Stereoselective Renal Tubular Secretion of Carbenicillin

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The stereoselective disposition of carbenicillin epimers was studied in healthy human volunteers. There was a difference between the two epimers in the extent of plasma protein binding in vitro, with the unbound fraction of the R epimer being greater than that of the S epimer. Renal clearance (CLR) of each epimer was greater than the glomerular filtration rate, suggesting renal tubular secretion of both epimers. Although the CLR was greater for the R epimer, renal tubular secretion was greater for the S epimer. When probenecid was coadministered, the CLR of each epimer was significantly reduced and was approximately equal to the glomerular filtration rate. The difference in CLR between the two epimers was simply due to differences in plasma protein binding. The observations in the present study suggest that both carbenicillin epimers are secreted by an organic anion transport system in the renal proximal tubule in humans and that the two epimers may be distinguished in the secretion process, resulting in the differences in the secretion rates.

Carbenicillin (CBPC) has been used clinically since the late 1960s, and its pharmacokinetics have been extensively studied both in humans (8, 12, 13, 17, 19, 20) and in animals (1, 9, 16, 21). However, it has been used as a mixture of two epimers (R-CBPC and S-CBPC) because of the chirality of a (carboxyphenylacetyl)amino group attached to the 6 position of a penicillin acid. The pharmacokinetics of each CBPC epimer have yet to be clarified, since almost all the previous studies used a microbiological assay method because of the lack of a reliable and convenient stereospecific analytical method. However, it is important to understand the pharmacokinetic behavior of each epimer because antimicrobial activity is different among the epimers of β-lactam antibiotics (4, 14). For example, the R epimer of sublenicillin has been reported to be about 40 times as potent as the S epimer (14). However, the difference in activity between CBPC epimers is not fully understood because of epimerization in the antimicrobial assay procedures, which usually uses incubation times of several hours to several days (4).

A stereospecific high-performance liquid chromatographic (HPLC) method has been developed in our laboratory for the analysis of CBPC epimers in biological fluids (11); this HPLC method enabled us to conduct the present study. In the present study, the stereoselectivities of CBPC epimers in plasma protein binding and renal tubular secretion were studied in healthy human volunteers, since renal excretion is the major elimination route for CBPC (8, 17).

MATERIALS AND METHODS

Volunteer study. Four male volunteers between 23 and 26 years of age participated in the present study. The study was approved by the Ethical Review Board of the School of Pharmaceutical Sciences, Kitasato University, and all the volunteers gave written informed consent.

The weight and surface area of the volunteers were 59.7 ± 3.8 kg and 1.71 ± 0.06 m² (mean ± standard deviation [SD]), respectively. They had no evidence of disease, as determined by physical examination, urinalysis, and blood chemical tests. Control and probenecid studies were conducted with a crossover study design with a 2-week washout period.

For the control study, 2 g of CBPC (2.71 g as CBPC disodium salt; R-CBPC:S-CBPC, 1.24:1; Geopen, Pfizer Pharmaceutical Co., Tokyo, Japan) was dissolved in 15 ml of saline and was injected into a forearm vein over an approximately 4-min injection time. Blood samples were collected in heparinized disposable syringes from the vein of the contralateral forearm before CBPC injection and at 0.25, 0.5, 0.75, 1, 1.5, 2.5, 3.5, and 5 h following injection of CBPC as a bolus. Plasma was immediately obtained by centrifugation and was stored at −70°C until it was analyzed. Urine was collected before CBPC injection as well as at the following time intervals after the injection: 0 to 0.5, 0.5 to 1, 1 to 2, 2 to 3, 3 to 4, 4 to 6, 6 to 12, and 12 to 24 h. Immediately after each collection, the urine volume was recorded and the urine was diluted 50-fold with deionized water, and the diluted urine sample was stored at −70°C until it was analyzed. The urine volume in a half-hour interval was 30.1 ± 16.1 ml (mean ± SD; n = 8).

For the probenecid study, 1 g of probenecid (Probenemid tablets; Banyu Pharmaceutical Co., Tokyo, Japan) was orally administered at 12 and 1 h before CBPC injection. CBPC was administered in the same manner as in the control study. Blood samples were collected before CBPC injection and at 0.25, 0.5, 0.75, 1, 1.5, 2.5, 3.5, 5, and 9 h after the injection. Urine samples were collected at the same time intervals as for the control study. The urine volume in a half-hour interval was 69.5 ± 47.8 ml (mean ± SD; n = 8).

Urine and plasma samples were stored in the same manner as for the control study. Probenecid concentrations in plasma were determined for each volunteer at 15 min before CBPC injection and at 2.5 and 9 h after the injection.

Sample preparation for HPLC analysis. A solid-phase extraction column (SAX-Bond Elut; Analytichem International, Harbor City, Calif.) was preconditioned with 2 ml of methanol and then 2 ml of distilled water. Plasma samples were filtered through a Cosmonice filter (pore size, 0.45 μm; type W; Nihon Millipore Kogyo, Kyoto, Japan), and an aliquot (0.5 ml) was mixed with 5 ml of 0.05 M ammonium acetate (AcONH₄). The mixture was loaded onto a preconditioned SAX column and was drawn through the column...
under vacuum. The column was flushed with 3 ml of a mixture consisting of 0.5 M AcOH-CH₃CN (1:1 [vol/vol]) and then with 2 ml of a mixture of 0.1 M AcONH₄-CH₂OH (1:1 [vol/vol]), which were discarded. This procedure was necessary to eliminate interfering substances on the chromatograph used for HPLC. The sample was then eluted with 0.5 ml of a mixture of 10% LiCl-CH₂OH (3:2), and a 20-μl portion of the final eluent was injected into the chromatograph for HPLC.

Five hundred microliters of a diluted urine sample was mixed with 5 ml of 0.05 M AcONH₄, and the sample was prepared for HPLC analysis by the same procedure used for the plasma samples. The detection limit was about 5 μg/ml for each epimer, and the day-to-day variability was low, with a coefficient of variation of less than 10% (11). No epimerization was observed in the procedures described above.

**HPLC conditions for CBPC determination.** A high-performance liquid chromatograph was used to determine R-CBPC and S-CBPC concentrations in plasma and urine. The HPLC system consisted of a dual-piston pump (model LC-9A), an UV detector (model SPD-6A), and an integrator (model C-R4A), all from Shimadzu Co., Kyoto, Japan. A Cosmosil analytical column was used (4.6 mm [inner diameter] by 250 mm; 5C₁₈AR; Nacalai Tesque Co., Kyoto, Japan) with a guard column (Cosmosil; 4.6 mm [inner diameter] by 10 mm). The mobile phase was a mixture consisting of 0.05 M AcONH₄-CH₂OH (9:1 [vol/vol]) with a flow rate of 1.2 ml/min. Both R-CBPC and S-CBPC were detected at 254 nm.

**Plasma protein binding study.** For both the control and the probenecid studies, plasma was obtained from each volunteer 15 min before the CBPC injection and was used for in vitro binding studies. Binding of CBPC in human plasma in vitro was measured by an ultrafiltration method. Amicon Centrifree was used as an ultrafiltration device with a type YMT membrane (Amicon Division, W. R. Grace & Co., Beverly, Mass.). Plasma (2.5 ml) was mixed with 125 μl of various concentrations of CBPC aqueous solution, and an aliquot (0.3 ml) was prepared for HPLC as described previously to determine the total (bound and unbound) concentration of each epimer. The remainder of the sample (ca. 2.2 ml) was centrifuged at 1,000 × g for 7 min at 37°C, and a 300-μl aliquot of the filtrate was prepared for HPLC analysis in the same manner as described above for the plasma sample in order to determine the unbound concentration of each epimer. A customized Himac 15D centrifuge (Hitachi, Tokyo, Japan) was used to control the temperature during ultrafiltration. The concentration of each epimer ranged from 20 to 200 μg/ml; the CBPC used for the binding study was purchased from Sigma Chemical Co. (St. Louis, Mo.); it had an R-CBPC to S-CBPC ratio of 1.1.

**HPLC methods for probenecid determinations.** One milliliter of 0.2 M phosphate buffer (pH 2.0) was added to a 0.1-ml plasma sample together with 10 μl of 1 mg fenbufen per ml in methanol (as an internal standard). The mixture was extracted twice with 5 ml of ether, and the combined ether layer was washed with 2 ml of 0.01 M phosphate buffer (pH 6.0). The ether layer was then evaporated to dryness under vacuum at 30°C with a rotary evaporator, and the residue was dissolved with 0.3 ml of a mobile phase. The sample was filtered through a Cosmonice filter and was injected into a chromatograph for HPLC. A Nucleosil analytical column (5C₁₈; 4 mm [inner diameter] by 250 mm; Sumika Analytical Service, Osaka, Japan) was used, with the mobile phase consisting of 0.01 M phosphate buffer (pH 7.0)-acetonitrile (80:20). The flow rate was 0.9 ml/min, and probenecid was detected at 254 nm. All other equipment was the same as that for CBPC determinations.

**Creatinine determination.** Creatinine concentrations in plasma and urine were determined with a Creatinine-Test Wako kit (Wako Pure Chemicals, Osaka, Japan).

**Pharmacokinetic analysis.** Plasma protein binding data were analyzed by the Scatchard equation with a single binding site. The unbound (free) fraction of each epimer in plasma was calculated on the basis of the parameter values obtained in in vitro binding studies. Parameters were estimated separately for the plasma obtained in the control and probenecid studies. The following equation was used to calculate the unbound fraction (fₜ) at each concentration:

\[
  f_u = -\frac{1 + nKP_t - (C_tK)}{2KC_t} + \sqrt{A}
\]

where \( A = (1 + nKP_t - C_tK)^2 + 4C_tK \), \( n \) is the number of binding sites, \( K \) is the binding constant, \( P_t \) is the albumin concentration in plasma (assumed to be 0.6 mM), and \( C_t \) is the total concentration (bound plus unbound) of each epimer in plasma.

CBPC concentration-in-plasma-versus-time curves were analyzed by a nonlinear least squares method (MULTI) (23) with a weight of 1/C² for each datum point, where \( C \) is the concentration of each epimer in plasma. In the control study, profiles of drug concentration in plasma were analyzed with a one-compartment open model which showed a better fit compared with that obtained with a two-compartment open model. In the probenecid study, however, profiles of drug concentrations in plasma were analyzed with a two-compartment open model. When probenecid was coadministered, a small distribution phase was observed and the two-compartment model gave smaller values for AIC (Akaike’s information criterion [23]). Total body clearance (CL) was calculated as the dose divided by the area under the curve in both the control and probenecid studies.

The amount of each CBPC epimer excreted in urine during each collection interval was determined as the concentration of each isomer in the urine multiplied by the volume. Renal clearance (CLR) was calculated as the renal excretion rate divided by the concentration in plasma at the midpoint of each urine collection interval (7, 18).

On the other hand, CLR can generally be expressed by equation 2: \( CLR = (f_u \cdot GFR + CL_{sec})(1 - R_{abs}) \) (equation 2), where \( CL_{sec} \) is the renal clearance, GFR is the glomerular filtration rate, \( f_u \) is the unbound fraction in plasma, \( CL_{sec} \) is the clearance by renal secretion, and \( R_{abs} \) is the extent of reabsorption (7, 18). However, \( R_{abs} \) can be neglected for CBPC, since it has been reported that CBPC is not reabsorbed in the renal tubule (19). This assumption was also supported by the results of the present study, because the CLR value was almost equal to \( f_u \cdot GFR \) when renal secretion was blocked with probenecid. Therefore, intrinsic clearance by renal tubular secretion (CLRint/sec) was calculated by using equations 3 and 4: \( CL_{int/sec} = CL_{R} - f_u \cdot GFR \) (equation 3) and \( CL_{int/sec} = (Q \cdot CL_{sec})/(f_u \cdot (Q - CL_{sec})) \) (equation 4), where \( Q \) is the renal plasma flow rate (7, 18).

**Statistical analysis.** Statistical analyses were conducted with a paired or an unpaired t test.

**RESULTS**

R-CBPC and S-CBPC concentration profiles in plasma following CBPC injection with and without probenecid coad-
ministration are shown in Fig. 1A and B. In the control study, R-CBPC concentrations in plasma were slightly greater than S-CBPC concentrations in plasma until approximately 1 h after CBPC injection, which was partly because the R-CBPC content was greater in the preparation that we administered. When the content of each epimer in the preparation was measured by HPLC, the R epimer to S epimer ratio was 1.24. However, R-CBPC and S-CBPC concentrations in plasma became similar as time progressed. This was reflected in the CL, which was slightly but significantly greater for the R epimer \( (P < 0.01) \). CL values were 157 \( \pm \) 16 and 129 \( \pm \) 13 ml/min (mean \( \pm \) SD; \( n = 4 \)) for R-CBPC and S-CBPC, respectively. The volume of distribution was also slightly greater for the R epimer (11.8 \( \pm \) 0.4 and 10.3 \( \pm \) 0.5 liters for R-CBPC and S-CBPC, respectively (mean \( \pm \) SD; \( n = 4 \)), which was probably due to the difference in plasma binding, as will be discussed below.

In the probenecid study, the profiles of drug concentration in plasma were analyzed with a two-compartment open model and CL values were 107 \( \pm \) 8 and 102 \( \pm \) 8 (mean \( \pm \) SD; \( n = 4 \)) for R-CBPC and S-CBPC, respectively. The CL values for both epimers were significantly lower \( (P < 0.01) \) than those in the control study, suggesting that elimination of both CBPC epimers from the body is inhibited by probenecid.

Probenecid concentrations in the plasma of each volunteer were 116 \( \pm \) 56, 150 \( \pm \) 22, and 97 \( \pm \) 18 \( \mu \)g/ml (mean \( \pm \) SD; \( n = 4 \)) at 0.25 h before and at 2.5 and 9 h after CBPC injection, respectively. Since probenecid concentrations in plasma exceeded 90 \( \mu \)g/ml over the 9-h period, the probenecid concentrations obtained in the present study appeared to be high enough to inhibit the renal tubular secretion of penicillins (10), which resulted in the slower elimination of CBPC.

The \( f_\text{u} \) measured in plasma in vitro was plotted against the concentration of each epimer (Fig. 2A and B), and the binding parameters calculated are listed in Table 1. There was a difference in the extent of plasma protein binding between the two epimers; that is, \( f_\text{u} \) was significantly greater \( (P < 0.01) \) for R-CBPC than for S-CBPC in the plasma of

**TABLE 1. Binding parameters of R-CBPC and S-CBPC in the plasma of control and probenecid-treated subjects obtained from Scatchard plots**

<table>
<thead>
<tr>
<th>Study group</th>
<th>Epimer</th>
<th>( n^* )</th>
<th>( K (M^{-1} \times 10^3) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without probenecid</td>
<td>R-CBPC</td>
<td>0.805</td>
<td>2.25</td>
</tr>
<tr>
<td></td>
<td>S-CBPC</td>
<td>1.10</td>
<td>3.12</td>
</tr>
<tr>
<td>With probenecid</td>
<td>R-CBPC</td>
<td>0.731</td>
<td>1.92</td>
</tr>
<tr>
<td></td>
<td>S-CBPC</td>
<td>0.984</td>
<td>2.79</td>
</tr>
</tbody>
</table>

\( ^* n \) is the number of binding sites.
TABLE 2. Urinary excretion rates of R-CBPC and S-CBPC following intravenous injection of 2 g of CBPC to healthy volunteers with and without probenecid coadministration

<table>
<thead>
<tr>
<th>Study group</th>
<th>Time period (h)</th>
<th>Urinary excretion rate (mg/min)*</th>
<th>R-CBPC</th>
<th>S-CBPC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without probenecid</td>
<td>0.5–1</td>
<td>8.21 ± 0.48</td>
<td>6.39 ± 0.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–2</td>
<td>4.81 ± 0.30</td>
<td>3.93 ± 0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2–3</td>
<td>2.65 ± 0.38</td>
<td>2.24 ± 0.28</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3–4</td>
<td>1.28 ± 0.20</td>
<td>1.09 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>With probenecid</td>
<td>0.5–1</td>
<td>5.52 ± 0.42</td>
<td>4.02 ± 0.32</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1–2</td>
<td>3.41 ± 0.32</td>
<td>2.54 ± 0.26</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2–3</td>
<td>2.35 ± 0.16</td>
<td>1.80 ± 0.08</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3–4</td>
<td>1.71 ± 0.18</td>
<td>1.30 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>

* Values are means ± SDs for four subjects in each group.

both control and probenecid-treated volunteers. In the plasma of control subjects, the ratio of $f_u$ for the R epimer to that for the S epimer [$f_u(R)/f_u(S)$] was approximately 1.4. The greater $f_u$ for the R epimer may at least partly contribute to the greater volume of distribution mentioned above.

The $f_u$ of each epimer in the plasma of control and probenecid-treated subjects was also different, with the $f_u$ of each epimer in the plasma of subjects treated with probenecid being slightly greater than that in the plasma of control subjects. This was reflected in the binding constants ($K$) in Table 1, where the $K$ values of both epimers were greater in the plasma of control subjects than in the plasma of those treated with probenecid.

Urinary excretion rates of CBPC for each time interval are summarized in Table 2 for each epimer with and without probenecid coadministration. Approximately 84% of the dose was excreted in urine within 12 h after CBPC injection in the control study, while the urinary excretion of CBPC was about 74% in 12 h with probenecid coadministration. These results suggest that urinary excretion is the major elimination pathway for CBPC. As shown in Table 2, urinary excretion rates of both R and S epimers were significantly reduced when probenecid was coadministered.

The urinary excretion rate was divided by the concentration of each epimer in plasma at the midpoint of each urine collection interval, and the values obtained are listed in Table 3 as CL$_R$. In the control study, CL$_R$ for the R epimer was greater than that for the S epimer, and the ratio of CL$_R$ for the R epimer to that for the S epimer [CL$_R$(R)/CL$_R$(S)] was approximately 1.2. This was consistent with the previous results with CL, in which the CL for the R epimer was greater than that for the S epimer. Also in the control study, CL$_R$ for each epimer tended to increase with a decrease in the concentration of drug in plasma as time progressed, suggesting that a saturable mechanism is involved in the renal excretion process.

When probenecid was coadministered, CL$_R$ was significantly reduced compared with that in control subjects, indicating that both epimers are normally secreted via an organic anion transporter in the renal tubules. Although CL$_R$ for the R epimer was greater than that for the S epimer, when CL$_R$ was divided by $f_u$, the values (CL$_R$/$f_u$) were almost identical between the epimers (108 ± 13 and 107 ± 14 ml/min [mean ± SD; n = 16] for R-CBPC and S-CBPC, respectively) and were approximately equal to the GFR (5). When creatinine clearance (CL$_\text{Cr}$) was measured in each subject, the CL$_\text{Cr}$ values were 110 ± 11 and 101 ± 7 (mean ± SD; n = 4) in the control and probenecid studies, respectively.

Also, in the probenecid study, the CL$_R$ for each epimer appeared to be consistent throughout the study, regardless of the plasma CBPC concentration. These results suggest that renal tubular secretion is almost completely inhibited by probenecid and that both epimers are excreted only by glomerular filtration. Therefore, the difference in CL$_R$ was simply due to the difference in plasma protein binding. Indeed, the clearance ratio of the two epimers [CL$_R$(R)/CL$_R$(S) = 1.4] in the probenecid study was equal to the $f_u(R)/f_u(S)$ ratio.

CL$_{\text{int-sec}}$ was calculated for each time interval according to equations 3 and 4. For the calculation of CL$_{\text{int-sec}}$, CL$_\text{Cr}$ in the control study (1.84 ml/min/kg) was used as the GFR and $Q$ was assumed to be 10 ml/min/kg (3). The CL$_{\text{int-sec}}$ values thus calculated are listed in Table 4. As shown in Table 4, CL$_{\text{int-sec}}$ for the S epimer was greater than that for the R epimer, suggesting that the S epimer is secreted faster in the renal proximal tubules. The ratio of CL$_{\text{int-sec}}$ for the R epimer to that for the S epimer was approximately 0.78, which was significantly different from unity. Moreover, the CL$_{\text{int-sec}}$ values for both epimers increased with a decrease in the concentration of drug in plasma as time progressed, indicating a concentration dependency of CBPC secretion. It should be noted that in the control study, CL$_\text{int-sec}$ was greater for the R epimer (Table 3), whereas CL$_{\text{int-sec}}$ was greater for the S epimer. These results suggest differences in the stereoselectivity at different steps in the whole renal excretion processes.

TABLE 3. CL$_R$ of R-CBPC and S-CBPC following intravenous injection of 2 g of CBPC to healthy volunteers with and without probenecid coadministration

<table>
<thead>
<tr>
<th>Study group</th>
<th>Time period (h)</th>
<th>CL$_R$(R) (ml/min)</th>
<th>CL$_R$(S) (ml/min)</th>
<th>CL$_R$(R)/CL$_R$(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without probenecid</td>
<td>0.5–1</td>
<td>112 ± 10</td>
<td>88.7 ± 7.9</td>
<td>1.26 ± 0.02b</td>
</tr>
<tr>
<td></td>
<td>1–2</td>
<td>127 ± 16</td>
<td>103 ± 14</td>
<td>1.23 ± 0.04b</td>
</tr>
<tr>
<td></td>
<td>2–3</td>
<td>155 ± 13</td>
<td>126 ± 12</td>
<td>1.23 ± 0.02b</td>
</tr>
<tr>
<td></td>
<td>3–4</td>
<td>169 ± 25</td>
<td>135 ± 20</td>
<td>1.25 ± 0.04b</td>
</tr>
<tr>
<td>With probenecid</td>
<td>0.5–1</td>
<td>65.3 ± 2.8</td>
<td>49.5 ± 2.8</td>
<td>1.32 ± 0.04b</td>
</tr>
<tr>
<td></td>
<td>1–2</td>
<td>56.4 ± 7.8</td>
<td>41.2 ± 6.2</td>
<td>1.37 ± 0.02b</td>
</tr>
<tr>
<td></td>
<td>2–3</td>
<td>50.5 ± 3.8</td>
<td>41.4 ± 3.8</td>
<td>1.21 ± 0.12b</td>
</tr>
<tr>
<td></td>
<td>3–4</td>
<td>60.4 ± 7.8</td>
<td>41.6 ± 5.4</td>
<td>1.45 ± 0.14b</td>
</tr>
</tbody>
</table>

* Values are means ± SDs for four subjects in each group.

b Significantly different from unity ($P < 0.01$).

c Significantly different from the control ($P < 0.01$).

TABLE 4. CL$_{\text{int-sec}}$ of R-CBPC and S-CBPC following intravenous injection of 2 g of CBPC to healthy volunteers without probenecid coadministration

<table>
<thead>
<tr>
<th>Time period (h)</th>
<th>CL$_{\text{int-sec}}$(R) (ml/min)</th>
<th>CL$_{\text{int-sec}}$(S) (ml/min)</th>
<th>CL$<em>{\text{int-sec}}$(R)/CL$</em>{\text{int-sec}}$(S)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5–1</td>
<td>112 ± 22</td>
<td>147 ± 22</td>
<td>0.759 ± 0.055b</td>
</tr>
<tr>
<td>1–2</td>
<td>158 ± 32</td>
<td>205 ± 40</td>
<td>0.770 ± 0.040b</td>
</tr>
<tr>
<td>2–3</td>
<td>240 ± 26</td>
<td>305 ± 39</td>
<td>0.788 ± 0.020b</td>
</tr>
<tr>
<td>3–4</td>
<td>289 ± 75</td>
<td>353 ± 87</td>
<td>0.818 ± 0.049b</td>
</tr>
</tbody>
</table>

* Values are means ± SDs for four subjects.

b Significantly different from unity ($P < 0.01$).
DISCUSSION

The extent of CBPC binding in plasma was different between the two epimers, as has been reported for an oxacine antibiotic, moxalactam (22). When the binding parameters were compared, binding affinity (K) was greater for S-CBPC than for R-CBPC. On the other hand, the extent of binding in rat plasma was approximately equal between the two CBPC epimers, whereas the unbound fraction was greater for the S epimer in rabbit plasma (data not shown). Therefore, there appears to be significant species differences in the stereoselectivity of CBPC binding in plasma, which makes it difficult to predict the stereoselectivity of CBPC in humans from animal data.

In the control study, the CLR of the R epimer was greater than that of the S epimer by a factor of about 1.2, because the difference in plasma protein binding plays a dominant role in the glomerular filtration process, which, in turn, overrides the difference in the secretion process. When CLR was divided by fun, the CLint/sec values were greater than the GFR for each epimer, suggesting the renal secretion of both epimers.

CBPC may be actively secreted by an organic anion transporter in the renal proximal tubule, since coadministered probenecid has been shown to increase CBPC concentrations in human plasma (12, 19). Indeed, CBPC has been shown to be actively secreted but not reabsorbed in the renal proximal tubule in rats (2). The results of the present study with probenecid coadministration are consistent with those previous observations.

When CLint/sec was calculated, the CLint/sec values were greater for the S epimer than for the R epimer by a factor of about 1.3, suggesting that the S epimer may be secreted more rapidly than the R epimer. However, CLint/sec values depend on Q, as shown in equation 4, and the Q values may differ from one subject to another. Since the Q values have been reported to vary with a coefficient of variation of about 15% in healthy subjects (6), CLint/sec values were calculated with a 30% smaller or greater Q value (7 or 13 ml/min/kg). The CLint/sec values thus calculated were different from the corresponding values in Table 4 by less than 10%. Moreover, the CLint/sec(R)/CLint/sec(S) ratios were almost equal to those listed in Table 4, with differences of less than 2%. These observations suggest that CLint/sec and CLint/sec(R)/CLint/sec(S) values are relatively insensitive to changes in Q and that the results in Table 4 may hold true even if the Q value differs among subjects.

Although it has been reported that quinidine is secreted faster than one of its diastereomers (quinine) via an organic cation transporter in the human kidney (15), the present study is the first to reveal differences in the renal secretion rates of organic anion stereoisomers in humans. From these observations, both anion and cation transporters in the human renal proximal tubule may be stereoselective in the secretion process. However, the differences in the renal secretion rates between CBPC epimers appear to be much smaller than those between quinidine and quinine, in which the CLR of quinidine was about six times as great as that of quinine.

The present study showed a difference in the extent of plasma protein binding between CBPC epimers. There was also a difference in the secretion rate between CBPC epimers in the human renal proximal tubule, suggesting that the organic anion transport system may distinguish CBPC epimers in the secretion process. In the whole renal excretion process, the faster glomerular filtration of the R epimer (because of its greater f un in plasma) overrides the faster tubular secretion of the S epimer, resulting in a greater CLR for the R epimer.

Since stereopharmacokinetics has become an important issue in recent years in order to fully understand the pharmacokinetics and pharmacodynamics of chiral drugs, the results obtained in the present study will contribute to a better understanding of the stereoselectivity in the renal excretion process. Studies are under way to examine the stereoselectivity of CBPC in plasma protein binding and in the renal excretion process in other animal species.

REFERENCES


