Stereoselective Disposition of Sulbenicillin in Humans

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Received 19 November 1996/Returned for modification 16 June 1997/Accepted 29 October 1997

Stereoselective disposition of sulbenicillin (SBPC) epimers in healthy human volunteers was studied in order to clarify the differences in pharmacokinetic behavior between the epimers. Stereosepecific high-performance liquid chromatography was used for the determination of SBPC epimers. Plasma protein binding was measured in vitro with an ultrafiltration method. The binding was stereoselective, with the unbound fraction (fub) of the R-epimer being approximately 1.3-fold greater than that of the S-epimer. SBPC was administered intravenously to human volunteers, and concentrations of SBPC in plasma and urinary excretion rates were measured. Renal clearance (CLR) for the unbound drug (approximately 400 ml/min) was greater than the glomerular filtration rate (GFR) (approximately 109 ml/min) for both epimers, suggesting that both epimers are secreted at the renal tubules. Renal tubular secretion appeared to be greater for the S-epimer. When probenecid was coadministered, the CLR values of both epimers were significantly reduced and were approximately equal to the GFR values. CLR was greater for the S-epimer (37.5 and 49.8 ml/min for R-SBPC and S-SBPC, respectively), which was simply due to the greater fub of the S-epimer in plasma. In contrast, total body clearance was greater for the R-epimer (67.8 and 56.3 ml/min for R-SBPC and S-SBPC, respectively) because of the stereoselective degradation of the R-epimer in plasma. It was revealed that stereoselective degradation in the body had significant influence on the disposition of SBPC epimers.

It has been revealed that stereoisomers are recognized by the body as distinct chemical entities and that they exhibit different pharmacological activities, toxicities, and pharmacokinetics. For many β-lactam antibiotics, stereoisomers exist because of chirality in the side chains. Some of these epimeric β-lactams are used as mixtures of stereoisomers, although there are differences in pharmacokinetics between the epimers. For example, stereoselectivity has been reported in the plasma protein binding of moxalactam (22, 23), carbenicillin (12), and cefitabuten (20). Intestinal absorption of cephalixin (15, 21) and cefitabuten (17, 27) is also stereoselective. Moreover, it has been reported that carbenicillin (1, 8, 10), ticarcillin (15, 21) and moxalactam (22, 23), carbenicillin (1, 8, 10), ticarcillin (15, 21) and moxalactam (22, 23) are secreted at the renal tubules. Renal tubular secretion appeared to be greater for the S-epimer. When probenecid was coadministered, the CLR values of both epimers were significantly reduced and were approximately equal to the GFR values. CLR was greater for the S-epimer (37.5 and 49.8 ml/min for R-SBPC and S-SBPC, respectively), which was simply due to the greater fub of the S-epimer in plasma. In contrast, total body clearance was greater for the R-epimer (67.8 and 56.3 ml/min for R-SBPC and S-SBPC, respectively) because of the stereoselective degradation of the R-epimer in plasma. It was revealed that stereoselective degradation in the body had significant influence on the disposition of SBPC epimers.

Sulbenicillin (SBPC) is a semisynthetic β-lactam antibiotic which has a broad antibacterial spectrum and is effective against both gram-positive and gram-negative bacteria. SBPC has been used clinically as a mixture of two epimers (R-SBPC and S-SBPC) because of the chirality of a (phenylsulfoacetyl) amino group attached to the 6 position of a penicillanic acid backbone. For example, stereoselectivity has been reported in the plasma protein binding of moxalactam (22, 23), carbenicillin (12), and cefitabuten (20). Intestinal absorption of cephalixin (15, 21) and cefitabuten (17, 27) is also stereoselective. Moreover, it has been reported that carbenicillin (1, 8, 10), ticarcillin (15, 21) and moxalactam (22, 23) are secreted at the renal tubules. Renal tubular secretion appeared to be greater for the S-epimer. When probenecid was coadministered, the CLR values of both epimers were significantly reduced and were approximately equal to the GFR values. CLR was greater for the S-epimer (37.5 and 49.8 ml/min for R-SBPC and S-SBPC, respectively), which was simply due to the greater fub of the S-epimer in plasma. In contrast, total body clearance was greater for the R-epimer (67.8 and 56.3 ml/min for R-SBPC and S-SBPC, respectively) because of the stereoselective degradation of the R-epimer in plasma. It was revealed that stereoselective degradation in the body had significant influence on the disposition of SBPC epimers.

1 h, which was obtained by a microbiologic assay method (7). However, the pharmacokinetic characteristics of each epimer have not been clarified to date, since almost all of the previous studies employed a microbiologic assay method due to a lack of reliable and convenient stereospecific analytical methods (2, 7, 25).

Stereospecific high-performance liquid chromatography (HPLC) methods have been developed in our laboratory for the analysis of some epimeric β-lactam antibiotics in biological fluids (11, 12, 14). Similar analytical methods were used in this study. Since many β-lactam antibiotics are secreted at the renal tubules, the effects of probenecid on the urinary excretion of SBPC epimers were also studied.

MATERIALS AND METHODS

Volunteer study protocol. This study was approved by the Ethical Review Board of the School of Pharmaceutical Sciences, Kitasato University. Four male volunteers participated in the present study, and all of them gave written informed consent. The volunteers were between 22 and 37 years old, with body weights of 63.0 ± 9.0 kg (mean ± standard deviation [SD]; n = 4). They had no evidence of disease, as determined by physical examination, urinalysis, and blood chemical tests. The following studies (control and probenecid studies) were conducted with a crossover study design and a 2-week washout period.

For the control study, 2 g of SBPC (R-SBPC/S-SBPC = 2.78) (Lilacillin; Takeda Pharmaceutical Industries Co., Osaka, Japan) was dissolved in 15 ml of saline and injected into a forearm vein over a 4-min injection time. Blood was collected in heparinized disposable syringes from the vein of the contralateral forearm before SBPC injection and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, and 10 h following injection of SBPC. Plasma was obtained immediately by centrifugation and stored at −80°C until analyzed. Urine was collected before SBPC injection as well as at the following time intervals after the injection: 0 to 0.5, 0.5 to 1, 1 to 1.5, 1.5 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 was diluted 50-fold with deionized water. The diluted urine samples were stored at −80°C until analyzed.

For the probenecid study, 1 g of probenecid (Probenemid tablets; Banyu Pharmaceutical Co., Tokyo, Japan) was administered orally at 12 and 1 h before SBPC injection. SBPC was administered in the same manner as in the control study. Blood was collected before SBPC injection and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 3.5, 5, and 9 h after injection. Urine was collected at the same time intervals as in the control study.
in the control study. Urine and plasma samples were stored in the same manner as in the control study.

**Sample preparation for HPLC analysis.** Solid-phase extraction methods, which were modifications of previously reported methods, were used to prepare plasma and urine samples for HPLC analysis. A solid-phase extraction column (Bond Elut-SAXX, Analytichem International, Harbor City, Calif.) was used for the plasma samples obtained in the control study and for the urine samples obtained both in the control and probenecid studies. Another type of solid-phase extraction column (Bond Elut-Certify II; Analytichem International) was used for the plasma samples obtained in the probenecid study, since the interfering substances could not be removed from the probenecid-treated plasma samples with the Bond Elut-SAX column.

A bond Elut-SAX column was preconditioned with 2 ml of methanol, followed by 2 ml of distilled water. A plasma sample was washed through a Cosmonice filter (pore size, 0.45 μm) type W; Nihon Millipore Kogyo, Tokyo, Japan), and an aliquot (0.5 ml) was mixed with 5 ml of 0.05 M CH₃COONH₄. One hundred microliters of carbencillin aqueous solution (100 μg/ml) was added as an internal standard. 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 CH₃COOH-CH₃CN (1:1 [vol/vol]) and then with 2 ml of a mixture of 0.1 M CH₃COONH₄-CH₃OH (1:1 [vol/vol]), and the flushing mixtures were discarded. This procedure was necessary to eliminate interfering substances on the HPLC chromatogram. The sample was then eluted with 0.5 ml of a mixture of 10% LiCl-CH₃OH (3:2 [vol/vol]), and a 20-μl portion of the final eluent was injected into the HPLC. For the preparation of urine samples, 30 μl of a diluted urine sample was mixed with 5 ml of 0.05M CH₃COONH₄, and the sample was prepared for HPLC analysis by the same procedure as that described above for plasma samples.

A Bond Elut-Certify II column was preconditioned with 5 ml of a mixture consisting of 10% LiCl-CH₃OH (3.2 [vol/vol]), followed by 2 ml of methanol, and then 2 ml of distilled water. An aliquot (0.5 ml) of the filtered plasma sample (as described above) was mixed with 2 ml of 0.05 M CH₃COONH₄. One hundred microliters of carbencillin aqueous solution (100 μg/ml) was added as an internal standard. The mixture was loaded onto a preconditioned Certify II column and was drawn through the column under vacuum. The column was flushed with 3 ml of a mixture consisting of 0.5 M CH₃COOH-CH₃CN (1:1 [vol/vol]) and then with 2 ml of a mixture of 0.1 M CH₃COONH₄-CH₃OH (1:1 [vol/vol]), and the flushing mixtures were discarded. The sample was then eluted with 1 ml of a mixture of 10% LiCl-CH₃OH (3.2 [vol/vol]), and a 40-μl portion of the final eluent was injected into the HPLC.

**HPLC conditions for SBPC determinations.** An HPLC was used to determine the concentrations of SBPC epimers. The HPLC system consisted of a dual piston pump (model LC10A), a UV detector (model SPD-10A), and an integrator (model C-R4A), all from Shimadzu Co., Kyoto, Japan. A Cosmolsil column (5C₁₈, 4.6 by 250 mm; Nacalai Tesque Co., Kyoto, Japan) was used as an analytical column. The mobile-phase compositions were 0.05 M phosphate buffer (pH 7.0)-CH₃OH (81:19 [vol/vol]) for the plasma samples in the control study and for the urine samples both in the control and probenecid studies, and 0.05 M CH₃COONH₄-H₂O (71:29 [vol/vol]) for the plasma samples in the probenecid study. The flow rate was 0.9 ml/min, and SBPC epimers were detected at 254 nm.

**Plasma protein binding study.** For both the control and probenecid studies, plasma was obtained from each volunteer 15 min before the SBPC injection and was used for in vitro binding studies. Binding of SBPC 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.). One milliliter of the plasma sample (pH adjusted to 7.4 ± 0.1 with 1 N HCl) was mixed with 50 μl of various concentrations of SBPC (R/S = 2.78) aqueous solution, and an aliquot (0.1 ml) was prepared for HPLC, as previously described, to determine the total (bound plus unbound) concentration of each epimer. The remainder of the sample (ca. 0.95 ml) was centrifuged at 1,000 × g for 4 min at 37°C, and a 100-μl aliquot of the filtrate was prepared for HPLC analysis in a manner similar to that described above for the plasma samples in order to determine the unbound concentration of each epimer. A Bond Elut-Certify II or SAX column was used for samples with and without probenecid pretreatment, respectively. A customized Himac 15D centrifuge (Hitachi, Tokyo, Japan) was used to control the temperature during ultrafiltration.

**Pharmacokinetic analysis.** Protein binding data were analyzed according to a Langmuir equation with a single class of binding sites:

\[
q = \frac{nKc}{1 + Kc} \tag{1}
\]

where \( r \) is the number of bound drug per albumin molecule, \( n \) is the number of binding sites, \( K \) is the binding constant, and \( C \) is the total plasma concentration (bound plus unbound) of each epimer in plasma. Equation 2 was derived by solving the equation for \( f_r \), which was reported by Martin (16), as a function of binding.

SBPC concentration in plasma versus time curves were analyzed by a nonlinear least-squares method (MULTI) (26) with a weight of 1/c² for each datum point, where \( c \) is the concentration in plasma. Total body clearance (CL) was calculated as the dose divided by the area under the curve.

The amount of each epimer excreted in urine during each collection interval was determined as the concentration of each epimer in the urine multiplied by the urine volume. Renal clearance (CLR) was calculated as the renal excretion rate divided by the concentration of plasma at the midpoint of each urine collection interval. Moreover, renal clearance for unbound drug (CLR(abs)) was calculated as CL abs/FC.

CLR can generally be expressed by the following equation:

\[
CL_{r} = \left( f_r \cdot GFR \cdot \frac{Q_{f}}{Q_{u}} \cdot \frac{K_{C_{f}}}{K_{C_{u}}} \right) \cdot (1 - R_{e}) \tag{4}
\]

where \( CL_{r} \) is the renal clearance, \( GFR \) is the glomerular filtration rate, \( f_r \) is the unbound fraction in plasma, \( Q_u \) is the renal plasma flow rate, \( CL_{int,sec} \) is the intrinsic clearance by renal tubular secretion, and \( R_{e} \) is the extent of reabsorption (6, 19). However, reabsorption was negligible (\( R_{e} = 0 \)) for both epimers, because the \( CL_{int,sec} \) values were almost equal to the GFR when renal secretion was blocked with probenecid. Therefore, intrinsic clearance by renal tubular secretion (\( CL_{int,sec} \)) was calculated with equation 4, which is obtained by solving equation 5 for \( CL_{int,sec} \). The same equation has been used for the analysis of carbencillin in humans (12):
respectively, over the range of 10 to 200 μg/ml. Intraday variabilities were 2.6 to 7.3% and 2.4 to 5.1% for R-SBPC and S-SBPC, respectively, over the range of 50 to 200 μg/ml.

The $f_u$ values were calculated for each subject, and the data were analyzed according to a Langmuir equation with a single class of binding sites since the Scatchard plots for both epimers were linear. As shown in Fig. 2, there was a difference in the extent of binding between the two epimers; that is, $f_u$ was significantly greater for S-SBPC than for R-SBPC in the plasma of both control and probenecid-treated volunteers ($P < 0.05$ [paired]). The $f_u$ of each epimer in the plasma of the probenecid-treated subjects was slightly less than that in the plasma of the control subjects. The binding parameters were calculated according to equation 1, and the values are listed in Table 1. The parameter values obtained for each subject were used to calculate $f_u$ of SBPC at a given concentration with equation 2.

R-SBPC and S-SBPC concentration profiles in plasma following SBPC injection with and without probenecid coadministration are shown in Fig. 3. In the control study, drug concentration profiles in plasma were analyzed with a one-compartment open model, which showed a better fit than that obtained with a two-compartment open model. In the probenecid study, drug concentration profiles in plasma were analyzed with a two-compartment open model. When probenecid was coadministered, a small distribution phase was observed and the two-compartment open model gave smaller values of AIC (Akaike's information criterion) (26).

In the control study, R-SBPC concentrations in plasma were approximately threefold greater than S-SBPC concentrations at all time points. The threefold difference between R-SBPC and S-SBPC concentrations reflected the R/S ratio in the administered preparations. The volumes of distribution ($V$) for R-SBPC and S-SBPC were 10.3 ± 0.5 and 13.1 ± 0.6 liters, respectively (mean ± SD; $n = 4$), with the $V$ of S-SBPC being slightly but significantly greater than that of R-SBPC ($P < 0.05$ [paired]). The difference in $V$ between the epimers reflected that in the $f_u$, as mentioned above. The CL of S-SBPC was significantly greater than that of R-SBPC (Table 2).

In the probenecid study, R-SBPC concentrations were approximately threefold greater than those of S-SBPC shortly after the injection. However, the concentration of R-SBPC in plasma became closer to that of S-SBPC, and the concentrations of R-SBPC and S-SBPC were almost equal at 9 h after the injection. This was reflected in the greater CL value of R-SBPC (Table 2). On the other hand, the CL values were significantly smaller than those in the control study, suggesting that elimination of both epimers from the body is inhibited by probenecid (Table 2). Urinary excretion profiles of SBPC epimers are shown in Fig. 4A and B for the control and probenecid studies, respectively. In the control study, all of the administered S-epimer was recovered in the urine within 24 h, whereas the urinary recovery of the R-epimer was ca. 80% in 24 h. The results suggested that urinary excretion is the major elimination pathway for both epimers.

In the probenecid study, more than 90% of the S-epimer was excreted in the urine within 24 h, while only 60% of the R-epimer was excreted in the urine during the same time period. When probenecid was coadministered, urinary excretion rates of both epimers were smaller than those in the control study. On the other hand, there were significant differences ($P < 0.05$ [paired]) in the urinary recovery of SBPC epimers both in the control and probenecid studies, and the difference was greater in the probenecid study.

The differences in urinary excretion between the epimers suggested a stereoselective elimination from the body, which was more marked when probenecid was coadministered.

![FIG. 2. Unbound fractions of R-SBPC (○) and S-SBPC (●) in plasma of control (A) and probenecid-treated (B) subjects. Binding was measured at nine different concentrations for each subject.](http://aac.asm.org/)

**TABLE 1. Binding parameters of R-SBPC and S-SBPC in plasma of control and probenecid-treated subjects**

<table>
<thead>
<tr>
<th>Study group</th>
<th>Epimer</th>
<th>$n^a$</th>
<th>$10^{-5} K$ (M$^{-1}$)$b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without probenecid</td>
<td>R-SBPC</td>
<td>1.29 ± 0.11</td>
<td>3.89 ± 0.43</td>
</tr>
<tr>
<td></td>
<td>S-SBPC</td>
<td>0.41 ± 0.07</td>
<td>8.58 ± 0.27</td>
</tr>
<tr>
<td>With probenecid</td>
<td>R-SBPC</td>
<td>1.11 ± 0.18</td>
<td>3.52 ± 0.53</td>
</tr>
<tr>
<td></td>
<td>S-SBPC</td>
<td>0.39 ± 0.09</td>
<td>5.09 ± 1.57</td>
</tr>
</tbody>
</table>

$a$ Number of binding sites. Values are means ± SDs for four subjects.

$b$ Binding constant.
The CLₚ values are listed in Table 2. In the control study, the CLₚ of S-SBPC was greater than that of R-SBPC, which was consistent with the greater CL of S-SBPC. Also, the CLₚ was slightly but significantly greater for S-SBPC in the probenecid study. This observation, however, disagreed with the fact that the CL of S-SBPC was smaller than that of R-SBPC in the probenecid study. The greater CL of R-SBPC was due to the greater nonrenal clearance (Table 2; also see Discussion).

The CLₚ was further divided by the f₁, which was calculated with equation 2, and the values obtained are shown as CLₚ,f₁ in Fig. 5. The CLₚ,f₁ values for both epimers were much greater than the GFR (109 ± 19 ml/min [mean ± SD; n = 4]), suggesting that both epimers are secreted at the renal tubules. When probenecid was coadministered, the CLₚ,f₁ values of both epimers were almost equal to the GFR values. The results suggested that secretion of both epimers is almost completely blocked by probenecid and that reabsorption is negligible for both epimers. Intrinsic clearance for renal tubular secretion (CLₚ,sec) was calculated for each time interval according to equation 4. For the calculation of CLₚ,sec, the renal plasma flow (Qₚ) was assumed to be 10 ml/min/kg of body weight (4). The CLₚ,sec values obtained were 378 ± 46 ml/min (mean ± SD; n = 4) for R-SBPC and S-SBPC, respectively. The CLₚ,sec values of S-SBPC were significantly greater than those of R-SBPC (P < 0.05 [paired]). Although equation 4 is widely accepted (6, 19), it may be more accurate to use Qₚ – GFR instead of Qₚ to calculate CLₚ,sec, since the plasma flow rate at the proximal renal tubules is represented more accurately by Qₚ – GFR. However, when the Qₚ – GFR value was used for the calculation of CLₚ,sec, the obtained CLₚ,sec values were only 3 to 7% greater than the values mentioned above.

SBPC was incubated with human plasma at 37°C, and the concentrations of the epimers were measured at appropriate times. The results are shown in Fig. 6. R-SBPC disappeared at a much higher rate than S-SBPC, indicating stereoselective degradation of R-SBPC. The degradation of R-SBPC appeared to be a first-order process.

**DISCUSSION**

Binding of SBPC epimers in human plasma was stereoselective, as has been reported for other epimeric β-lactam antibiotics such as moxalactam (22) and carbenicillin (12). However, the stereoselectivity in the binding of SBPC was opposite to those of moxalactam and carbenicillin; the f₁ of the R-epimer is greater for moxalactam and carbenicillin. Since the concentrations of R-SBPC are approximately threefold greater than those of S-SBPC in the present binding studies, there is a possibility that S-SBPC may be displaced by R-SBPC from the binding sites, which may result in the greater f₁ of S-SBPC. However, when the binding was measured with isolated SBPC

**TABLE 2. CL, CLₚ, and CLₚ,sec of R-SBPC and S-SBPC following intravenous injection of 2 g of SBPC to healthy volunteers with and without probenecid coadministration**

<table>
<thead>
<tr>
<th>Study group</th>
<th>CL (ml/min/163 kg)</th>
<th>CLₚ (ml/min/163 kg)</th>
<th>CLₚ,sec (ml/min/163 kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R-SBPC</td>
<td>S-SBPC</td>
<td>R-SBPC</td>
</tr>
<tr>
<td>Without probenecid</td>
<td>129 ± 11</td>
<td>157 ± 9</td>
<td>112 ± 11</td>
</tr>
<tr>
<td>With probenecid</td>
<td>67.8 ± 7.0</td>
<td>56.3 ± 6.2</td>
<td>37.5 ± 7.6</td>
</tr>
<tr>
<td></td>
<td>18 ± 7</td>
<td></td>
<td>30.2 ± 8.3</td>
</tr>
</tbody>
</table>

* Each value is the mean ± SD for four subjects in each group.

* Calculated as the mean of four urine collection intervals (0.5 to 1, 1 to 2, 2 to 3, and 3 to 4 h).

* Calculated as CL minus CLₚ.

* Significantly greater than R-SBPC (P < 0.01) according to a paired t test.

* This value was not calculated, since the CLₚ value was slightly greater than the CL value.

* Significantly different from the control (P < 0.01) according to a paired t test.
epimers in our preliminary study, the $f_u$ values were similar to those observed in the present study (data not shown). The results suggested that the greater $f_u$ of $S$-SBPC is not because of the displacement by $R$-SBPC with threefold-greater concentrations and that the observed stereoselectivity may indeed reflect the intrinsic stereoselectivity in the binding of SBPC epimers.

The parameter values listed in Table 1 may be apparent values, because the binding of each epimer was measured in the presence of the other epimer. According to our study, serum albumin is the predominant binding component for the binding of SBPC in plasma (data not shown), and only a small portion (less than 20%) of the binding sites are occupied by SBPC epimers at therapeutic concentrations. Under these conditions, mutual displacement between the epimers is not significant (13). Indeed, the $f_u$ values were successfully calculated at therapeutic concentrations with the present parameter values.

The $f_u$ values in the probenecid study may be overestimated because the protein binding was measured with plasma samples shortly before SBPC administration. The probenecid concentration in plasma should decrease following SBPC administration, which may result in reduced effects on the binding of SBPC. However, considering the relatively long half-life of probenecid (6 to 12 h in humans), the influence of probenecid on the binding of SBPC may not change drastically during the course of the administration study.

On the other hand, potential misestimation of $f_u$ values may influence the calculation of $CL_{int,sec}$ but not the calculation of the $CL$ and $CL_{R}$ values. The $CL$ values were calculated from the plasma concentration profiles, and the $CL_{R}$ values were calculated from the plasma concentration and urinary excretion data. Therefore, the difference between the $CL$ and $CL_{R}$ in the probenecid study truly resulted from the degradation of $R$-SBPC (see below), not from the misestimation of $f_u$ values.

Since the $CL_{R,u}$ values of both SBPC epimers were much
greater than the GFR values in the control study (Fig. 5), it was confirmed that SBPC epimers are actively secreted in the renal tubules. This was further supported by the observations in the probenecid study, in which the CL\textsubscript{R} values were similar to the GFR values. The results suggest that the secretion of SBPC epimers by the organic anion transport system is almost completely blocked by probenecid and that reabsorption of the epimers is negligible. These observations are consistent with previous results on the disposition of carbenicillin, which is also a dianionic \( \beta \)-lactam. In humans, carbenicillin was secreted but not reabsorbed from the renal tubules, and the secretion was almost completely blocked by probenecid under the same conditions (12).

Recovery of \( S \)-SBPC in the 24-h urine appeared to be slightly greater than 100% in the control study (Fig. 4A), which may be partly due to the epimerization of R-SBPC in the body. In our preliminary study, approximately 2.5% of R-SBPC was converted to S-SBPC in 10 h in the presence of 4% human serum albumin (HSA) at 37°C (data not shown). Since R-SBPC concentrations in plasma were approximately threefold greater than those of S-SBPC following intravenous injection, the 2.5% conversion of R-SBPC corresponds to 7.5% of S-SBPC being generated in the body.

In the control study, the nonrenal clearance (CL\textsubscript{NR}) of S-SBPC (CL\textsubscript{NR}(S)) was negligible since the CL of S-SBPC was almost equal to the CL\textsubscript{R} of S-SBPC, whereas the CL\textsubscript{NR} of R-SBPC (CL\textsubscript{NR}(R)) was 18 ml/min (Table 2). In the probenecid study, the CL\textsubscript{NR}(S) was 6.5 ml/min. Although this value was not significantly different from zero \((P > 0.05 \text{ [Student's } t \text{ test]})\), the increased CL\textsubscript{NR}(S) may be because of the greater contribution of other elimination pathways (e.g., bile excretion, etc.) in the disposition of SBPC, which resulted from the increase in \( f_a \). On the other hand, the CL\textsubscript{NR}(R) in the probenecid study was 30.2 ml/min (Table 2). Assuming that the contribution of other elimination pathways to CL\textsubscript{NR}(R) is also 6.5 ml/min, 23.7 ml/min of CL\textsubscript{NR}(R) is still unaccounted for in the probenecid study. This value is similar to the CL\textsubscript{NR}(R) in the control study (18 ml/min). Therefore, it is suggested that approximately 20 ml/min of stereoselective elimination clearance for R-SBPC exists both in the control and probenecid studies.

When SBPC was incubated in human plasma, it was observed that the \( R \)-epimer degraded stereoselectively (Fig. 6). The degradation was facilitated by HSA and was dependent on the HSA concentration (data not shown). The apparent first-order rate constant for the degradation of R-SBPC was 0.178 h\(^{-1}\), which was almost equal to that observed in 4% HSA solution (pH 7.4; 37°C). The extent of epimerization of R-SBPC was much lower than the extent of degradation; less than 3% of the disappeared \( R \)-epimer was converted to the \( S \)-epimer in the presence of 4% HSA in our preliminary study (data not shown).

In order to estimate the influence of the stereoselective degradation on the disposition of R-SBPC, the following assumptions were made: (i) SBPC epimers are distributed only in plasma and interstitial fluids, and (ii) the first-order degradation rate constant of R-SBPC is proportional to the HSA concentration. Since it has been reported that most \( \beta \)-lactams are distributed only in plasma and interstitial fluids (22), the first assumption may hold true for SBPC. The second assumption is also likely to hold true because the degradation of R-SBPC was dependent on HSA concentration in our preliminary study.

With these assumptions, the clearance for the degradation of R-SBPC \([\text{CL}_{\text{deg}}(R)]\) was calculated by the following equation:

\[
\text{CL}_{\text{deg}}(R) = K_{\text{deg}}(R) \cdot V_p + \sum_i \left( K_{\text{deg}}(R) \cdot V_{ip} \cdot AR \right)
\]

where \( K_{\text{deg}}(R) \) is the first-order degradation rate constant in 4% HSA solution, \( V_p \) is the plasma volume in the body, \( V_{ip} \) is the volume of the interstitial fluid of the \( i \)th tissue sample, and AR is the reported ratio of the albumin concentration in the interstitial fluid to that in plasma in the \( i \)th tissue sample (19). The \( V_p \) value was calculated as 63 kg · (1/13) · 0.55 = 2.67 liters, where the ratio of the plasma volume to the whole blood volume was assumed to be 0.55. Clearance for the degradation of R-SBPC calculated for each tissue type is listed in Table 3, together with other physiological parameters. Calculation was conducted for a 63-kg human, since the average weight of the volunteers was 63 kg. The CL\textsubscript{deg}(R) value calculated according to equation 5 was 23.0 ml/min (Table 3), which was similar to the stereoselective elimination clearance for R-SBPC (approximately 20 ml/min), as mentioned above. Therefore, it is very likely that at least approximately 20 ml/min of CL\textsubscript{NR}(R) results from the degradation of R-SBPC in the body.

**TABLE 3. Physiological parameters and degradation clearance values of R-SBPC in various tissues of a 63-kg human**

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>( V_p ) or ( V_{ip} ) (ml)</th>
<th>AR</th>
<th>( K_{\text{deg}}(R) \cdot V_p ) (or ( V_{ip} ), AR (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pool</td>
<td>2.670(^b)</td>
<td>1.0</td>
<td>7.9</td>
</tr>
<tr>
<td>Lung</td>
<td>108</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Heart</td>
<td>36</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Muscle</td>
<td>3,240</td>
<td>0.6</td>
<td>5.8</td>
</tr>
<tr>
<td>Skin</td>
<td>810</td>
<td>1.0</td>
<td>2.4</td>
</tr>
<tr>
<td>Bone</td>
<td>864</td>
<td>1.0</td>
<td>2.6</td>
</tr>
<tr>
<td>Gut</td>
<td>90</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>Liver</td>
<td>90</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>41</td>
<td>0.5</td>
<td>0.1</td>
</tr>
<tr>
<td>Carcass</td>
<td>2,421</td>
<td>0.5</td>
<td>3.6</td>
</tr>
<tr>
<td>Total [CL\textsubscript{deg}(R)](^c)</td>
<td></td>
<td></td>
<td>23.0</td>
</tr>
</tbody>
</table>

\(^a\) Physiological parameters were obtained from reference 18, with the \( V_{ip} \) adjusted for a 63-kg human.

\(^b\) \( V_p \).

\(^c\) CL\textsubscript{deg}(R) calculated with equation 5 (see text).
Steroselective degradation of R-SBPC may also account for the reduced urinary excretion of R-SBPC. The ratios of $\text{CL}_{\text{deg}}(R)/[\text{CL}_{\text{deg}}(R) + \text{CL}_{\text{deg}}(S)]$ were 0.83 and 0.62 for the control and probenecid studies, respectively, similar to the observed urinary recoveries of approximately 0.87 and 0.66, respectively.

The present study revealed that the $R$-epimer of SBPC degrades stereoselectively in plasma, which plays a significant role in the stereoselective disposition of SBPC epimers. Since many chiral drugs are currently used as mixtures of stereoisomers with little information on stereoselective behavior in the body, it is important to clarify the differences in pharmacokinetics and pharmacodynamics between stereoisomers. The results obtained in the present study should provide valuable information for understanding the stereoselective dispositions of chiral drugs.

ACKNOWLEDGMENT

Part of this study was financially supported by the Japan Research Foundation for Clinical Pharmacology.

REFERENCES