Effects of *Escherichia coli* Lipopolysaccharide on Telithromycin Pharmacokinetics in Rats: Inhibition of Metabolism via CYP3A

Joo H. Lee, Yu K. Cho, Young S. Jung, Young C. Kim, and Myung G. Lee*

College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea

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It has been reported that telithromycin is metabolized primarily via hepatic microsomal cytochrome P450 (CYP) 3A1/2 in rats and that the expression of hepatic and intestinal CYP3A decreases in rats pretreated with *Escherichia coli* lipopolysaccharide (ECLPS rats; an animal model of inflammation). Thus, it is possible that the area under the plasma concentration-time curve from 0 h to infinity (AUC$_{0\text{--}\infty}$) of intravenous and oral telithromycin is greater for ECLPS rats than for the controls. To assess this, the pharmacokinetic parameters of telithromycin were compared after intravenous and oral administration (50 mg/kg). After intravenous administration of telithromycin, the AUC$_{0\text{--}\infty}$ was significantly greater (by 83.4%) in ECLPS rats due to a significantly lower nonrenal clearance (by 44.5%) than in the controls. This may have been due to a significantly decreased hepatic metabolism of telithromycin in ECLPS rats. After oral administration of telithromycin, the AUC$_{0\text{--}\infty}$ in ECLPS rats was also significantly greater (by 140%) than in the controls and the increase was considerably greater than the 83.4% increase after intravenous administration. This could have been due to a decrease in intestinal metabolism in addition to a decreased hepatic metabolism of telithromycin in ECLPS rats.

Telithromycin, a ketolide antibiotic, is the first of a new class of semisynthetic agents derived from erythromycin by the replacement of the sugar cladinose at position C-3 with a keto group. This alteration resulted in both improved pharmacokinetic properties and an improved spectrum of activity against community-acquired upper and lower respiratory tract pathogens compared to those of erythromycin (4). Telithromycin inhibits bacterial protein synthesis via two mechanisms, first by directly blocking the translation of mRNA and second by interfering with the assembly of new ribosomal units (5). Telithromycin has potent activities both in vitro and in vivo against common respiratory tract pathogens, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and group A beta-hemolytic streptococci, irrespective of their beta-lactam or macrolide susceptibility (1). Its spectrum of activity also extends to atypical and intracellular pathogens (23). Lotter et al. (17) reported that telithromycin has anti-inflammatory properties like those of conventional macrolides due to the inhibition of production of proinflammatory cytokines, which leads to a decreased formation of nitric oxide in *Escherichia coli* lipopolysaccharide (ECLPS)-treated mice.

LPS is an active component in the outer membrane of gram-negative bacteria. ECLPS has been used as a classical inflammatory model for rats (9, 27). Changes in the expression and mRNA levels of hepatic microsomal cytochrome P450 (CYP) isozymes have been reported for rats pretreated with ECLPS (ECLPS rats). For example, the expression and mRNA levels of hepatic CYP2C11, -2E1, and -3A2 decreased in male rats of the Fisher 344 or Sprague-Dawley strain 24 h after intraperitoneal injection of ECLPS (20, 24, 25). Maezono et al. (18) also reported that intraperitoneal injection of ECLPS significantly reduced the activity of intestinal epithelial CYP3A by 41%, as assessed by nifedipine oxidation in male Wistar rats. Therefore, one might expect the pharmacokinetics of telithromycin to change after intravenous or oral administration to ECLPS rats.

Although changes in the pharmacokinetics of drugs in ECLPS rats have been reported (13, 26), the effects of ECLPS on the pharmacokinetics of telithromycin, which is metabolized primarily via CYP3A1/2 in rats (15), have not been published to date. The aim of this study was to assess the pharmacokinetic changes of telithromycin after intravenous or oral administration to ECLPS rats with respect to changes in hepatic and intestinal CYP3A. Changes in the expression of hepatic and intestinal CYP3A in ECLPS rats were also reported.

**MATERIALS AND METHODS**

**Chemicals.** Telithromycin was supplied by sanofi-aventis (Paris, France). Quinine hydrochloride (an internal standard for the high-performance liquid chromatography [HPLC] analysis of telithromycin), NADPH (as a tetrasodium salt), Tris buffer, EDTA, ECLPS (serotype O127:B8), primary monoclonal antibody for beta-actin, and Kodak X-Omat film were purchased from Sigma-Aldrich Corporation (St. Louis, MO). Anti-human polyclonal 3A antibody and horseradish peroxidase-conjugated goat anti-rabbit antibody were products fromDetroit R&D (Detroit, MI) and Bio-Rad Laboratories (Hercules, CA), respectively. Enhanced chemiluminescence reagents were obtained from Amersham Biosciences Corporation (Piscataway, NJ). Other chemicals were of reagent grade or HPLC grade.

**Animals.** The protocol for this animal study was approved by the Institutional Animal Care and Use Committee of Seoul National University, Seoul, South Korea. Male Sprague-Dawley rats, 6 to 8 weeks old and weighing 240 to 300 g, purchased from Taconic Farms, Inc. (Sanmaku Bio Korea, O-San, South Korea), were maintained according to previously reported methods (14, 15).

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* Corresponding author. Mailing address: College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, San 56-1, Shinlim-Dong, Kwanak-Gu, Seoul 151-742, South Korea. Phone: 82 2 8807855. Fax: 82 2 8898693. E-mail: leemg@snu .ac.kr.

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Treatment with ECLPS. The rats were randomly divided into two groups: control rats and ECLPS rats. ECLPS (dissolved in an injectable solution of 0.9% NaCl to produce a concentration of 1 mg/ml at a dose of 1 mg/kg was injected intraperitoneally into the ECLPS rats (25). The same volume of injectable 0.9% NaCl solution was injected into the controls.

Preparation of rat hepatic and intestinal microsomal fractions. The procedures used for the preparation of hepatic (12) and intestinal (22) microsomal fractions were similar to previously reported methods (3).

Measurement of \(V_{\text{max}}\), \(K_m\), and the intrinsic clearance \(\text{Cl}_{\text{int}}\) for the elimination of telithromycin from the hepatic and intestinal microsomal fractions of control and ECLPS rats. The maximum rates of metabolism \(V_{\text{max}}\) and the apparent Michaelis-Menten constants \(K_m\); the concentration at which the rate is one-half of the \(V_{\text{max}}\); for the elimination of telithromycin from the hepatic and intestinal microsomal fractions were determined after incubation of the following in a water bath shaker (37°C, 500 oscillations/min): (i) the above-described microsomal fractions (equivalent to 1 mg protein for both hepatic and intestinal microsomes); (ii) either a 10-μl aliquot of microsomal buffer solution containing 0.01, 0.02, 0.05, 0.075, 0.1, 0.2, 0.5, or 1 μM telithromycin or a 20-μl aliquot of intestinal microsomal buffer solution containing 0.05, 0.075, 0.1, 0.2, 0.5, or 1 μM telithromycin; and (iii) either a 50-μl (for hepatic microsomes) or a 20-μl (for intestinal microsomes) aliquot of 0.1 M phosphate buffer (pH 7.4) containing 1.2 mM NADPH in a final volume of 300 μl (for hepatic microsomes) or 600 μl (for intestinal microsomes) by the addition of 0.1 M phosphate buffer (pH 7.4). All of the above-described microsomal incubation conditions were linear.

Intravenous study. Telithromycin (dissolved in distilled water with a few drops of acetic acid) at a dose of 50 mg/kg was infused (total infusion volume, approximately 0.6 ml) over 1 min via the jugular vein of control (n = 9) and ECLPS (n = 9) rats. A blood sample (approximately 220 μl) was collected via the carotid artery at 0, 5, 15, 30, 60, 120, 180, 240, 360, 480, and 600 min after the start of the intravenous infusion of telithromycin. After centrifugation of the blood sample, a 100-μl aliquot of distilled water containing 50 μg/ml of quinine hydrochloride (an internal standard) was added.

The kinetic constants \(K_m\) and \(V_{\text{max}}\) for the elimination of telithromycin from the hepatic and intestinal microsomes were calculated using a nonlinear regression method (7). The \(\text{Cl}_{\text{int}}\) for the elimination of telithromycin from the hepatic or intestinal microsomes was calculated by dividing the respective \(V_{\text{max}}\) by the respective \(K_m\).

Western blot analysis. The microsomal suspension (10 or 20 μg of protein per lane for the hepatic or intestinal microsomes, respectively) was loaded onto the Western blot apparatus. Other procedures were similar to a previously reported method (11).

 Pretreatment of rats for intravenous and oral studies. In the early morning of the day after treatment with ECLPS or the injectable 0.9% NaCl solution, the body weights of the rats were measured. Then, the carotid artery (for blood sampling) and the jugular vein (for drug administration for intravenous study only) of each rat were cannulated with a polyethylene tube (inside diameter, 0.76 mm; outside diameter, 1.22 mm) (Clay Adams, Parsippany, NJ) while the 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. Thus, the rats were not restrained during the present study. A heparinized injectable 0.9% NaCl solution (15 units/ml, 0.3 ml) containing 0.05, 0.075, 0.1, 0.2, 0.5, or 1 μM telithromycin was used to flush the cannulas to prevent blood clotting. Each rat was housed in an individual cage allowing free movement of the rats. Thus, the rats were not restrained during the present study.

RESULTS

Measurement of \(V_{\text{max}}, K_m\), and \(\text{Cl}_{\text{int}}\) for the elimination of telithromycin from the hepatic and intestinal microsomal fractions. The \(V_{\text{max}}, K_m\), and \(\text{Cl}_{\text{int}}\) values for the elimination of telithromycin from the hepatic and intestinal microsomal fractions of both groups of rats are given in Table 1. In ECLPS rats, the \(V_{\text{max}}\) and \(K_m\) values for the livers were higher (by 70.5%; \(P = 0.177\)) and greater (by 119%; \(P = 0.114\)), respectively, than those of the controls. Thus, the \(\text{Cl}_{\text{int}}\) for the livers of ECLPS rats was significantly lower (by 18.5%) than that of the controls. The \(V_{\text{max}}\) and \(K_m\) values for the intestines were lower (by 30.1%; \(P = 0.421\)) and smaller (by 24.6%; \(P = 0.227\)), respectively, than those of the controls. Thus, the \(\text{Cl}_{\text{int}}\) for the intestines of ECLPS rats was significantly lower (by 10.0%) than that of the controls. The above-mentioned data suggest that the \(V_{\text{max}}\) for the elimination (primarily due to metabolism) of telithromycin from the liver and intestine and the affinity for telithromycin of the enzyme(s) in the liver and intestine were not significantly altered by ECLPS. However, the metabolism of telithromycin in the liver and intestine was significantly lower in ECLPS rats.

Western blot analysis. The levels of hepatic and intestinal CYP3A measured by Western blot analysis with the hepatic and intestinal microsomes prepared from both groups of rats are shown in Fig. 1. In ECLPS rats, the expression of hepatic

<table>
<thead>
<tr>
<th>Microsomal fraction and rat type</th>
<th>(V_{\text{max}}) (nmol/min/mg protein)</th>
<th>(K_m) (μM)</th>
<th>(\text{Cl}_{\text{int}}) (ml/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic</td>
<td></td>
<td></td>
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<tr>
<td>Control (n = 4)</td>
<td>0.0129 ± 0.00677</td>
<td>1.81 ± 0.824</td>
<td>0.00702 ± 0.000737</td>
</tr>
<tr>
<td>ECLPS (n = 4)</td>
<td>0.0220 ± 0.00987</td>
<td>3.96 ± 2.17</td>
<td>0.00357 ± 0.000658</td>
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<tr>
<td>Intestinal</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Control (n = 5)</td>
<td>0.0092 ± 0.0256</td>
<td>7.10 ± 1.72</td>
<td>0.0140 ± 0.000574</td>
</tr>
<tr>
<td>ECLPS (n = 5)</td>
<td>0.00693 ± 0.0364</td>
<td>5.35 ± 2.43</td>
<td>0.0126 ± 0.00104</td>
</tr>
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</table>

\(a\) Values are means ± standard deviations. 
\(b\) \(n\), number of rats.
\(c\) \(P < 0.05\) compared to respective control.
and intestinal CYP3A was significantly lower (by 53.2 and 52.6%, respectively) than that in the controls.

**Pharmacokinetics of telithromycin after intravenous administration.** For the intravenous administration of telithromycin to control and ECLPS rats, the mean arterial plasma concentration-time profiles are shown in Fig. 2A, and relevant pharmacokinetic parameters are given in Table 2. After intravenous administration of telithromycin, changes in pharmacokinetic parameters for ECLPS rats compared to those for the controls were as follows: the AUC<sub>0-24h</sub> became significantly greater (by 83.4%); the CL (by 40.1%), CLR (by 19.3%), and CLNR (by 44.5%) became significantly lower; the V<sub>ss</sub> became significantly smaller (by 33.7%); and the percentage of the intravenous dose of telithromycin excreted in the urine at 24 h as unchanged drug (Ae<sub>0-24h</sub>) became significantly greater (by 44.2%).

**Pharmacokinetics of telithromycin after oral administration.** For the oral administration of telithromycin to control and ECLPS rats, the mean arterial plasma concentration-time profiles are shown in Fig. 2B, and relevant pharmacokinetic parameters are given in Table 2. After oral administration of telithromycin, the levels of absorption of telithromycin from the rat gastrointestinal tract were high for both groups of rats; telithromycin was detected in plasma at the first blood sampling time (5 min). After oral administration of telithromycin, changes in pharmacokinetic parameters in ECLPS rats compared to those in the controls were as follows: the AUC<sub>0-24h</sub> became significantly greater (by 140%), the C<sub>max</sub> became significantly higher (by 114%), and the Ae<sub>0-24h</sub> became significantly greater (by 99.6%).

**DISCUSSION**

The pharmacokinetic changes of drugs (compounds) seemed to be dependent on the gender and species of the rats (CD, Wistar, or Sprague-Dawley rats), the source (Escherichia coli or Klebsiella pneumoniae) and dose (50, 250, 500, or 1,000 mg/kg) of LPS species, and the starting time of the experiment after LPS administration (2, 6, 10, 24, or 96 h) (reference 2 and references therein). For most studies, ECLPS was administered at a dose of 1 mg/kg and the experiment was started 24 h after the intraperitoneal injection of ECLPS into male rats (20, 25). Thus, the same protocol was employed for this study.

After intravenous administration of telithromycin, the contribution of the CL<sub>int</sub> to the CL of telithromycin was not considerable in rats; the values were 16.3% and 22.0% for control and ECLPS rats, respectively (Table 2), indicating that most of
the intravenous telithromycin is extracted via the nonrenal (CLNR) route in rats. The contribution of gastrointestinal (including biliary) excretion of unchanged telithromycin to the CLNR of telithromycin was also not considerable; the total amount of the dose recovered from the entire gastrointestinal tract (including contents of the tract and feces) at 24 h (Gl24 h) was less than 8.53% of the intravenous dose of telithromycin for all rats studied (Table 2). The small Gl24 h values, less than 8.53%, were not likely due to chemical and enzymatic degradation of telithromycin in the rats’ gastric fluids. Lee and Lee (14) reported that telithromycin was stable in various buffer solutions having pHs ranging from 1 to 13 (more than 92.0% of the spiked amount of telithromycin was recovered), except at pH 4 for up to 48 h of incubation (83.7% was recovered) and in three rats’ gastric juices (pHs of 1, 2.5, and 3, respectively) for up to 4 h of incubation (more than 90.5% was recovered). The above-mentioned data suggest that the CLNR of telithromycin given in Table 2 could represent the metabolic clearance of the drug. Additionally, changes in the CLNR of telithromycin could be due to changes in the metabolic clearance of the drug in rats.

The AUC values for telithromycin were dose dependent after both intravenous and oral administration of doses of 20, 50, and 100 mg/kg to rats (14). Thus, an intravenous and oral dose of telithromycin of 50 mg/kg was arbitrarily chosen for the study.

After intravenous administration of telithromycin to ECLPS rats, the significantly greater AUC0–ss could have been due to a significantly lower CL (Table 2). The lower CL was attributable to lower CLNR and lower CLR values in ECLPS rats than in the controls (Table 2). The lower CLNR might result from a decrease in the expression of hepatic CYP3A (Fig. 1) in ECLPS rats, because telithromycin was metabolized via CYP3A1/2 in rats (15). Decreased expression of rat hepatic 3A2 has also been reported (24, 25). The hepatic first-pass effect of telithromycin was almost negligible following absorption into the portal vein, based on the AUC0–ss difference following intravenous and intraportal administration to male Sprague-Dawley rats (14). Because telithromycin is a drug with a low hepatic-extraction ratio, its hepatic clearance depends more on the Clint for the elimination of telithromycin than on the hepatic blood flow rate (28). The significantly lower CLNR of telithromycin in ECLPS rats (Table 2) might be supported by the significantly lower Clint in the liver (Table 1). The free fractions (unbound to plasma protein) of telithromycin in plasma were comparable between the two groups of rats. Nolan and O’Connell (21) reported that the hepatic blood flow rate was lower in ECLPS rats.

After intravenous administration of telithromycin, the Vss was significantly smaller in ECLPS rats than in the controls (Table 2). Although the exact reason is not clear, the smaller Vss of telithromycin in ECLPS rats was not due to the significant decrease in the free fraction of telithromycin in rat plasma; the plasma protein binding levels of telithromycin were not significantly different between the two groups of rats. The protein binding levels of telithromycin to plasma from control and ECLPS rats at a telithromycin concentration of 5 μg/ml were not significantly different (70.0% ± 12.5% and 66.4% ± 7.60%, respectively), as determined by a previously reported equilibrium dialysis process (14).
After oral administration of telithromycin to rats, the AUC<sub>0→∞</sub> was also significantly greater in ECLPS rats than in the controls (Table 2). This was possibly due to a decreased hepatic metabolism. After oral administration, the AUC<sub>0→∞</sub> was also significantly greater in ECLPS rats than in the controls (Table 2), possibly due to a decreased intestinal metabolism combined with a decreased hepatic metabolism of telithromycin in ECLPS rats.

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REFERENCES


