Effect of First-Pass Metabolism on Enantioselective Pharmacokinetics after Oral Administration of (+)-, (−)- and Racemic Homochlorcyclizine to Rats

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The enantioselective relationship between the pharmacokinetics and hepatic metabolism of homochlorcyclizine hydrochloride (HCZ) was investigated using rats. There were no significant differences in blood concentrations between the three forms after intravenous administration (5 mg/kg) of (+)-, (−)- and racemic HCZ. On the other hand, there were significant differences in the pharmacokinetics between (−)- and (+)-HCZ and between (−)- and racemic HCZ after oral administration (50 mg/kg) of these three forms. The Cmax and AUC0−∞ of (−)-HCZ were lower than those of (+)-isomer and racemate. The (−)-isomer and racemate showed no significant differences in their pharmacokinetic parameters. At a lower dose (10 mg/kg), however, no enantiomeric differences were found in the pharmacokinetic parameters of (+)- and (−)-HCZ. Also examined was the cytochrome p-450-dependent-oxidative metabolism of (+), (−)- and racemic HCZ in vitro using rat liver 9000 × g supernatant fraction. The in vitro metabolism of (−)-HCZ was extremely fast, compared with those of the (+)-isomer and the racemate. The Vmax in vitro showed a good correlation with the CLo in vivo after oral administration (50 mg/kg) of all three forms of HCZ.

In vitro study of enantiomeric inhibition of the metabolism showed that (+)-HCZ was a competitive inhibitor of (−)-HCZ metabolism, with a Ki of 6.96 μM. (−)-HCZ was also a competitive inhibitor of (+)-HCZ metabolism, with a Ki of 20.4 μM. This is consistent with the observation that the (+)/(-) ratio of AUC0−∞ after dosing with a racemic mixture was clearly lower than after dosing with the individual enantiomers. Moreover, the blood concentrations of racemic HCZ were similar to those of (+)-HCZ rather than intermediate between those of (+)- and (−)-HCZ, probably because of the stronger inhibitory effect of (+)-HCZ on (−)-HCZ than vice versa.

These results suggest that the enantiomeric differences in the pharmacokinetics of HCZ after oral administration were caused by enantioselective first-pass metabolism in the liver, and that the pharmacokinetics of HCZ after the administration of its racemate was affected by the enantiomeric inhibitory interactions in the hepatic metabolism.

Keywords homochlorcyclizine; enantioselectivity; first-pass metabolism; pharmacokinetics; rat

Although enantioselectivity is frequently observed in the absorption, distribution, metabolism and excretion of enantiomeric drugs, many such drugs are clinically used as racemates.1–5)

Homochlorcyclizine hydrochloride (HCZ), a potent antihistamine, is marketed as a racemate, although the activity of the (−)-isomer is about 100 times more potent than that of the (+)-isomer in isolated guinea pig ileum tests.6) Moreover, there are significant pharmacokinetic differences in humans between the enantiomers after oral administration of racemic HCZ7) as well as after oral administration of either (+)- or (−)-HCZ alone.8)

Our previous studies9) showed that the concentrations of (+)-HCZ in urine, blood and nearly all tissues were significantly higher than those of (−)-HCZ after oral administration of racemic HCZ to rats. Also, in vitro studies showed that these results were not caused by enantioselective absorption or distribution, but rather by preferential first-pass metabolism of (−)-HCZ in rat liver. However, the enantiomeric differences in the blood concentrations were smaller after racemic HCZ dosing than after individual enantiomer dosing.6) It can therefore be presumed that the enantiomeric interactions occurred after racemic HCZ dosing.

In order to better understand the effect of first-pass metabolism on the enantioselective pharmacokinetics of HCZ, we carried out both in vivo and in vitro experiments using (+), (−)- and racemic HCZ. First, these three forms of HCZ were administered intravenously and orally to rats, and in vivo pharmacokinetic parameters were obtained. Next, the cytochrome p-450-dependent-oxidative metabolism of these three forms in vitro using rat liver 9000 × g supernatant fraction was investigated. Metabolic interactions between the enantiomers of HCZ in vitro were also examined.

MATERIALS AND METHODS

Materials  Racemic HCZ and diphenhydramine hydrochloride were purchased from Nippon Bulk Yakuhin Co., Ltd. (Osaka). Both (+)- and (−)-HCZ were prepared as described earlier.5) All other chemicals were of analytical reagent grade.

Animals  Male Sprague-Dawley rats (7–9 weeks old) were used for in vivo studies, and 7-week-old rats were used for in vitro metabolic studies.

In Vivo Pharmacokinetic Studies  Rats were cannulated with silicone tubing in the jugular vein.10) They were kept in individual cages and were fasted overnight after operation. They were given 10 or 50 mg/kg of (+)-,
(−)- and racemic HCZ via a stomach tube for oral administration or 5 mg/kg of (+)-, (−)- and racemic HCZ by injection into the jugular vein for intravenous administration, and they were not allowed to drink water for 4 h after the administration. Blood samples (0.25 ml) were collected from the jugular vein at definite times after drug administration and immediately analyzed by a nonchiral HPLC method.

**In Vitro Metabolic Studies** Rats were fasted for 24 h before the experiments and sacrificed by decapitation. The liver was perfused with ice-cold 1.15% KCl-phosphate buffer (pH 7.4) to dehematize it, then quickly removed. It was homogenized with four volumes of the buffer. The incubation mixture consisted of 1 ml of 9000 × g supernatant (200 mg of liver), 2–40 μM of (+)-, (−)- and racemic HCZ, 90 μM of NADP, 4 mM of glucose-6-phosphate, 10 mM of MgCl2, and 1.5 mM of nicotinamide in a total volume made up to 5 ml with 0.1 M phosphate buffer (pH 7.4). The samples were incubated at 37 °C at definite times, and the reaction was stopped by immersing the sample tubes in boiling water. The amount of unchanged HCZ was determined by a nonchiral HPLC method.

**Effect of Enantiomers on Enantioselective Metabolism in Vitro** For Lineweaver-Burk plots and enantiomeric inhibition studies, 5 ml samples of the incubation mixture were subjected to testing as described above with 10 and 40 μM of (−)-HCZ and 4 and 10 μM of (+)-HCZ, respectively. After the reactions had been conducted for definite times, the amounts of (+)- and (−)-HCZ were determined by a chiral HPLC method.

**Sample Preparations and HPLC Methods** To 0.25 ml of each sample, 0.2 ml of internal standard solution (diphenhydramine hydrochloride 3 μg/ml), 3 ml of purified water and 0.2 ml of 4 m sodium hydroxide were added with vigorous mixing. The drugs were then extracted with 5 ml of n-hexane. The extract was evaporated to dryness at 40 °C and dissolved in the mobile phase (120 μl). HCZ in the samples (100 μl) was analyzed by the chiral or nonchiral HPLC methods described earlier. An OVM column (Ultrion ES-OVM, 150 mm × 4.6 mm i.d.) (Shinwa Chemical Industries, Kyoto) and a Shim-pack CLC-CN column (150 mm × 6.0 mm i.d.) (Shimadzu, Kyoto) were used for the chiral and nonchiral HPLC methods, respectively.

**Data Analysis** The maximum blood concentration (Cmax) and the time required to reach the maximum blood concentration (Tmax) were read directly from the concentration–time profile. The area under the blood concentration–time curve (AUC0−∞) was calculated by standard linear trapezoidal integration with extrapolation to infinite time. The absolute bioavailability (BA) was calculated as follows: BA = (AUC0−∞(p.o.)) / (dose(p.o.)) · (AUC0−∞(i.v.)). The values for total body clearance per body weight (CLb) after intravenous and oral administration were expressed as: CLb(i.v.) = dose / AUC0−∞(i.v.), for intravenous administration and CLb(p.o.) = BA · dose / AUC0−∞(p.o.) for oral administration. The apparent oral clearance per body weight (CLO) after oral administration was derived from dose / AUC0−∞(p.o.). Enantiomeric differences in the results were analyzed for their significance by Student’s t-test.

**RESULTS**

**In Vitro Pharmacokinetic Studies** Figure 1 shows the blood concentrations versus time profiles of HCZ after intravenous administration (5 mg/kg) of (+)-, (−)- and racemic HCZ to rats. The AUC0−∞ and CLb of these three forms of HCZ were about the same (Table I).

Figure 2 shows the blood concentrations versus time profiles of HCZ after oral administration (50 mg/kg) of (+)-, (−)- and racemic HCZ to rats. The pharmacokinetic parameters of HCZ are listed in Table II. The blood concentrations of the (−)-isomer in all samples were markedly lower than that of the (+)-isomer and the
TABLE II. Pharmacokinetic Parameters after Oral Administration of (+)−, (−)− and Racemic HCZ (50 mg/kg) to Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(+)-HCZ</th>
<th>Racemic HCZ</th>
<th>(−)-HCZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (μg/ml)</td>
<td>0.90 ± 0.06a</td>
<td>0.86 ± 0.07a</td>
<td>0.21 ± 0.10</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>1.67 ± 0.47</td>
<td>1.33 ± 0.47</td>
<td>1.40 ± 0.49</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (μg h/ml)</td>
<td>5.42 ± 0.54a</td>
<td>4.65 ± 0.98a</td>
<td>1.24 ± 0.13</td>
</tr>
<tr>
<td>$CL_{a}$ (l/h/kg)</td>
<td>9.33 ± 0.98a</td>
<td>11.2 ± 2.1a</td>
<td>40.9 ± 4.4</td>
</tr>
<tr>
<td>$CL_{r}$ (l/h/kg)</td>
<td>2.27 ± 0</td>
<td>2.14 ± 6</td>
<td>2.25 ± 0</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>24.4 ± 2.4a</td>
<td>19.8 ± 4.2a</td>
<td>5.58 ± 0.59</td>
</tr>
</tbody>
</table>

Each data represents the mean ± S.D. of 3−5 rats. a) Significantly different from (−)-HCZ, assessed by Student’s t-test ($p < 0.01$).

Fig. 3. (+)/(−) Ratio of Blood Concentrations after Oral Administration of HCZ (50 mg/kg) to Rats

The blood concentrations of (+)− and (−)-HCZ were taken from Fig. 2, and those of racemic HCZ were obtained from our previous paper. The (+)- and (−)-HCZ administration, ▲, racemic HCZ administration.

TABLE III. Pharmacokinetic Parameters after Oral Administration of (+)- and (−)-HCZ (10 mg/kg) to Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(+)-HCZ</th>
<th>(−)-HCZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{max}$ (μg/ml)</td>
<td>0.11 ± 0.01</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>0.50 ± 0</td>
<td>1.00 ± 0</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (μg h/ml)</td>
<td>0.32 ± 0.12</td>
<td>0.26 ± 0.01</td>
</tr>
<tr>
<td>$CL_{a}$ (l/h/kg)</td>
<td>32.7 ± 9.2</td>
<td>38.8 ± 1.8</td>
</tr>
<tr>
<td>$CL_{r}$ (l/h/kg)</td>
<td>2.40 ± 0</td>
<td>2.26 ± 0</td>
</tr>
<tr>
<td>Bioavailability (%)</td>
<td>7.33 ± 2.77</td>
<td>5.81 ± 0.26</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 3 rats.

Fig. 4. Percentage of Remaining HCZ after Incubation of (+)-, (−)- and Racemic HCZ (20 μM) with Liver 9000 × g Supernatant Fraction ●, (+)-HCZ; ▲, racemic HCZ; ■, (−)-HCZ. Each point and bar represent the mean ± S.E.M. of 3 rats.

TABLE IV. Kinetic Parameters of the in Vitro Metabolism of HCZ by Liver 9000 × g Supernatant Fraction from Rats

<table>
<thead>
<tr>
<th>Substrate</th>
<th>$K_m$ (μM)</th>
<th>$V_{max}$ (nmol/g liver/min)</th>
<th>$V_{max}/K_m$ (ml/g liver/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-HCZ</td>
<td>4.58</td>
<td>50.13</td>
<td>10.95</td>
</tr>
<tr>
<td>Racemic HCZ</td>
<td>5.34</td>
<td>62.96</td>
<td>11.79</td>
</tr>
<tr>
<td>(−)-HCZ</td>
<td>11.41</td>
<td>152.24</td>
<td>13.35</td>
</tr>
</tbody>
</table>

Each data represents the mean of 3−5 experiments.

Fig. 5. Correlation between $CL_{a}$ and $V_{max}$ for Enantiomeric HCZ. $CL_{a}$ and $V_{max}$ were taken from Tables II and IV, respectively. $r = 0.998$ ($p < 0.05$). ●, (+)-HCZ; ▲, racemic HCZ; ■, (−)-HCZ.

In Vitro Metabolic Studies. Figure 4 shows the in vitro degradation of HCZ during incubation of 20 μM of (+)-, (−)- or racemic HCZ with the liver 9000 × g supernatant fraction. (−)-HCZ was more rapidly metabolized than the others, with the metabolic rates of the (+)-isomer and the racemate being almost the same. The apparent maximum metabolic rate ($V_{max}$) and the Michaelis constant ($K_m$) were determined from double-reciprocal plots using various concentrations of (+), (−)- and racemic HCZ, and the results are summarized in Table IV. The $K_m$ of (+)-HCZ was the lowest of these three forms, indicating high affinity to metabolic enzymes. The $V_{max}$ clearly increases in the order of (−)-isomer > racemate > (+)-
isomer. The $V_{\text{max}}$ in vitro showed a good correlation with $CL_o$ in vivo after oral administration (50 mg/kg) of (+)-, (−)- and racemic HCZ (Fig. 5).

The $V_{\text{max}}/K_m$ was also in the order of (−)-isomer > racemate ≥ (+)-isomer, but the differences were minimal (Table IV). The (−)/(+) ratio of the $V_{\text{max}}/K_m$ was approximately 0.82 and agreed with that of the $CL_o$ in vivo after oral administration (10 mg/kg) of (+)- and (−)-HCZ (0.84).

Effect of Enantiomers on Enantioselective Metabolism

Although the (+)/(−) ratio of $AU_{\text{UO}}$ after individual enantiomer dosing was 4.37, it was only 1.39 after racemic HCZ dosing. This difference in enantioselectivity led us to further study enantiomeric inhibition. The results, shown in Fig. 6 as Lineweaver-Burk plots, reveal that the (+)- and (−)-isomers competitively inhibit the metabolism of each other.

From the plots of $K_m/V_{\text{max}}$ versus inhibitor concentrations (Fig. 7), the inhibition constant ($K_i$) values of the (+)- and (−)-isomers were estimated to be 6.96 and 20.4 μM, respectively.

**DISCUSSION**

Several studies have demonstrated enantiomeric pharmacokinetics due to enantioselective metabolism in the liver.\textsuperscript{11−13} The pharmacokinetics after oral administration of (+)-, (−)- and racemic HCZ distinctly differed and also varied with the dose. Enantiomeric difference in the metabolic rate of the cytochrome p-450-dependent oxidation in vitro was also found. The metabolic rate of HCZ, $v$, can be expressed by the Michaelis-Menten equation\textsuperscript{14}:

$$v = \frac{V_{\text{max}} \cdot C}{K_m + C}$$

where $C$ is the concentration of HCZ in the incubation mixture. HCZ is a high presystemic clearance drug,\textsuperscript{9} and the apparent threshold dose of racemic HCZ metabolism is 31.6 mg/kg (unpublished data). Absorption of HCZ from the small intestine is extremely rapid without an enantioselective process.\textsuperscript{9} When the three forms of HCZ were administered to rats at a high dose (50 mg/kg), the free drug concentrations in the liver seemed to be extremely high ($C \gg K_m$). The above equation can be simplified to:

$$v = \frac{V_{\text{max}}}{K_m}$$

This may be why, in this study, the enantiomeric relationship between the $V_{\text{max}}$ in vitro and $CL_o$ in vivo after oral administration of the three forms of HCZ at 50 mg/kg showed a statistically significant correlation as revealed in Fig. 5. On the other hand, when the free drug concentration in the liver was low ($C \ll K_m$), $v$ can be expressed as follows:
\( v = \frac{V_{\text{max}}}{K_m} \)

The \((+)/(−)\) ratio of \(CL_{\alpha}\) after oral administration at a lower dose (10 mg/kg) of each enantiomer agreed with that of the \(V_{\text{max}}/K_m\) obtained \textit{in vitro}. The binding of the \((−)\)-isomer to plasma protein was slightly higher than that of the \((+)\)-isomer in rats.\(^9\) However, no significant difference was found between the enantiomers in the free plasma concentration and the distribution.\(^9\) These results suggest that the enantiomeric differences in pharmacokinetic profiles of HCZ were due to enantioselectivity of the cytochrome p-450-dependent-oxidative metabolism, especially the first-pass effect in the liver.

As shown in Fig. 6, \((+)-\) and \((−)-\)HCZ were competitive metabolic inhibitors of each other. The inhibitory effect of \((+)\)-HCZ was approximately 3 times as potent as that of \((−)-\)HCZ. This is consistent with the observation that the blood concentration profiles after racemic HCZ dosing clearly differed from those after dosing with the \((−)\)-isomer, but not after that with the \((+)\)-isomer. The metabolic rate with competitive inhibition can be expressed as follows:

\[
v = \frac{V_{\text{max}}}{K_m(1 + i/K_i)} + C
\]

where \(i\) is the inhibitor concentration in the incubation mixture. The racemic drug is a 1:1 mixture of the enantiomers, that is, including 50% of the inhibitor. The \((+)/(−)\) ratio of the metabolic rate calculated from the above expression was about 0.75. This agreed with the \((+)/(−)\) ratio (0.72) of \(CL_{\alpha}\) after oral administration of racemic HCZ (50 mg/kg).\(^9\) Therefore, the \((+)/(−)\) ratio of \(AUC_{\alpha−\alpha}\) was lower after racemic HCZ dosing than after individual enantiomer dosing. These results demonstrated that the enantiomeric inhibitory interactions in the hepatic metabolism caused the marked enantiomeric differences in the pharmacokinetics between racemic HCZ and its individual enantiomers.

REFERENCES