Pharmaceutical Research, Vol. 25, No. 8, August 2008 (© 2008)
DOI: 10.1007/s11095-008-9610-7

Research Paper

Telithromycin Pharmacokinetics in Rat Model of Diabetes Mellitus Induced by Alloxan or Streptozotocin

Joo H. Lee[1] and Myung G. Lee[1,2]

Received December 7, 2007; accepted April 24, 2008; published online May 14, 2008

Purpose. It has been reported that telithromycin is primarily metabolized via hepatic CYP3A1/2 in rats, the expression and/or mRNA level of hepatic CYP3A1/2 increase in rat model of diabetes mellitus induced by alloxan (DMIA) or streptozotocin (DMIS), and intestinal CYP3A1/2 enzyme activity decreases in rat model of DMIS. Thus, the pharmacokinetic changes of telithromycin in both models of diabetes mellitus compared with those in the control rats were evaluated.
Methods. Telithromycin was administered (50 mg/kg) intravenously or orally to both rat models of diabetes and their respective control rats.
Results. After intravenous administration of telithromycin to both models of diabetes, the non-renal clearance (CL_{NR}) was significantly faster (32.3 and 53.1% increase for rat models of DMIA and DMIS, respectively) and the AUC was significantly smaller (25.0 and 33.8% decrease, respectively) than those in their respective controls. However, after oral administration of telithromycin, the AUC was comparable to that in their respective controls.
Conclusions. The faster CL_{NR} after intravenous administration was due to increased hepatic CYP3A1/2 in both models of diabetes. The comparable AUC after oral administration was mainly due to decreased intestinal CYP3A1/2 activity. Alloxan and streptozotocin appear to influence some pharmacokinetics of telithromycin in a different fashion.

KEY WORDS: diabetes mellitus; hepatic and intestinal CYP3A1/2; pharmacokinetics; rats; telithromycin.

INTRODUCTION

Telithromycin, a ketolide, is the first of a new class of semisynthetic agents derived from erythromycin. It was developed for the treatment of upper and lower community-acquired respiratory tract infections. It inhibits bacterial protein synthesis *via* two mechanisms; the first by directly blocking translation of mRNA and the second by interfering with the assembly of new ribosomal units (1). Telithromycin has potent activities both *in vitro* and *in vivo* against common respiratory tract pathogens (2), and its spectrum of activity also extends to atypical and intracellular pathogens (3). The clearance, the volume of distribution, the half-life, the plasma protein binding, the urinary excretion, and the extent of absolute oral bioavailability (F) of telithromycin in humans are 12–16 ml/min/kg, 2.1–4.5 l/kg, 7–23 h, 70%, 19–27% of the dose, and 41–112%, respectively (4–6). Recently, Lee and Lee (7) reported that telithromycin is primarily metabolized via hepatic microsomal cytochrome P450 (CYP) 3A1/2 in male Sprague–Dawley rats.

Kim *et al*. (8) reported that in male Sprague–Dawley rats with diabetes mellitus induced by either alloxan (rat model of DMIA) or streptozotocin (rat model of DMIS), the protein expression and mRNA level of hepatic CYP3A1 increase than those in the control rats. The increase in hepatic CYP3A1 (9,10) and 3A2 (10,11) in rat model of DMIS based on the Western analysis and/or various enzyme activity tests have also been reported. Borbás *et al*. (10) reported that there is a significant decrease (50.7% decrease) in the intestinal testosterone 6β-hydroxylase (a CYP3A1/2 marker) activity in rat model of DMIS than that in the control rats. Thus, it could be expected that the pharmacokinetic parameters of intravenous or oral telithromycin would alter in both rat models of DMIA and DMIS.

Watkins and Sanders (12) reported that major differences exist in the diabetogenic effects of streptozotocin and alloxan. 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.

Diabetes mellitus is associated with an increased susceptibility to infection and is the most frequently recognized coexisting illness in patients with community-acquired respiratory tract infections (13). Thus, we studied telithromycin in both rat models of DMIA and DMIS.

The purpose of this study was to examine changes in the telithromycin pharmacokinetics after its intravenous or oral

[1] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul, 151-742, South Korea.
[2] To whom correspondence should be addressed. (e-mail: leemg@snu.ac.kr)

administration at a dose of 50 mg/kg to both rat models of DMIA and DMIS with respect to changes in hepatic (8–11) and intestinal (10) CYP3A1/2 in rat model of diabetes compared with those of respective control rats. It has been reported (14) that the total area under the plasma concentration–time curve from time zero to time infinity (AUC) values of telithromycin are dose-dependent after intravenous (20, 50, and 100 mg/kg; each dose was significantly different) or oral (20, 50, and 100 mg/kg; each dose was also significantly different) administration to rats. Thus, the intravenous or oral dose of telithromycin, 50 mg/kg, was arbitrarily chosen for the study.

MATERIALS AND METHODS

Chemicals

Telithromycin was donated from Sanofi-Aventis (Paris, France). Quinine hydrochloride [internal standard for the high-performance liquid chromatographic (HPLC) analysis of telithromycin], 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; as a disodium salt) were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Other chemicals were of reagent grade or HPLC grade.

Animals

Protocols for the animal studies were approved by the Institutional Animal Care and Use Committee of the Seoul National University, Seoul, South Korea. Male Sprague–Dawley rats (7–9 weeks old and weighing 200–310 g) were purchased from the Taconic Farms Inc. (Samtako Bio Korea, O-San, 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 (07:00–19:00) and dark (19:00–07:00) cycles, and a relative humidity of 50±5%. The rats were housed in metabolic cages (Tecniplast, Varese, Italy) under filtered, pathogen-free air, with food (Agribrands Purina Korea, 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, rat models of DMIA and DMIS, and their respective control rats. Freshly prepared alloxan, 40 mg/kg (approximately 0.3 ml), was injected to the overnight-fasted rats via the tail vein for two consecutive days (8). An equal volume of 0.9% NaCl-injectable solution was injected into the control rats. Freshly prepared streptozotocin, 45 mg/kg (approximately 0.3 ml), was injected once to the overnight-fasted rats via the tail vein (8,15). An equal volume of citrate buffer (pH 4.5) was injected into the control rats. On the fourth day after the first alloxan administration (rat model of DMIA) or 0.9% NaCl-injectable solution (control rats for rat model of DMIA), and on the seventh day after intravenous administration of streptozotocin (rat model of DMIS) or citrate buffer, pH 4.5 (control rats for rat model of DMIS), 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.

Preparation of Rat Hepatic or Intestinal Microsomal Fractions

The procedures used for the preparation of hepatic microsomal fractions were similar to a reported method (8). The livers of each rat model of diabetes mellitus and their respective control rats (n=5; each) 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 microsomal pellet was resuspended in the buffer of 0.154 M KCl/50 mM Tris–HCl in 1 mM EDTA (pH 7.4). Microsomal preparations were stored at −70°C (Model DF8517; Ilshin Laboratory Company, Seoul, South Korea) until use.

The procedures used for the preparation of intestinal microsomal fractions were similar to a reported method (16) with a minor modification. The small intestines of overnight fasted each rat model of diabetes mellitus and their respective control rats (n=4 or 5; each) were cut and washed two times with ice-cold 0.154 M NaCl plus 1 mM dithiothreitol using a squeeze bottle whose tip can fit in the proximal end of the small intestine and the solution was squeezed into the lumen until the content in the lumen was removed. The end of the lumen was tied and filled with solution A [8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 1.5 mM KCl, 96 mM NaCl, 27 mM sodium citrate, and 0.04 mg/ml phenylmethylsulfonyl fluoride (PMSF)], and then the other end tied. The lumen was put into a water-bath (37°C, 15 min) and the solution was discarded. One end of the intestine was kept tied up and filled with ice-cold solution B (8 mM KH_2PO_4, 5.6 mM Na_2HPO_4, 1.5 mM EDTA, 0.5 mM dithiothreitol, and 0.04 mg/ml PMSF). Then, the lumen was wound around the fingers and tapped gently with fingers to release upper villus cells into the solution B. This process was repeated three times for each small intestine and the solution B was collected in a centrifuge tubes. After centrifugation (1,000×g, 5 min) at 4°C, the supernatant was discarded. Then, approximately 20 ml ice-cold solution C (5 mM of histidine, 0.25 M of sucrose, 0.5 mM of EDTA, and 0.04 mg/ml PMSF) was added, and each tube was inverted twice. After centrifugation (1,000×g, 10 min) at 4°C, the supernatant was discarded and approximately 5 ml ice-cold solution C was left. The cells in solution C were homogenized manually using a 15 ml Pyrex glass homogenizer (Ultra-Turrax T25; Janke and Kunkel, IKA-Labortechnik, Staufeni, Germany) and the homogenates were collected and centrifuged (10,000×g, 20 min) at 4°C. The supernatant was collected, and the fat layer and pellet were discarded. The microsomes were pelleted by ultracentrifugation (100,000×g, 65 min) at 4°C. The resulting microsomes were resuspended in buffer of 10 mM KH_2PO_4, 250 mM sucrose, 1 mM EDTA, and 0.04 mg/ml PMSF, and stored at −70°C until use. Protein contents in the hepatic or intestinal microsomal fractions were measured using a reported method (17).

Measurement of V_{max}, K_m, and CL_{int} for the Disappearance of Telithromycin in Hepatic or Intestinal Microsomal Fractions of Rat Models of DMIA and DMIS, and Their Respective Control Rats

The V_{max} (the maximum velocity) and the K_m (the apparent Michaelis–Menten constant; the concentration at which the rate is one-half of the V_{max}) for the disappearance of telithromycin in hepatic or intestinal microsomal fractions were determined after incubating the above hepatic or intestinal microsomal fractions (equivalent to 1 mg protein for both hepatic and intestinal microsomes), a 10-μl or 20-μl aliquot (for hepatic or intestinal microsomes, respectively) of microsomal buffer solution containing telithromycin, and a 50-μl or 20-μl (for hepatic or intestinal microsomes, respectively) aliquot of 0.1 M phosphate buffer (pH 7.4) containing 1.2 mM NADPH in a final volume of 300 μl or 600 μl (for hepatic or intestinal microsomes, respectively) by adding 0.1 M phosphate buffer (pH 7.4), in a water-bath shaker [37°C, 500 oscillations/min (opm)]. The final incubation concentrations of telithromycin were 0.01, 0.02, 0.05, 0.075, 0.1, 0.2, 0.5, and 1 μM for hepatic microsomes and 0.05, 0.075, 0.1, 0.2, 0.5, and 1 μM for intestinal microsomes. All of the above microsomal incubation conditions were linear.

The reaction was terminated by addition of 300 μl or 500 μl (for hepatic or intestinal microsomes, respectively) of acetonitrile after 30 min incubation. Then, a 50-μl aliquot of distilled water containing 50 μg/ml of quinine hydrochloride (internal standard) was added. Concentrations of telithromycin in the above hepatic or intestinal microsomal samples were determined using an HPLC method (18).

The kinetic constants (the K_m and the V_{max}) for the disappearance of telithromycin in the hepatic or intestinal microsomes were calculated using a non-linear regression method (19). The intrinsic clearance (CL_{int}) for the disappearance of telithromycin in the hepatic or intestinal microsomes was calculated by dividing the V_{max} by the K_m.

Pretreatment of Rats for Intravenous or Oral Study

The procedures used for the pretreatment of rats including the cannulation of the jugular vein (for drug administration in the intravenous study) and the carotid artery (for blood sampling) were similar to a reported method (20). Early in the morning on the fourth day after starting the treatment with alloxan (rat model of DMIA) or 0.9% NaCl-injectable solution (control rats for rat model of DMIA), or on the seventh day after streptozotocin (rat model of DMIS) or citrate buffer, pH 4.5 (control rats for rat model of DMIS), the carotid artery and the jugular vein of both rat models of diabetes and their respective control rats were cannulated with a polyethylene tube (Clay Adams, Parsippany, NJ) while each rat was under light ether anesthesia. 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). Both silastic tubes were covered with a wire sheath to allow free movement of the rats. A heparinized 0.9% NaCl-injectable solution (15 units/ml), 0.3 ml, was used to flush the cannula to prevent blood clotting. 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. Thus, each rat was not restrained in the present study.

Intravenous Study

Telithromycin (dissolved in distilled water adjusted to pH 3 with acetic acid) at a dose of 50 mg/kg (approximately 0.6 ml) was infused over 1 min via the jugular vein to rats in each group (n=7, 8, 10, and 8 for the rat model of DMIA and their controls, and rat model of DMIS and their controls, respectively). A blood sample (approximately 220 μl) was collected via the carotid artery at 0 (control), 1 (at the end of the infusion), 5, 15, 30, 60, 120, 180, 240, 360, 480, and 600 min after the start of the intravenous infusion of telithromycin. After centrifugation of blood sample, a 100-μl aliquot of plasma sample was stored at −70°C until used for the HPLC analysis of telithromycin (18). At the end of 24 h, each metabolic cage was rinsed twice with 15 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 output and the combined urine sample, two 100-μl aliquots of the combined urine sample were stored at −70°C until used for the HPLC analysis of telithromycin (18). At the same time (24 h), each rat was exsaguinated and 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 100 ml of methanol (to facilitate the extraction of telithromycin), and cut into small pieces using scissors. After manual shaking and stirring with a glass rod for 1 min, two 100-μl aliquots of the supernatant fractions were collected from each beaker and stored at −70°C until used for the HPLC analysis of telithromycin (18).

Oral Study

Telithromycin (the same solution used in the intravenous study) at a dose of 50 mg/kg (approximately 1.5 ml) was administered orally using a feeding tube to rats in each group (n=9, 9, 10, and 9 for the rat model of DMIA and their control rats, and rat model of DMIS and their control rats, respectively) after overnight fasting with free access to water. Blood sample was collected via the carotid artery at 0, 5, 15, 30, 60, 90, 120, 180, 240, 360, 480, and 600 min after oral administration of telithromycin. Other procedures were similar to those for the intravenous study.

Measurement of Rat Plasma Protein Binding of Telithromycin Using Equilibrium Dialysis

It has been reported that the binding values of telithromycin to 4% human serum albumin was independent of telithromycin concentrations ranging from 1 to 10 μg/ml; the mean value was 26.4% (14). Thus, a telithromycin concentration of 5 μg/ml was arbitrarily chosen for this plasma protein binding study. Protein binding of telithromycin to fresh plasma from rat models of DMIA and DMIS and their respective control rats (n=5 for each group except n=4 for rat model of DMIS) was determined using equilibrium dialysis (14). Plasma (1 ml) was dialyzed against 1 ml of isotonic

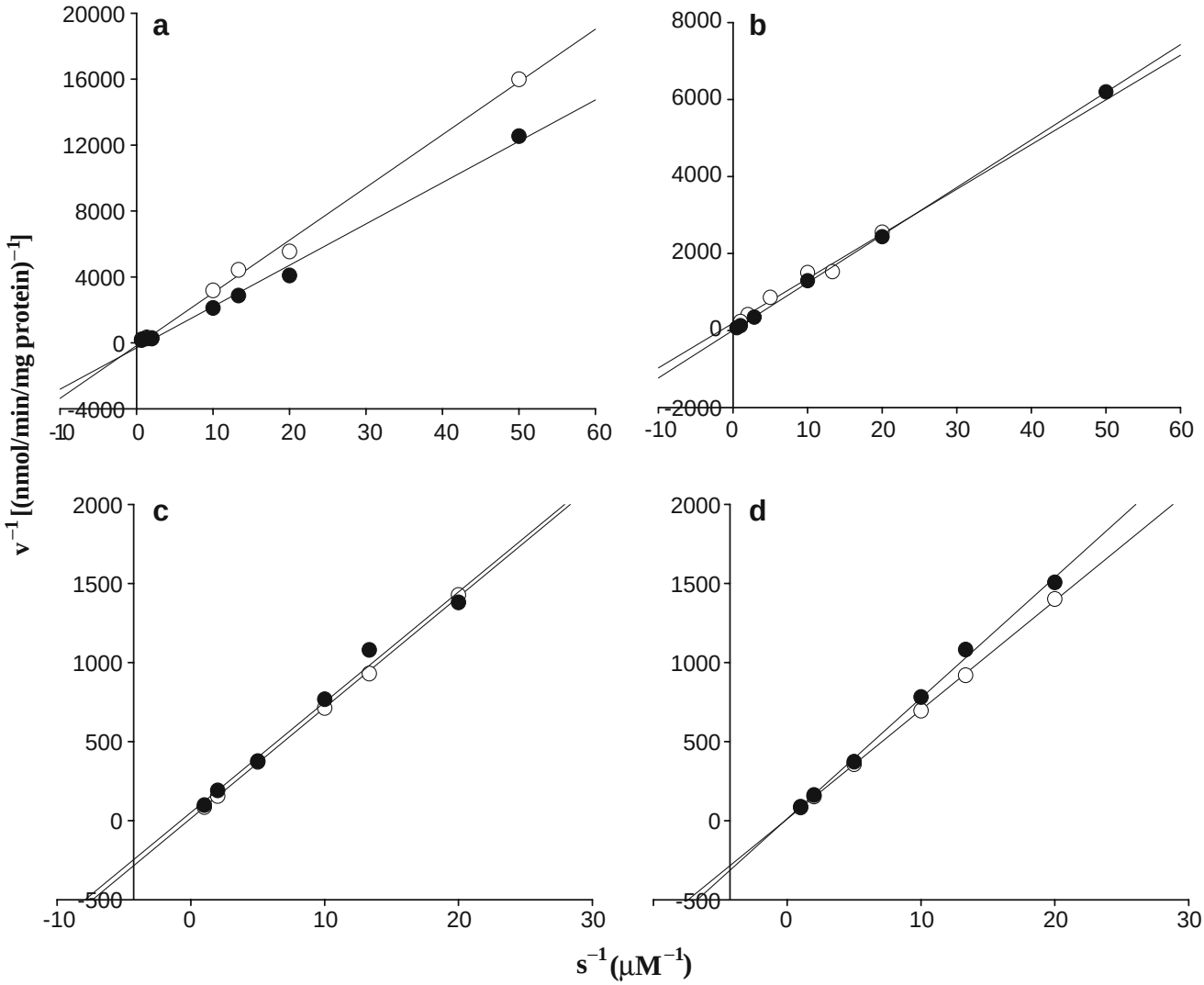

Fig. 1. Lineweaver–Burk plots for the disappearance of telithromycin in hepatic (**a**) and intestinal (**c**) microsomal fractions of rat model of DMIA (*solid circles*) and their control rats (*open circles*) and hepatic (**b**) and intestinal (**d**) microsomal fractions of rat model of DMIS (*solid circles*) and their control rats (*open circles*). The '*s*' and '*v*' represent the telithromycin concentration and the velocity for the disappearance of telithromycin, respectively.

Table I. V_{max}, K_m, and CL_{int} for the Disappearance of Telithromycin after Incubation of Telithromycin with Hepatic or Intestinal Microsomal Fractions of Rat Models of DMIA and DMIS, and Their Respective Control Rats

Parameter	Control for DMIA	DMIA	Control for DMIS	DMIS
A) Hepatic	(*n*=5)	(*n*=5)	(*n*=5)	(*n*=5)
V_{max} (nmol/min/mg protein)	0.00836±0.00128	0.0128±0.00692	0.0101±0.00532	0.0528±0.0214[a]
K_m (μM)	1.12±0.205	1.43±0.825	1.43±0.659	6.34±2.27[a]
CL_{int} (ml/min/mg protein)	0.00756±0.00111	0.00924±0.000933[b]	0.00694±0.000688	0.00834±0.00114[b]
B) Intestinal	(*n*=4)	(*n*=5)	(*n*=4)	(*n*=5)
V_{max} (nmol/min/mg protein)	0.0699±0.0219	0.0647±0.0180	0.0829±0.0454	0.0766±0.0276
K_m (μM)	4.97±1.72	5.56±2.46	5.74±3.21	5.69±2.18
CL_{int} (ml/min/mg protein)	0.0142±0.000647	0.0122±0.00191	0.0145±0.000339	0.0135±0.000311[a]

Data represent means ± standard deviations
[a] The value was significantly different ($p<0.01$) from respective control
[b] The value was significantly different ($p<0.05$) from respective control

Sørensen phosphate buffer (pH 7.4) containing 3% (*w*/*v*) dextran ('the buffer') to minimize volume shift (21) in a 1 ml dialysis cell (Fisher Scientific, Fair Lawn, NJ) using a Spectra/Por 4 membrane (mol. wt. cut off 12–14 KDa; Spectrum Medical Industries Inc., Los Angeles, CA). To reduce equilibrium time, telithromycin was spiked into plasma side (22). The spiked dialysis cell was incubated in a water-bath shaker (37°C, 50 opm). After 24-h incubation, two 100-µl aliquots were collected from each compartment and stored at −70°C until used for the HPLC analysis of telithromycin (18).

HPLC Analysis of Telithromycin

Concentrations of telithromycin in the samples were determined using a slight modification of a reported HPLC method (18); quinine hydrochloride instead of RU 66260 was used as internal standard. Briefly, to a 100-µl aliquot of biological sample, a 25-µl aliquot of distilled water containing 100 µg/ml of quinine hydrochloride (internal standard) and a 300-µl aliquot of acetonitrile were added. After vortex-centrifugation (12,000 rpm, 5 min), the organic layer was collected and dried (Dry thermobath, Eyela, Tokyo, Japan) under a gentle stream of nitrogen gas at 40°C. A 100-µl aliquot of the mobile phase was added to reconstitute the residue and a 50-µl aliquot was directly injected onto a reversed-phase (C_{18}) HPLC column.

The mobile phase, ammonium acetate (0.05 M): methanol: acetonitrile (52: 29: 24, *v*/*v*/*v*), was run at a flow-rate of 0.9 ml/min, and the column eluent was monitored using a fluorescence detector at an excitation wavelength of 264 nm and an emission wavelength of 460 nm at room temperature. The retention times of internal standard (quinine hydrochloride) and telithromycin were approximately 4.3 and 9.5 min, respectively. The detection limits of telithromycin in the rat plasma, urine, and gastrointestinal samples were all 0.1 µg/ml. The coefficients of variation of the assay were below 5.70, 7.94, and 6.31% for the rat plasma, urine, and gastrointestinal samples, respectively. The relative recoveries of telithromycin compared with those in water were 92.6–110% (0.5–100 µg/ml), 83.4–86.0% (10–100 µg/ml), and 85.9–101% (0.5–5 µg/ml) for rat plasma, urine, and gastrointestinal tract samples, respectively. The corresponding values for internal standard were 90.8–108% (0.5–100 µg/ml), 116–120% (10–100 µg/ml), and 99.7–117% (0.5–5 µg/ml) for rat plasma, urine, and gastrointestinal tract samples, respectively.

The HPLC system consisted of a model 717 autosampler (Waters Corporation, Milford, MA), a model L-6000 pump (Hitachi, Tokyo, Japan), a reversed-phase column (C_{18}; 15 cm, *l.* × 4.6 mm, i.d.; particle size, 5 µm; CAPCELL PAK, Shiseido, Tokyo, Japan), a fluorescence detector (SpectraSYSTEM® FL3000, Thermo Scientific, MA), and a data system (Autochro-2000, Young Lin Instrument Company, Ltd., Seoul, South Korea).

Pharmacokinetic Analysis

The AUC was calculated using the trapezoidal rule-extrapolation method (23). The area from the last datum point to time infinity was estimated by dividing the last measured concentration in plasma by the terminal-phase rate constant.

Standard methods (24) were used to calculate the following pharmacokinetic parameters using a non-compartmental analysis (WinNonlin®; Pharsight Corporation, Mountain View, CA); the time-averaged total body, renal, and non-renal clearances (CL, CL_R, and CL_{NR}, respectively), 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 F (20). The peak plasma concentration (C_{max}) and the time to reach C_{max} (T_{max}) were directly read from the experimental data.

Statistical Analysis

A p-value <0.05 was deemed to be statistically significant using a t-test between the two means for the unpaired data. All results are expressed as means±standard deviations except medians (ranges) for T_{max}.

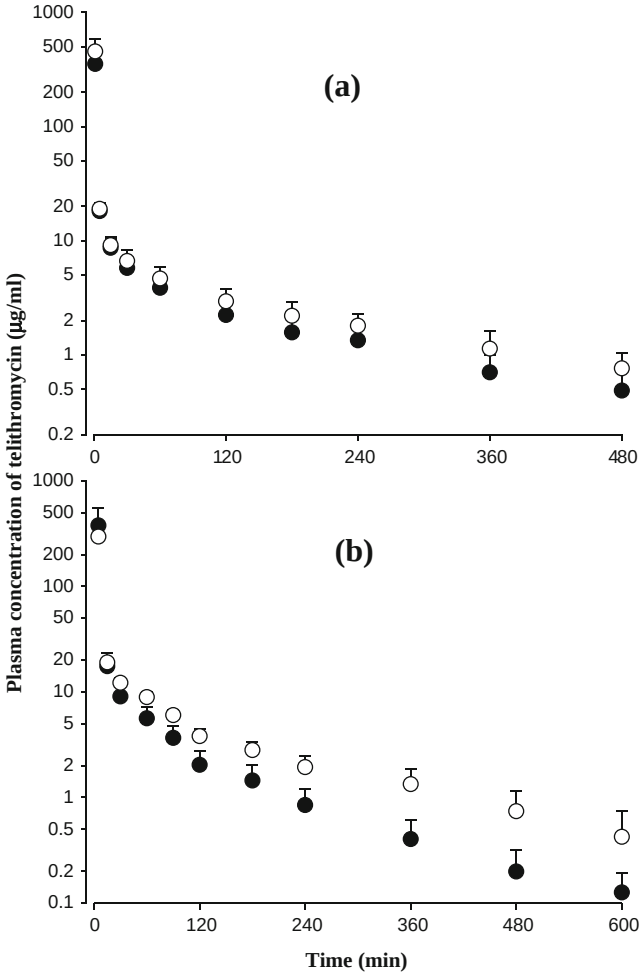

Fig. 2. Mean arterial plasma concentration–time profiles of telithromycin after its intravenous infusion at a dose of 50 mg/kg to rat model of DMIA (*solid circles*; n=7) and their control rats (*open circles*; n=8) (**a**), and rat model of DMIS (*solid circles*; n=10) and their control rats (*open circles*; n=8) (**b**). *Vertical bars* represent standard deviations.

RESULTS

V_{max}, K_m, and CL_{int} for the Disappearance of Telithromycin in Hepatic or Intestinal Microsomal Fractions of Rat Models of DMIA and DMIS, and Their Respective Control Rats

Lineweaver–Burk (25) plots for the disappearance of telithromycin in hepatic or intestinal microsomal fractions of rat models of DMIA and DMIS, and their respective control rats are shown in Fig. 1(a–d), respectively. The V_{max}, the K_m, and the CL_{int} for the disappearance of telithromycin in the hepatic or intestinal microsomal fractions of all rats studied are listed in Table I. In rat model of DMIA, the V_{max} and the K_m for the disappearance of telithromycin in both hepatic and intestinal microsomal fraction were not significantly different compared with those in their control rats, suggesting that the disappearance (primarily metabolism) of telithromycin and affinity of the enzyme(s) for the telithromycin in the liver were not altered by alloxan. In rat model of DMIA, the CL_{int} for the disappearance of telithromycin in hepatic microsomes was significantly faster (22.2% increase) than that in the control rats, suggesting that hepatic metabolism of telithromycin was significantly faster in rat model of DMIA. Although the intestinal CL_{int} values for the disappearance of telithromycin were statistically comparable, possibly due to the limited number of rats used (n=5), there was a tendency (14.1% decrease; p=0.0858) that the intestinal metabolism of telithromycin decreased in rat model of DMIA (from 0.0142 to 0.0122 ml/min/mg protein).

In rat model of DMIS, the V_{max} and the K_m for the disappearance of telithromycin in the hepatic microsomal fraction were significantly faster (423% increase) and higher (343% increase), respectively, than those in the control rats, suggesting that the disappearance of telithromycin and affinity of the enzyme(s) for the telithromycin in the liver significantly increased and decreased, respectively, than those in the control rats. However, the V_{max} and the K_m for the disappearance of telithromycin in the intestinal microsomal fraction were comparable between two groups of rats. In rat model of DMIS, the CL_{int} for the disappearance of telithromycin in hepatic or intestinal microsomal fraction became significantly faster (20.2% increase) or slower (6.90% decrease), respectively, than those in the control rats, suggesting that the metabolism of telithromycin increased or decreased by streptozotocin in the liver or intestine, respectively.

Pharmacokinetics of Telithromycin after Its Intravenous Administration to Rats

For the intravenous administration of telithromycin at a dose of 50 mg/kg to both rat models of diabetes and their respective control rats, the mean arterial plasma concentration–time profiles are shown in Fig. 2, and the relevant pharmacokinetic parameters are listed in Table II. After intravenous administration of telithromycin to rat models of DMIA and DMIS, changes in the pharmacokinetic parameters of telithromycin compared with those in their respective control rats are as follows; the AUC became significantly smaller (25.0 and 33.8% decrease for the DMIA and DMIS rats, respectively), and the CL (32.0 and 52.7% increase, respectively) and the CL_{NR} (32.3 and 53.1% increase, respectively) became significantly faster than those in the control rats. In rat model of DMIS, the MRT and the CL_R were significantly shorter (44.8% decrease) and faster (49.2% increase), respectively, than those in the control rats. The terminal half-life, the V_{ss}, the percentages of the intravenous dose of telithromycin excreted in the 24-h urine as an unchanged drug ($Ae_{0-24\ h}$; less than 19.0% of the dose), and the percentages of the dose

Table II. Pharmacokinetic Parameters of Telithromycin after Its Intravenous Administration at a Dose of 50 mg/kg to Rat Models of DMIA and DMIS, and Their Respective Control Rats

Parameter	Control for DMIA (n=8)	DMIA (n=7)	Control for DMIS (n=8)	DMIS (n=10)
Body weight (g)				
Initial[a]	269±6.23	276±9.00	268±4.58	273±5.89
Final[b]	297±11.0	267±20.0[c]	302±12.8	215±30.9[d]
Blood glucose (mg/dL)	156±30.2	327±123[c]	144±15.2	403±63.3[d]
Urine output (ml/24-h)	13.4±2.30	21.4±9.43[e]	9.00±5.01	17.2±9.70[e]
Telithromycin				
AUC (μg min/ml)	2160±536	1620±389[e]	2,280±438	1,510±310[d]
Terminal half-life (min)	180±47.9	134±40.6	147±53.3	130±40.4
MRT (min)	143±40.5	102±36.0	143±56.6	79.0±15.5[c]
V_{ss} (ml/kg)	3,340±641	3,180±1090	3,090±733	2,710±710
CL (ml/min/kg)	24.4±5.59	32.2±6.73[e]	22.6±3.72	34.5±6.82[d]
CL_R (ml/min/kg)	4.52±1.09	6.03±2.03	2.97±0.933	4.43±1.50[e]
CL_{NR} (ml/min/kg)	19.8±5.28	26.2±5.53[e]	19.6±3.89	30.0±5.79[d]
$Ae_{0-24\ h}$ (% of the dose)	19.0±4.53	18.7±4.14	13.6±4.73	12.8±2.99
$GI_{24\ h}$ (% of the dose)	5.15±1.49	4.66±1.22	5.22±3.17	5.50±4.06

Data represent means±standard deviations
[a] Measured just before treatment
[b] Measured just before experiment
[c] The value was significantly different (p<0.01) from respective control
[d] The value was significantly different (p<0.001) from respective control
[e] The value was significantly different (p<0.05) from respective control

of telithromycin recovered from the entire gastrointestinal tract (including its contents and feces) at 24 h as unchanged drug ($GI_{24\ h}$; less than 5.50% of the dose) were comparable between both rat model of diabetes and their respective control rats. And the MRT and the CL_R were comparable between rat model of DMIA and the control rats.

Pharmacokinetics of Telithromycin after Its Oral Administration to Rats

For the oral administration of telithromycin at a dose of 50 mg/kg to both rat models of diabetes and their respective control rats, the mean arterial plasma concentration–time profiles of telithromycin are shown in Fig. 3, and the relevant pharmacokinetic parameters are listed in Table III. Absorption of telithromycin from the rat gastrointestinal tract was rapid; the drug was detected in plasma from the first blood sampling time (5 min) for all rats studied. The pharmacokinetic parameters of telithromycin listed in Table III, such as the AUC, the C_{max}, the T_{max}, the CL_R, the $Ae_{0–24\ h}$ (less than 9.60% of the dose), and the $GI_{24\ h}$ (less than 7.90% of the dose), were comparable (not significantly different) between each rat model of diabetes and their respective control rats except significantly shorter terminal half-life (32.2% decrease) in rat model of DMIS than that in the control rats.

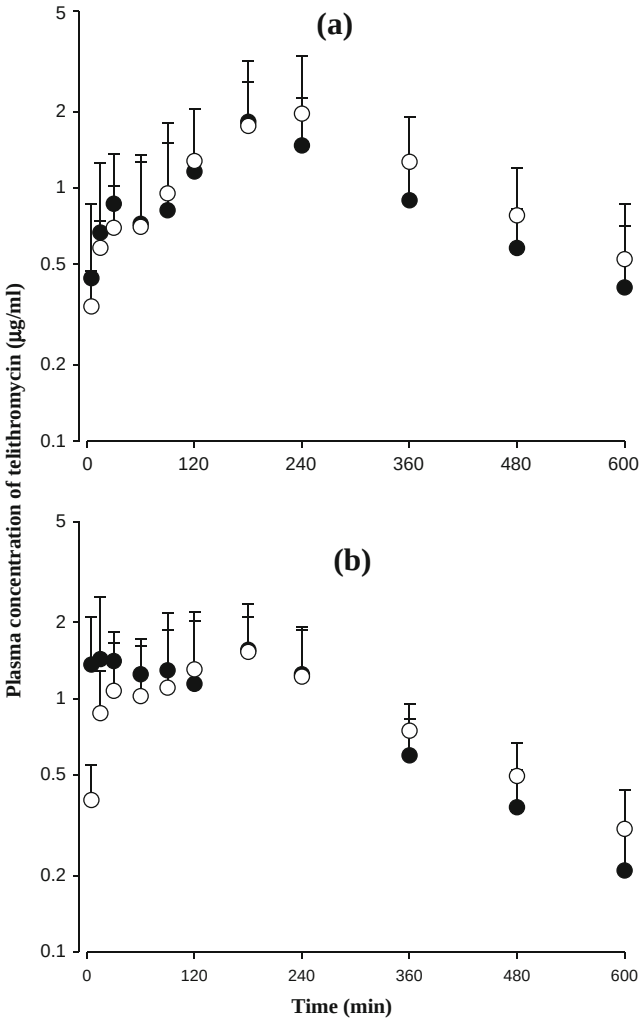

Fig. 3. Mean arterial plasma concentration–time profiles of telithromycin after its oral administration at a dose of 50 mg/kg to rat model of DMIA (*solid circles*; $n=9$) and their control rats (*open circles*; $n=9$) (**a**), and rat model of DMIS (*solid circles*; $n=10$) and their control rats (*open circles*; $n=9$) (**b**). Vertical bars represent standard deviations.

Rat Plasma Protein Binding of Telithromycin

Protein binding values of telithromycin to fresh rat plasma from rat model of DMIA and their control rats were 63.2±18.3 and 68.0±15.2%, respectively; they were not significantly different. The corresponding values were 54.8±5.03 and 70.0±12.5% for the rat model of DMIS and the control rats, respectively; they were also not significantly different.

DISCUSSION

Induction of diabetes mellitus in rats by alloxan or streptozotocin was evident based on the significantly higher blood glucose level, larger 24-h urine output, and decrease in body weight gain (Tables II and III).

The contribution of the gastrointestinal (including the biliary) excretion of unchanged telithromycin to the CL_{NR} of the drug did not seem to be considerable in each rat model of diabetes and their respective control rats; the $GI_{24\ h}$ values were less than 5.50% of the dose (Table II). However, the small values of $GI_{24\ h}$, less than 5.50% of the dose, were not likely due to chemical and enzymatic degradation of the drug in rats' gastric fluids; Lee and Lee (14) reported that telithromycin is stable in various buffer solutions having pHs ranging from 1 to 13 (more than 92.0% of the spiked amounts of telithromycin were recovered) except at pH 4 (83.7% was recovered) for up to 48 h incubation and in three rats' gastric juices (pHs of 1, 2.5, and 3, respectively) for up to 4 h incubation (more than 90.5% was recovered). Thus, the above data suggest that the CL_{NR} of telithromycin listed in Table II could represent the metabolic clearance of the drug. Additionally, changes in the CL_{NR} of telithromycin could represent changes in the hepatic metabolic clearance of the drug in rats.

After intravenous administration of telithromycin to both rat models of DMIA and DMIS, the significantly smaller AUC than that in respective control rats could have been due to significantly faster CL (Table II). The faster CL was primarily attributable to a significantly faster CL_{NR} than that in the control rats, because the contribution of the CL_R to the CL of telithromycin was not considerable; the ratios were 18.5, 18.7, 13.1 and 12.8% for the control rats for rat model of DMIA, rat model of DMIA, the control rats for rat model of DMIS, and rat model of DMIS, respectively (Table II). Telithromycin was metabolized via CYP3A1/2 in rats (7); in rats pretreated with dexamethasone phosphate or troleandomycin (a main inducer or a main inhibitor of CYP3A1/2 in rats, respectively), the CL_{NR} of telithromycin was significantly faster or slower (129% increase or 31.3% decrease, respectively), respectively, than that in the control rats. The significantly faster CL_{NR} of telithromycin could have been due to increased CYP3A1/2 in rat model of DMIA or DMIS (8–11). Because telithromycin is a low hepatic extraction ratio drug in rats (14), its hepatic clearance depends more on the

Table III. Pharmacokinetic Parameters of Telithromycin after Its Oral Administration at a Dose of 50 mg/kg to Rat Models of DMIA and DMIS, and Their Respective Control Rats

Parameter	Control for DMIA (n=9)	DMIA (n=9)	Control for DMIS (n=9)	DMIS (n=10)
Body weight (g)				
Initial[a]	242±6.61	241±3.00	221±9.82	227±7.09
Final[b]	259±8.46	234±17.4[c]	241±12.2	192±18.0[d]
Blood glucose (mg/dl)	108±12.2	184±55.4[d]	91.6±5.20	328±83.0[d]
Urine output (ml/24-h)	8.64±4.52	16.1±10.9[e]	8.11±5.11	15.0±6.38[e]
Telithromycin				
AUC (μg min/ml)	846±363	707±309	608±188	559±183
Terminal half-life (min)	203±56.6	204±142	208±74.7	141±39.1[e]
C_{max} (μg/ml)	2.44±1.19	2.07±1.34	1.87±0.646	2.31±0.806
T_{max} (min)[f]	180 (60–480)	180 (90–360)	180 (15–240)	90 (15–240)
CL_R (ml/min/ kg)	6.61±3.77	5.69±2.43	5.23±1.39	5.16±2.80
$Ae_{0-24\ h}$ (% of the dose)	9.60±3.32	7.01±1.54	6.60±3.32	5.67±3.62
$GI_{24\ h}$ (% of the dose)	5.66±4.04	7.90±3.35	5.44±2.44	6.86±4.32
F (%)	39.2	43.6	26.7	37.2

Data represent means±standard deviations
[a] Measured just before treatment
[b] Measured just before experiment
[c] The value was significantly different (p<0.01) from respective control
[d] The value was significantly different (p<0.001) from respective control
[e] The value was significantly different (p<0.05) from respective control
[f] T_{max} was expressed as median (ranges)

intrinsic clearance (CL_{int}) for the disappearance of telithromycin rather than on the hepatic blood flow rate (26). The faster CL_{NR} of telithromycin in both rat models of diabetes (Table II) could be supported by the significantly faster *in vitro* hepatic CL_{int} for the disappearance of telithromycin than that in the control rats (Table I). Even though Sato *et al.* (27) reported that the hepatic plasma flow rate was faster (67.0% increase) in male Wistar rat model of DMIS, its effect on the hepatic clearance of telithromycin is negligible as above mentioned.

After oral administration of telithromycin to both rat models of diabetes, the AUC of telithromycin was comparable to that in their respective control rats (Table III). However, this was not likely due to decreased gastrointestinal absorption of telithromycin for both rat models of diabetes. For comparison, the mean 'true' fraction of the oral dose of telithromycin unabsorbed in this study could be estimated by a reported equation (28); the values thus estimated were 5.87, 3.64, 4.81, and 4.05% for the rat model of DMIA and their control rats, and rat model of DMIS and their control rats, respectively. Thus, more than 94% of the oral dose of telithromycin was absorbed for all groups of rats. Although the AUC of intravenous telithromycin significantly decreased due to increased hepatic metabolism of the drug (Table II), the AUC values of oral telithromycin were comparable between each model of diabetes and their respective control rats (Table III). This could have been due to decreased intestinal metabolism of telithromycin in rat model of DMIS. The intestinal first-pass effect of telithromycin was considerable, 63.4%, after oral administration of telithromycin at a dose of 50 mg/kg to controls rats (14). Because telithromycin is an intermediate intestinal extraction ratio drug, its intestinal clearance depends on the intestinal blood flow rate, the CL_{int} for the disappearance of telithromycin, and the free fraction of the drug in plasma, assuming that the hepatic clearance concept (26) could be applied to the intestine. The decreased intestinal clearance of telithromycin in rat model of diabetes could be supported by the slower CL_{int} for the disappearance of telithromycin in the intestine (Table I). However, the contribution of the intestinal blood flow rate and the free fraction of telithromycin in plasma to the significantly decreased intestinal metabolism of the drug did not seem to be considerable. Because, it has been reported that the intestinal blood flow rate increased in rat model of DMIA and DMIS (29,30) and the free fraction of telithromycin in plasma was comparable between rat models of diabetes and their respective control rats as above mentioned. Thus, the slower CL_{int} in both rat models of diabetes (Table I) could mainly be due to the decrease in intestinal CYP3A1/2 in rat model of DMIS (10).

The terminal half-lives of telithromycin were comparable between each rat model of diabetes and their respective control rats after intravenous administration to rat models of DMIA and DMIS (Table II) and oral administration to rat model of DMIA (Table III). However, the terminal half-life of telithromycin was significantly shorter after oral administration to rat model of DMIS than that in control rats although the AUC values were comparable between two groups of rats (Table III). Although the exact reason is not clear, the similar results have been obtained from zopolrestat (31) and omeprazole (32).

Some pharmacokinetic parameters of telithromycin were significantly different between rat models of DMIA and DMIS compared with those in their respective control rats; the V_{max} and the K_m in hepatic microsomes, and the CL_{int} in intestinal microsomes (Table I), the MRT and the CL_R after intravenous administration (Table II), and the terminal half-life after oral administration (Table III). Although the exact reason is not clear, this could be partly due to the major difference existing in the diabetogenic effects of streptozotocin and alloxan (12).

Kim *et al.* (8) reported that in rats pretreated with streptozotocin (rat model of DMIS), the protein expression and mRNA level of hepatic CYP1A2, 2B1/2, 2E1, and 3A1

increased, whereas CYP2C11 decreased compared with those in the control rats. Similar results were also obtained from rats pretreated with alloxan (rat model of DMIA) (8), although the streptozotocin and alloxan are structurally dissimilar. Shimojo *et al*. (11) reported that increased hepatic CYP2A1, 2C6, 2C7, 3A2, and 4A3 in rat model of DMIS were restored to the levels in the control rats by treatment with insulin. In type I and type II diabetes patients, the mRNA level of CYP2E1 increased (33) and in type I diabetes patients, CYP1A2 increased (34) as shown in rat models of DMIA and DMIS (8). Thus, the pharmacokinetic changes of telithromycin in both rat models of diabetes (Tables II and III) could be due to changes in CYP3A1/2 induced by diabetes mellitus and not due to streptozotocin or alloxan, itself.

In conclusion, after intravenous administration of telithromycin to both rat models of diabetes, the CL_{NR} (AUC) was significantly faster (smaller) than that in the respective control rats (Table II). This could have been due to increased expression and/or mRNA level of hepatic CYP3A1/2 in rat model of DMIA and DMIS (8), because telithromycin was primarily metabolized *via* hepatic CYP3A1/2 in rats (7). After oral administration of telithromycin, however, the AUC was comparable between each rat model of diabetes and their respective control rats. This could have mainly been due to decreased intestinal first-pass effect of telithromycin in rat models of DMIS caused by decreased intestinal CYP3A1/2 activity in rat model of DMIS (10). Alloxan and streptozotocin appear to influence some pharmacokinetics of telithromycin in a different fashion after both intravenous and oral administration.

ACKNOWLEDGEMENTS

The authors thank to Sanofi-Aventis group for the supply of telithromycin. This study was supported in part by 2007 BK21 Project for Applied Pharmaceutical Life Sciences.

REFERENCES

1. W. S. Champney, and C. L. Tober. Inhibition of translation and 50S ribosomal subunit formation in *Staphylococcus aureus* cells by 11 different ketolide antibiotics. *Curr. Microbiol.* **37**:418–425 (1998).
2. C. Agouridas, A. Bonnefoy, and J. F. Chantot. *In vivo* antibacterial activity of HMR3647, a novel ketolide highly active against respiratory pathogens, In *Fourth International Conference on Macrolides, Azalides, Streptogramins and Ketolides*. Barcelona, Spain, 1998, pp. 21–23.
3. P. M. Roblin, and M. R. Hammerschlag. *In vitro* activity of a new ketolide antibiotic, HMR 3647, against *Chlamydia pneumoniae*. *Antimicrob. Agents Chemother.* **42**:1515–1516 (1998).
4. C. Perret, B. Lenfant, E. Weinling, D. H. Wessels, H. E. Scholtz, G. Montay, and E. Sultan. Pharmacokinetics and absolute oral bioavailability of an 800-mg oral dose of telithromycin in healthy young and elderly volunteers. *Chemotherapy*. **48**:217–223 (2002).
5. G. G. Zhanel, M. Walters, A. Noreddin, L. M. Vercaigne, A. Wierzbowski, J. M. Embil, A. S. Gin, S. Douthwaite, and D. J. Hoban. The ketolides: a critical review. *Drugs*. **62**:1771–1804 (2002).
6. K. E. Thummel, D. D. Shen, N. Isoherranen, and H. E. Smith. Appendices II. Design and optimization of dosage regimens: pharmacokinetic data. In L. L. Brunton, J. S. Lazo, and K. L. Parker (eds.), *Goodman and Gilman's The Pharmacological Basis of Therapeutics*, 11McGraw-Hill, New York, 2006, p. 1876.
7. J. H. Lee, and M. G. Lee. Effects of acute renal failure on the pharmacokinetics of telithromycin in rats: negligible effects of increase in CYP3A1 on the metabolism of telithromycin. *Biopharm. Drug Dispos.* **28**:157–166 (2007).
8. Y. C. Kim, A. K. Lee, J. H. Lee, I. Lee, D. C. Lee, S. H. Kim, S. G. Kim, and M. G. Lee. Pharmacokinetics of theophylline in diabetes mellitus rats: induction of CYP1A2 and CYP2E1 on 1,3-dimethyluric acid formation. *Eur. J. Pharm. Sci.* **26**:114–123 (2005).
9. L. A. Reinke, S. J. Stohs, and H. Rosenberg. Altered activity of hepatic mixed-function mono-oxygenase enzymes in streptozotocin-induced diabetic rats. *Xenobiotica*. **8**:611–619 (1978).
10. T. Borbás, B. Benkö, B. Dalmadi, I. Szabó, and K. Tihanyi. Insulin in flavin-containing monooxygenase regulation. Flavin-containing monooxygenase and cytochrome P450 activities in experimental diabetes. *Eur. J. Pharm. Sci.* **28**:51–58 (2006).
11. N. Shimojo, T. Ishizaki, S. Imaoka, Y. Funae, S. Fujii, and K. Okuda. Changes in amounts of cytochrome P450 isozymes and levels of catalytic activities in hepatic and renal microsomes of rats with streptozocin-induced diabetes. *Biochem. Pharmacol.* **46**:621–627 (1993).
12. J.B. Watkins Jr, and R. A. Sanders. Diabetes mellitus-induced alterations of hepatobiliary function. *Pharmacol. Rev.* **47**:1–23 (1995).
13. M. Falguera, M. Martin, A. Ruiz-Gonzalez, R. Pifarre, and M. Garcia. Community-acquired pneumonia as the initial manifestation of serious underlying diseases. *Am. J. Med.* **118**:378–383 (2005).
14. J. H. Lee, and M. G. Lee. Dose-dependent pharmacokinetics of telithromycin after intravenous and oral administration to rats: contribution of intestinal first-pass effect to low bioavailability. *J Pharm Pharm Sci.* **10**:37–50 (2007).
15. J. M. Park, C. H. Moon, and M. G. Lee. Pharmacokinetic changes of methotrexate after intravenous administration to streptozotocin-induced diabetes mellitus rats. *Res. Commun. Mol. Pathol. Pharmacol.* **93**:343–352 (1996).
16. J. Z. Peng, R. P. Remmel, and R. K. Sawchuk. Inhibition of murine cytochrome P4501A by tacrine: *in vitro* studies. *Drug Metab. Dispos.* **32**:805–812 (2004).
17. M. M. Bradford. 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 (1976).
18. P. H. Edelstein, and M. A. Edelstein. *In vitro* activity of the ketolide HMR 3647 (RU 6647) for *Legionella* spp., its pharmacokinetics in guinea pigs, and use of the drug to treat guinea pigs with *Legionella pneumophila* pneumonia. *Antimicrob. Agents Chemother.* **43**:90–95 (1999).
19. R. G. Duggleby. Analysis of enzyme progress curves by nonlinear regression. *Methods Enzymol.* **249**:61–90 (1995).
20. S. H. Kim, Y. M. Choi, and M. G. Lee. Pharmacokinetics and pharmacodynamics of furosemide in protein-calorie malnutrition. *J. Pharmacokinet. Biopharm.* **21**:1–17 (1993).
21. F. D. Boudinot, and W. J. Jusko. Fluid shifts and other factors affecting plasma protein binding of prednisolone by equilibrium dialysis. *J. Pharm. Sci.* **73**:774–780 (1984).
22. S. Øie, and T. W. Guentert. Comparison of equilibrium time in dialysis experiments using spiked plasma or spiked buffer. *J. Pharm. Sci.* **71**:127–128 (1982).
23. W. L. Chiou. 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 (1978).
24. M. Gibaldi, and D. Perrier. *Pharmacokinetics*. second edn. Marcel-Dekker, New York, 1982.
25. H. Lineweaver, and D. Burk. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* **56**:658–666 (1934).
26. G. R. Wilkinson, and D. G. Shand. A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther.* **18**:377–390 (1975).
27. H. Sato, T. Terasaki, K. Okumura, and A. Tsuji. Effect of receptor-up-regulation on insulin pharmacokinetics in streptozotocin-treated diabetic rats. *Pharm. Res.* **8**:563–569 (1991).
28. M. G. Lee, and W. L. Chiou. Evaluation of potential causes for the incomplete bioavailability of furosemide: gastric first-pass metabolism. *J. Pharmacokinet. Biopharm.* **11**:623–640 (1983).

29. P. D. Lucas, and J. M. Foy. Effects of experimental diabetes and genetic obesity on regional blood flow in the rat. *Diabetes*. **26**:786–792 (1977).
30. M. A. Hill, and R. G. Larkins. Alterations in distribution of cardiac output in experimental diabetes in rats. *Am. J. Physiol.* **257**:H571–H580 (1989).
31. P. B. Inskeep, A. E. Reed, and R. A. Ronfeld. Pharmacokinetics of zopolrestat, a carboxylic acid aldose reductase inhibitor, in normal and diabetic rats. *Pharm. Res.* **8**:1511–1515 (1991).
32. D. Y. Lee, M. G. Lee, H. S. Shin, and I. Lee. 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. *J. Pharm. Pharm. Sci.* **10**:420–433 (2007).
33. Z. Wang, S. D. Hall, J. F. Maya, L. Li, A. Asghar, and J. C. Gorski. Diabetes mellitus increases the *in vivo* activity of cytochrome P450 2E1 in humans. *Br J Clin Pharmacol.* **55**:77–85 (2003).
34. G. R. Matzke, R. F. Frye, J. J. Early, R. J. Straka, and S. W. Carson. Evaluation of the influence of diabetes mellitus on antipyrine metabolism and CYP1A2 and CYP2D6 activity. *Pharmacotherapy* **20**:182–190 (2000).