreatment of rats including the cannulation of the carotid artery (for blood sampling) and the jugular vein (for drug administration for intravenous study only) were similar to previously reported methods (Kim et al., 1993; Lee et al., 2006). Since Watanabe et al. (2002) reported that immobilization stress could change the pharmacokinetics of omeprazole in rats, they were not restrained in the present study. Heparinized 0.9% NaCl-injectable solution (15 units/ml), 0.25 ml, was used to flush the cannula to prevent blood clotting. After surgical suture, each rat was housed individually in a rat metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from anesthesia for 4–5 h before the commencement of experiment.

Omeprazole (the same solution that was used in the plasma protein binding study) at a dose of 20 mg/kg was infused (total infusion volume of 2 ml/kg) over a 1-min via the jugular vein of control ($n = 7$) and cirrhotic ($n = 8$) rats. An approximately 0.22-ml aliquot of blood sample was collected via the carotid artery at 0 min (to serve as a control), 1 min (at the end of the infusion), 3, 7, 15, 30, 45, 60, 70, 80, and 90 min after starting of the intravenous infusion of omeprazole. Blood samples were centrifuged immediately, and a 100-μl aliquot of each plasma sample was stored in a −70 °C freezer until HPLC analysis of omeprazole (Kang et al., 1999). The heparinized 0.9% NaCl-injectable solution, 0.3 ml, was used to flush the cannula immediately after each blood sampling. At the end of experiment (24 h), each metabolic cage was rinsed with 5 ml of distilled water and the rinsings were combined with 24-h urine sample. After measuring the exact volume of combined urine sample, a 100-μl aliquot of the combined urine sample was stored in a −70 °C freezer until HPLC analysis of omeprazole (Kang et al., 1999). At the same time (24 h), as much blood as possible was collected via the carotid artery and each rat was sacrificed through cervical dislocation. And then, its entire gastrointestinal tract (including its contents and feces) was removed, transferred into a beaker that contained 50 ml of methanol (to facilitate the extraction of omeprazole) and cut into small pieces using scissors. After stirring with a glass rod for 1 min, two 100-μl aliquots of the supernatant were collected from each beaker and stored in a −70 °C freezer until HPLC analysis of omeprazole (Kang et al., 1999). The following experiment was also performed in additional cirrhotic ($n = 10$) rats to calculate the ascite/plasma ratios of omeprazole in cirrhotic rats. Omeprazole at a dose of 20 mg/kg was intravenously administered to cirrhotic ($n = 10$) rats. At 60 min after intravenous administration of omeprazole, a 100-μl aliquot of plasma and ascite was collected after an abdominal operation and they were stored in a −70 °C freezer until HPLC analysis of omeprazole (Kang et al., 1999). Stability test of omeprazole was also performed in the ascite of cirrhotic rats (without administration of omeprazole) using the reported methods (Yu et al., 2003).

Omeprazole (the same solution that was used in the intravenous study) at a dose of 40 mg/kg was administered orally (total orally volume of 5 ml/kg) using a feeding tube to control ($n = 9$) and cirrhotic ($n = 9$) rats. Blood samples were collected at 0, 5, 15, 30, 60, 75, 90, 105, 120, 135, 150, 180, and 240 min after oral administration of omeprazole. Other procedures were similar to those of the intravenous study.

2.8. HPLC analysis of omeprazole

Concentrations of omeprazole in the above samples were determined by the slight modification of the reported HPLC method (Kang et al., 1999); torasemide instead of lansoprazole was used as an internal standard. In a 2.2-ml eppendorf tube that contained a 100-μl aliquot of a sample, a 50-μl aliquot

of methanol that contained an internal standard (torasemide; 50 μg/ml) and a 50-μl aliquot of 0.2 M phosphate buffer (pH 7.0) were added. The mixture was then extracted with 1 ml of diethylether. The organic layer was transferred into a clean eppendorf tube and evaporated under a gentle stream of nitrogen gas at 50 °C. The residue was reconstituted in a 125-μl aliquot of the mobile phase and a 50-μl aliquot was injected directly onto a reversed-phase (C_8) HPLC column. The mobile phase, phosphate buffer (0.2 M KH_2PO_4 of pH 7.0):acetonitrile (77:23; v/v) was run at a flow rate of 1.4 ml/min and the column effluent was monitored by an ultraviolet detector set at a 302 nm. The retention times of omeprazole and the internal standard were approximately 10.2 and 8.1 min, respectively. The detection limits of omeprazole in rat plasma and urine samples were 20 and 50 ng/ml, respectively. Coefficients of variation of omeprazole in plasma and urine samples were below 5.34 and 7.90%, respectively

2.9. Pharmacokinetic analysis

The AUC was calculated using the trapezoidal rule-extrapolation method; this method uses the logarithmic trapezoidal rule for the calculation of the area during the declining plasma-level phase (Chiou, 1978) and the linear trapezoidal rule for the rising plasma-level phase. The area from the last datum point to time infinity was estimated by dividing the last measured plasma concentration by the terminal phase rate constant.

Standard methods (Gibaldi and Perrier, 1982) were used to calculate the following pharmacokinetic parameters using noncompartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, CA); the time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, terminal half-life, first moment of AUC (AUMC), mean residence time (MRT), apparent volume of distribution at steady state (V_{ss}), and F (Kim et al., 1993). The peak plasma concentration (C_{max}) and time to reach a C_{max} (T_{max}) were read directly from the experimental data.

Glomerular filtration rate (GFR) was estimated by measuring the creatinine clearance (CL_{CR}) assuming that kidney function was stable during the experimental period (24 h). The CL_{CR} was measured by dividing the total amount of unchanged creatinine excreted in 24-h urine by the $AUC_{0–24\,h}$ of creatinine in plasma.

The harmonic mean method was used to calculate the mean values of V_{ss} (Chiou, 1979), terminal half-life (Eatman et al., 1977), and each clearance (Chiou, 1980).

2.10. Statistical analysis

A $P < 0.05$ was considered to be statistically significant using an unpaired t-test. All data are expressed as mean ± standard deviation (S.D.).

3. Results

3.1. Preliminary study

Body weight, plasma chemistry data, plasma protein binding of omeprazole, CL_{CR}, and relative liver and kidney weights in control and cirrhotic rats are listed in Table 1. In cirrhotic rats, the plasma levels of total proteins (33.1% decrease) and albumin (40.7% decrease) were significantly lower, the levels of GOT (180% increase), GPT (174% increase), total bilirubin (298% increase), and ALP (91.6% increase) were significantly higher, and relative liver weight was significantly lighter (27.2% decrease) than the controls. The relative kidney weight was significantly heavier (32.0% increase) than the controls. However, the plasma levels of urea nitrogen and CL_{CR} were not significantly different between two groups of rats. The above data suggest that liver function seemed to be impaired in cirrhotic rats and this could be supported by the liver microscopy; there was extensive hepatocellular degeneration with bridging fibrosis. However, no significant findings were observed in the liver of control rats. It was reported (Yamashita et al., 1998) that the serum level of hyaluronate increased in rats with dimethylnitrosamine-induced cirrhotic liver with prolongation of prothrombin time, which indicates disorder of liver function. The above data suggest that kidney function seemed not to be impaired considerably in cirrhotic rats and this could be supported by the kidney microscopy; there were no significant findings in the kidneys of both groups of rats. In cirrhotic rats, the plasma protein binding of omeprazole was significantly smaller (50.4% decrease) than the controls. Note that body weight gain decreased significantly in cirrhotic rats (from 198 to 274 g) compared with the controls (from 190 to 354 g).

Table 1
Body weight, plasma chemistry data, plasma protein binding of omeprazole, creatinine clearance, and relative liver and kidney weights in control rats and cirrhotic rats induced by dimethylnitrosamine[a]

Parameter	Control ($n=5$)	Cirrhosis ($n=4$)
Body weight (g)		
Initial	190 ± 15.4	198 ± 9.57
Final	354 ± 23.8	274 ± 19.7[b]
Plasma		
Total proteins (g/dl)	6.28 ± 0.179	4.20 ± 1.00[b]
Albumin (g/dl)	3.76 ± 0.0894	2.23 ± 0.602[c]
Urea nitrogen (mg/dl)	9.68 ± 2.82	14.8 ± 4.51
GOT (IU[d]/l)	164 ± 109	459 ± 220[e]
GPT (IU/l)	39.0 ± 12.1	107 ± 61.7[e]
Total bilirubin (mg/dl)	0.220 ± 0.0447	0.875 ± 0.330[b]
ALP (IU/l)	179 ± 18.4	343 ± 146[e]
Protein binding of omeprazole (%)	78.9 ± 5.22	39.1 ± 13.8[b]
CL_{CR} (ml/min/kg)	3.19 ± 0.521	2.82 ± 1.28
Liver weight (% of body weight)	3.68 ± 0.140	2.68 ± 0.326[c]
Kidney weight (% of body weight)	0.679 ± 0.0705	0.896 ± 0.0721[b]

[a] Each value represents the mean ± S.D.
[b] Significantly different ($P<0.01$) from control.
[c] Significantly different ($P<0.001$) from control.
[d] International unit.
[e] Significantly different ($P<0.05$) from control.

3.2. Measurement of V_{max}, K_m, and CL_{int} for the disappearance of omeprazole in hepatic microsomal fractions

The V_{max}, K_m, and CL_{int} for the disappearance of omeprazole in hepatic microsomal fractions of control and cirrhotic

Table 2
V_{max}, K_m, and CL_{int} for the disappearance of omeprazole in hepatic microsomes of control rats and cirrhotic rats induced by dimethylnitrosamine[a]

Parameter	Control ($n=5$)	Cirrhosis ($n=4$)
V_{max} (nmol/min/mg protein)	6.03 ± 2.09	3.26 ± 0.422[b]
K_m (μM)	42.4 ± 15.5	32.4 ± 4.65
CL_{int} (ml/min/mg protein)	0.144 ± 0.0172	0.102 ± 0.0124[c]

[a] Each value represents the mean ± S.D.
[b] Significantly different ($P<0.05$) from control.
[c] Significantly different ($P<0.01$) from control.

rats are listed in Table 2. In cirrhotic rats, the V_{max} for the disappearance of omeprazole was significantly slower (45.9% decrease) than the controls, suggesting that maximum velocity for the disappearance (mainly due to metabolism) of omeprazole was significantly slower than the controls. However, the K_m values were not significantly different between control and cirrhotic rats, suggesting that the affinity of omeprazole to the enzyme(s) is not changed in cirrhotic rats. Hence, the intrinsic clearance (CL_{int}) for the disappearance of omeprazole in cirrhotic rats was significantly slower (29.2% decrease) than the controls, suggesting that the metabolism of omeprazole decreased in cirrhotic rats due to decrease in the expressions of CYP1A2 and 3A1.

3.3. Pharmacokinetics of omeprazole after intravenous administration of the drug to rats

After intravenous administration of omeprazole at a dose of 20 mg/kg to control and cirrhotic rats, the mean arterial plasma concentrations–time profiles of the drug are shown in Fig. 1, and some relevant pharmacokinetic parameters are listed in Table 3. In cirrhotic rats, the changes in the pharmacokinetic parameters of omeprazole are as follows: the AUC was significantly greater (149% increase), terminal half-life and MRT were significantly longer (103 and 371% increase, respectively), V_{ss} was significantly larger (86.1% increase), CL and CL_{NR} were significantly slower (58.0 and 58.9% decrease, respectively), and percentage of intravenous dose of omeprazole excreted in 24-h urine as unchanged drug ($Ae_{0-24\,h}$) was significantly greater (485% increase) than the controls. Omeprazole was below the detection limit in the gastrointestinal tract (including its contents and feces) at 24 h ($GI_{24\,h}$) for both groups of rats. Note that body weight gain also decreased significantly in cirrhotic rats (from 201 to 262 g) compared with the controls (from 198 to 353 g).

Table 3
Pharmacokinetic parameters of omeprazole after intravenous administration at a dose of 20 mg/kg to control rats and cirrhotic rats induced by dimethylnitrosamine[a]

Parameter	Control ($n=7$)	Cirrhosis ($n=8$)
Body weight (g)		
Initial	198 ± 15.4	201 ± 8.34
Final	353 ± 20.8	262 ± 26.9[b]
AUC (μg min/ml)	474 ± 147	1180 ± 102[b]
Terminal half-life (min)	16.6 ± 2.46	33.7 ± 29.2[c]
MRT (min)	9.86 ± 2.37	46.4 ± 5.67[b]
V_{ss} (ml/kg)	389 ± 243	724 ± 245[c]
CL (ml/min/kg)	42.6 ± 13.2	17.9 ± 5.48[b]
CL_R (ml/min/kg)	0.195 ± 0.0622	0.276 ± 0.655
CL_{NR} (ml/min/kg)	42.3 ± 13.2	17.4 ± 5.07[b]
$Ae_{0-24\,h}$ (% of dose)	0.504 ± 0.261	2.95 ± 3.49[d]

[a] Each value represents the mean ± S.D.
[b] Significantly different ($P<0.001$) from control.
[c] Significantly different ($P<0.05$) from control.
[d] Significantly different ($P<0.01$) from control.

The ascite/plasma ratios of omeprazole at 60 min after 1-min intravenous infusion of the drug at a dose of 20 mg/kg to cirrhotic rats were 0.324 ± 0.106. Omeprazole was stable (almost

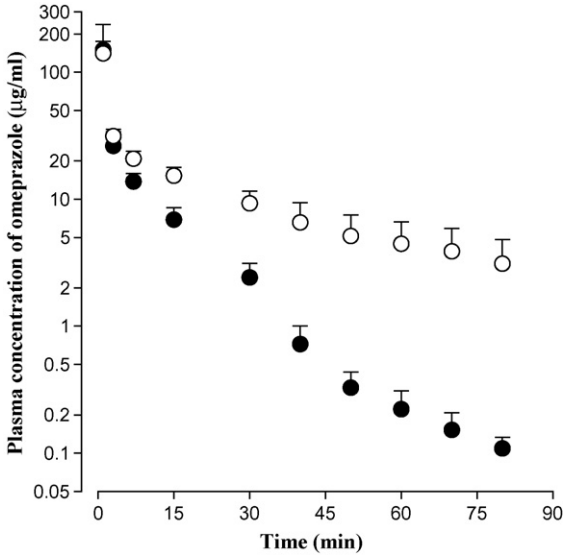

Fig. 1. Mean arterial plasma concentration–time profiles of omeprazole after 1-min intravenous administration at a dose of 20 mg/kg to control rats (●; $n=8$) and cirrhotic rats induced by dimethylnitrosamine (◯; $n=8$). Vertical bars represent standard deviation.

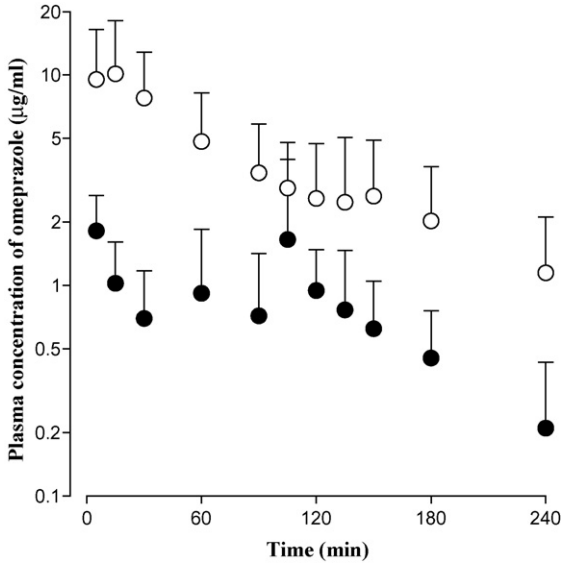

Fig. 2. Mean arterial plasma concentration–time profiles of omeprazole after oral administration at a dose of 40 mg/kg to control rats (●; $n=8$) and cirrhotic rats induced by dimethylnitrosamine (◯; $n=8$). Vertical bars represent standard deviation.

Table 4
Pharmacokinetic parameters of omeprazole after oral administration at a dose of 40 mg/kg to control rats and cirrhotic rats induced by dimethylnitrosamine[a]

Parameter	Control ($n=9$)	Cirrhosis ($n=9$)
Body weight (g)		
Initial	223 ± 4.67	221 ± 14.1
Final	316 ± 11.9	238 ± 20.2[b]
AUC (μg min/ml)	185 ± 86.2	1020 ± 466[b]
C_{max} (μg/ml)	2.66 ± 2.00	11.6 ± 8.32[c]
T_{max} (min)	40.6 ± 49.1	11.1 ± 8.58
Terminal half-life (min)	24.3 ± 42.4	72.0 ± 60.9[d]
CL_R (ml/min/kg)	0.895 ± 0.785	0.290 ± 0.840
$Ae_{0-24\,h}$ (% of dose)	0.422 ± 0.0917	1.12 ± 0.581[c]
$GI_{24\,h}$ (% of dose)	1.10 ± 0.523	3.83 ± 3.77
F (%)	9.75	43.5

[a] Each value represents the mean ± S.D.
[b] Significantly different ($P<0.001$) from control.
[c] Significantly different ($P<0.01$) from control.
[d] Significantly different ($P<0.05$) from control.

complete recovery) up to 4-h incubation in ascite of cirrhotic rats.

3.4. Pharmacokinetics of omeprazole after oral administration to rats

After oral administration of omeprazole at a dose of 40 mg/kg to control and cirrhotic rats, the mean arterial plasma concentrations–time profiles of omeprazole are shown in Fig. 2, and some relevant pharmacokinetic parameters are listed in Table 4. After oral administration of omeprazole, absorption of the drug was rapid; omeprazole was detected in plasma from the first blood sampling time (5 min) and the plasma concentration of the drug reached rapidly its peak (T_{max}) at 11.1–40.6 min for both groups of rats. In cirrhotic rats, the changes in the pharmacokinetic parameters of omeprazole are as follows: the AUC was significantly greater (451% increase), C_{max} was significantly higher (336% increase), terminal half-life was significantly longer (196% increase), and $Ae_{0-24\,h}$ was significantly greater (165% increase) than the controls. In cirrhotic rats, the F was considerably greater than the controls (346% increase). Note that body weight gain again decreased significantly in cirrhotic rats (from 221 to 238 g) compared with the controls (from 223 to 316 g).

4. Discussion

Induction of cirrhosis by dimethylnitrosamine in rats was proven as follows. The ascite was identified in every cirrhotic rat. The plasma levels of GOP, GPT, total bilirubin, and ALP became significantly higher, however, the total proteins and albumin became significantly lower, relative liver weight became significantly lighter (Table 1), and body weight gain became significantly smaller (Tables 1, 3 and 4) than the controls. Liver microscopy also supports the impaired liver function in cirrhotic rats.

After intravenous administration of omeprazole at doses of 2.5, 5, and 10 mg/kg to rats, the $AUC_{0-2\,h}$ of the drug was dose-proportional and terminal half-life, V_{ss}, and CL of the drug were also dose-independent (Watanabe et al., 1994). In the preliminary study, the $AUC_{0-2\,h}$ of omeprazole after intravenous administration of the drug at a dose of 20 mg/kg to control rats was approximately two-time of $AUC_{0-2\,h}$ obtained after intravenous administration of the drug at a dose of 10 mg/kg to rats (Watanabe et al., 1994). After oral administration of omeprazole at doses of 10, 20, and 40 mg/kg to rats, the pharmacokinetic parameters of the drug including $AUC_{0-3\,h}$, C_{max}, T_{max}, and terminal half-life were also dose-independent (Watanabe et al., 1994). Hence, the intravenous and oral doses of omeprazole, 20 and 40 mg/kg, respectively, were arbitrarily chosen in the present study.

After intravenous administration of omeprazole, the contribution of CL_R to CL of the drug was almost negligible; the values were less than 1.54% for both groups of rats (Table 3). This suggests that almost all of the intravenously administrated omeprazole is eliminated via the nonrenal route (CL_{NR}). The contribution of biliary excretion of omeprazole to CL_{NR} of the drug was also negligible; it was reported (Lee et al., 2006) that only 0.0436 ± 0.0159% of dose was excreted as unchanged omeprazole in 24-h bile after intravenous administration of the drug at a dose of 20 mg/kg to 10 control rats with bile duct cannulation. This suggests that omeprazole is almost completely metabolized in rats. Two major metabolites of omeprazole, omeprazole sulphone and omeprazole sulphide, were formed in rats (Webster et al., 1985). Hence, the CL_{NR} of omeprazole listed in Table 3 could represent the metabolic clearance of omeprazole in rats. Therefore, the changes in the CL_{NR} of omeprazole could be due to changes in the metabolism of omeprazole in rats.

After intravenous administration of omeprazole to cirrhotic rats, the significantly greater AUC of the drug could be due to significantly slower CL than the controls (Table 3). The slower CL could be due to significantly slower CL_{NR} in cirrhotic rats since the CL_R was comparable between two groups of rats (Table 3). The slower CL_{NR} in cirrhotic rats (Table 3) could be due to decreased metabolism of omeprazole caused by decreased expressions of CYP1A2 and 3A1 in cirrhotic rats, because omeprazole was mainly metabolized via CYP1A1/2, 2D1, and 3A1/2 in rats (Lee et al., 2006). It was reported that liver was the main metabolizing organ for omeprazole in humans (Karam et al., 1996) and in rats (Watanabe et al., 1994). Since omeprazole is an intermediate hepatic extraction ratio drug (hepatic first-pass effect of approximately 60% in rats; Watanabe et al., 1994), the hepatic clearance of the drug in rats depends on the intrinsic clearance of omeprazole (CL_{int}), free (unbound to plasma proteins) fractions of omeprazole in plasma, and hepatic blood flow rate (Wilkinson and Shand, 1975). The significantly slower CL_{NR} of omeprazole in cirrhotic rats (Table 3) could be supported by significantly slower *in vitro* CL_{int} for the disappearance of omeprazole (Table 2) and significantly slower hepatic blood flow rate. Goeting et al. (1986) reported that hepatic blood flow rate was slower in cirrhotic rats induced by carbon tetrachloride. Since the free fractions of omeprazole in plasma significantly increased in cirrhotic rats (189% increase), its contribution to significantly slower CL_{NR} of omeprazole in cirrhotic rats seemed to be not considerable. Note that the expression of

CYP2D1 was not investigated in cirrhotic rats. Hence, the role of CYP2D1 on the CL_{NR} of omeprazole in cirrhotic rats should be studied.

If pleural or peritoneal cavity expanded by ascite or pleural effusion, they may act as a site of storage and release of some drugs including methotrexate (MTX), with resultant prolonged elevation of plasma concentrations and more severe toxicity. It was reported that after intravenous administration of MTX at a dose of 12 g/m^2 body surface area for 6 h to six patients with pleural effusion, the MTX concentration in pleural fluid were considerably higher (the pleural fluid/plasma ratio was 4.81–25.0 during 12–48 h after MTX administration) than that in plasma and its half-life was longer (about two times) than patients without pleural effusion (Evans and Pratt, 1978). However, in the present study, the omeprazole concentration in ascite was considerably lower than that in plasma (the ascite/plasma ratio was only 0.324). Although the exact volume of ascite could not be measured, the volume was no more than about 3 ml. Therefore, the potential effect of the ascite on the pharmacokinetics of omeprazole seemed to be not considerable.

After intravenous administration of omeprazole, the CL_R of the drug was estimated as free fractions of omeprazole in plasma based on CL_R (Table 3) and plasma protein binding values. The values thus estimated were 0.924 and 0.453 ml/min/kg for control and cirrhotic rats, respectively. The 0.924 and 0.453 ml/min/kg were considerably slower than the CL_{CR} (Table 1). The above data indicate that omeprazole is mainly reabsorbed in the renal tubules for both groups of rats. The renal extraction ratios of omeprazole (CL_R of omeprazole/renal plasma flow rate; only for urinary excretion of unchanged omeprazole) were estimated based on the CL_R of omeprazole (Table 3), reported kidney blood flow rate of 36.8 ml/min/kg in control rats [(Davies and Morris, 1993); the value was not changed in cirrhotic rats induced by carbon tetrachloride (Fujiya et al., 1989; Atucha et al., 1993)], and hematocrit values (Bae et al., 2004). The values thus estimated were 0.994 and 1.18% for control and cirrhotic rats, respectively. The above data indicate that omeprazole is extracted poorly via rat kidney (a poor renal extraction ratio drug) for both control and cirrhotic rats. It was also reported that omeprazole was poorly excreted via the kidney (Regardh et al., 1985).

After intravenous administration of omeprazole to cirrhotic rats, the V_{ss} of the drug was significantly larger than the controls (Table 3), and this could be due to significant increase in free fractions of omeprazole in plasma; the free fractions were 21.1 and 60.9% for control and cirrhotic rats, respectively. Similar results were also reported in rats; the V_{ss} of oltipraz was significantly larger in cirrhotic rats due to increase in free fractions of the drug in plasma (Bae et al., 2004). The increase in free fractions (decrease in plasma protein binding) of omeprazole in plasma of cirrhotic rats may be due to significantly lower level of plasma albumin (Table 1) and α_1-acid glycoprotein (AAG). The plasma concentrations of albumin and AAG decreased in cirrhotic rats induced by carbon tetrachloride (Fouad et al., 1996). Omeprazole considerably binds to albumin and AAG; the binding values of omeprazole at a concentration of 0.2 μM to human serum albumin at a concentration of 1.8×10^{-4} M alone, AAG at a concentration of 0.8 mg/ml alone, and both at 37 °C were 92.4, 91.9, and 93.4%, respectively (Regardh et al., 1985). The corresponding values of omeprazole at a concentration of 2.0 μM were 86.3, 76.1, and 93.7% (Regardh et al., 1985).

After oral administration of omeprazole to cirrhotic rats, the AUC of the drug was also significantly greater than the controls (Table 4). However, this was not due to increase in gastrointestinal absorption of omeprazole in cirrhotic rats, since it was reported that omeprazole is absorbed almost completely from rat gastrointestinal tract in control rats (Watanabe et al., 1994). Moreover, the $GI_{24\,h}$ values were almost negligible; 1.10 and 3.83% of oral dose for control and cirrhotic rats, respectively (Table 4). Note that after oral administration of omeprazole to cirrhotic rats, the AUC of the drug increased 451% (Table 4), which was greater than 149% after intravenous administration (Table 3). Hence, only decreased hepatic metabolism of omeprazole in cirrhotic rats could not fully explain the 451% difference after oral administration (Table 4). Hence, the greater AUC of oral omeprazole in cirrhotic rats (Table 4) could be due to significantly slower metabolism of omeprazole in rat intestine as well as liver by decrease in the expressions of CYP1A2 and 3A1 in cirrhotic rats. It was reported that CYP1A2 and 3A1 are expressed in small intestine of male Sprague–Dawley rats (Kaminsky and Fasco, 1991; Hakkak et al., 1993). The intestinal first-pass effect of omeprazole was considerable in control rats; it was estimated that the intestinal first-pass effect of omeprazole was approximately 72.4% in control rats (Watanabe et al., 1994). The decrease in intestinal first-pass effect of omeprazole in cirrhotic rats may also explain the considerably greater F than the controls (Table 4). The F value of 12.6% was reported from other control rats (Watanabe et al., 1994).

5. Conclusions

After intravenous administration of omeprazole to cirrhotic rats, the CL_{NR} of omeprazole was significantly slower than the controls (Table 3) due to decrease in the expressions of CYP1A2 and 3A1 in cirrhotic rats. Slower CL_{NR} of omeprazole in cirrhotic rats could be supported by significantly slower *in vitro* CL_{int} for the disappearance of omeprazole and slower hepatic blood flow rate in cirrhotic rats. After oral administration of omeprazole to cirrhotic rats, the AUC difference of the drug compared with the controls was considerably greater than that after intravenous administration. This could be due to decrease in the first-pass effect of omeprazole in the intestine as well as in the liver in cirrhotic rats.

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