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Faster clearance of omeprazole in rats with acute renal failure induced by uranyl nitrate: contribution of increased expression of hepatic cytochrome P450 (CYP) 3A1 and intestinal CYP1A and 3A subfamilies

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Abstract

It has been reported that omeprazole is mainly metabolized via hepatic cytochrome P450 (CYP) 1A1/2, CYP2D1 and CYP3A1/2 in male Sprague–Dawley rats, and the expression of hepatic CYP3A1 is increased in male Sprague–Dawley rats with acute renal failure induced by uranyl nitrate (U-ARF rats). Thus, the metabolism of omeprazole would be expected to increase in U-ARF rats. After intravenous administration of omeprazole (20 mg kg^{-1}) to U-ARF rats, the area under the plasma concentration–time curve from time zero to infinity (AUC) was significantly reduced (371 vs 494 μg min mL^{-1}), possibly due to the significantly faster non-renal clearance (56.6 vs 41.2 mL min^{-1} kg^{-1}) compared with control rats. This could have been due to increased expression of hepatic CYP3A1 in U-ARF rats. After oral administration of omeprazole (40 mg kg^{-1}) to U-ARF rats, the AUC was also significantly reduced (89.3 vs 235 μg min mL^{-1}) compared with control rats. The AUC difference after oral administration (62.0% decrease) was greater than that after intravenous administration (24.9% decrease). This may have been primarily due to increased intestinal metabolism of omeprazole caused by increased expression of intestinal CYP1A and 3A subfamilies in U-ARF rats, in addition to increased hepatic metabolism.

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Introduction

Omeprazole, 5-methoxy-2-[(4-methoxy-3,5-dimethyl-2-pyridinyl)-methylsulfoxide]-1H-benzimidazole, is a gastric parietal cell proton pump inhibitor. The drug has greater antisecretory activity than histamine H_2-receptor antagonists and has widely been used in the treatment of peptic ulcer, efflux oesophagitis and Zollinger–Ellison syndrome (Berglindh & Sachs 1985; Im et al 1985). Lee et al (2006a) reported that omeprazole is mainly metabolized via hepatic microsomal cytochrome P450 (CYP) 1A1/2, CYP2D1 and CYP3A1/2 (not via CYP2C11 and CYP2E1) in male Sprague–Dawley rats. Moon et al (2003) reported the following hepatic CYP isozyme changes in male Sprague–Dawley rats with acute renal failure induced by uranyl nitrate (U-ARF rats). For example, in U-ARF rats, the expression (based on Western blot analysis) and mRNA levels (based on Northern blot analysis) of CYP1A2 were not altered, but the expression of CYP3A1 increased compared with control rats. However, the mRNA levels of CYP3A1 and CYP3A2 were not altered in U-ARF rats. This may be as a result of protein stabilization (i.e. a decrease in protein turnover). However, to date, no studies on the expression and/or mRNA level of the hepatic CYP2D subfamily in U-ARF rats have been reported. Although intestinal CYP1A and CYP3A subfamilies are reported to be the most abundant CYP subfamilies in rats (Kaminsky & Fasco 1991) and their role in the metabolism of some drugs including omeprazole (Lee et al 2007) has been reported to be important, no studies on the expression and/or mRNA levels of the intestinal CYP1A and 3A subfamily in U-ARF rats have been reported to date.

It is well known that patients with renal failure may suffer from various types of upper gastrointestinal tract lesions such as oesophagitis, erosive or atrophic gastritis, gastro-duodenal

ulcers, and polyps (Iwabuchi & Matsuzaki 2002). It has also been reported that disease states such as renal insufficiency and liver disease, age, drugs and genetic factors could change the CYP isozyme(s) and these changes sometimes lead to adverse drug reactions, even with medications such as omeprazole, which has a good safety profile (Borras-Blasco et al 2001; Lutz et al 2002). Although pharmacokinetic changes of some drugs in U-ARF rats have previously been reported (Lee M. G. et al 2006), changes with respect to hepatic and intestinal CYP isozymes have received little attention. Changes in the expression and mRNA levels of hepatic CYP isozymes in U-ARF rats have been reported (Moon et al 2003), but studies on changes in the hepatic CYP2D subfamily and intestinal CYP isozymes in U-ARF rats have not yet been reported. To our knowledge, no studies on the pharmacokinetics of omeprazole in U-ARF rats or in humans with acute renal failure have been reported.

The present study reports changes in the expression of hepatic CYP2D subfamily proteins and intestinal CYP1A and 3A subfamily proteins in U-ARF rats based on Western blot analysis, and pharmacokinetic changes of omeprazole after intravenous (20 mg kg^{-1}) or oral (40 mg kg^{-1}) administration to U-ARF rats with respect to hepatic and intestinal CYP isozyme changes.

Materials and Methods

Chemicals

Omeprazole and torasemide, the internal standard for the high-performance liquid chromatographic (HPLC) analysis of omeprazole, were donated from Yungjin Pharmaceutical Company (Seoul, South Korea) and Roche Pharmaceutical Company (Mannheim, Germany), respectively. The reduced form of β-nicotinamide adenine dinucleotide phosphate (NADPH; as a tetrasodium salt), β-actin, Kodak X-OMAT film, tris(hydroxymethyl) aminomethane (Tris) buffer, and ethylenediamine tetraacetic acid (EDTA; as a disodium salt) were purchased from Sigma-Aldrich Corporation (St Louis, MO, USA). The primary monoclonal anti-rabbit antibody for β-actin was purchased from Cell Signaling Technology (Danvers, MA, USA). Uranyl nitrate was a product from BDH Chemicals (Poole, UK). Anti-rat polyclonal CYP1A, 2D and 3A antibodies and horseradish peroxidase-conjugated goat anti-rabbit antibody were products from Detroit R&D (Detroit, MI, USA) and Bio-Rad Laboratories (Hercules, CA, USA), respectively. Enhanced chemiluminescence reagents were products from Amersham Biosciences Corporation (Piscataway, NJ, USA). Other chemicals were of reagent grade or HPLC grade.

Animals

Protocols for the animal studies were approved by the Animal Care and Use Committee of the College of Pharmacy of Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats (7–8 weeks old, 230–290 g) were purchased from the Charles River Company Korea (Orient, Seoul, South Korea). The rats were randomly divided into two groups: control and U-ARF rats. They were maintained in a clean-room (Animal Center for Pharmaceutical Research, College of Pharmacy, Seoul National University) at a temperature of 20 to 23°C, with a 12-h light–dark cycle (lights on: 0700–1900 hours) and a relative humidity of 50 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered, pathogen-free air, with food (Samyang Company, Pyeongtaek, South Korea) and water freely available.

Induction of acute renal failure in rats by uranyl nitrate injection

Uranyl nitrate (freshly dissolved in 0.9% NaCl-injectable solution to make 0.5%) at a dose of 5 mg kg^{-1} (1 mL) was injected once via the tail vein to induce acute renal failure (Moon et al 2003). The same volume of 0.9% NaCl-injectable solution was injected into control rats.

Measurement of liver and kidney function

The following study was performed in control and U-ARF rats (n = 5 in each group) at Day 5 after intravenous administration of uranyl nitrate or 0.9% NaCl-injectable solution, to measure liver and kidney function. A 24-h urine sample was collected for the measurement of the creatinine level. A plasma sample was also collected for the measurement of total protein, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), free fatty acid (FFA), triglycerides and creatinine levels (analysed by the Green Cross Reference Lab., Seoul, South Korea), and protein binding of omeprazole using equilibrium dialysis. 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 10% neutral phosphate-buffered formalin and then processed for routine histological examination with haematoxylin–eosin staining.

Preparation of hepatic or intestinal microsomal fractions

The procedures used for the preparation of hepatic (Ahn et al 2003; Chung et al 2003; Moon et al 2003) and intestinal (Peng et al 2004) microsomal fractions were similar to reported methods, with a minor modification. On Day 5, the livers of control and U-ARF rats (n = 5 in each group) were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) in ice-cold buffer of 0.154 M KCl/50 mM Tris-HCl in 1 mM EDTA (pH 7.4). The homogenate was centrifuged (10 000 *g*, 30 min) and the supernatant fraction was further centrifuged (100 000 *g*, 90 min).

The small intestines of control and U-ARF rats (n = 3 each) were excised and rinsed with ice-cold solution of 0.154 M NaCl and 1 mM dithiothreitol. Then, the intestine was filled with solution A (1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, 8 mM KH_2PO_4, 5.6 mM Na_2HPO_4 and 40 μg mL^{-1} phenylmethylsulfonyl fluoride (PMSF)) and incubated in a water bath (37°C, 15 min). After incubation, solution A was discarded and the intestine was filled with ice-cold solution B (12 mM Na_2HPO_4, 0.274 mM NaH_2PO_4, 123 mM NaCl, 1.5 mM EDTA, 0.5 mM dithiothreitol and 40 μg mL^{-1} PMSF). The lumen was

wounded and tapped against the finger. Solution B including upper villus cells was centrifuged (10 000 *g*, 5 min) and ice-cold solution C (5 mM histidine, 0.25 mM dithiothreitol, 0.5 mM EDTA and 40 μg mL^{-1} PMSF) was added to the precipitate and centrifuged (20 000 *g*, 15 min). The pellets were resuspended in ice-cold solution C and centrifuged (10 000 *g*, 20 min). The supernatant fraction was further centrifuged (100 000 *g*, 65 min). Protein content in the hepatic or intestinal microsomes was measured using a reported method (Bradford 1976).

Western blot analysis

The procedures used were similar to the method reported by Kim et al (2001). The above hepatic or intestinal microsomal fractions (10 μg protein per lane) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on a 7.5% gel, transferred to a nitrocellulose membrane (Bio-Rad, Inc., Hercules, CA, USA), and blocked for 1 h in 5% milk powder in PBS-T (0.05% v/v Tween 20 in phosphate-buffered saline solution). For immunodetection, blots were incubated overnight with rabbit anti-rat CYP1A, 2D, and 3A antibodies (diluted 1:10 000 in 5% bovine serum albumin in PBS-T) at 4°C, followed by incubation with goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (diluted 1:10 000 in 5% milk powder in PBS-T) for 1 h at room temperature. Proteins were detected by enhanced chemiluminescence on Kodak X-OMAT film and scanning densitometry was performed with a microcomputer imaging device (model M1; Imaging Research, St Catharines, Ontario, Canada). β-Actin was used as a loading control.

Measurement of kinetic parameters for the disappearance of omeprazole in hepatic microsomal fractions of control and U-ARF rats

The maximum velocity (V_{max}) and the apparent Michaelis–Menten constant (K_m; the concentration at which the rate is one-half of the V_{max}) for the disappearance of omeprazole were determined after incubating the above hepatic microsomal fractions (equivalent to 0.5 mg protein), a 5-μL aliquot of 0.1 M carbonate buffer (pH 9.8) containing the final omeprazole concentration of 1, 2.5, 5, 10 and 20 μM, and a 50-μL aliquot of Tris-HCl buffer (pH 7.4) containing 1 mM NADPH in a final volume of 0.5 mL by adding 0.1 M phosphate buffer (pH 7.4), in a water-bath shaker (37°C, 500 oscillations min^{-1}). All of the above microsomal incubation conditions were linear. The reaction was terminated by the addition of 1 mL of diethyl ether after 5 min incubation. The kinetic constants (K_m and V_{max}) for the disappearance of omeprazole were calculated using a non-linear regression method (Duggleby 1995). The intrinsic clearance (CL_{int}) for the disappearance of omeprazole was calculated by dividing the V_{max} by the K_m.

Measurement of rat plasma protein binding of omeprazole

Protein binding of omeprazole to fresh plasma from control and U-ARF rats (n = 4 each) was determined using equilibrium dialysis (Lee D. Y. et al 2006). Plasma (1 mL) was dialysed against 1 mL of isotonic Sørensen phosphate buffer (pH 7.4) containing 3% (w/v) dextran (the buffer), in a 1-mL dialysis cell (Fisher Scientific, Fair Lawn, NJ, USA) using a Spectra/Por 4 membrane (molecular weight cutoff 12–14 kDa; Spectrum Medical Industries Inc., Los Angeles, CA, USA). Omeprazole (dissolved in 0.1 M carbonate buffer pH 9.8) was added into the plasma side at a concentration of 10 μg mL^{-1}. The dialysis cell to which omeprazole was added was incubated for 8 h in a water-bath shaker (37°C, 50 oscillations min^{-1}). The binding of omeprazole to 4% human serum albumin was constant, 91.7 ± 0.785%, at omeprazole concentrations ranging from 1 to 200 μg mL^{-1} (Lee D. Y. et al 2006). Thus, an omeprazole concentration of 10 μg mL^{-1} was arbitrarily chosen for this plasma protein binding study.

Pretreatment of rats for the intravenous or oral study

The procedures used were similar to a previously reported method (Kim et al 1993). Early in the morning, the carotid artery (for blood sampling) and the jugular vein (for drug administration in the intravenous study) of each rat were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ, USA) while each rat was under light diethyl ether anaesthesia. Both tubes were inserted into a wire coil to allow free movement of the rat. Both cannulas were exteriorized to the dorsal side of the neck where each cannula was terminated with a long Silastic tube (Dow Corning, Midland, MI, USA). A heparinized 0.9% NaCl-injectable solution (15 units mL^{-1}; 0.25 mL) was used to flush the cannula to prevent blood clotting. Then, each rat was housed individually in a metabolic cage (Daejong Scientific Company, Seoul, South Korea) and allowed to recover from anaesthesia for 4–5 h before beginning the experiment. Watanabe et al (2002) reported that immobilization stress could change the pharmacokinetics of omeprazole in rats and so the rats were not restrained in the present study.

Intravenous study

Omeprazole (the same solution used in the plasma protein binding study) at a dose of 20 mg kg^{-1} (2 mL) was infused over 1 min via the jugular vein of control (n = 14) and U-ARF (n = 9) rats. A heparinized 0.9% NaCl-injectable solution (0.3 mL) was used to flush the cannula immediately after each blood sampling. A blood sample (approx. 0.22 mL) was collected via the carotid artery at time 0 (control) and 1 (at the end of the infusion), 3, 7, 15, 30, 45, 60, 70, 80 and 90 min after the start of the intravenous infusion of omeprazole. Blood samples were centrifuged immediately and a 100-μL aliquot of each plasma sample was stored at −70°C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC, USA) until used for the HPLC analysis of omeprazole (Kang et al 1999). At the end of the experiment (24 h), each metabolic cage was rinsed with 5 mL of distilled water and the rinsings were combined with the 24-h urine sample. After measuring the exact volume of the 24-h urine and the combined urine samples, two 100-μL aliquots of the combined urine sample were stored at −70°C until used for the 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 killed by cervical dislocation. Then, the abdomen was opened and the entire gastrointestinal tract (including its contents and faeces) of each rat was removed, transferred into a beaker containing 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 at −70°C until used for the HPLC analysis of omeprazole (Kang et al 1999).

The following experiment was also performed in additional control (n = 4) and U-ARF (n = 5) rats to determine if the timed-interval renal clearance of omeprazole is dependent on the urine flow rate in both groups of rats. A priming dose (280 μg kg^{-1}) of omeprazole was administered as an intravenous bolus and followed by intravenous infusion (1200 μg h^{-1}; 0.5 mL) for 3 h with the assistance of an infusion pump (Model 2400-006; Harvard Instrument, Southnatick, MA, USA) to reach a plateau plasma concentration of omeprazole of approximately 1 μg mL^{-1}. Priming and maintenance doses of omeprazole were estimated based on the intravenous data. A 0.9% NaCl-injectable solution was infused at rates of 0.2–2.0 mL min^{-1} to control the urine flow rate.

Oral study

Omeprazole (the same solution used in the intravenous study) at a dose of 40 mg kg^{-1} (5 mL) was administered orally using a feeding tube in control (n = 10) and U-ARF (n = 9) rats. Blood samples were collected at 0, 5, 15, 30, 60, 75, 90, 105, 120, 135, 150, 180 and 240 min after the oral administration of omeprazole. Other procedures were similar to those for the intravenous study.

In-vitro disappearance of omeprazole in rat tissue homogenates

The procedures used were similar to reported methods (Litterst et al 1975; Yu et al 2003). Approximately 1 g each of stomach, small intestine and large intestine of control (n = 4) and U-ARF (n = 5) rats was excised after cervical dislocation, rinsed with cold 0.9% NaCl-injectable solution, blotted dry with tissue paper and weighed. Metabolic activity was initiated by adding 300-μL of the 9000 *g* supernatant fraction of each tissue homogenate (each tissue was homogenized with 4 vols of 0.25 M sucrose solution) to an Eppendorf tube containing 280 μL of Tris buffer, 10 μL of 0.1 M carbonate buffer (pH 9.8) containing 10 μg of omeprazole, and 10 μL of Tris buffer containing 1 mM NADPH. To terminate enzyme activity, 1 mL of diethyl ether was added after 30 min incubation (37°C, 500 oscillations min^{-1}) in a thermomixer (Thermomixer 5436; Eppendorf, Hamburg, Germany).

HPLC analysis of omeprazole

Concentrations of omeprazole in the samples were determined by a slight modification of the HPLC method reported by Kang et al (1999): torasemide instead of lansoprazole was used as internal standard. Briefly, to a 2.2-mL microfuge tube containing a 100-μL of the sample, a 50-μL aliquot of methanol containing torasemide (50 μg mL^{-1}; internal standard) 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 diethyl ether. 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 125 μL of the mobile phase and a 50-μL aliquot was directly injected onto a reversed-phase HPLC column (C_8; 150 mm length × 4.6 mm, i.d.; particle size, 5 μm; Waters, Milford, MA, USA). The mobile phase comprising phosphate buffer (0.2 M KH_2PO_4, pH 7.0)/acetonitrile (77:23 v/v), was run at a flow rate of 1.4 mL min^{-1}, and the column eluent was monitored using a UV detector at 302 nm. The retention times of omeprazole and torasemide (internal standard) were approximately 10.2 and 8.1 min, respectively. The detection limits of omeprazole in the rat plasma and urine samples were 20 and 50 ng mL^{-1}, respectively. The coefficients of variation of omeprazole in the rat plasma and urine samples were below 5.34 and 7.90%, respectively.

Pharmacokinetic analysis

The total area under the plasma concentration–time curve from time zero to infinity (AUC) was calculated using the trapezoidal rule-extrapolation method (Chiou 1978). 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 & Perrier 1982) were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis (WinNonlin; Pharsight Corporation, Mountain View, CA, USA); the time-averaged total body, renal and non-renal clearance (CL, CL_R and CL_{NR}, respectively), the timed-interval renal clearance, the terminal half-life, the first moment of AUC (AUMC), the mean residence time (MRT), the apparent volume of distribution at steady state (V_{ss}), and the extent of absolute oral bioavailability (F) (Kim et al 1993). The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were read directly from the experimental data.

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

Statistical analysis

A P value less than 0.05 was deemed to be statistically significant using an unpaired t-test. All data are expressed as the mean ± s.d.

Results

Liver and kidney function

Bodyweight, plasma chemistry data, plasma protein binding of omeprazole, CL_{CR} and relative liver and relative kidney weights in control and U-ARF rats are listed in Table 1. In

Table 1 Bodyweight, plasma chemistry data, plasma protein binding of omeprazole, creatinine clearance and relative liver and kidney weight in control rats and rats with acute renal failure induced by uranyl nitrate (U-ARF)

Parameter	Control	U-ARF
Initial bodyweight (g)	254 ± 11.4	275 ± 7.07*
Final bodyweight (g)	277 ± 7.58	261 ± 22.2
Plasma		
Total proteins (g dL^{-1})	5.54 ± 0.182	5.32 ± 0.581
Albumin (g dL^{-1})	3.48 ± 0.130	3.26 ± 0.297
Urea nitrogen (mg dL^{-1})	12.0 ± 0.876	144 ± 63.6*
GOT (IU L^{-1})	56.2 ± 5.59	136 ± 18.5**
GPT (IU L^{-1})	22.4 ± 6.73	14.0 ± 5.29
FFA (μEq L^{-1})	1560 ± 424	1650 ± 608
Triglyceride (mg dL^{-1})	98.0 ± 48.1	81.8 ± 25.8
Protein binding (%)	83.2 ± 2.85	73.6 ± 3.01*
CL_{CR} (mL min^{-1} kg^{-1})	2.58 ± 0.457	0.217 ± 0.103**
Liver weight (% of bodyweight)	3.60 ± 0.257	3.49 ± 0.109
Kidney weight (% of bodyweight)	0.778 ± 0.0473	1.08 ± 0.0590**

GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; FFA, free fatty acid; CL_{CR}, creatinine clearance. Values are mean ± s.d., n = 5. *$P < 0.01$ and **$P < 0.001$ significantly different compared with control rats.

U-ARF rats, impaired kidney function was observed: the plasma level of urea nitrogen became significantly greater (1100% increase), the CL_{CR} became significantly slower (91.6% decrease), and the relative kidney weight became significantly greater (38.8% increase) compared with control rats. The plasma level of urea nitrogen in U-ARF rats was higher than reported values (5.0–29.0 mg dL^{-1}) in control rats (Mitruka & Rawnsley 1981). Impaired kidney function in U-ARF rats was also supported by kidney histology; the distal convoluted tubule underwent extensive acute tubular necrosis. In contrast, no significant findings were observed based on the kidney histology of control rats. Hepatic function, however, seemed not to be seriously impaired in U-ARF rats; the plasma levels of total protein, albumin, GPT, FFA and triglyceride, and the relative liver weight were not significantly different between the two groups of rats, although the plasma level of GOT became significantly higher compared with that in control rats (142% increase). The plasma level of GOT in U-ARF rats was higher than reported values (45.7–80.8 IU L^{-1}) in control rats (Mitruka & Rawnsley 1981). That hepatic function in U-ARF rats was not seriously impaired was supported by the liver histology: minimal to mild hepatocellular swelling (hydropic change) was observed. However, there were no significant findings based on the liver histology of control rats. Similar results have been reported in other studies (Ahn et al 2003; Chung et al 2003). Plasma protein binding of omeprazole in U-ARF rats became significantly less than that in control rats (11.5% decrease). The bodyweight gain significantly decreased in U-ARF rats (from 275 to 261 g) compared with that in control rats (from 254 to 277 g), as reported in other studies (Ahn et al 2003; Chung et al 2003).

Western blot analysis

The levels of the hepatic CYP2D subfamily and intestinal CYP1A and 3A subfamilies measured by Western blot analysis with the hepatic and intestinal microsomes prepared from two groups of rats are shown in Figure 1. In U-ARF rats, the expression of the hepatic CYP2D subfamily significantly decreased (26.3%) compared with that in control rats. However, in U-ARF rats, the expression of the intestinal CYP1A and 3A subfamilies significantly increased (200 and 55.8% increase, respectively) compared with that in control rats.

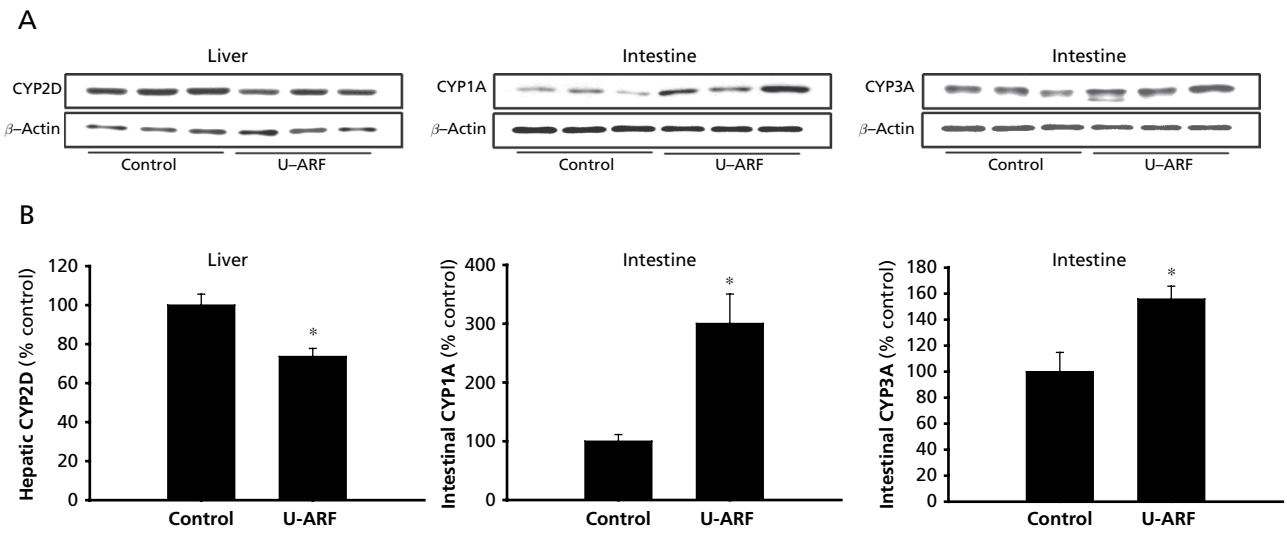

Figure 1 Immunoblotting of hepatic CYP2D and intestinal CYP1A and 3A in rats with acute renal failure induced by uranyl nitrate (U-ARF) and control rats (A), and expressed in terms of % of control rats (100%) (B). β-Actin was used as a loading control. Vertical bars represent s.d. *$P < 0.05$ significantly different compared with the control (each value was significantly different).

V_{max}, K_m and CL_{int} for the disappearance of omeprazole in hepatic microsomal fractions of control and U-ARF rats

The V_{max} values for the disappearance of omeprazole were 2.29 ± 0.461 and 4.26 ± 0.427 nmol min^{-1} (mg protein)$^{-1}$ for control and U-ARF rats, respectively; the value in U-ARF rats became significantly faster (86.0% increase) compared with that in control rats. The above data suggest that the maximum velocity for the disappearance (primarily metabolism) of omeprazole became significantly faster compared with that in control rats. However, the K_m values were not significantly different between two groups of rats (18.2 ± 6.16 and 25.3 ± 3.94 mM for control and U-ARF rats, respectively), suggesting that the affinity of the enzyme(s) for omeprazole was not altered in U-ARF rats. Thus, the CL_{int} for the disappearance of omeprazole in U-ARF rats became significantly faster (29.8% increase) compared with that in control rats (0.131 ± 0.0223 and 0.170 ± 0.0212 mL min^{-1} (mg protein)$^{-1}$, respectively). The above data suggest that metabolism of omeprazole increased in U-ARF rats. In U-ARF rats, the total protein in the hepatic microsome was significantly smaller compared with that in control rats (30.0% decrease; 174 ± 26.0 and 122 ± 15.1 mg (total liver weight)$^{-1}$ for control and U-ARF rats, respectively).

Table 2 Pharmacokinetic parameters of omeprazole after intravenous administration at a dose of 20 mg kg^{-1} to control rats and rats with acute renal failure induced by uranyl nitrate (U-ARF)

Parameter	Control	U-ARF
Initial bodyweight (g)	267 ± 19.7	266 ± 17.1
Final bodyweight (g)	297 ± 7.96	258 ± 14.1***
Urine output (mL 24-h^{-1})	18.4 ± 8.04	2.22 ± 1.72***
AUC (μg min mL^{-1})	494 ± 63.5	371 ± 79.9***
V_{ss} (mL kg^{-1})	301 ± 81.3	394 ± 208†
CL (mL min^{-1} kg^{-1})	41.2 ± 5.70	56.6 ± 13.4***
CL_R (mL min^{-1} kg^{-1})	0.351 ± 0.241	0.0640 ± 0.0350*
CL_{NR} (mL min^{-1} kg^{-1})	40.8 ± 5.74	55.6 ± 16.3**
$Ae_{0-24\,h}$ (% of dose)	0.880 ± 0.674	0.0640 ± 0.0350*
$GI_{24\,h}$ (% of dose)	BD	BD

AUC, area under the plasma concentration–time curve from time zero to infinity; V_{ss}, the apparent volume of distribution at steady state; CL, CL_R and CL_{NR}, the time-averaged total body, renal and non-renal clearance, respectively; $Ae_{0-24\,h}$, percentage of omeprazole dose excreted in the 24-h urine as unchanged drug; $GI_{24\,h}$, percentage of omeprazole dose in the gastrointestinal tract at 24 h; BD, below the detection limit. Values are mean ± s.d., n = 14 for control rats and n = 9 for U-ARF rats. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ †$P = 0.0813$, significantly different compared with control rats.

Pharmacokinetics of omeprazole after its intravenous administration to rats

The mean arterial plasma concentration–time profiles of omeprazole after intravenous administration at a dose of 20 mg kg^{-1} to control and U-ARF rats are shown in Figure 2, and the relevant pharmacokinetic parameters are listed in Table 2. Compared with control rats, the AUC became significantly smaller (24.9% decrease), the CL (43.4% increase) and the CL_{NR} (36.3% increase) became significantly faster, the CL_R became significantly slower (81.8% decrease), and the percentage of the intravenous dose of omeprazole excreted in the 24-h urine as unchanged drug ($Ae_{0-24\,h}$; 92.7% decrease) and the 24-h urine output (87.9% decrease) became significantly smaller in U-ARF rats. In U-ARF rats, the V_{ss} became considerably ($P = 0.0813$) larger compared with that in control rats (30.9% increase). Omeprazole was below the detection limit in the gastrointestinal tract at 24 h for all rats studied. Bodyweight gain also significantly decreased in U-ARF rats compared with that in control rats.

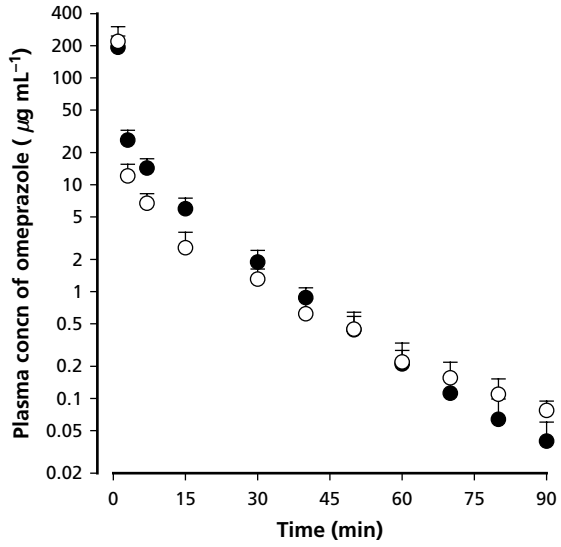

Figure 2 Mean arterial plasma concentration–time profiles of omeprazole after intravenous administration at a dose of 20 mg kg^{-1} to control rats (•; n = 14) and rats with acute renal failure induced by uranyl nitrate (U-ARF) (○; n = 9). Vertical bars represent the s.d.

The urine flow rate-dependent timed-interval renal clearances of omeprazole in both control and U-ARF rats were evident. There was a straight line between 1/timed-interval renal clearance and 1/urine flow rate (Chiou 1986) in both control and U-ARF rats (data not shown): the less urine evacuated, the less omeprazole was excreted in the urine.

Pharmacokinetics of omeprazole after oral administration to rats

The mean arterial plasma concentration–time profiles of omeprazole after oral administration at a dose of 40 mg kg^{-1} to control and U-ARF rats are shown in Figure 3, and the relevant pharmacokinetic parameters are listed in Table 3. After oral administration of omeprazole, absorption of the drug from the gastrointestinal tract was rapid; omeprazole was detected in plasma from the first blood sampling time (5 min) and rapidly reached C_{max} (T_{max} values of 37.0 and 40.6 min for control and U-ARF rats, respectively). Compared with control rats, the AUC (62.0% decrease), the $Ae_{0-24\,h}$ (98.0% decrease), and the 24-h urine output (75.0% decrease) became significantly smaller, the CL_R became significantly

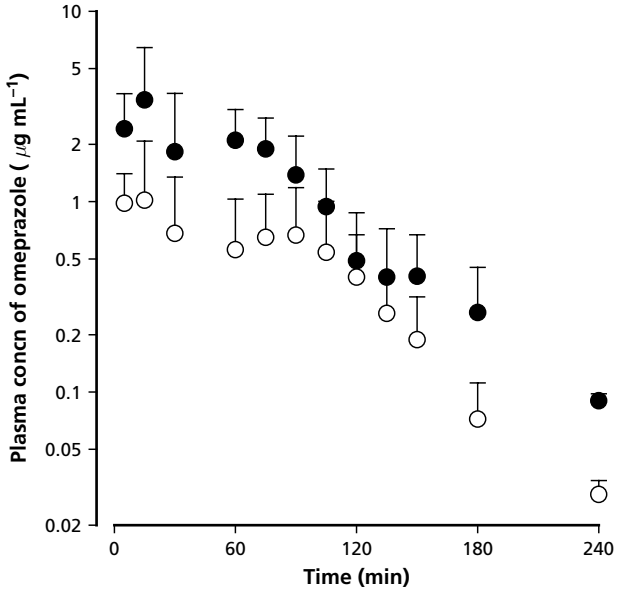

Figure 3 Mean arterial plasma concentration–time profiles of omeprazole after oral administration at a dose of 40 mg kg^{-1} to control rats (•; n = 10) and rats with acute renal failure induced by uranyl nitrate (U-ARF) (○; n = 9). Vertical bars represent the s.d.

Table 3 Pharmacokinetic parameters of omeprazole after oral administration at a dose of 40 mg kg^{-1} to control rats and rats with acute renal failure induced by uranyl nitrate (U-ARF)

Parameter	Control	U-ARF
Initial bodyweight (g)	264 ± 19.6	266 ± 17.1
Final bodyweight (g)	277 ± 11.3	250 ± 13.0***
Urine output (mL 24-h^{-1})	8.00 ± 4.64	2.00 ± 6.89*
AUC (μg min mL^{-1})	235 ± 71.6	89.3 ± 32.3***
C_{max} (μg mL^{-1})	4.37 ± 2.45	1.53 ± 0.863**
T_{max} (min)	37.0 ± 33.7	40.6 ± 41.6
Terminal half-life (min)	22.4 ± 11.3	22.9 ± 8.44
CL_R (mL min^{-1} kg^{-1})	1.68 ± 1.12	0.0740 ± 0.0721*
$Ae_{0-24\,h}$ (% of dose)	0.881 ± 0.533	0.0180 ± 0.0147**
$GI_{24\,h}$ (% of dose)	2.63 ± 2.33	1.47 ± 0.951
F (%)	23.8	12.0

AUC, area under the plasma concentration–time curve from time zero to infinity; C_{max}, the peak plasma concentration; T_{max}, the time to reach C_{max}; CL_R, time-averaged total renal clearance; $Ae_{0-24\,h}$, percentage of omeprazole dose excreted in the 24-h urine as unchanged drug; $GI_{24\,h}$, percentage of omeprazole dose in the gastrointestinal tract at 24 h; F, bioavailability; Values are mean ± s.d., n = 10 for control rats and n = 9 for U-ARF rats. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ significantly different compared with control rats.

slower (95.6% decreased), and the C_{max} became significantly lower (65.0% decrease) in U-ARF rats. The F values were 23.8 and 12.0% for control and U-ARF rats, respectively. Bodyweight gain was again significantly decreased in U-ARF rats compared with that in control rats.

In-vitro disappearance of omeprazole in rat tissue homogenates

In both control and U-ARF rats, the stomach (86.7 ± 6.82 and 73.0 ± 7.76% of the spiked amounts of omeprazole remained for control and U-ARF rats, respectively), the small intestine (80.8 ± 5.55 and 70.8 ± 6.67%, respectively), and the large intestine (96.0 ± 3.45 and 75.2 ± 10.8%, respectively) had some metabolic activity for omeprazole, with the exception of the large intestine of control rats. In U-ARF rats, the metabolic activity of stomach, small intestine and large intestine was significantly increased compared with that in control rats.

Discussion

It has been reported that the AUC values of omeprazole were dose-proportional after intravenous (doses of 2.5–20 mg kg^{-1}) or oral (doses of 10–40 mg kg^{-1}) administration to rats (Watanabe et al 1994; Lee D. Y. et al 2006). Thus, intravenous and oral doses of 20 and 40 mg kg^{-1}, respectively, were arbitrarily chosen for this study.

The CL_R/CL ratios of omeprazole were less than 0.570% (Table 2) and the 24-h biliary excretion of omeprazole was only 0.0436 ± 0.0159% of the dose (Lee D. Y. et al 2006). This suggests that omeprazole is almost completely metabolized in rats. Two major metabolites of omeprazole, omeprazole sulfone and omeprazole sulfide, were formed in rats (Webster et al 1985). Thus, the CL_{NR} of omeprazole listed in Table 2 could represent the metabolic clearance of the drug in rats. Additionally, changes in the CL_{NR} of omeprazole could represent changes in the hepatic metabolism of the drug in rats.

After intravenous administration of omeprazole to U-ARF rats, the CL became significantly faster, possibly due to the significantly faster CL_{NR}, because the CL_R became significantly slower than that in control rats (Table 2). The faster CL_{NR} of omeprazole in U-ARF rats (Table 2) could have been due primarily to increased hepatic metabolism of the drug caused by increased expression of hepatic CYP3A1 (Moon et al 2003), because the expression and mRNA level of hepatic CYP1A2 were not altered (Moon et al 2003), and the expression of the CYP2D subfamily was decreased (Figure 1) in U-ARF rats. This suggests that the contribution of decreased expression of the CYP2D subfamily to increased hepatic metabolism of omeprazole in U-ARF rats is not considerable compared with that of increased CYP3A1 expression. Since omeprazole is mainly metabolized in the liver and is an intermediate hepatic extraction ratio drug (Watanabe et al 1994), its hepatic clearance depends on the intrinsic clearance for the disappearance of the drug (CL_{int}), the free (unbound to plasma protein) fraction of omeprazole in plasma, and the hepatic blood flow rate in rats (Wilkinson & Shand 1975). The significantly faster CL_{NR} of omeprazole in U-ARF rats (Table 2) may have been supported by the significantly faster in-vitro CL_{int} for the disappearance of the drug and significantly greater free fraction of the drug in plasma (57.1% increase) compared with control rats (Table 1). However, the contribution of the hepatic blood flow rate to the significantly faster CL_{NR} of omeprazole in U-ARF rats did not

seem to be considerable, because the hepatic blood flow rate was rather slower in U-ARF rats induced by glycerol (Kishimoto et al 1989).

After intravenous administration of omeprazole to U-ARF rats, the V_{ss} became larger than that in control rats. This may have been due, at least in part, to the significantly greater free fraction of omeprazole in plasma in U-ARF rats (57.1% increase). Similar results have also been reported in other studies. For example, in U-ARF rats, the V_{ss} of azosemide (Park et al 1998) and phenytoin (Itoch et al 1988) became larger due to the greater free fraction of the drugs in plasma compared with that in control rats. The significantly reduced plasma protein binding of omeprazole in U-ARF rats compared with that in control rats (Table 1) could have been due to the accumulation of endogenous binding inhibitors in ARF (Bowmer & Lindup 1979).

Interestingly, treatment of rats with subcutaneous injections of recombinant human growth hormone (rGH) (5 units kg^{-1}, twice daily; U-ARFGH rats) for 1 day on Day 4 or 10% (w/v) glucose in drinking water for 5 days (U-ARFG rats) after uranyl nitrate injection partially restored both the expression and mRNA level of CYP2E1, which were induced by U-ARF compared with control rats (Chung et al 2002). These restorative effects on CYP2E1 may have been due to glucose utilization, which is enhanced by growth hormone (Waxman et al 1989; Peng & Coon 1998; Son et al 2000), an important determinant for CYP2E1 expression. These restoration effects on CYP2E1 have been demonstrated (Ahn et al 2003; Chung et al 2003) by the pharmacokinetics of 6-hydroxychlorzoxazone (a metabolite of chlorzoxazone), which is mainly formed via CYP2E1 in rats (Rockich & Blouin 1999; Ahn et al 2003; Chung et al 2003; Moon et al 2003). In order to determine if the increase in the expression of CYP3A1 in U-ARF rats is also restored to control levels by glucose feeding or rGH injection, the same dose of omeprazole was administered intravenously to U-ARFG (n = 8) and U-ARFGH (n = 7) rats. The pharmacokinetic parameters of omeprazole in U-ARFG and U-ARFGH rats were not significantly different compared with those in U-ARF rats (data not shown). This suggests that the expression of CYP3A1 seemed not to be restored to control levels by glucose feeding or rGH injection.

After oral administration of omeprazole to U-ARF rats, the AUC became significantly smaller than that in control rats (Table 3). However, this was unlikely to be due to decreased gastrointestinal absorption of omeprazole in U-ARF rats, because it has been reported that omeprazole is absorbed almost completely from the rat gastrointestinal tract (Watanabe et al 1994). After oral administration of omeprazole, the AUC in U-ARF rats was significantly smaller than that in control rats (62.0% decrease; Table 3). The 62.0% decrease was greater than the 24.9% decrease after intravenous administration of omeprazole (Table 2). Thus, increased hepatic metabolism of omeprazole alone could not fully explain the 62.0% decrease after oral administration in U-ARF rats (Table 3). The smaller AUC of omeprazole after oral omeprazole in U-ARF rats (Table 3) could have been primarily due to the significantly faster intestinal metabolism (greater intestinal first-pass effects) of the drug than that in control rats caused by increased expression of intestinal CYP1A and 3A subfamilies (Figure 1). Kaminsky & Fasco (1991) reported that CYP1A and 3A subfamilies are most abundant in rat intestine; the CYP2D subfamily is expressed at a very low level in the intestine. It has been estimated that the intestinal first-pass effect of omeprazole is considerable, being approximately 72.4% of the oral dose in rats (Watanabe et al 1994). Thus, the hepatic first-pass effect of omeprazole (59% after absorption into the portal vein) in rats is equivalent to 15.9% of the oral dose considering the 72.4% intestinal first-pass effect. Therefore, the contribution of the increased hepatic first-pass effect to the smaller AUC after oral administration did not seem to be considerable compared with that of the intestinal first-pass effect. The increased metabolizing activity of intestine in U-ARF rats was also proven by the results of in-vitro rat gastrointestinal tissue homogenate studies. In the present study, the F value in U-ARF rats (12.0%) was less than the 23.8% in control rats (49.6% decrease; Table 3). The low F value in U-ARF rats could also be a result of the significantly increased gastrointestinal first-pass effect of omeprazole in U-ARF rats.

Conclusion

After intravenous administration of omeprazole to U-ARF rats, the CL_{NR} of the drug became significantly faster compared with that in control rats, and this could have been due to increased expression of hepatic CYP3A1 in U-ARF rats (Moon et al 2003). After oral administration of omeprazole to U-ARF rats, the AUC of the drug became significantly smaller (62.0% decrease) compared to that in control rats; the 62.0% decrease was greater than the 29.4% decrease after intravenous administration. This could have been primarily due to increased intestinal metabolism caused by increased expression of intestinal CYP1A and 3A subfamilies in U-ARF rats.

References

Ahn, C. Y., Kim, E. J., Lee, I., Kwon, J. W., Kim, W. B., Kim, S. G., Lee, M. G. (2003) Effects of glucose on the pharmacokinetics of intravenous chlorzoxazone in rats with acute renal failure induced by uranyl nitrate. *J. Pharm. Sci*. **92**: 1604–1613

Berglindh, T., Sachs, G. (1985) Emerging strategies in ulcer therapy: pumps and receptors. *Scand. J. Gastroenterol*. **108**: 7–14

Borras-Blasco, J., Navarro-Ruiz, A., Navarro-Blasco, F., Tovar-Beltran, J., Gonzalez-Delgado, M. (2001) Erythrodermia induced by omeprazole. *Int. J. Clin. Pharmacol. Ther*. **39**: 219–223

Bowmer, C. J., Lindup, W. E. (1979) Investigation of the drug-binding defect in plasma from rats with glycerol-induced acute renal failure. *J. Pharmacol. Exp. Ther*. **210**: 440–445

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*. **72**: 248–254

Chiou, W. L. (1978) Critical evaluation of the potential error in pharmacokinetic studies using the linear trapezoidal rule method for the calculation of the area under the plasma level–time curve. *J. Pharmacokinet. Biopharm*. **6**: 539–546

Chiou, W. L. (1986) A new simple approach to study the effect of changes in urine flow and/or urine pH on renal clearance and its applications. *Int. J. Clin. Pharmacol. Ther. Toxicol*. **24**: 519–527

Chung, H. C., Kim, S. H., Lee, M. G., Kim, S. G. (2002) Increase in urea in conjunction with L-arginine metabolism in the liver leads to induction of cytochrome P450 2E1 (CYP2E1): the role of urea in CYP2E1 induction by acute renal failure. *Drug Metab. Dispos*. **30**: 739–746

Chung, W. -S., Kim, E. J., Lee, I., Kim, S. G., Lee, M. G., Kim, S. H. (2003) Effects of recombinant human growth hormone on the pharmacokinetics of intravenous chlorzoxazone in rats with acute renal failure induced by uranyl nitrate. *Life Sci*. **73**: 253–263

Duggleby, R. G. (1995) Analysis of enzyme progress curves by non-linear regression. *Methods Enzymol*. **249**: 61–90

Gibaldi, M., Perrier, D. (1982) *Pharmacokinetics*, 2nd edn. Marcel-Dekker, New York

Im, W., Blakeman, D. P., Davis, J. P. (1985) Irreversible inactivation of rat gastric (H^+-K^+)-ATPase in vivo by omeprazole. *Biochem. Biophys. Res. Commun*. **126**: 78

Itoch, T., Sawada, Y., Lin, T. H., Iga, T., Hanano, M. (1988) Kinetic analysis of phenytoin disposition in rats with experimental renal and hepatic disease. *J. Pharmacobiodyn*. **11**: 289–308

Iwabuchi, H., Matsuzaki, K. (2002) The treatment of peptic ulcer in elderly CRF patients. *Nippon Rinsho* **60**: 1601–1605

Kaminsky, L. S., Fasco, M. J. (1991) Small intestinal cytochromes P450. *Crit. Rev. Toxicol*. **21**: 407–422

Kang, W. K., Kim, D. S., Kwon, K. I. (1999) Advanced method for determination of omeprazole in plasma by HPLC. *Arch. Pharm. Res*. **22**: 86–88

Kim, S. H., Choi, Y. M., Lee, M. G. (1993) Pharmacokinetics and pharmacodynamics of furosemide in protein-calorie malnutrition. *J. Pharmacokinet. Biopharm*. **21**: 1–17

Kim, S. G., Kim, E. J., Kim, Y. G., Lee, M. G. (2001) Expression of cytochrome P-450s and glutathione S-transferases in the rat liver during water deprivation: effects of glucose supplementation. *J. Appl. Toxicol*. **21**: 123–129

Kishimoto, T., Sakamoto, W., Nakatani, T., Ito, T., Iwai, K., Kim, T., Abe, Y. (1989) Cardiac output, renal blood flow and hepatic blood flow in rats with glycerol-induced acute renal failure. *Nephron* **53**: 353–357

Lee, D. Y., Shin, H. S., Bae, S. K., Lee, M. G. (2006) Effects of enzyme inducers and inhibitors on the pharmacokinetics of intravenous omeprazole in rats. *Biopharm. Drug Dispos*. **27**: 209–218

Lee, D. Y., Kim, J. W., Lee, M. G. (2007) Pharmacokinetic interaction between oltipraz and omeprazole in rats: competitive inhibition of metabolism of oltipraz by omeprazole via CYP1A1 and 3A2, and of omeprazole by oltipraz via CYP1A1/2, 2D1/2, and 3A1/2. *Eur. J. Pharm. Sci*. **32**: 328–329

Lee, M. G., Lee, J. H., Oh, J. M. (2006) Pharmacokinetic changes of drugs in rat model of acute renal failure induced by uranyl nitrate: correlation between drug metabolism and hepatic microsomal cytochrome P450 isozymes. *Curr. Clin. Pharmacol*. **1**: 193–205

Litterst, C. L., Mimnaugh, E. G., Regan, R. L., Gram, T. E. (1975) Comparison of *in vitro* drug metabolism by lung, liver, and kidney of several common laboratory species. *Drug Metab. Dispos*. **3**: 259–265

Lutz, M., Schwab, M., Griese, E. U., Marx, C., Muller-Oerlinghausen, B., Schonhofer, P. S., Meisner, C., Gleiter, C. H., Eichelbaum, M., Morike, K. (2002) Visual disorders associated with omeprazole and their relation to CYP2C19 polymorphism. *Pharmacogenetics* **12**: 73–75

Mitruka, B. M., Rawnsley, H. M. (1981) *Clinical biochemical and hematological reference values in normal experimental animals and normal humans*, 2nd edn. Masson Publishing USA Inc., New York

Moon, Y. J., Lee, A. K., Chung, H. J., Kim, E. J., Lee, S. H., Lee, D. C., Lee, I., Kim, S. G., Lee, M. G. (2003) Effects of acute renal failure on the pharmacokinetics of chlorzoxazone in rats. *Drug Metab. Dispos*. **31**: 776–784

Park, K. J., Yoon, W. H., Kim, S. H., Shin, W. G., Lee, M. G. (1998) Pharmacokinetics and pharmacodynamics of azosemide after intravenous and oral administration of azosemide to uranyl nitrate-induced acute renal failure rats. *Biopharm. Drug Dispos*. **19**: 141–146.

Peng, H. M., Coon, M. J. (1998) Regulation of rabbit cytochrome P450 2E1 expression in HepG2 cells by insulin and thyroid hormone. *Mol. Pharmacol*. **54**: 740–747

Peng, J. Z., Remmel, R. P., Sawchuk, R. K. (2004) Inhibition of murine cytochrome P4501A by tacrine: *in vitro* studies. *Drug Metab. Dispos*. **32**: 805–812

Rockich, K., Blouin, R. (1999) Effect of the acute-phase response on the pharmacokinetics of chlorzoxazone and cytochrome P-450 2E1 *in vitro* activity in rats. *Drug Metab. Dispos*. **27**: 1074–1077

Son, M. H., Kang, K. W., Kim, E. J., Ryu, J. H., Cho, H., Kim, S. H., Kim, W. B., Kim, S. G. (2000) Role of glucose utilization in the restoration of hypophysectomy-induced hepatic cytochrome P450 2E1 by growth hormone in rats. *Chem. Biol. Interact*. **127**: 13–28

Watanabe, K., Furuno, K., Eto, K., Oishi, R., Gomita, Y. (1994) First-pass metabolism of omeprazole in rats. *J. Pharm. Sci*. **83**: 1131–1134

Watanabe, K., Matsuka, N., Okazaki, M., Hashimoto, Y., Araki, H., Gomita, Y. (2002) The effect of immobilization stress on the pharmacokinetics of omeprazole in rats. *Acta Med. Okayama* **56**: 19–23

Waxman, D. J., Morrissey, J. J., Leblanc, G. A. (1989) Hypophysectomy differentially alters P-450 protein levels and enzyme activities in rat liver: pituitary control of hepatic NADPH cytochrome P-450 reductase. *Mol. Pharmacol*. **35**: 519–525

Webster, L. K., Jones, D. B., Mihaly, G. W., Morgan, D. J., Smallwood, R. A. (1985) Effect of hypoxia on oxidative and reductive pathways of omeprazole metabolism by the isolated perfused rat liver. *Biochem. Pharmacol*. **34**: 1239–1245

Wilkinson, G. R., Shand, D. G. (1975) A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther*. **18**: 377–390

Yu, S. Y., Bae, S. K., Kim, E. J., Kim, Y. G., Kim, S. O., Lee, D. H., Lim, H., Lee, M. G. (2003) Dose-independent pharmacokinetics of a new reversible proton pump inhibitor, KR-60436, after intravenous and oral administration to rats: gastrointestinal first-pass effect. *J. Pharm. Sci*. **92**: 1592–1603