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Involvement of the Drug Transporters P Glycoprotein and Multidrug Resistance-Associated Protein Mrp2 in Telithromycin Transport

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The present study aims to investigate the role of P glycoprotein and multidrug resistance-associated protein (Mrp2) in the transport of telithromycin, a newly developed ketolide antibiotic, in vitro and in vivo. The in vitro experiments revealed that the intracellular accumulation of telithromycin in adriamycin-resistant human chronic myelogenous leukemia cells (K562/ADR) overexpressing P glycoprotein was significantly lower than that in human chronic myelogenous leukemia cells (K562/S) not expressing P glycoprotein. Cyclosporine significantly increased the intracellular accumulation of telithromycin in K562/ADR cells. When telithromycin was coadministered intravenously with cyclosporine in Sprague-Dawley (SD) rats, cyclosporine significantly delayed the disappearance of telithromycin from plasma and decreased its systemic clearance to 60% of the corresponding control values. Hepatobiliary excretion experiments revealed that cyclosporine almost completely inhibited the biliary clearance of telithromycin, suggesting that telithromycin is a substrate of P glycoprotein and a potential substrate of Mrp2. Moreover, the biliary clearance of telithromycin was significantly decreased by 80% in Eisai hyperbilirubinemic mutant rats with a hereditary deficiency in Mrp2, indicating that Mrp2, as well as P glycoprotein, plays an important role in the biliary excretion of telithromycin. When the effect of telithromycin on the biliary excretion of doxorubicin, a substrate of P glycoprotein and Mrp2, was examined in SD rats, telithromycin significantly decreased the biliary clearance of doxorubicin by 80%. Results obtained from this study indicate that telithromycin is a substrate of both P glycoprotein and Mrp2, and these transporters are involved in the hepatobiliary transport of telithromycin.

P glycoprotein, a member of the ATP-binding cassette (ABC) transport proteins, is known to act as an efflux pump for various drugs such as *Vinca* alkaloid and anthracycline anticancer drugs, calcium channel blockers, and immunosuppressive agents (5, 27, 34–36). This transporter is located not only in anticancer drug resistance cells but also in normal tissues, including the bile canalicular membrane of hepatocytes, the brush border membrane of renal proximal tubule cells, intestinal epithelial cells, and the blood-brain barrier (23, 24, 33). Another known transporter, multidrug resistance-associated protein 2 (Mrp2), like P glycoprotein, is also presented in almost the same tissues as P glycoprotein and acts as an important role in excretion of various organic anion drugs, such as glutathione, glucuronate, and sulfate conjugates by an ATP-dependent mechanism (3, 12, 18). Thus, both drug transporters appear to play an important role in the disposition of various drugs and have a protective function for endogenous and exogenous compounds. It is suggested that P-glycoprotein substrate and Mrp2 substrate overlap (1, 10, 14).

Telithromycin, a semisynthetic antibiotic, is one of a new class of antibiotics called ketolides that have been developed for the treatment of upper and lower respiratory tract infections (16, 22, 26, 29). Telithromycin has a chemical structure similar to a 14-ring member macrolide antibiotic, erythromycin, and has high activity against β-lactam, macrolide, and fluoroquinolone reduced-susceptibility pathogens (22, 29). We previously reported that many macrolide antibiotics, including erythromycin, azithromycin, and clarithromycin could overcome P-glycoprotein-dependent anticancer drug resistance and cause profound alterations in the pharmacokinetics of doxorubicin, which is a substrate for P glycoprotein (11, 30, 39). On the other hand, it is well known that P-glycoprotein and cytochrome P450 (CYP) 3A4 substances overlap (10, 37), and the macrolide antibiotics erythromycin and clarithromycin inhibit not only CYP3A4 but also P glycoprotein (8, 20, 38, 40). Telithromycin is a substrate for CYP3A4 and also inhibits CYP3A4 (2, 16, 28). On the basis of these findings, it is possible that telithromycin is a substrate for P glycoprotein. Seral and colleagues (25) reported that influx of telithromycin is adversely influenced by the activity of P glycoprotein in J774 macrophages, resulting in suboptimal drug accumulation. Pachot and colleagues (19) also reported that the P-glycoprotein-mediated efflux mechanism is involved in the transport of telithromycin by using a Caco-2 cell model. However, no in vivo data are available that specify which drug transporters are related to the transport of telithromycin. It is necessary to investigate if P glycoprotein and/or Mrp2 is involved in the excretion of telithromycin.

The present study aims to clarify the involvement of the drug transporters P glycoprotein and/or Mrp2 in the hepatobiliary

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excretion of telithromycin in rats. First, we measured the in vitro cellular accumulation of telithromycin in a human chronic myelogenous leukemia cell line (K562/S) and its adriamycin-resistant subline (K562/ADR). Second, the effect of cyclosporine on the systemic and hepatobiliary excretion of telithromycin in normal rats was investigated. Third, we studied the role of Mrp2 in the hepatobiliary excretion of telithromycin using Eisai hyperbilirubinemic mutant rats (EHBRs), which have a hereditary deficiency in Mrp2 (31, 32). Finally, we investigated the effect of telithromycin on the hepatobiliary excretion of doxorubicin.

MATERIALS AND METHODS

Chemicals. Telithromycin was extracted with dichloromethane from a telithromycin tablet, which was purchased from Sankyo Co., Ltd. (Tokyo, Japan), and the purity was verified by high-performance liquid chromatography (HPLC) and thin-layer chromatography (H. Nosaka et al., unpublished data). Cyclosporine was purchased from Novartis Pharma Co., Ltd. (Tokyo, Japan). All other reagents are commercially available and were of analytical grade and used without further purification. Telithromycin was dissolved in 1 M phosphoric acid and adjusted at pH 4 with 1 N NaOH.

Animals. Male Sprague-Dawley (SD) rats (270 to 280 g) and EHBRs (270 to 280 g) were obtained from the Japan SLC, Inc. (Hamamatsu, Japan). The rats were housed under controlled environmental conditions (temperature of 23 ± 1°C and humidity of 55% ± 5%) with a commercial food diet and water freely available to the animals. All animal experiments were carried out in accordance with the guidelines of Aichi Medical University for the Care and Use of Laboratory Animals.

Cell culture and intracellular accumulation of telithromycin in K562/S and K562/ADR cells. The human chronic myelogenous leukemia cell line (K562/S) and its adriamycin-resistant subline (K562/ADR) were kindly provided by Ken-ichi Miyamoto (Kanazawa University School of Medicine). These cells were grown in RPMI medium supplemented with 1% penicillin-streptomycin and 10% fetal calf serum (Gibco Biocult) at 37°C in a 5% CO_2 humidified atmosphere. Adriamycin (500 nM) was added to the culture medium of K562/ADR cells. The resistant K562/ADR cells were grown for 7 days in the presence of adriamycin prior to use in the experiments. Cell viability was assessed by trypan blue dye exclusion. K562/S and K562/ADR cells (2×10^6 cells/ml) were suspended in phosphate-buffered saline solution (pH 7.2). Dose-dependent intracellular accumulation of telithromycin in both cells was carried out at a concentration range from 50 to 500 μM at 4°C and 37°C. The effect of cyclosporine (1 and 10 μM) on the intracellular accumulation of telithromycin (50 μM) in both cells was also performed at 4°C and 37°C. After incubation, the cells were washed three times with cold phosphate-buffered saline solution, and the obtained cell pellets were kept at −30°C until analysis. For measurement of intracellular telithromycin, the pellets were suspended in 300 μl of water and then ultrasonicated with an ultrasonic disrupter (UD-2000; Tomy Seiko, Tokyo, Japan). The concentration of telithromycin in ultrasonicated solution was measured by HPLC.

Effect of cyclosporine on plasma concentration-time curve of telithromycin in SD rats. To investigate the effects of cyclosporine on the pharmacokinetics of telithromycin, rats under anesthesia by intraperitoneal injection of sodium pentobarbital (25 mg/kg) were cannulated with polyethylene tubes in the right jugular vein for drug administration and blood sampling. The rats received a single intravenous injection of telithromycin (10 mg/kg) after awakening. Telithromycin was administered 10 min after a single intravenous injection of cyclosporine (20 mg/kg of body weight). Control rats received isotonic saline (0.2 ml/100 g). Blood samples were collected at designated intervals (5, 10, 20, 30, 45, 60, 90, 120, 180, and 240 min after injection of telithromycin). Plasma samples were obtained from the blood samples by centrifugation at $1,200 \times g$ for 5 min at 4°C and stored −30°C until analysis.

Effect of cyclosporine on biliary clearance of telithromycin in SD rats. To investigate the effect of cyclosporine on biliary excretion of telithromycin, rats under light anesthesia with sodium pentobarbital (40 mg/kg) were cannulated in the right jugular vein, left carotid artery, and bile duct for drug administration, blood sampling, and bile collection, respectively. After surgical preparations, the rats received a bolus injection of telithromycin in a loading dose of 1.4 mg/kg, followed by a constant-rate infusion, with a Harvard infusion pump (PHD 2000; South Natick, Mass.), of a saline solution delivering 0.42 mg of telithromycin per h at a rate of 2 ml/h until the end of the study. The loading and maintenance doses of telithromycin were determined by using pharmacokinetic parameters obtained from systemic clearance experiments. After a 60-min infusion, bile samples were collected at 20-min intervals for 60 min. After a 120-min infusion, cyclosporine (30 mg/kg) was administered intravenously. Bile samples were collected at 20-min intervals from 120 to 220 min. Blood samples were collected at the midpoints of the bile collection periods (70, 90, 110, 130, 150, 170, 190, and 210 min after the infusion was started). Plasma samples were obtained by immediate centrifugation of blood samples. The volume of bile samples was measured gravimetrically with specific gravity assumed to be 1.0. Plasma and bile samples were stored at −30°C until analysis. This experiment was done under anesthesia with pentobarbital, and the body temperature of the animals was maintained at 37°C with a heat lamp.

Hepatobiliary excretion of telithromycin in SD rats and EHBRs. To further clarify the contribution of Mrp2 to the biliary exertion of telithromycin, normal rats (SD rats) and EHBRs received cannulation and an injection of telithromycin as described above. Bile samples were collected at 20-min intervals for 60 min after 60 min of infusion. Blood samples were collected at the midpoints of the bile collection periods (70, 90, and 110 min after the infusion was started). Sample treatment and the experiment were done as described above.

Effect of telithromycin on hepatobiliary excretion of doxorubicin in SD rats. To investigate whether telithromycin modifies the biliary excretion of doxorubicin, rats under anesthesia with sodium pentobarbital (40 mg/kg) were cannulated with polyethylene tubes into the carotid artery, the jugular vein, and the bile duct. The rats received a loading dose of 0.26 mg/kg and a maintenance dose of 156 μg/h at a rate of 2 ml/h of doxorubicin until the end of the study. The loading and maintenance doses of doxorubicin used in this study are from our previous study (1). After a 60-min infusion, bile samples were collected at 20-min intervals for 60 min. Blood samples were collected at the midpoints of the bile collection periods (70, 90, and 110 min after the infusion was started). After a 120-min infusion, telithromycin (30 mg/kg) was administered intravenously. Bile samples were collected at 20-min intervals from 120 to 220 min. Blood samples were collected at the midpoints of the bile collection periods (130, 150, 170, 190, and 210 min after the infusion was started). Samples were treated as described above.

Drug analysis. Concentrations of telithromycin and doxorubicin in plasma, bile, and cells were determined by HPLC. Bile samples were appropriately diluted with distilled water. For telithromycin, each sample (50 μl) and 200 μl of acetonitrile were mixed and centrifuged at $12,000 \times g$ for 10 min. After centrifugation, the supernatant (200 μl) was dried under nitrogen gas at 45°C. The residue was dissolved in 200 μl of mobile phase and injected into the HPLC system. For doxorubicin, each sample (50 μl) and 300 μl of methanol containing an internal standard of daunorubicin (0.2 μg/ml) were mixed and centrifuged at $12,000 \times g$ for 10 min. After centrifugation, the supernatant (100 μl) was subjected directly to the HPLC system. The apparatus used for HPLC was a Shimadzu LC-10A system (Kyoto, Japan) equipped with a fluorescence detector (RF-10AXL; Shimadzu) (for telithromycin, excitation was 263 nm and emission was 460 nm; for doxorubicin, excitation was 480 nm and emission was 560 nm) consisting of an LC-10A liquid pump and a SIL-10A autoinjector. The conditions were as follows: column, a Cosmocil $5C_{18}$ column (4.6 by 150 mm; Nacalai Tesque, Kyoto, Japan); mobile phase, 50 mM ammonium acetate-methanol-acetonitrile (52:29:24 [vol/vol]) for telithromycin and 10 mM phosphate buffer (pH 4.0)-methanol (40:60 [vol/vol]) for doxorubicin; column temperature (OTC-6A, Shimadzu), 40°C; flow rate, 1.0 ml/min. These assays were shown to be linear for the concentrations studied with a correlation coefficient of 0.999. No interference with the peak of telithromycin and doxorubicin was observed in any samples. The within- and between-day coefficients of variation for this assay were less than 8%.

Pharmacokinetic analysis. Plasma concentration-time data for telithromycin after a single administration were analyzed using a noncompartmental model. The area under the curve (AUC) and the area under the first-moment curve (AUMC) were calculated by the trapezoidal rule with extrapolation to infinity. The system clearance (CL_{SYS}) was determined as dose/AUC. The mean residence time (MRT) was calculated as MRT = AUMC/AUC. The volume of distribution at steady state (V_{SS}) was calculated as $V_{SS} = CL_{SYS} \times MRT$. The biliary clearance (CL_{BILE}) of telithromycin was calculated by dividing the biliary excretion rate by the steady-state concentration in plasma (C_{SS}) determined for that collection period.

Statistical analysis. The experiments were performed at least three times, and the results are expressed as means ± standard errors. Statistical comparisons were assessed by Student's *t* test or one-way analysis of variance. When *F* ratios were significant ($P < 0.05$), Scheffe's post hoc tests between the groups were done, and *P* values of <0.05 were considered statistically significant differences. Stat View software (version 4.5; Abacus Concepts Inc., Berkeley, Calif.) was used for the analysis.

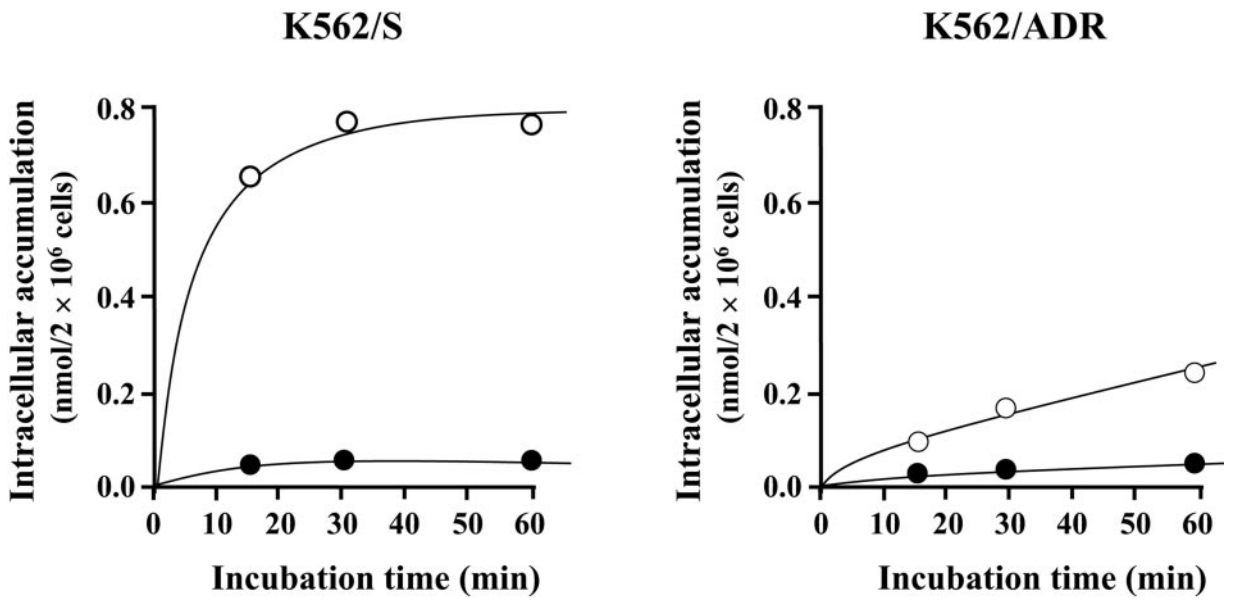

FIG. 1. Time-dependent intracellular accumulation of telithromycin in K562/ADR and K562/S cells at 4°C or 37°C. The mean uptake of telithromycin in K562/S and K562/ADR cells for 60 min was 0.77 and 0.28 nmol/2 × 10^6 cells, respectively. The data are the means ± standard errors ($n = 4$). When the standard error is small, it is included in the symbol. Symbols: ○, 37°C; ●, 4°C. Significant differences were observed at all time points between 4°C and 37°C.

RESULTS

In vitro accumulation of telithromycin in K562/S and K562/ADR cells. P glycoprotein was overexpressed in K562/ADR cells, but not K562/S cells, as reported previously (1). In the in vitro uptake experiments, dose-dependent intracellular accumulation of telithromycin was observed in both cells (data not shown). The time courses of the intracellular accumulation of telithromycin (50 μM) into K562/S and K562/ADR cells are shown in Fig. 1. As shown in Fig. 1, the concentration of telithromycin accumulated in both cells was higher at 37°C than at 4°C and the amount of telithromycin accumulated in K562/S cells at 60 min was approximately threefold higher than that in K562/ADR cells (0.77 and 0.28 nmol/2 × 10^6 cells, respectively). Figure 2 shows the effect of cyclosporine on the intracellular accumulation of telithromycin in K562/S and K562/ADR cells. As shown in Fig. 2, cyclosporine (10 μM) significantly increased the intracellular accumulation of telithromycin in K562/ADR cells. However, no effect of cyclosporine

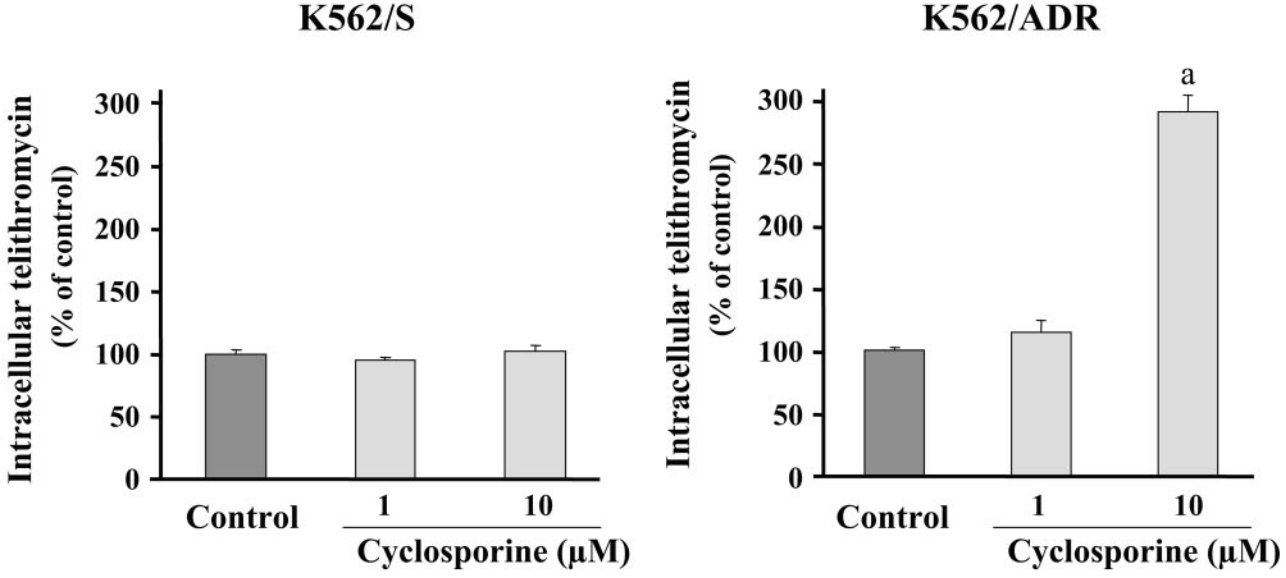

FIG. 2. Effect of cyclosporine (1 and 10 μM) on intracellular accumulation of telithromycin in K562/S and K562/ADR cells. The data are the means ± standard errors ($n = 3$). "a" indicates values that are significantly different from the control values ($P < 0.05$).

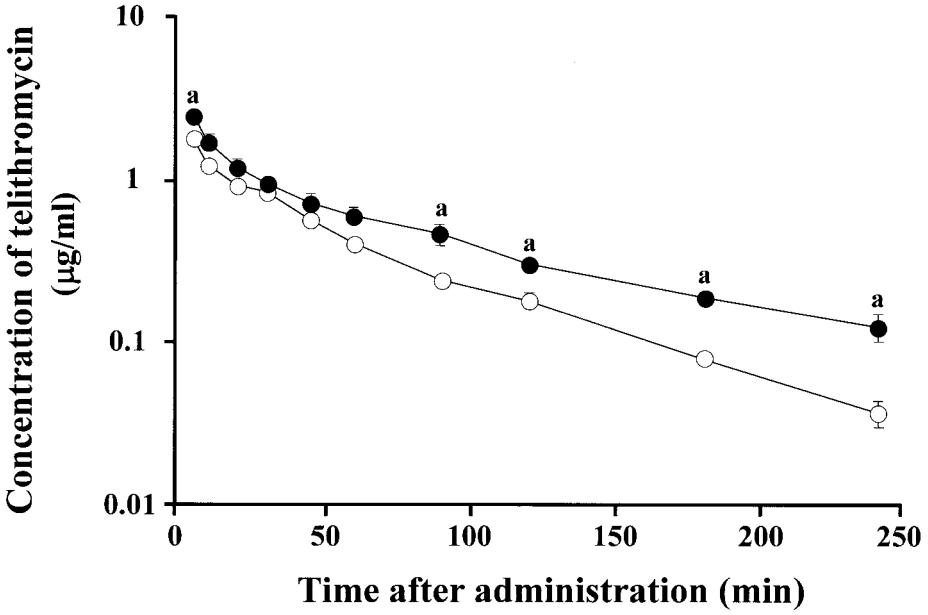

FIG. 3. Effect of cyclosporine on plasma concentration of telithromycin after a single intravenous injection of telithromycin in SD rats. Cyclosporine (20 mg/kg) was administered intravenously 10 min before injection of telithromycin (10 mg/kg). The data are the means ± standard errors ($n = 3$). When the standard error is small, it is included in the symbol. Symbols: ○, control; ●, cyclosporine. "a" indicates values that are significantly different from the control values ($P < 0.05$).

on the intracellular accumulation of telithromycin was observed in K562/S cells.

Effect of cyclosporine on plasma concentration-time curve of telithromycin. Mean semilogarithmic plots of plasma concentration-time data for telithromycin after a single intravenous injection (10 mg/kg) with or without cyclosporine are illustrated in Fig. 3. Pretreatment with cyclosporine (20 mg/kg, 10 min earlier) significantly delayed the disappearance of telithromycin from plasma and increased the concentrations in plasma. The corresponding pharmacokinetic parameters of telithromycin are summarized in Table 1. Pretreatment with cyclosporine significantly decreased the systemic clearance of telithromycin to 60% of the control value with no change in the volume of distribution at steady state.

Effect of cyclosporine on hepatobiliary excretion of telithromycin in SD rats. The effect of cyclosporine (30 mg/kg) on the hepatobiliary excretion of telithromycin was investigated under steady-state conditions obtained by the continuous infusion. The control values of the biliary excretion rate and the CL_{BILE} and C_{SS} values of telithromycin in SD rats were 1.06 ± 0.04 μg/min, 4.41 ± 0.21 ml/min, and 0.25 ± 0.01 μg/ml, respectively. An intravenous injection of cyclosporine significantly decreased the biliary excretion rate of telithromycin (0.11 ± 0.02 μg/ml). As shown in Fig. 4, cyclosporine significantly decreased the CL_{BILE} of telithromycin to 10% of the control values: the overall mean values of the CL_{BILE} of telithromycin were significantly decreased by cyclosporine from 4.40 ± 0.21 ml/min to 0.35 ± 0.03 ml/min. The overall mean concentration in plasma (C_{SS}) of telithromycin was also significantly increased by injection of cyclosporine from 0.25 ± 0.01 μg/ml to 0.40 ± 0.01 μg/ml.

TABLE 1. Effect of cyclosporine on pharmacokinetic parameters of telithromycin in Sprague-Dawley rats[a]

Parameter	Control	Cyclosporine
CL_{SYS} (liters/h/kg)	6.97 ± 0.22	4.43 ± 0.58[b]
V_{SS} (liters/kg)	7.03 ± 0.36	6.77 ± 0.45
MRT (h)	1.01 ± 0.06	1.55 ± 0.10[b]

[a] CL_{SYS}, V_{SS}, and MRT represent systemic clearance, volume of distribution at steady state, and mean residence time, respectively. Each value represents the means ± the standard errors ($n = 3$).
[b] Values are significantly different from the control values ($P < 0.05$).

Hepatobiliary excretion of telithromycin in SD rats and EHBRs. To confirm whether telithromycin could be transported via Mrp2, the hepatobiliary excretion of telithromycin was determined in SD rats with Mrp2 and EHBRs lacking Mrp2. The CL_{BILE} of telithromycin in SD rats and EHBRs are shown in Fig. 5. The CL_{BILE} values of telithromycin in EHBRs were significantly lower than those in SD rats (1.00 ± 0.08 ml/min and 4.41 ± 0.21 ml/min, respectively). The biliary excretion rate of telithromycin in EHBRs was delayed compared to that in SD rats (0.32 ± 0.03 μg/min and 1.07 ± 0.04 μg/min, respectively). The C_{SS} values of telithromycin in EHBRs were significantly higher than those in SD rats (0.33 ± 0.01 μg/ml and 0.25 ± 0.01 μg/ml, respectively).

Inhibitory effect of telithromycin against hepatobiliary excretion of doxorubicin in SD rats. To clarify whether telithromycin inhibits P-glycoprotein- and Mrp2-mediated biliary excretion of doxorubicin, in vivo biliary clearance experiments were performed in SD rats. As shown in Fig. 6, an intravenous injection of telithromycin significantly decreased the CL_{BILE} of doxorubicin by 80% compared to the control values (during the first 60 min) with no change in the C_{SS} of doxorubicin (0.14 ± 0.00 and 0.15 ± 0.01 μg/ml): the overall mean biliary

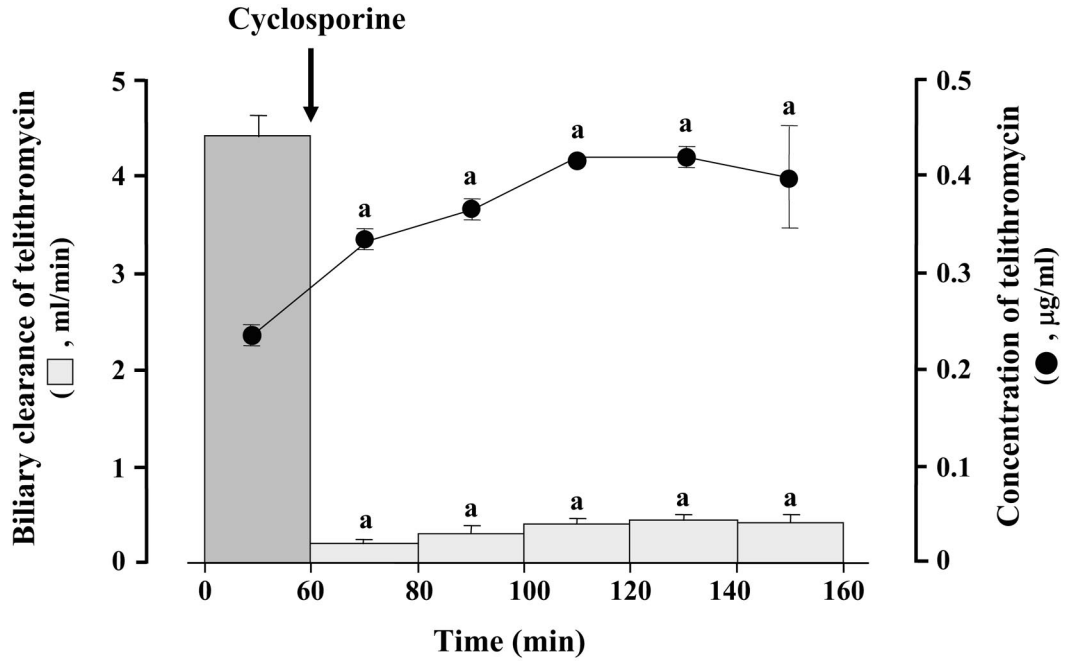

FIG. 4. Effect of cyclosporine on the biliary clearance and steady-state plasma concentration of telithromycin in SD rats. The overall mean values of CL_{BILE} of telithromycin in control rats and cyclosporine-treated rats were 4.40 ± 0.21 ml/min and 0.35 ± 0.03 ml/min, respectively. The overall mean values of C_{SS} of telithromycin were 0.25 ± 0.10 μg/ml and 0.40 ± 0.01 μg/ml, respectively. The data are the means ± standard errors ($n = 5$). At the time point indicated by the arrow, cyclosporine was administered intravenously. "a" indicates values that are significantly different from the control values ($P < 0.05$).

clearance of doxorubicin decreased from 3.18 ± 0.30 ml/min to 0.67 ± 0.09 ml/min. The overall biliary excretion rate of doxorubicin was significantly decreased by injection of telithromycin from 0.43 ± 0.04 μg/min to 0.06 ± 0.01 μg/min.

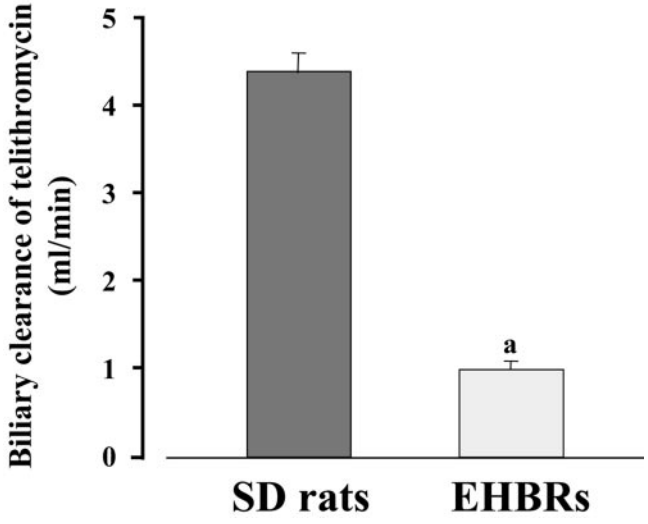

FIG. 5. Biliary clearance of telithromycin in SD rats and EHBRs. The overall mean values of biliary clearance of telithromycin in SD rats and EHBRs were 4.41 ± 0.21 ml/min and 1.00 ± 0.08 ml/min, respectively. The data are the means ± standard errors ($n = 5$). "a" indicates values that are significantly different from the control values ($P < 0.05$).

DISCUSSION

It is reported that the liver is the major organ for the metabolism and elimination of telithromycin (16), thus, CYP and drug transporters such as P glycoprotein and Mrp2 might be involved in the metabolism and elimination of telithromycin. Telithromycin is a substrate of CYP3A4 (2, 16, 41), and more recently, we have found that telithromycin significantly inhibits the metabolism of theophylline by reducing the activity and expression of hepatic CYP3A2 and CYP1A2 (Nosaka et al., unpublished). However, to our knowledge, there is no in vivo evidence that telithromycin is a substrate for P glycoprotein and/or Mrp2 and that the hepatobiliary excretion of telithromycin is mediated by P-glycoprotein- and/or Mrp2-mediated transport systems. In the present study, we performed the in vitro and in vivo experiments to clarify whether telithromycin is a substrate for P glycoprotein and/or Mrp2 and the role of the drug transporters P glycoprotein and Mrp2 in its hepatobiliary excretion.

First, we measured the in vitro accumulation of telithromycin in K562/ADR cells and K562/S cells to clarify whether telithromycin is a substrate for P glycoprotein. However, we did not perform the in vitro experiments using cells expressing only Mrp2 since we could not obtain these cells. We previously confirmed that P glycoprotein, but not Mrp2, is overexpressed in K562/ADR cells and not in K562/S cells by Western blot analysis (1). The in vitro experiments showed that the concentration of telithromycin accumulated in both cells was increased in a dose-dependent manner (data not shown) and that the amount of telithromycin accumulated in K562/S cells

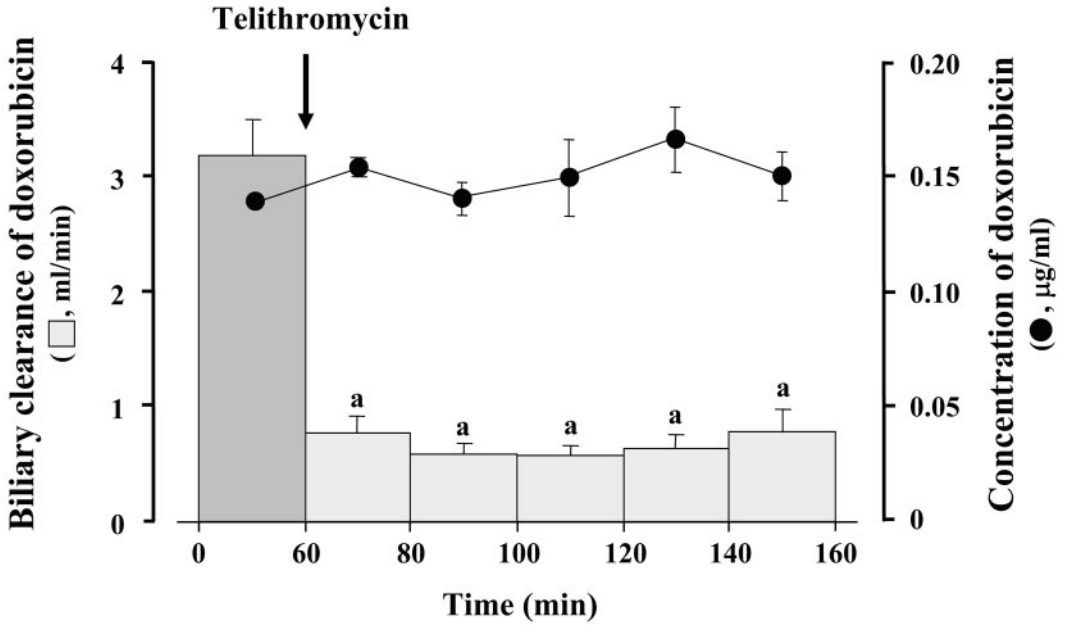

FIG. 6. Effect of telithromycin on the biliary clearance and steady-state plasma concentration of doxorubicin. The mean control biliary clearance of doxorubicin (0 to 60 min) was 3.18 ± 0.30 ml/min. The mean steady-state control plasma concentration of doxorubicin was 0.14 ± 0.01 μg/ml. The data are the means ± standard errors ($n = 5$). "a" indicates values that are significantly different from the control values ($P < 0.05$).

was much higher than that in K562/ADR cells. It was also found that cyclosporine significantly increased the intracellular accumulation of telithromycin in K562/ADR cells. These results suggest that telithromycin is pumped out of K562/ADR cells via P glycoprotein and that it is a substrate for P glycoprotein. These results agreed well with a report by Seral and colleagues (25) who reported that verapamil and GF120918, typical P-glycoprotein inhibitors, markedly increased the cellular accumulation of telithromycin in J774 murine macrophages. Based on these findings, it can be concluded that telithromycin may be a substrate for P glycoprotein.

Second, we examined the in vivo effect of cyclosporine, a typical inhibitor of P glycoprotein, on the pharmacokinetics of telithromycin after a single intravenous injection in SD rats. This experiment revealed that treatment with cyclosporine significantly delayed the disappearance of telithromycin from plasma and decreased its CL_{SYS}. However, no change in the V_{SS} of telithromycin was observed. These results suggest that cyclosporine delays disappearance of telithromycin from plasma by inhibiting cyclosporine-sensitive transporters expressed in various tissues. Considering that cyclosporine inhibits the function of both P glycoprotein and Mrp2 (4, 10), it is likely that P glycoprotein and/or Mrp2 contributes to the elimination of telithromycin.

It is reported that hepatobiliary excretion is an important elimination route for telithromycin in humans (16) and that both P glycoprotein and Mrp2 exist in the bile canalicular membrane of hepatocytes (3, 12). Third, we performed the hepatobiliary clearance experiments for telithromycin by using SD rats. Our results revealed that cyclosporine dramatically decreased the CL_{BILE} of telithromycin by 90% and increased the C_{SS} of telithromycin, suggesting that P glycoprotein and/or Mrp2 contributes largely to the hepatobiliary excretion of telithromycin. On the basis of these results, we note that telithromycin is a substrate of P glycoprotein and a potential substrate of Mrp2. It is possible that the increased C_{SS} of telithromycin by cyclosporine is due in part to competitive inhibition of CYP3A4-mediated metabolism of telithromycin, in addition to inhibition of P glycoprotein and Mrp2. These findings proved the results obtained in the in vitro uptake experiments of telithromycin using K562/S and K562/ADR cells. Moreover, these results were partly supported by a case report that telithromycin in combination with verapamil caused adverse effects such as hypotension and bradyarrhythmia, due to an increase in plasma concentration of verapamil (21). Considering that verapamil is known to be a typical substrate of P glycoprotein, it is likely that the increased plasma concentration of verapamil by coadministration of telithromycin would be partly induced by inhibition of P glycoprotein, in addition to the inhibition of CYP3A4 systems as mentioned by the authors.

It is well known that Mrp2 mainly exists in the liver, as well as in the kidney and gut (3, 9, 13), it functions as a potent efflux pump, and its function is inhibited by cyclosporine at a high dose (4). Considering that the substrate specificities of P glycoprotein and Mrp2 overlap (10), we presume that cyclosporine also inhibited Mrp2-mediated hepatobiliary excretion of telithromycin to some extent. Fourth, to make sure whether and to what extent Mrp2 contributes to the hepatobiliary excretion of telithromycin, the CL_{BILE} of telithromycin in EHBRs lacking Mrp2 was compared with that in SD rats possessing Mrp2. The values of CL_{BILE} for telithromycin in EHBRs were approximately 20% of those in SD rats, confirming that Mrp2 also plays an important role in the hepatobiliary excretion of telithromycin. In addition, it can be concluded that telithromycin is a substrate of Mrp2 as well as P glycoprotein, and the contribution of Mrp2 to the hepatobiliary excretion of telithromycin is larger than that of P gly-

coprotein. On the other hand, it has been reported that CYP3A2 activity in liver microsomes is significantly lower in EHBRs than in SD rats (15, 17). From these findings, it is possible that the increase in the C_{SS} of telithromycin in EHBRs may be in part caused by lower CYP3A2 activity than that in SD rats. Consequently, the present study suggests that telithromycin is, at least in part, transported via Mrp2.

We previously reported that P-glycoprotein- and Mrp2-mediated hepatobiliary excretion of doxorubicin was inhibited by either erythromycin or azithromycin and that both macrolide antibiotics had a similar inhibitory effect on the uptake of doxorubicin in K562/ADR cells (11, 30). On the basis of these findings, telithromycin might inhibit the hepatobiliary excretion of doxorubicin. Finally, we further investigated the in vivo effect of telithromycin on P-glycoprotein- and Mrp2-mediated hepatobiliary excretion of doxorubicin in SD rats. Our results revealed that telithromycin significantly decreased the CL_{BILE} of doxorubicin by 80%, suggesting that telithromycin strongly inhibits P-glycoprotein- and Mrp2-mediated hepatobiliary excretion of doxorubicin. We previously reported that the CL_{BILE} of doxorubicin was almost completely inhibited by erythromycin (11) and inhibited by 25% by azithromycin (30). In the present study, telithromycin was shown to have the same inhibitory effect as erythromycin against the hepatobiliary excretion of doxorubicin and a much stronger effect than azithromycin. It is likely that the different inhibitory effects of telithromycin, azithromycin, and erythromycin are due to their different chemical structures and their different affinities to P glycoprotein and Mrp2. Telithromycin inhibits CYP3A4 and CYP2D6 (the pharmaceutical interview form, no. 876149), and doxorubicin is metabolized by CYP2B1 (7). Based on these findings and the present data, the influence of the metabolic activity on the hepatobiliary excretion of doxorubicin might be very small.

In conclusion, the present study is, to our knowledge, the first demonstration that telithromycin is a substrate for P glycoprotein and Mrp2, that these drug transporters play an important role in the hepatobiliary excretion of telithromycin, and that telithromycin can inhibit the hepatobiliary excretion of doxorubicin by inhibiting these drug transporters. Although the data obtained in the present study cannot be extrapolated directly to humans, these results may provide some useful information for designing drug regimens in patients with bacterial infections. Telithromycin can be expected to overcome P-glycoprotein- and/or Mrp2-dependent anticancer drug-resistant tumor cells.

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