Biliary and Renal Excretions of Cefpiramide in Eisai Hyperbilirubinemic Rats

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Eisai hyperbilirubinemic mutant rats (EHBRS) with conjugated hyperbilirubinemia were recently derived from Sprague-Dawley rats (SDRS). The pharmacokinetic characteristics of the β-lactam antibiotic cefpiramide (CPM), which is mainly excreted into bile, were investigated in 10- and 20-week-old EHBRS and were compared with those in 20-week-old healthy SDRS. The pharmacokinetic parameters of CPM after an intravenous administration of 20 mg/kg of body weight were estimated for each rat by noncompartmental methods. When compared with age-matched healthy SDRS, significant decreases (by approximately 30%) in the systemic clearance of CPM were observed in 20-week-old EHBRS. The biliary clearance of CPM in 20-week-old EHBRS markedly decreased to less than 10% of that in age-matched healthy SDRS, while total urinary recovery of unchanged CPM increased to threefold and renal clearance doubled. However, no significant differences in any of the pharmacokinetic parameters of CPM were observed between the two groups of EHBRS. There were no significant differences among the three groups in the steady-state volume of distribution of CPM. The present study indicates that hyperbilirubinemia induces an increase in the urinary excretion ability of CPM in return for a reduction in the biliary excretion.

Hyperbilirubinemia in rats is known to modify the pharmacokinetics of certain drugs as a result of the impairment of the process of biliary excretion (13, 25, 26, 30). Hyperbilirubinemic mutant TR − and GY rats derived from Wistar strain rats have generally been used as experimental animal models for hyperbilirubinemia because they exhibit abnormalities in the biliary excretion of various organic anions and glucuronidated and sulfated bile acids, which are similar to the symptoms of Dubin-Johnson syndrome in humans (8, 11, 16, 24).

Eisai hyperbilirubinemic mutant rats (EHBRS), which possess much higher concentrations of conjugated bilirubin in plasma, were derived from Sprague-Dawley rats (SDRS) (7, 19). Several investigators have recently reported that hyperbilirubinemia in EHBRS decreases the biliary secretion of various organic anions by impairing the canicular membrane transport (26, 28) and that the canicular membrane vesicles isolated from EHBRSs and TR − rats lack the ATP-dependent canicular transport capability of organic anions (6, 9). These observations suggest that the biliary secretory characteristics of organic anions in EHBRSs closely resemble those in TR − and GY rats (6, 26, 28) and in humans with Dubin-Johnson syndrome (4). Thus, EHBRSs can be used as an animal model for studying hyperbilirubinemia in humans.

Cepiramide (CPM), an anionic β-lactam antibiotic, exhibits antibacterial activity against gram-positive and gram-negative bacteria, in particular, to Pseudomonas aeruginosa, which is resistant to several antibiotics. CPM has been shown to be eliminated mainly into the bile in unchanged form by active biliary secretion in both humans and animals (18, 22, 31), suggesting that CPM could be prescribed as the preferred drug for the treatment of hepatobiliary infections. Some studies support the biliary excretion mechanism of CPM and the effect of obstructive jaundice on its pharmacokinetic behavior in experimental animals (13, 29). Tamai et al. (29) reported that CPM is transported as an organic anion by the bile canalicular membrane in healthy rats. Kimura et al. (13) reported that rats with obstructive jaundice exhibited a reduction in the biliary excretion of CPM while urinary excretion increased. However, little information is available on the influence of the dysfunctions in EHBRSs on the biliary excretion and renal excretion of CPM.

The study described here was conducted as part of a program for the development of guidelines for the safe use of CPM in patients with hepatobiliary infections. We investigated changes in the pharmacokinetics of CPM, in particular, its biliary and urinary excretions, in EHBRSs.

MATERIALS AND METHODS

Chemicals. CPM was kindly donated by Sumitomo Chemical and Industries (Osaka, Japan). 3-Butylxanthine was synthesized in our laboratory and was identical to that used previously (20, 21). All other chemical reagents used were obtained commercially and were used without further purification.

Animals. Ten- and 20-week-old male EHBRSs (weights, 346 to 370 and 492 to 550 g, respectively), maintained by inbreeding male homozygotic and female heterozygotic rats at Eisai Laboratory (Gifu, Japan), and 20-week-old healthy SDRS (weight, 492 to 540 g; Nippon SLC, Hamamatsu, Japan) were used for all experiments. A preliminary experiment showed a typical hyperbilirubinemic state was attained in 20-week-old EHBRSs, with the estimated conjugated bilirubin concentration being almost identical to that reported previously (21). In the present study, bilirubin concentrations in plasma obtained from each group were not measured, since these concentrations had already been recorded in 9- and 19-week-old EHBRSs and age-matched healthy SDRSs; the conjugated bilirubin concentration (n = 5) was >1.4 mg/dl for 9-week-old EHBRSs, >5.7 mg/dl for 19-week-old EHBRSs, and <0.3 mg/dl for 19-week-old healthy SDRSs (21).

Animal experiments. To evaluate the pharmacokinetic characteristics of CPM in EHBRSs, rats under sodium pentobarbital anesthesia were cannulated in the right jugular vein, urinary bladder, and bile duct with polyethylene tubes for drug administration and blood sampling, urine collection, and bile collection, respectively. After the surgical preparations were complete, rats were placed in plastic metabolic cages (Natsume, Tokyo, Japan). Body temperatures were maintained at 37°C with a heat lamp throughout the experiment. Under these conditions, CPM was administered intravenously at a dose of 20 mg/kg of body weight. Urine samples were collected at 30-min intervals (0 to 30, 30 to 60, 60 to 90, and 90 to

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120 min) over a period of 120 min after the administration of CPM. Bile samples were collected at 15-min intervals (0 to 15, 15 to 30, 30 to 45, 45 to 60, 60 to 75, 75 to 90, 90 to 105, and 105 to 120 min) over a period of 120 min. Blood samples (0.25 ml) were collected at designated intervals (5, 10, 20, 30, 45, 60, 90, and 120 min) after the administration of CPM. Urine and bile samples were collected in preweighed tubes, and the volume of each was measured gravimetrically by assuming a specific gravity of 1.0. Plasma, urine, and bile samples were stored at −40°C until analysis.

Drug analysis. Concentrations of CPM in plasma, urine, and bile were measured by high-pressure liquid chromatography (HPLC). Urine and bile samples were appropriately diluted with distilled water. The HPLC apparatus was an LC-6A system (Shimadzu, Kyoto, Japan) consisting of an LC-6A liquid pump, an SPD-6A variable-wavelength spectrophotometric detector, and an SIL-6A autoinjector. The UV detector was set at 279 nm and a Cosmosil 5C18 column (4.6 by 150 mm; Nacalai Tesque, Kyoto, Japan) was used with a column oven (OTC-6A) heated to 40°C. The mobile phase consisted of 0.03 M isotonic phosphate buffer (pH 5.0) and methanol (80:20 [vol/vol]), and a flow rate of 1.5 ml/min was used. Fifty microliters of each sample, 50 μl of 10% perchloric acid, and 50 μl of phosphate buffer containing the internal standard (3-butylxanthine) were well mixed and centrifuged at 11,000 × g for 10 min. The supernatant was added to 0.24 ml of 1 M sodium acetate solution and was injected into the HPLC apparatus. For calculations, standard curves for CPM were measured over a range of 0.5 to 300 μg/ml and were proved to be linear with a coefficient of correlation of 0.999. The intra- and interday coefficients of variation for the desired concentrations (5 and 50 μg/ml) were less than 5%. Recoveries of CPM from plasma, urine, and bile ranged from 96 to 102%. Blank plasma, urine, and bile samples did not interfere with any of the peaks corresponding to each compound.

Data analysis. The plasma concentration-time data for CPM in each rat were independently analyzed by noncompartmental methods. The area under the plasma concentration-time curve (AUC) was calculated by the trapezoidal rule method up to the last measured concentration in plasma and was extrapolated to infinity by adding the following: the value of the last measured concentration in plasma divided by the terminal elimination rate constant (kₚ). kₚ was calculated by determining the slope of the least-squares regression line from the terminal portion of the log concentration-time data. Systemic clearance (CLsys) was calculated as the dose divided by AUC. The renal clearance (CLR) was estimated by dividing the terminal concentration in urine by the terminal rate of urine flow. The mean residence time (MRT) was calculated as AUMC/AUC, where AUMC represents the area under the first moment curve. The steady-state volume of distribution (Vss) was determined as CLsys × MRT. Renal clearance (CLR) was calculated by dividing the total amount of CPM excreted into the urine within the collection period (120 min) by the corresponding AUC (AUC/CLR). Bile clearance (CLbile) was likewise determined by dividing the total amount of CPM excreted into the bile for 120 min by AUCbile. All computer analyses were performed by the nonlinear least-squares regression program MULTI (36), weighting the data with the reciprocal of the concentrations.

Statistical analysis. Results are expressed as means ± standard deviations for the indicated number of experiments. Statistical comparisons between the groups were assessed by one-way analysis of variance. When statistically significant differences were found, pairwise comparisons were performed by Scheffe’s multiple comparison test, with P < 0.05 being the minimum level of significance.

RESULTS

Figure 1 shows the mean semilogarithmic plasma concentration-time data for CPM in 10- and 20-week-old EHBRs and 20-week-old healthy SDRs following intravenous administration of a single dose of 20 mg/kg. The elimination was delayed in both EHBR groups in comparison with that in healthy SDRs. The corresponding pharmacokinetic parameters of CPM are summarized in Table 1. There were no significant differences among the three groups in the Vss of CPM. The CLsys of CPM in 20-week-old EHBRs decreased to approximately 70% of that in age-matched healthy SDRs, although there were no significant differences in the parameters between the two EHBR groups. The MRT significantly increased in both EHBR groups.

Figures 2 and 3 show the cumulative biliary excretion of CPM and bile flow rate, respectively, during the collection periods (120 min) after administration. The cumulative amount of CPM excreted into the bile in 20-week-old EHBRs (5.7% of the dose) was much smaller than that observed in age-matched healthy SDRs (57.8% of the dose); however, no significant differences in the percentage of biliary excretion of CPM were noted between the two EHBR groups (Fig. 2). The biliary excretion of CPM in the two EHBR groups was 5% less than that observed in healthy SDRs during the first sampling period (15 min) (Fig. 2). The bile flow rate in both EHBRs and SDRs remained nearly constant, while the rate in both EHBR groups was approximately half that in healthy SDRs (28.3 ± 1.0 μl/min) (Fig. 3). The urinary excretion of CPM in both EHBR groups was approximately twice that in healthy SDRs during the first collection period (30 min) (Fig. 4). Table 2 shows the percentage of the CPM dose recovered in the urine over 120 min. Values obtained for both EHBR groups reflected significant increases of approximately three times that for healthy SDRs.

The CLR of CPM nearly doubled in 20-week-old EHBRs, indicating a significant increase, while the parameters remained for both EHBR groups (Table 2). However, the CLbile of CPM markedly decreased in 10- and 20-week-old EHBRs (1/7 and 1/15, respectively, of that in healthy SDRs). As shown in Fig. 5, the ratio of CLbile to CLsys for CPM (CLbile/CLsys) in 10- and 20-week-old EHBRs (0.13 ± 0.06 and 0.07 ± 0.02, respectively) dropped to 1/5 and 1/9 of that in healthy SDRs (0.64 ± 0.05, respectively), but no significant differences were observed between the two EHBR groups, although the ratio tended to be lower in the older group. However, the CLR/CLsys values increased in both EHBR groups (0.81 ± 0.06 and 0.81 ± 0.09, respectively) to three times that in healthy SDRs (0.27 ± 0.09).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Vss (liter/kg)</th>
<th>CLsys (liter/h/kg)</th>
<th>MRT (h)</th>
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<tr>
<td>SDRs</td>
<td>0.323 ± 0.070</td>
<td>0.489 ± 0.059</td>
<td>0.638 ± 0.109</td>
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<td>10-wk-old EHBRs</td>
<td>0.333 ± 0.020</td>
<td>0.347 ± 0.004</td>
<td>0.923 ± 0.057</td>
</tr>
<tr>
<td>20-wk-old EHBRs</td>
<td>0.335 ± 0.027</td>
<td>0.321 ± 0.031</td>
<td>0.998 ± 0.030</td>
</tr>
</tbody>
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* Each value represents the mean ± standard deviation (n = 4).  
  b Significantly different from those for healthy SDRs (P < 0.05).
DISCUSSION

It is known that the biliary excretion of organic anions is impaired in typical hyperbilirubinemic mutant strain TR and GY rats. In particular, TR rats exhibit a deficiency in the ATP-dependent canalicular membrane transport of organic anions (14), activity which resembles that in EHBRs. Recently, EHBRs with a high concentration of conjugated bilirubin in plasma have been shown to possess a deficiency in the transport of organic anions (26, 28), suggesting the possibility that hyperbilirubinemia can modify the distribution and biliary and urinary excretions of organic anions which would otherwise be excreted in large amounts into the bile.

Anumber of articles on the canalicular membrane transport mechanism of organic anions, including β-lactam antibiotics, have been published (5, 15, 23–26, 28, 29). In vitro experiments by Tsuji and colleagues (29, 32, 33), who used rat liver bile canalicular membrane vesicles, showed that the uptake of CPM into the hepatocytes occurs by a carrier-mediated transport system and is excreted into the bile through the bile canalicular membrane via a carrier-mediated mechanism, which is the same transport system used for other β-lactam antibiotics, bile acids, and bromosulfophthalein. It has also been reported that the uptake of CPM into the membrane vesicles is strongly inhibited in a competitive manner by β-lactam antibiotics known to be mainly excreted into bile (1, 29, 35). For these reasons, CPM was selected as a model drug for studying β-lactam antibiotics because it is excreted into the bile at high concentrations (18) and possesses a relatively low affinity and high capacity for the carrier systems in bile canalicular membranes (29). To better understand the effect of the biliary excretion dysfunction in EHBRs on the pharmacokinetic behaviors of drugs, we investigated the pharmacokinetic characteristics of CPM, in particular, the biliary and urinary excretions of CPM in 10- and 20-week-old EHBRs, and compared the findings with those for 20-week-old healthy SDRs.

In the present study, the percentage of the dose of CPM excreted into the bile obtained in 20-week-old healthy SDRs (58%) was consistent with the value (60%) obtained in healthy SDRs reported previously (18). We found marked decreases in the CLbile of CPM in EHBRs; the CLbile values of CPM dropped in 10- and 20-week-old EHBRs to 1/7 and 1/15, respectively, of that in healthy SDRs, although the differences between the two EHBR groups failed to reach the 5% level of statistical significance. Age-related changes may be responsible for the biliary excretion of CPM because this biliary excretion...
tended to decrease as the conjugated bilirubin concentration increased. The present study also demonstrated significant decreases in the $CL_{\text{bile}}/CL_{\text{sys}}$ for CPM and increases in the $CL_{\text{r}}/CL_{\text{sys}}$ in EHBRs without inducing changes in the $V_{\text{ss}}$ of CPM. These findings indicate that CPM, which is excreted in large amounts into the bile, is principally excreted into the urine in EHBRs. On the other hand, nonbiliary and nonrenal clearances for CPM did not change among the three groups and contributed little to the elimination of CPM from the body (6 to 12% of $CL_{\text{sys}}$). It has been reported that CPM is almost completely recovered in the urine and bile in the unchanged form (>90%) and that the amount of its metabolite that is recovered is negligible (18). The results from the present study also indicate that the increased urinary excretion of CPM in EHBRs compensates for a reduction in the biliary excretion ability, although this compensation is not complete. It is also likely that the increases in the MRT of CPM, as observed in both EHBR groups, are responsible for the decreases in the $CL_{\text{bile}}$.

It is known that plasma protein binding is a limiting factor in drug disposition, since only the unbound drug portion is capable of being diffused across various biological membranes to be distributed in the body and is subject to metabolism and renal excretion. Bilirubin has been shown to bind strongly to albumin in plasma (2, 3, 10). More recent studies on the effect in EHBRs of hyperbilirubinemia on the pharmacokinetics of enprofylline, which has a high degree of protein binding (20, 34), have demonstrated that hyperbilirubinemia in 19-week-old EHBRs significantly increased the $V_{\text{ss}}$ for enprofylline by changing protein-binding behavior when compared with that in age-matched healthy SDRs, but the $V_{\text{ss}}$ for unbound enprofylline did not change (21). However, the present study showed that there were no significant differences in the $V_{\text{ss}}$ for CPM among the three groups, indicating the absence of the effect of hyperbilirubinemia in EHBRs on the protein-binding behavior of CPM. CPM has been reported to exhibit much weaker protein binding (46%) than enprofylline (18, 27). Moreover, there is an interesting report of bile duct-ligated rats showing that there were no significant changes in the $V_{\text{ss}}$ or the extent of protein binding of CPM (13), which is supported by the data from the present study.

The mechanism responsible for the increase in urinary excretion of CPM in both EHBR groups may be from one or more sources, as explained below, that cause an increase in glomerular filtration, an acceleration of active tubular secretion, and an inhibition of tubular reabsorption. First, the renal excretion mechanism for CPM consists of both glomerular filtration and tubular reabsorption (18). Second, no changes in the glomerular filtration rate were found between the 19-week-old EHBRs and the age-matched healthy SDRs, indicating that hyperbilirubinemia in EHBRs has no effect on the glomerular filtration rate (21). Third, the $CL_{\text{r}}$ for unbound CPM ($CL_{\text{r}}$) in both EHBR groups and the healthy SDRs were recalculated by using the value of unbound fraction ($f_u$) as reported previously (21), and the resulting values ($CL_{\text{r}} = CL_{\text{r}}/f_u$) were 0.24, 0.52, and 0.48 liters/h/kg for 20-week-old healthy SDRs and 10- and 20-week-old EHBRs, respectively. The value of $CL_{\text{r}}$ in 20-week-old healthy SDRs is comparable to the value in healthy SDRs reported previously (18). These values in the three groups were less than the value of the glomerular filtration rate (0.62 liters/h/kg) reported previously (21), indicating that CPM is partially reabsorbed in the proximal tubular cells. Earlier studies by Kimura et al. (13) with rats with obstructive jaundice assumed that the increase in the $CL_{\text{r}}$ of CPM is caused by the increase in clearance by glomerular filtration but not by any inhibition of tubular reabsorption or enhancement of active tubular secretion. This discrepancy in results could be explained by reviewing the differences in rat strains or the disease states between EHBRs and Wistar rats with obstructive jaundice. On the basis of these observations, the primary cause for the increases in the $CL_{\text{r}}$ of CPM in both EHBR groups may be from an inhibition of tubular reabsorption of CPM, most probably by conjugated bilirubin. The renal excretion of CPM may also accelerate when CPM is coadministered with other $\beta$-lactam antibiotics which are typically excreted into the bile through the canalicular membrane via a carrier-mediated mechanism. Further studies will be necessary to clarify why the tubular reabsorption of CPM is inhibited in EHBRs.

### REFERENCES