Disposition of Enantiomers of Sultopride in a Human, Rats and Rabbits

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Pharmacokinetics of sultopride enantiomers was examined following a single dose in a human, rabbits and rats. Pharmacokinetic profiles were similar between (+)- and (-)-enantiomers of sultopride in human, whereas the serum concentrations of (-)-sultopride were slightly higher than those of (+)-sultopride after i.v. administration of 50 mg/kg of racemic sultopride to rats and rabbits.

Keywords sultopride; enantiomer; stereoselective disposition; human; rat; rabbit

Most drugs containing chiral center(s) are marketed as racemic mixtures, primarily for economic reasons. The dramatic progress in analytical technology in recent years has focused attention on the implications of stereoselectivity and stereochemistry of drugs in relation to pharmacokinetics and pharmacodynamics.1–5

Sultopride (Fig. 1) is structurally classified as a substituted benzamide, which selectively inhibits D2 dopaminergic receptors and is distinct from typical neuroleptics such as phenothiazines and butyrophenones.6–8

Sultopride has one chiral center at its pyrrolidine ring. Although clinically used as a racemate, binding studies of 3H-labeled sultopride in rats indicated that (-)-sultopride is 70 times more active than (+)-sultopride.7

Recently, a considerable number of chiral stationary phases (CSPs) have been commercially available which separate enantiomers of drugs employing high performance liquid chromatography (HPLC). The chromatographic techniques are developed to isolate the enantiomers and to quantitatively analyze them in biological fluids.9–12

We investigated the pharmacokinetic behavior of sultopride enantiomers after administration of racemic sultopride in a human, rabbits and rats.

Materials and Methods

Reagents and Materials Sultopride hydrochloride as a racemate was generously supplied by Mitsubishi Pharmaceuticals (Tokyo, Japan). All reagents were of analytical reagent grade and purchased from Wako Pure Chemical Industries (Osaka, Japan).

Human Study A healthy male volunteer (body weight 57 kg; age 35 years) participated in the study after giving his informed consent. The subject received an oral dose of 200 mg sultopride (1 tablet of Barneitil®) at 10:00 a.m., after an overnight fast. Blood samples were collected at predetermined time intervals.

Animal Studies Male Wistar rats (body weight 250–320 g) and male New Zealand white rabbits (body weight 2–3 kg) were anesthetized with pentobarbital. Their jugular veins and urinary bladders were cannulated with polyethylene tubing. Sultopride hydrochloride was dissolved in saline at a concentration of 25 mg/ml as a racemate. The solution (50 mg/kg) was injected into the femoral vein in an anesthetized state. No additional pentobarbital was administered in this study. Blood samples were collected through another jugular cannula at 0, 0.5, 1, 2, 3, 4, 6 and 8 h after administration. Blood samples were centrifuged and the serum portion was separated. All samples were immediately frozen at –20°C until analysis.

Assay Sultopride was extracted from serum and urine according to the previous report.13 To the 1.0 and 0.2 ml portion of serum or urine in humans and rats, respectively, in a 10 ml glass test-tube, 0.3 ml of 0.5 n sodium hydroxide solution saturated with sodium chloride and 3 ml of chloroform were added. The mixture was agitated for 10 min by a reciprocal shaker and centrifuged for 5 min at 3000 x g. Two ml of organic phase was then transferred into another test tube and evaporated to dryness using a rotary vacuum evaporator. The residue was dissolved in 50 μl chloroform and 25 μl aliquots of the resulting solution were injected into a HPLC system.

The chromatographic system consisted of a LC-6A pump (Shimadzu, Kyoto, Japan) and a variable-wavelength UV detector (Shimadzu) set at 250 nm. For quantitation of the sultopride enantiomers in serum and urine, a Chiralcel OD column, 250 × 4.6 mm i.d. (Daicel Chemical Ind., Tokyo, Japan) was used for the chiral separation. A typical chromatogram is shown in Fig. 2. The mobile phase (hexane: isopropanol = 8:2 containing 0.1% diethylamine) was pumped at a flow rate of 0.5 ml/min. (+)- and (-)-sultopride were identified by measurement of optical rotation employing a sodium lamp at a wavelength of 589 nm at room temperature (DIP-360, Jasco, Tokyo). The resolution factor of sultopride enantiomers was found to be 1.27 under this condition. Linear calibration curves for (+)- and (-)-sultopride were obtained at the serum concentration range of 0.1–1.0 μg/ml with correlation coefficients of >0.999 and were generated in each set of studies.

Pharmacokinetic Analysis Although sultopride was administered orally, the Cmax values were achieved before the first sampling point in human study. Thus, half-lives of absorption phase could not be calculated in this study. The serum concentration–time profiles were fitted to a two compartment open model with rapid intravenous injection using a nonlinear regression analysis technique. An area under the serum drug con-

Fig. 1. Chemical Structure of Sultopride

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centration–time curve from 0 to 8 h \( [AUC_{0-8}] \) was calculated by the trapezoidal rule. The area to infinity \( (AUC) \) was calculated by adding the \( AUC_{0-8} \) to the area obtained by dividing the concentration at 8 h by the terminal elimination rate constant. The elimination half-life was estimated by least-squares regression analysis of the terminal concentration–time curve. Renal clearance \( (CL_u) \) of sultopride was calculated from the following equation:

\[
CL_u = \frac{U_{t_1-t_2}}{AUC_{t_1-t_2}}
\]

where \( U_{t_1-t_2} \) is the amount of sultopride excreted in urine over the period from time \( t_1 \) to \( t_2 \) after administration, and \( AUC_{t_1-t_2} \) is the corresponding area under the serum sultopride concentration curve over the same interval.

The Student's \( t \) test was used for statistical analysis, taking \( p \) of 0.05 as the level of significance.

**Results**

Sultopride enantiomers in serum and urine were completely separated under the HPLC condition employed in this study (Fig. 2). Optical rotation was measured after fractions and concentration of elute from HPLC drain on the basis of retention times of both enantiomers. Serum concentration–time profiles of sultopride enantiomers after oral administration of 200 mg racemic sultopride in human are shown in Fig. 3 and their pharmacokinetic parameters are shown in Table I. Concentration–time profiles and pharmacokinetic parameters, i.e., \( AUC \), mean residence times \( (MRT) \), \( t_{1/2} \), \( V_c \) and \( CL_{tot} \) were similar between the two enantiomers.

However, as shown in Figs. 4 and 5, slightly higher \((-\)-sultopride concentrations were observed in serum and urine following intravenous administration of racemic sultopride in rats. The mean \( AUC \) values of \((-\)-sultopride were approximately 1.2-fold greater than those of \((+\)-sultopride (Table II). The mean cumulative amount of \((-\)-enantiomer excreted in urine was 1.3-fold greater than that of \((+\)-enantiomer (Fig. 5). No pharmacokinetic parameter was significantly different \( (p>0.05) \). Similar results were observed in rabbits (Figs. 6 and 7 and Table II).

**Discussion**

Jenner et al.\(^{14}\) reported that apomorphine-induced

\[
\text{Fig. 4. Serum Concentration–Time Curves of \((+\)-}\text{ and \((-\)-Sultopride (\(\triangle\)) in Rats after Intravenous Administration of 50 mg/kg (\(\pm\)-Sultopride (Mean ± S.E.M., \(n = 5\))}
\]

\[
\text{Fig. 5. Cumulative Amounts of Unchanged \((+\)-\text{ and \((-\)-Sultopride (\(\triangle\)) Excreted in Urine of Rats after Intravenous Administration of 50 mg/kg (\(\pm\)-Sultopride (Mean ± S.E.M., \(n = 5\))}
\]

\[
\text{Fig. 6. Serum Concentration–Time Curves of \((+\)-\text{ and \((-\)-Sultopride (\(\triangle\)) in Rabbits after Intravenous Administration of 50 mg/kg (\(\pm\)-Sultopride (Mean ± S.E.M., \(n = 4\))}
\]

\[
<table>
<thead>
<tr>
<th>Parameter</th>
<th>((+)-Sultopride</th>
<th>((-)-Sultopride</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{121}), h(^{-1})</td>
<td>0.39</td>
<td>0.42</td>
</tr>
<tr>
<td>(k_{21}), h(^{-1})</td>
<td>0.28</td>
<td>0.28</td>
</tr>
<tr>
<td>(t_{1/2}), h</td>
<td>8.19</td>
<td>8.0</td>
</tr>
<tr>
<td>(V_c/F), l/kg</td>
<td>1.35</td>
<td>1.3</td>
</tr>
<tr>
<td>(AUC_{0-8}), ((\mu)g/ml)·h</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>(AUC), ((\mu)g/ml)·h</td>
<td>6.0</td>
<td>5.9</td>
</tr>
<tr>
<td>(CL_{tot}/F), ml/min</td>
<td>278</td>
<td>282</td>
</tr>
</tbody>
</table>

\(a) V_c\) volume of distribution at central compartment; \(F\), fraction absorbed.
TABLE II. Pharmacokinetic Parameters of (+) - and (-)-Sultopride after Intravenous Administration of 50 mg/kg (+)-Sultopride to Rats (n = 5) and Rabbits (n = 4) (Mean ± S.E.M.)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(+)-Sultopride</th>
<th>(-)-Sultopride</th>
<th>(+)-Sultopride</th>
<th>(-)-Sultopride</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{12}$, h$^{-1}$</td>
<td>0.59 ± 0.12</td>
<td>0.62 ± 0.21</td>
<td>0.52 ± 0.22</td>
<td>0.59 ± 0.12</td>
</tr>
<tr>
<td>$k_{21}$, h$^{-1}$</td>
<td>0.53 ± 0.12</td>
<td>0.59 ± 0.14</td>
<td>0.55 ± 0.055</td>
<td>0.63 ± 0.63</td>
</tr>
<tr>
<td>$t_{1/2}$, h</td>
<td>2.8 ± 0.99</td>
<td>2.4 ± 0.62</td>
<td>3.1 ± 0.15</td>
<td>3.3 ± 0.26</td>
</tr>
<tr>
<td>$P_{r}$, ml/kg</td>
<td>2.2 ± 0.49</td>
<td>2.0 ± 0.43</td>
<td>1.7 ± 0.13</td>
<td>1.6 ± 0.17</td>
</tr>
<tr>
<td>$AUC_{10-80}$, μg/ml-h</td>
<td>9.0 ± 1.3</td>
<td>10 ± 1.5</td>
<td>23 ± 2.8</td>
<td>26 ± 2.4</td>
</tr>
<tr>
<td>$AUC$, μg/ml-h</td>
<td>9.3 ± 1.3</td>
<td>11 ± 1.4</td>
<td>26 ± 3.4</td>
<td>29 ± 3.2</td>
</tr>
<tr>
<td>$CL_{air}$, ml/min/kg</td>
<td>45 ± 7.0</td>
<td>38 ± 5.9</td>
<td>16 ± 3.4</td>
<td>14 ± 3.6</td>
</tr>
<tr>
<td>$CL_{air}$, ml/min/kg</td>
<td>3.2 ± 0.34</td>
<td>3.3 ± 0.34</td>
<td>7.7 ± 1.9</td>
<td>7.5 ± 1.9</td>
</tr>
</tbody>
</table>

a) $V_c$, volume of distribution at central compartment.

Fig. 7. Cumulative Amounts of Unchanged (+)- (○) and (-)-Sultopride (△) Excreted in Urine of Rabbits after Intravenous Administration of 50 mg/kg (+)-Sultopride (Mean ± S.E.M., n = 4)

Locomotor activity in reserpine-pretreated mice and apomorphine- and amphetamine-induced stereotyped behavior in rats were antagonized by (-)-sultopride but not by the (+)-enantiomer. Mizuchi et al.7 also reported that (-)-sultopride is the neuroleptically active form of sultopride.

In the present study, we examined disposition of sultopride enantiomers in a human, rat, and rabbits. Bressolle and Bres15 demonstrated the pharmacokinetic profile of racemic sultopride after oral and intravenous administration of racemate to man, which was in good agreement with our results expressed as a racemate. In the human, stereoselectivity was not observed, whereas serum concentrations and urinary excretion rates of (-)-enantiomer were slightly higher than those of (+)-enantiomer in rats and rabbits.

The extent of protein binding of sultopride in man, rabbit and rat has been reported to be approximately 10%,16 but no study has been made of protein binding of sultopride enantiomers. Species differences in renal and non-renal clearances of the two enantiomers were not significant in rats and rabbits. Although sultopride is excreted almost entirely unchanged in urine in man, it is metabolized by oxidation at the alpha position of the pyrrolidine ring, N-deethylation, O-demethylation and hydrolysis of the amide bond in rats and rabbits.16 Therefore, species difference in stereoselectivity is considered related to the species difference in sultopride metabolism.

References