Changes in omeprazole pharmacokinetics in rats with diabetes induced by alloxan or streptozotocin: Faster clearance of omeprazole due to induction of hepatic CYP1A2 and 3A1

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ABSTRACT. Purpose. To investigate the effect of diabetes mellitus induced by alloxan (DMIA) or streptozotocin (DMIS) on the pharmacokinetics of omeprazole in rats. It has been reported that omeprazole is primarily metabolized via hepatic CYP1A2, 2D1, and 3A1 in rats. The expression and mRNA levels of hepatic CYP1A2 and 3A1 increases in DMIA and DMIS rats, but the expression of hepatic CYP2D1 does not change in DMIS rats. In addition, the metabolic activities of intestinal CYP3A1/2 decreases in DMIS rats. Thus, it could be expected that the pharmacokinetics of omeprazole would be affected by changes in both DMIA and DMIS. **Methods.** Omeprazole was administered intravenously (20 mg/kg) and orally (40 mg/kg) to DMIA and DMIS rats and their respective controls. **Results.** After intravenous administration of omeprazole, the CL_{NR} of the drug was significantly faster in DMIA (52.6 versus 67.4 mL/min/kg) and DMIS (50.2 versus 73.0 mL/min/kg) rats than the respective controls. However, after oral administration of omeprazole, the AUC was comparable between each type of diabetic rat and the respective controls. **Conclusions.** The significantly faster CL_{NR} of intravenous omeprazole could be due to increased expression and mRNA levels of hepatic CYP1A2 and 3A1 in both types of diabetic rat. The comparable AUC of oral omeprazole could be due to a decrease in the intestinal first-pass effect of omeprazole caused by decreased intestinal CYP3A1/2 in diabetic rats. Following both intravenous and oral administration in DMIA and DMIS rats, the pharmacokinetics of omeprazole were similarly altered.

INTRODUCTION

Omeprazole, 5-methoxy-2-[{(4-methoxy-3,5-dimethyl-2-pyridinyl)-methyl}sulfoxide]-1H-benzimidazole, is a gastric parietal cell proton pump inhibitor. The drug has greater anti-secretory activity than histamine H_2-receptor antagonists and has been widely used in the treatment of peptic ulcers, efflux esophagitis, and Zollinger–Ellison syndrome (1, 2). Recently, it was reported that omeprazole is primarily metabolized via hepatic microsomal cytochrome P450 (CYP) 1A1/2, 2D1, and 3A1/2, but not via CYP2B1/2, 2C11, or 2E1, in male Sprague–Dawley rats (3).

Kim et al. (4) reported that in rats with diabetes mellitus induced by both alloxan (DMIA) and streptozotocin (DMIS), the expression and mRNA levels of hepatic CYP1A2 and 3A1 increased compared to their respective control male Sprague–Dawley rats. Results consistent with these CYP isozyme changes have been reported in other studies: hepatic CYP1A1, 1A2, 3A1, or 3A2 increased in DMIA or DMIS rats based on Western blot analysis or various enzyme activity tests (5–9). However, it has also been reported that expression of hepatic CYP2D1 did not change in DMIS rats (9). Changes in intestinal CYP isozymes in DMIS rats have also been reported. For example, testosterone 6-β-hydroxylase activity (a CYP3A1/2 marker in rats) in the small intestine of DMIS rats was reduced to half that of controls (6). However, 7-ethoxycoumarin-O-deethylase activity (a CYP1A1/2 marker in rats) in the intestine of DMIS rats increased (10). The CYP1A and 3A subfamilies are abundantly expressed in rat intestine (11). In addition to changes in hepatic and intestinal CYP isozymes, decreased bile flow rate and altered bile composition (12), hepatotoxicity (13), and impaired kidney function (14, 15) have been reported in DMIS rats.

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Formation of glucuronide, glutathione, and sulfate conjugates are also profoundly affected in DMIA and DMIS (16, 17). Thus, it could be expected that the pharmacokinetics of omeprazole would be altered in diabetic (DMIS, DMIA) rats.Masuda et al. (18) found that the incidence of peptic ulcers in diabetic patients was significantly elevated. It was also reported that disease states can cause changes in CYP isozyme(s) and these changes can sometimes lead to adverse drug reaction, even with medications such as omeprazole, which has a good safety profile (19, 20). Thus, we examined omeprazole in this study.

Although pharmacokinetic changes for some drugs have previously been reported in DMIA or DMIS rats, changes with respect to hepatic and intestinal CYP isozyme changes have received little attention (4, 15, 21–23 and references therein). To our knowledge, no study on omeprazole has yet been reported, likely because the hepatic CYP isozymes responsible for the metabolism of omeprazole have only recently been reported (3).

Major differences exist in the diabetogenic effects of streptozotocin and alloxan (24). Structural alteration in pancreatic beta cells (total degranulation) occurs within 48 h after administration of streptozotocin and lasts for up to 4 months. Alloxan causes a decrease in hepatic glycogen within 24 to 72 h, an effect that is partially reversible by insulin. Alloxan generally produces greater cytotoxicity because of its conversion to anionic radicals. We studied both DMIA and DMIS rats.

The aim of this study was to examine changes in omeprazole pharmacokinetics after intravenous (20 mg/kg) and oral (40 mg/kg) administration to DMIA or DMIS rats with respect to increased expression and mRNA levels of hepatic CYP1A1/2 and 3A1/2 (4) and decreased levels of intestinal CYP3A1/2 (6).

Abbreviations:

HPLC, high-performance liquid chromatography; 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; CL_{CR}, time-averaged creatinine clearance; Vd_{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; C_{max}, peak plasma concentration; T_{max}, time to reach a C_{max}; $Ae_{0–24\ h}$, percentage of dose excreted in 24 h urine; $GI_{24\ h}$, percentage of dose recovered from the gastrointestinal tract (including its contents and feces) at 24 h; F, extent of absolute oral bioavailability.

METHODS AND MATERIALS

Chemicals

Omeprazole and torasemide [an internal standard for the high-performance liquid chromatographic (HPLC) analysis of omeprazole] were donated by Yungjin Pharmaceutical Company (Seoul, South Korea) and Roche Pharmaceutical Company (Mannheim, Germany), respectively. Alloxan, streptozotocin, the 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.

Animals

The protocol for this animal study was approved by the Animal Care and Use Committee of the College of Pharmacy of Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats, 6–7 weeks old and weighing 230–295 g, were purchased from the Charles River Company Korea (Orient, Seoul, South Korea), 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 12-h light (0700–1900) and dark (1900–0700) cycles and a relative humidity of 50 ± 5%. Rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered pathogen-free air and with food (Samyang Company, Pyeongtaek, South Korea) and water available *ad libitum*.

Induction of Diabetes Mellitus in Rats by Alloxan or Streptozotocin Injection

Rats were randomly divided into four groups, DMIA, DMIS, and their respective control groups. Freshly prepared alloxan (40 mg/kg) was administered (total injection volume of approximately 0.25 mL) to overnight-fasted rats via the tail vein for two consecutive days (4, 21–23). An equal volume of a 0.9% NaCl-injectable solution was injected into the controls. Freshly prepared streptozotocin (45 mg/kg) was administered (total injection volume of approximately 0.3 mL) once to overnight-fasted rats via the tail vein (4, 15, 23). An equal volume of a citrate buffer (pH 4.5) was injected into the controls. On the fourth day after the first alloxan administration (DMIA rats) or the 0.9% NaCl-

injectable solution (controls for DMIA rats), and on the seventh day after administration of streptozotocin (DMIS rats) or the citrate buffer, pH 4.5 (controls for DMIS rats), blood glucose levels were measured using the Medisense Optium Kit (Abbott Laboratories, Bedford, MA) and rats with blood glucose levels higher than 250 mg/dL were selected as being diabetic.

Preliminary Study

The following preliminary study was performed at the fourth day (DMIA rats and their controls; n = 4, each) and the seventh day (DMIS rats and their controls; n = 4, each) to measure liver and kidney function. A 24-h urine sample was collected for the measurement of creatinine levels. Plasma was collected for the measurement of total protein, albumin, urea nitrogen, glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), and creatinine levels (analyzed by Green Cross Reference Lab., Seoul, South Korea). The whole kidney and liver of each rat were excised, rinsed with a 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 hematoxylin–eosin staining.

Measurement of V_{max}, K_m, and CL_{int} for the Disappearance of Omeprazole in Hepatic Microsomal Fractions

The procedures used were similar to reported methods (4). The livers (6–7 g) of DMIA (n = 5), DMIS (n = 4), and their control (n = 5) rats were homogenized (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) in 15 mL of ice-cold buffer [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). Protein content was measured using a reported method (25). 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 0.1 M carbonate buffer (pH 9.8) that contained 1, 2.5, 5, 10, 20, or 50 µM omeprazole, and a 50-µL aliquot of 1 mM 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 (kept at 37 ^{o}C, 500 oscillations/min). All of the above microsomal incubation conditions were linear. The reaction was terminated by addition of 1 mL of diethyl ether after 5 min incubation. Omeprazole was measured by a reported HPLC method (26). The kinetic constants (K_m and V_{max}) for the disappearance of omeprazole were calculated using a nonlinear regression method (27). The intrinsic clearance (CL_{int}) for the disappearance of omeprazole was calculated by dividing the respective V_{max} by the respective K_m.

Measurement of Rat Plasma Protein Binding of Omeprazole Using Equilibrium Dialysis

Protein binding of omeprazole to fresh rat plasma from DMIA, DMIS, and their respective controls (n = 5, each) was determined using equilibrium dialysis (28). Plasma (1 mL) was dialyzed against 1 mL of isotonic Sørensen phosphate buffer (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 Dalton; Spectrum Medical Industries). Omeprazole [dissolved in 0.1 M carbonate buffer (pH 9.8)] was spiked into the plasma compartment at an omeprazole concentration of 10 µg/mL. After 8 h incubation, two 100-µL aliquots were collected from each compartment and stored at –70 ^{o}C (Revco ULT 1490 D-N-S; Western Mednics, Asheville, NC) until used for the HPLC analysis of omeprazole (26). In a preliminary study, binding of omeprazole to 4% human serum albumin was constant, 91.7 ± 0.785%, at omeprazole concentrations ranging from 1 to 200 µg/mL. Thus, an omeprazole concentration of 10 µg/mL was arbitrarily chosen for this plasma protein binding study.

Intravenous Study

In the early morning of the fourth day after starting the treatment with alloxan (DMIA rats) or the 0.9% NaCl-injectable solution (controls for DMIA rats), or on the seventh day after streptozotocin (DMIS rats) or the citric buffer, pH 7.4 (controls for DMIS rats), the carotid artery (for blood sampling) and the jugular vein (for drug administration) of DMIA, DMIS, and their respective controls were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ) while each rat was under light diethyl ether anesthesia (29). Heparinized 0.9% NaCl-injectable solution (15 units/mL), 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 anesthesia for 4–5 h, before beginning the experiment. Because Watanabe et al. (30) reported that immobilization stress could change the pharmacokinetics of omeprazole in rats, the rats were not restrained in the present study. Other procedures were similar to previously reported methods (4, 29). Omeprazole (the same solution used in the plasma protein binding study) at a dose of 20 mg/kg was infused (total infusion volume of 2 mL/kg) over 1 min via the jugular vein to rats in each group (n = 8, 8, 9, and 8 for DMIA rats and their controls, and DMIS rats and their controls, respectively). A blood sample (approximately 0.22 mL) was collected via the carotid artery at 0 (control), 1 (at the end of the infusion), 3, 7, 15, 30, 45, 60, 70, 80, and 90 min after that start of the intravenous infusion of omeprazole. A heparinized 0.9% NaCl-injectable solution (0.3 mL) was used to flush the cannula immediately after each blood sampling. Blood samples were centrifuged immediately and a 100-μL aliquot of each plasma sample was stored at −70 °C until used for the HPLC analysis of omeprazole (26). 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 (26). At the same time (24 h), as much blood as possible was collected via the carotid artery and each rat was sacrificed by cervical dislocation. Then, the abdomen was opened and the entire gastrointestinal tract (including its contents and feces) 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 (26).

Oral Study

Omeprazole (the same solution used in the intravenous study) at a dose of 40 mg/kg was administered orally (total oral volume of 5 mL/kg) using a feeding tube to rats in each group (n = 7, 8, 9, and 8 for DMIA rats and their controls, and DMIS rats and their controls, respectively). 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 for the intravenous study.

HPLC Analysis of Omeprazole

Concentrations of omeprazole in the above samples were determined by a slight modification of a reported HPLC method (26); torasemide instead of lansoprazole was used as an internal standard. In a 2.2-mL microfuge tube containing a 100-μL aliquot of a sample, a 50-μL aliquot of methanol containing torasemide (an internal standard; 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 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 a 125-μL aliquot of the mobile phase and a 50-μL aliquot was injected directly onto a reversed-phase HPLC column (C_8; 150 mm × 4.6 mm; particle size, 5μm; Waters, Milford, MA). The mobile phase, 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, and the column eluent was monitored using an ultraviolet detector at 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. The coefficients of variation of omeprazole in 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 time infinity (AUC) was calculated using the trapezoidal rule–extrapolation method (31). 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 (32) were used to calculate the following pharmacokinetic parameters, using a non-compartmental analysis (WinNonlin 2.1; Pharsight Corp., Mountain View, CA); the time-averaged total body (CL), renal (CL_R), and nonrenal (CL_{NR}) clearances, the terminal half-life, the first moment of AUC (AUMC), the mean residence time (MRT), the apparent volume of distribution at steady state (Vd_{ss}), and the extent of absolute oral bioavailability (F) (29). The peak plasma

concentration (C_{max}) and time to reach C_{max} (T_{max}) were directly read from the experimental data.

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

Statistical Analysis

A p value < 0.05 was deemed to be statistically significant. The unpaired Student's *t*-test was used for comparison between two means. For comparison of more than two means the Duncan's multiple range test of the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL), *posteriori* analysis of variance (ANOVA) was used. All data are expressed as means ± standard deviations.

RESULTS

Preliminary Study

Body weight, blood glucose levels, 24-h urine output, plasma chemistry data, $CL_{CR,}$ and relative liver and kidney weights in DMIA, DMIS, and their respective controls are listed in Table 1. In DMIS rats, the plasma levels of total protein (13.5% decrease) and albumin (15.5% decrease) were significantly lower, and GOT (64.2% increase) and GPT (116% increase) were significantly higher than in the control rats. These parameters were not significantly different between DMIA rats and their controls. The plasma levels of total protein, albumin, GOT, and GPT listed in Table 1 are in the reported ranges for control rats (33). The relative liver weight was not significantly different between either type of diabetic rat and the respective controls. The above data suggest that hepatic function was not seriously impaired in either type of diabetic rat. Consistent with the liver histology, no significant findings were detected in the livers of either type of diabetic rat or their respective controls. However, the plasma levels of urea nitrogen in DMIA and DMIS rats were significantly higher (152 and 82.7% increase, respectively) than those in the respective controls as reported in other studies (15). The plasma levels of urea nitrogen in both diabetic rat groups were higher than the reported values (5.0–29.0 mg/dL) in control rats (33). The CL_{CR} values in DMIA and DMIS rats were not significantly different from their respective controls, as reported in other studies (15). Relative kidney weights were significantly heavier in DMIA and DMIS rats (37.7 and 33.3% increase, respectively) than in their respective controls as reported in other studies (15). These findings suggest that kidney function was somewhat impaired in both types of diabetic rat, although no significant findings were detected in the kidneys in both types of diabetic rat or their respective controls based on histology. Impaired kidney function in diabetic rats has also been reported in other studies (4, 14, 15, 22, 34). Moreover, it has also been reported that nephropathy was induced in DMIS rats (35, 36).

Measurement of V_{max}, K_m, and CL_{int} for the Disappearance of Omeprazole in Hepatic Microsomal Fractions of DMIA, DMIS, and Their Controls

The V_{max}, K_m, and CL_{int} in hepatic microsomal fractions of all rats studied are listed in Table 2. In DMIA and DMIS rats, the V_{max} for the disappearance of omeprazole was significantly faster (100 and 91.1% increase, respectively) than in the control rats; they were not significantly different between DMIA and DMIS rats. This suggests that the maximum velocity for the disappearance (primarily metabolism) of omeprazole was significantly faster in both types of diabetic rat than the control rats. However, the K_m values were not significantly different among the three groups of rats, suggesting that the affinity for omeprazole of the enzyme(s) did not change in either diabetic rat. Hence, the CL_{int} in DMIA and DMIS rats was significantly faster (30.3 and 23.5% increase, respectively) than the control rats, but no significant difference was observed between DMIA and DMIS rats, suggesting that the metabolism of omeprazole increased in both types of diabetic rat compared to the control rats. No difference was detected in protein content in liver microsomes among the three groups of rat.

Plasma Protein Binding of Omeprazole in Rats

The plasma protein binding values of omeprazole in DMIA and the control rats were 44.6 ± 15.3 and 77.0 ± 5.51%, respectively, and the corresponding values in DMIS and the control rats were 76.3 ± 5.26 and 76.8 ± 3.19%, respectively. The value in DMIA rats was significantly smaller than in the controls, but the value in DMIS rats was comparable to that of the controls.

Pharmacokinetics of Omeprazole after Intravenous Administration

For the intravenous administration of omeprazole at a dose of 20 mg/kg to both types of diabetic rat and the respective controls, the mean arterial plasma concentration–time profiles are shown in Figure 1, and relevant pharmacokinetic parameters are listed in Table 3. In DMIA and DMIS rats, the AUC of omeprazole was significantly smaller (21.2 and 26.2% decrease in DMIA and DMIS rats, respectively), while the CL (27.4 and 46.6% increase, respectively), CL_R (192 and 343% increase, respectively), and CL_{NR} (28.1 and 45.4% increase, respectively) were significantly faster. In addition, percentages of the intravenous dose of omeprazole excreted in the 24-h urine as unchanged drug ($Ae_{0–24\ h}$) were significantly greater (120 and 233% increase, respectively) than those of the respective controls. In DMIA rats, the Vd_{ss} was significantly larger (46.1% increase) than in the controls. Omeprazole was below the detection limit in the gastrointestinal tract (including its contents and feces) at 24 h ($GI_{24\ h}$) for all rats studied.

Pharmacokinetics of Omeprazole after Oral Administration

For the oral administration of omeprazole at a dose of 40 mg/kg to both types of diabetic rat and their respective controls, the mean arterial plasma concentration–time profiles are shown in Figure 2 and 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 reached its peak (T_{max}) rapidly; the T_{max} values were 10.0–26.1 min for all groups of rats studied. In DMIA and DMIS rats, the CL_R was significantly faster (717 and 105% increase, respectively), $Ae_{0–24\ h}$ was significantly greater (422 and 104% increase, respectively), and $GI_{24\ h}$ was significantly smaller (57.5 and 58.9% decrease, respectively) than in the respective controls. In DMIA rats, the C_{max} was significantly lower (32.6% decrease) than in the controls. In DMIS rats, the terminal half-life was significantly shorter (47.7% decrease) than in the controls.

DISCUSSION

Induction of diabetes mellitus in rats by alloxan or streptozotocin was evident based on the significantly higher blood glucose levels, increased 24-h urine output, and decreased final body weight (body weight gain; Tables 1, 3, and 4).

After intravenous administration of omeprazole at doses of 2.5, 5, and 10 mg/kg in rats, the $AUC_{0–2\ h}$ of the drug were dose-proportional, and the terminal half-life, Vd_{ss}, and CL of the drug were dose-independent (37). In preliminary studies using control rats, the $AUC_{0–2\ h}$ of omeprazole after intravenous administration of the drug at a dose of 20 mg/kg was approximately two times that of the $AUC_{0–2\ h}$ obtained after intravenous administration of the drug at a dose of 10 mg/kg to rats (37). 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 (37). Thus, 20 and 40 mg/kg intravenous and oral doses of omeprazole, respectively, were arbitrarily chosen for the study. After intravenous administration of omeprazole, the $Ae_{0–24\ h}$ of the drug was less than 1.63% of the dose for all groups of rats (Table 3), indicating that almost all of the intravenous omeprazole was eliminated via a nonrenal (CL_{NR}) route in rats. It has been reported that the contribution of biliary excretion of omeprazole to the CL_{NR} of the drug is almost negligible; only 0.0436 ± 0.0159% of the dose was excreted as unchanged drug in 24-h bile juices after intravenous administration of the drug at a dose of 20 mg/kg to ten control rats after bile duct cannulation (3). This suggests that omeprazole is almost completely metabolized in rats. It has been reported that the liver is the main metabolizing organ for omeprazole in humans (38) and in rats (37). Thus, the CL_{NR} of omeprazole listed in Table 3 could represent the metabolic clearance of the drug. Additionally, the changes in the CL_{NR} of omeprazole could represent changes in metabolism of the drug.

After intravenous administration of omeprazole, the significantly smaller AUC of the drug in both types of diabetic rat could have been due to significantly faster CL than in their respective controls (Table 3). The faster CL was attributable to a significantly faster CL_R and CL_{NR} in both types of diabetic rat. The faster CL_{NR} in both diabetic rat groups (Table 3) could have been due to increased metabolism of omeprazole caused by the increased expression and mRNA levels of hepatic CYP1A1/2 and 3A1/2 (4), because the expression of CYP2D1 does not change in DMIS rats (9). The hepatic first-pass effect of omeprazole has been estimated to be 59% in other rat studies following intravenous and intraportal administration (37).

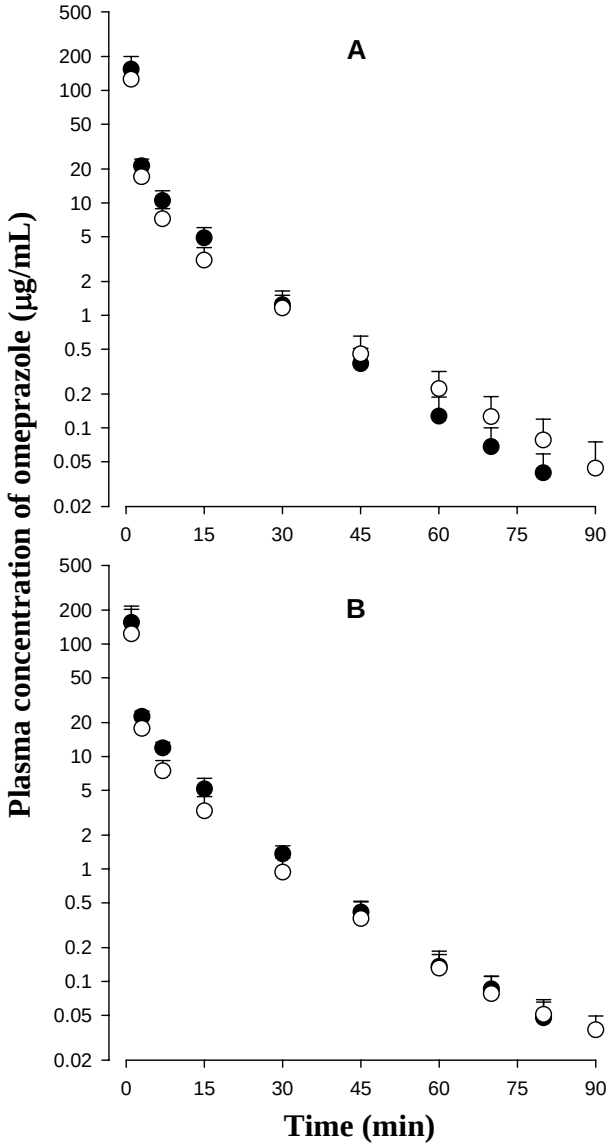

Figure 1: (A) Mean arterial plasma concentration–time profiles of omeprazole after 1 min intravenous administration at a dose of 20 mg/kg to DMIA rats (○; n = 8) and the control rats (●; n = 8). (B) The profiles to DMIS rats (○; n = 9) and their control rats (●; n = 8). Bars represent standard deviations

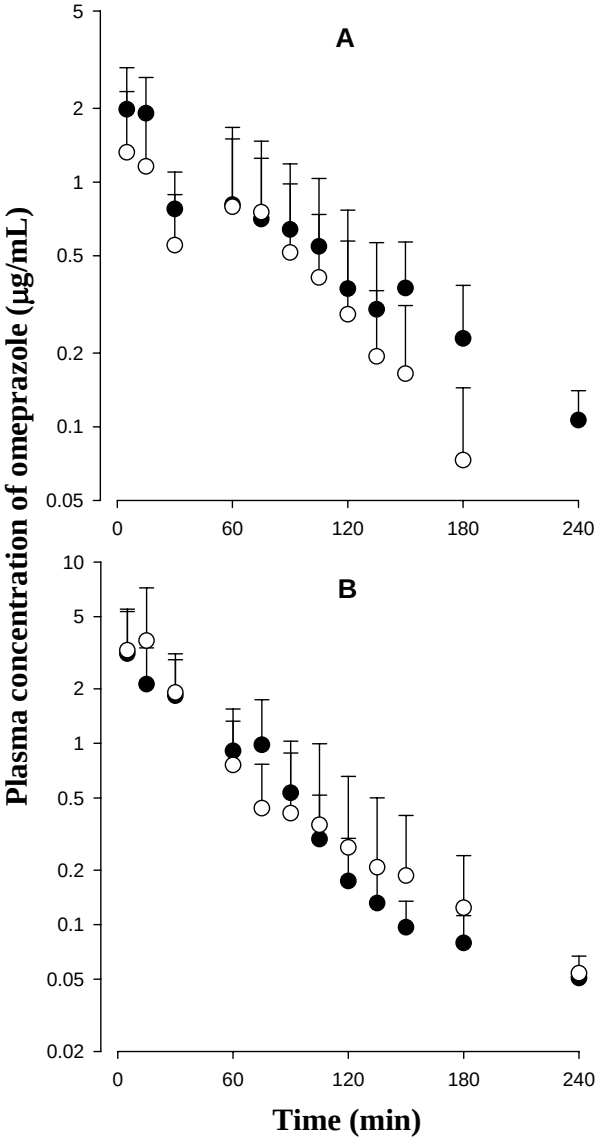

Figure 2: (A) Mean arterial plasma concentration–time profiles of omeprazole after oral administration at a dose of 40 mg/kg to DMIA rats (○; n = 7) and the control rats (●; n = 8). (B) The profiles to DMIS rats (○; n = 9) and their control rats (●; n = 8) (B). Bars represent standard deviations.

Because omeprazole is an intermediate hepatic extraction ratio drug, its hepatic clearance depends on the hepatic blood flow rate, the free (unbound to plasma proteins) fraction of omeprazole in plasma, and the CL_{int} for the disappearance of omeprazole in rats (39). The significantly faster CL_{NR} of omeprazole in both groups of diabetic rat (Table 3) could be supported by significantly faster *in vitro* CL_{int} for the disappearance of omeprazole than in the controls (Table 2), significantly greater (141% increase) free fraction of omeprazole in DMIA rats, as noted earlier, and faster hepatic blood flow rate in DMIS rats (40).

Although the CL_R of omeprazole was almost negligible compared to the CL of the drug, the significantly faster CL_R in both diabetic rat groups could have been due to a significantly greater $Ae_{0–24\ h}$ (120 and 233% increase in DMIA and DMIS rats, respectively), because the

decrease in AUC (21.2 and 26.2% decrease in DMIA and DMIS rats, respectively) was considerably smaller than the increase in $Ae_{0–24\ h}$ (Table 3). The greater $Ae_{0–24\ h}$ could have been due to urine flow rate-dependent timed-interval renal clearance of omeprazole in rats; it has been demonstrated that the $Ae_{0–24\ h}$ of omeprazole increases with increasing urine flow rate in rats (41). In the present diabetic rats, the 24-h urine output was significantly larger than that of their respective controls (Tables 1 and 3). The contribution of the CL_R to the CL of omeprazole was almost negligible, less than 1.61% (Table 3). Thus, changes in the CL_R of omeprazole in either type of diabetic rat could not greatly impact the pharmacokinetics of the drug.

In addition to changes in hepatic CYP isozymes, changes in the bile flow rate and altered bile composition (12) and formation of glucuronide, glutathione, and sulfate conjugates (16, 17) in diabetic rats might also affect the pharmacokinetics of omeprazole in these rats. However, these factors seem to be of little importance because omeprazole is poorly excreted via the biliary route (3) and is negligibly conjugated in rats (42).

After intravenous administration of omeprazole, the CL_R of the drug was estimated from the free fraction of the drug in plasma based on the CL_R (Table 3) and the free fraction of omeprazole in plasma. The values thus estimated were 1.97, 1.62, 3.86, and 0.892 mL/min/kg for DMIA rats and their controls, and DMIS rats and their controls, respectively. The 0.892–1.97 mL/min/kg range, without the DMIS rats, was considerably slower than the CL_{CR} (Table 1). This suggests that omeprazole is primarily reabsorbed in the renal tubules of all rats studied, except the DMIS rats. The renal extraction ratios (CL_R of omeprazole / renal plasma flow rate for the urinary excretion of unchanged omeprazole) of omeprazole were estimated based on the CL_R of the drug (Table 3), reported kidney blood flow rates of 36.8 mL/min/kg (43), 44.3 mL/min/kg (44), and 97.5 mL/min/kg (45) for the control, diabetic rats with 7-day alloxan (50 mg/kg), and DMIS rats, respectively, and hematocrit of approximately 45% in the control rats (33). It has been reported that the hematocrit values are not significantly different between control and DMIS rats (46). The hematocrit values in control and DMIA rats treated with 0.1% NaCl-injectable solution were 44 ± 1 and 41 ± 1% respectively (47). The renal extraction ratios thus estimated were 4.47, 1.84, 1.71, and 1.02% for DMIA rats and their controls, and DMIS rats and their controls, respectively. These results suggest that omeprazole is poorly excreted via the kidney (a poor renal extraction ratio drug) in all the rats studied, as has been reported in previously (48).

After intravenous administration of omeprazole to DMIA rats, the Vd_{ss} of the drug was significantly larger than in the control rats (Table 3). This could have been to an increase in the free fraction of omeprazole in plasma; the free fractions were 55.4 and 23.0% for DMIA and control rats, respectively. A similar result has been reported for torasemide; the significantly larger Vd_{ss} (40.1% increase) may have arisen due to the significantly greater free fraction of torasemide in plasma (36.3% increase) in DMIA rats (23). The decrease in plasma protein binding of omeprazole in DMIA rats could have been attributable to glycosylation of plasma proteins. Day et al. (49) reported that serum proteins including albumin and hemoglobin are nonenzymatically glycosylated in DMIA rats. It has also been reported that glycosylation of serum proteins can decrease the plasma protein binding of drugs (50).

After oral administration of omeprazole, the AUC of the drug was not significantly different between each type of diabetic rat and the respective controls (Table 4), although the AUC of omeprazole after intravenous administration of the drug to each diabetic rat was significantly smaller than in the respective controls (Table 3). However, this was not likely due to increased gastrointestinal absorption of omeprazole in either type of diabetic rat because it has been reported that omeprazole is absorbed almost completely from the gastrointestinal tract of control rats (37). Thus, the comparable AUC of oral omeprazole between each diabetic rat group and the respective controls (Table 4) could have been due to the significantly slower metabolism of omeprazole in rat intestine (smaller intestinal first-pass effect) via the CYP3A subfamily, the most abundant intestinal CYP isozyme (11). It has been reported that omeprazole is subject to a considerable intestinal first-pass effect; the estimated value was approximately 72.4% in control rats (37) and the metabolic activity of intestinal CYP3A1/2 significantly decreased in DMIS rats (8).

Although additional studies would be required to investigate the contribution of intestinal CYP1A1/2 and 2D1 to the pharmacokinetic changes of omeprazole in diabetic rats, it appeared that the contribution of increased intestinal CYP1A1/2 in DMIS rats (10) and changes in CYP2D1 in diabetic rats were not large compared to that of intestinal CYP3A1/2. It has been reported that CYP2D1 is expressed at a very low level in rat intestine (50).

Table 1: Mean (± standard deviation) body weight, blood glucose level, 24-h urine output, plasma chemistry data, CL_{CR}, and relative liver and kidney weights in DMIS and DMIA rats, and the respective control rats.

Parameter	DMIA control (n = 4)	DMIA (n = 4)	DMIS control (n = 4)	DMIS (n = 4)
Body weight (g)				
Initial	288 ± 6.45	281 ± 11.1	291 ± 4.79	270 ± 11.5
Final	304 ± 14.9	250 ± 31.6[a]	308 ± 21.0	230 ± 8.16[b]
Blood glucose (mg/dL)	122 ± 12.6	369 ± 96.0[c]	127 ± 13.3	299 ± 36.2[b]
Urine output (mL/24-h/kg)	23.8 ± 6.59	215 ± 132[a]	27.2 ± 12.5	380 ± 243[a]
Plasma				
Total protein (g/dL)	5.55 ± 0.265	5.23 ± 0.971	5.35 ± 0.387	4.63 ± 0.330[a]
Albumin (g/dL)	3.48 ± 0.189	3.18 ± 0.544	3.35 ± 0.238	2.83 ± 0.206[a]
Urea nitrogen (mg/dL)	19.4 ± 3.85	48.9 ± 18.9[a]	19.6 ± 3.44	35.8 ± 12.3[a]
GOT (IU[d]/L)	55.3 ± 5.44	50.8 ± 8.18	53.0 ± 3.92	87.0 ± 21.0[a]
GPT (IU/L)	22.3 ± 3.95	25.5 ± 9.40	20.8 ± 1.26	45.0 ± 18.4[a]
CL_{CR} (mL/min/kg)	2.46 ± 0.831	2.72 ± 0.574	3.02 ± 1.10	4.06 ± 2.53
Liver weight (% of body weight)	3.15 ± 0.160	3.34 ± 0.362	3.19 ± 0.174	3.51 ± 0.242
Kidney weight (% of body weight)	0.770 ± 0.0376	1.06 ± 0.150[c]	0.780 ± 0.0233	1.04 ± 0.0708[b]

[a] *Significantly different ($p < 0.05$) from respective control group.*
[b] *Significantly different ($p < 0.001$) from respective control group.*
[c] *Significantly different ($p < 0.01$) from respective control group.*
[d] *International unit*

Table 2: Mean (± standard deviation) V_{max}, K_m, and CL_{int} for the disappearance of omeprazole in hepatic microsomes of DMIA and DMIS rats, and the control rats.

Parameter	Control (n = 5)	DMIA (n = 5)	DMIS (n = 4)
V_{max} (nmol/min/mg protein)	2.25 ± 0.787	4.51 ± 1.53[*]	4.30 ± 1.43[a]
K_m (μM)	19.2 ± 7.18	29.1 ± 8.54	29.4 ± 9.35
CL_{int} (mL/min/mg protein)	0.119 ± 0.0106	0.155 ± 0.0268[*]	0.147 ± 0.0144[a]

[a] Significantly different ($p < 0.05$) from control group.

Table 3: Mean (± standard deviation) pharmacokinetic parameters of omeprazole after intravenous administration of the drug at a dose of 20 mg/kg to DMIA and DMIS rats, and the respective control rats.

Parameter	**DMIA control (*n* = 8)**	**DMIA (*n* = 8)**	**DMIS control (*n* = 8)**	**DMIS (*n* = 9)**
Body weight (g)				
Initial	258 ± 6.75	252 ± 12.4	241 ± 4.97	237 ± 5.62
Final	301 ± 11.2	228 ± 19.0[a]	308 ± 13.1	229 ± 19.0[a]
Blood glucose (mg/dL)	120 ± 11.4	386 ± 76.1[a]	128 ± 37.0	312 ± 47.0[a]
Urine output (mL/24-h/kg)	49.6 ± 17.7	143 ± 102[b]	37.3 ± 9.50	173 ± 120[c]
AUC (μg * min/mL)	387 ± 63.4	305 ± 56.3[b]	405 ± 70.9	299 ± 95.4[b]
Terminal half-life (min)	14.6 ± 6.78	14.1 ± 3.64	10.2 ± 0.478	16.6 ± 12.6
MRT (min)	6.73 ± 0.822	7.70 ± 1.48	7.45 ± 1.08	7.43 ± 1.69
CL (mL/min/kg)	53.0 ± 8.64	67.5 ± 12.2[b]	50.4 ± 7.26	73.9 ± 24.4[b]
CL_R (mL/min/kg)	0.373 ± 0.288	1.09 ± 0.627[b]	0.207 ± 0.0786	0.916 ± 0.687[b]
CL_{NR} (mL/min/kg)	52.6 ± 8.71	67.4 ± 12.8[b]	50.2 ± 7.29	73.0 ± 24.7[b]
Vd_{ss} (mL/kg)	356 ± 69.0	520 ± 137[b]	380 ± 88.3	565 ± 260
$Ae_{0-24\ h}$ (% of dose)	0.741 ± 0.699	1.63 ± 0.922[b]	0.429 ± 0.203	1.43 ± 1.08[b]
$GI_{24\ h}$ (% of dose)	BD[d]	BD	BD	BD

[a] *Significantly different ($p < 0.001$) from respective control group.*
[b] *Significantly different ($p < 0.05$) from respective control group.*
[c] *Significantly different ($p < 0.01$) from respective control group.*
[d] Below the detection limit.

Table 4: Mean (± standard deviation) pharmacokinetic parameters of omeprazole after oral administration of the drug at a dose of 40 mg/kg to DMIA and DMIS rats, and the respective control rats.

Parameter	**DMIA control (*n* = 8)**	**DMIA (*n* = 7)**	**DMIS control (*n* = 8)**	**DMIS (*n* = 9)**
Body weight (g)				
Initial	243 ± 5.65	236 ± 6.39	246 ± 7.38	245 ± 5.49
Final	273 ± 21.4	211 ± 18.0[a]	283 ± 12.8	212 ± 20.5[b]
Blood glucose (mg/dL)	92.7 ± 18.2	285 ± 87.1[b]	91.2 ± 8.42	208 ± 67.9[b]
Urine output (mL/24-h/kg)	22.7 ± 9.38	116 ± 43.1[a]	22.2 ± 14.0	85.6 ± 44.2[c]
AUC (μg * min/mL)	126 ± 55.9	114 ± 47.2	147 ± 65.4	153 ± 57.6
C_{max} (μg/mL)	2.61 ± 0.553	1.76 ± 0.754[b]	3.47 ± 2.00	5.20 ± 3.15
T_{max} (min)	10.0 ± 5.35	25.7 ± 29.2	16.3 ± 24.2	26.1 ± 26.0
Terminal half-life (min)	39.9 ± 14.6	70.9 ± 77.2	53.0 ± 25.6	27.7 ± 15.5[b]
CL_R (mL/min/kg)	0.552 ± 0.605	4.51 ± 2.46[c]	0.522 ± 0.362	1.07 ± 0.387[b]
$Ae_{0-24\,h}$ (% of dose)	0.201 ± 0.252	1.05 ± 0.840[a]	0.182 ± 0.192	0.371 ± 0.131[a]
$GI_{24\,h}$ (% of dose)	1.52 ± 0.843	0.646 ± 0.442[a]	0.654 ± 0.392	0.269 ± 0.0761[b]
F (%)	16.3	18.7	18.1	25.6

[a] *Significantly different ($p < 0.05$) from respective control group.*
[b] *Significantly different ($p < 0.001$) from respective control group.*
[c] Significantly different ($p < 0.01$) from respective control group

The decrease in the intestinal first-pass effect of oral omeprazole in DMIS rats caused by a decreased metabolic activity of intestinal CYP3A1/2 (6) could explain the somewhat greater *F* in DMIS rats than in the controls (Table 4). Although similar changes in hepatic CYP isozymes in DMIA and DMIS rats has been reported (4, 5, 51), to our knowledge, no information on changes in intestinal CYP isozymes in DMIA rats have yet been reported. Based on the present data alone, it is hard to extrapolate from DMIS to DMIA rats. Further study is required to examine the reason for the greater *F* in DMIA rats. The pharmacokinetic parameters of omeprazole were not significantly different between DMIA and DMIS rats after intravenous (Table 3) and oral (Table 4) administration, which suggests that despite major differences in the diabetogenic effects of streptozotocin and alloxan (28), their effects on the pharmacokinetic parameters of omeprazole were similar.

CONCLUSIONS

After intravenous administration of omeprazole to rats with both types of experimental diabetes, we observed an faster CL_{NR} of omeprazole and increased expression of hepatic CYP1A1/2 and 3A1/2 and their respective mRNAs. This was associated with a significantly faster *in vitro* CL_{int} and increased plasma unbound fraction of omeprazole. The hepatic blood flow rate was also faster in DMIS rats. However, after oral administration of omeprazole, the AUC of the drug was comparable between diabetic and control animals. This may be explained by the observed decreased intestinal first-pass effect of omeprazole; decreased intestinal CYP3A1/2 has previously been reported in DMIS rats. Overall, DMIA and DMIS appear to influence the pharmacokinetics of omeprazole in a similar fashion after both intravenous and oral administration.

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