Effect of Probenecid on Disposition of Cefpiramide in Rat. The Application of Population Pharmacokinetics

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The effect of probenecid on the disposition of cefpiramide was studied in rats. Cefpiramide (50 mg/kg) was injected into rats through a femoral vein. Probenecid (75 or 150 mg/kg) was administered simultaneously with cefpiramide. The plasma concentration of cefpiramide and the amount excreted into the bile were monitored with a high-performance liquid chromatograph (HPLC). Population pharmacokinetics with Akaike’s information criterion (AIC) was applied to analyze the effect of probenecid on the disposition of cefpiramide. The result of the AIC method was compared with that of the χ² test. It was concluded that probenecid exclusively inhibits the biliary excretion of cefpiramide. The presence of probenecid does not influence the volume of distribution of cefpiramide or the non-biliary elimination rate. The administration of 75 mg/kg of probenecid markedly suppresses the biliary excretion of cefpiramide. However, the effect of 150 mg/kg of probenecid on the elimination rate of cefpiramide is almost the same as that of 75 mg/kg.

Keywords—probenecid; cefpiramide; population pharmacokinetics; MULTI (ELS); bile excretion; MULTI; extended least-squares

Introduction

Cefpiramide (Fig. 1) is a new semisynthetic cephalosporin antibiotic which has a broad spectrum and high activity against gram-positive and gram-negative bacteria including Pseudomonas aeruginosa.1) Cefpiramide shows much longer plasma half-lives in rabbits, dogs and rhesus monkeys2) and humans3) as compared with other cephalosporin antibiotics. This antibiotic has the peculiarity that the major elimination route is not the urinary excretion but biliary excretion as the intact drug.2) Cefpiramide, therefore, is considered to be a potentially useful in research on membrane transport.

It is well known that the administration of probenecid with many penicillins and cephalosporins results in prolongation of the half-lives of these drugs in the body.4) The pharmacological action of probenecid is believed to be inhibition of the renal tubular secretion of organic acids5) which are mainly eliminated through the urinary excretion. It is of interest in connection with our understanding of membrane transport to investigate the effect of probenecid on cefpiramide elimination.

Fig. 1. Structure of Cefpiramide
Population pharmacokinetics, which deals with plural time courses altogether, has attracted growing attention since the proposal of Sheiner et al.\(^6\) The theoretical basis of population pharmacokinetics is an extended nonlinear least-squares method. This analysis has usually been used in the field of clinical pharmacokinetics.\(^7,8\) The present report is concerned with an attempt to apply population pharmacokinetics to the evaluation of the pharmacodynamic effect of probenecid on the disposition of cefpiramide in rats. Akaike’s information criterion (AIC) is shown to be effective in the population pharmacokinetic analysis.

**Experimental**

**Reagents and Materials**—Cefpiramide was a gift from Yamanouchi Pharmaceutical Co., Ltd. (Tokyo, Japan). Probenecid was provided by Sigma Chemical Company (MO, U.S.A.). Heparin was obtained from Novo Ind. (Denmark). Sodium pentobarbital solution (Nebutal for animal injection, Abbott Lab., IL, U.S.A.) was used to anesthetize rats. Trichloroacetic acid for the precipitation of protein was obtained from Wako Pure Chem. Ind. Ltd. (Osaka, Japan). Acetonitrile and the other chemicals for high-performance liquid chromatographic (HPLC) analysis were commercial products of reagent grade. Water was purified by distillation. The prepared mobile phase for HPLC was degassed before use.

**Determination of Cefpiramide by HPLC**——A high-performance liquid chromatograph (LC-3A, Shimadzu Co., Kyoto, Japan) equipped with a variable-wavelength UV detector (SPD-2A, Shimadzu Co., Kyoto, Japan) was used with a stationary phase of Chemosorb 7-ODS-H (50 × 4.6 mm i.d., Chemco Co., Osaka, Japan). A short precolumn (50 × 4.6 mm i.d.) packed with LiChrosorb RP-2 (E. Merck Co.) was attached to guard the main column. The detection wavelength was set at 254 nm. The flow rate of the mobile phase was set at 1.5 ml/min. The peak area was recorded with a Chromatopac C-R1B (Shimadzu, Kyoto, Japan). Column temperature was 30 °C. The mobile phase composition for the analysis of cefpiramide in plasma and bile was phosphate buffer (pH = 7.0, 1/15 v/v)–acetonitrile (85 : 15 v/v). The pretreatments before HPLC injection are described in the following section.

**Rat Experiments**——Under anesthesia by i.p. administration of pentobarbital (30—40 mg/kg), male Wistar rats weighing 200—250 g received 50 mg/kg cefpiramide alone (treatment I), 50 mg/kg cefpiramide with 75 mg/kg probenecid (treatment II), or 50 mg/kg cefpiramide with 150 mg/kg probenecid (treatment III). Cefpiramide and probenecid dissolved in warm pH 7.4 isotonic phosphate buffer (2.54% NaH\(_2\)PO\(_4\)-2H\(_2\)O-4.41% Na\(_2\)HPO\(_4\)-12H\(_2\)O) were rapidly injected into a femoral vein. Blood samples (0.2 ml) were collected from a jugular vein at 5, 10, 15, 20, 40, 60 and 120 min after the injection. Test tubes used to collect blood were heparinized. After centrifugation of the blood at 3000 rev/min for 5 min, 25 µl of 0.1 M phosphate buffer (pH 7.4) and 450 µg of a 5% aqueous solution of trichloroacetic acid were added to 25 µl of plasma. After centrifugation and precipitation of protein, 5 µl of the supernatant was injected into the liquid chromatograph. Rats were opened with a midline incision and the bile duct was cannulated with PE-50 polyethylene tubing (Clay Adams, NJ, U.S.A.), before the injection of cefpiramide and probenecid into a femoral vein. Bile samples were collected at 30, 60, 90 and 120 min after the injection of cefpiramide and probenecid. The collected bile was diluted with 0.1 M phosphate buffer (pH 7.4) to 2 ml. Then 450 µl of an aqueous solution of 5% trichloroacetic acid was added to the bile sample. After centrifugation of the sample at 3000 rev/min for 5 min, 5 µl of supernatant was injected into the liquid chromatograph. Calibration curves for plasma and bile samples were freshly prepared by spiking plasma and bile with appropriate amounts of cefpiramide.

**Data Analysis**——Prior to application of the extended nonlinear least-squares (ELS) method, the plasma and bile data on cefpiramide were evaluated by MULTI, which is based on an ordinary nonlinear least-squares (OLS)\(^9\) approach, in order to reduce the number of population model candidates. Since all time courses of plasma concentration excepting that of one rat showed monoexponential decrease on the testing by AIC, a one-compartment model was adopted for the analysis of plasma and bile data. When both plasma and bile data for a rat were available, the simultaneous least-squares method was applied using the following equations:

\[
C_p = \frac{D}{V_d} \exp(-k_e t) 
\]

\[
F_b = F_{b^*} (1 - \exp(-k_e t)) 
\]

where \(C_p\) is plasma concentration, \(D\) is the dose, \(k_e\) is the elimination rate constant, \(F_b\) is the biliary recovery ratio of cefpiramide, and \(F_{b^*}\) is the recovery ratio at infinite time. The biliary excretion rate constant \(k_b\) and non-biliary elimination constant \(k_{ob}\) are calculated by means of the following equations.

\[
k_b = F_{b^*} k_e 
\]

\[
k_{ob} = (1 - F_{b^*}) k_e 
\]
The estimated pharmacokinetic parameters were compared among treatments I, II and III by the one-way analysis of variance (ANOVA) with a paired t-test. With reference to the results of ANOVA, some population pharmacokinetic models were constructed for the time course data of treatments I, II and III. The effect of probenecid on the disposition of cefpiramide was evaluated by using MULTI(ELS), which is based on the extended least-squares method. The results of the AIC method for extended least-squares were compared with those of the $\chi^2$ test.

**Results and Discussion**

Figure 2 presents the time course of plasma concentration of cefpiramide without probenecid (treatment I), with 75 mg/kg probenecid (treatment II) and with 150 mg/kg probenecid (treatment III). When the data points of treatments II (△) and III (□) are too close, those of treatment III are shifted to the right in Fig. 2. Figure 3 shows the time course of the biliary recovery ratio $F_b$. It appears that the presence of probenecid inhibits the elimination of cefpiramide from the plasma and the excretion into the bile. Table I shows the pharmacokinetic parameters estimated by MULTI and the results of ANOVA. The volume of distribution ($V_d$) and the non-biliary elimination rate constant ($k_{nb}$) are independent of the presence of probenecid. Nakagawa et al. showed that 60% and 35% of administered

![Fig. 2. Time Courses of Cefpiramide Plasma Concentrations with Probenecid Treatments I, II and III](image)

![Fig. 3. Time Courses of Cefpiramide Biliary Excretions with Probenecid Treatments I, II and III](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$(N_p)$</th>
<th>$V_d$ (ml/kg)</th>
<th>$k_e$ (h)</th>
<th>$(N_b)$</th>
<th>$F_b$ (%)</th>
<th>$k_b$ (h)</th>
<th>$k_{nb}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>(3) mean 294</td>
<td>0.0310</td>
<td>(3)</td>
<td>53.9</td>
<td>0.0168</td>
<td>0.0142</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.D. 44</td>
<td>0.0056</td>
<td>2.3</td>
<td>0.0037</td>
<td>0.0019</td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>(4) mean 317</td>
<td>0.0161</td>
<td>(2)</td>
<td>25.0</td>
<td>0.00441</td>
<td>0.0135</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.D. 35</td>
<td>0.0037</td>
<td>1.5</td>
<td>0.00085</td>
<td>0.0036</td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>(5) mean 324</td>
<td>0.0187</td>
<td>(4)</td>
<td>13.1</td>
<td>0.00269</td>
<td>0.0182</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.D. 44</td>
<td>0.0069</td>
<td>2.5</td>
<td>0.00072</td>
<td>0.0055</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ANOVA (5%) NS
Paired t-test (5%) I ≠ II I ≠ II I ≠ II I ≠ III I ≠ III I ≠ III II = III II ≠ III II = III

$N_p$, number of rats for which plasma data are available; $N_b$, number of rats for which bile data are available; $V_d$, volume of distribution; $k_e$, elimination rate constant; $F_b$, recovery ratio of cefpiramide into bile; $k_b$, biliary excretion rate constant, $k_{nb}$, non-biliary elimination rate constant; NS, not significant at the 5% level; S, significant at the 5% level.
Cefpiramide are excreted into the bile and the urine of rats, respectively. Therefore, $k_{ob}$ approximately represents the urinary excretion rate constant. ANOVA shows that the differences of $k_e$, $F_b^\infty$ and $k_b$ among the treatments are significant at the 5% level. The following paired $t$-test at the 5% significance level revealed that the values of $k_e$, $F_b^\infty$ and $k_b$ decrease in the presence of probenecid. The difference of $k_e$ (or $k_b$) between treatments II and III is insignificant, but the difference of $F_b^\infty$ between treatments II and III is significant. Based on the results of OLS, the four population models were selected.

**Model 1**

The population time course data of treatments I, II and III are expressed by the following population model.

\[
C_p = D(V_d + \eta_\nu)\exp(-(k_e + \eta_k)t) + \epsilon_c 
\]

\[
F_b = (F_b^\infty + \eta_F)(1 - \exp(-(k_e + \eta_k)t)) + \epsilon_F
\]

where $C_p$ is plasma concentration, $F_b$ is the recovery ratio in the bile, $D$ is the dose, $V_d$ is the volume of distribution, $\eta_\nu$ is the inter-individual variation around $V_d$, $k_e$ is elimination rate constant, $\eta_k$ is the inter-individual variation around $k_e$, $F_b^\infty$ is the bile recovery ratio at infinite time, $\eta_F$ is the inter-individual variation around $F_b^\infty$, $\epsilon_c$ is the intra-individual variation of plasma concentration, and $\epsilon_F$ is the intra-individual variation of bile recovery ratio. In model 1, the population parameters are assumed to be the same among the treatments (i.e. probenecid has no influence on the disposition of cefpiramide).

**Model 2**

$F_b^\infty$ and $k_e$ of treatment I are different from those of treatments II and III. These parameters are the same between treatments II and III.

**Model 3**

The value of $k_e$ of treatment I is different from those of treatments II and III. The values of $F_b^\infty$ are different between treatments I and II, and between treatments II and III. This model corresponds to the results of ANOVA.

**Model 4**

Both $k_e$ and $F_b^\infty$ are different among treatments I and II, and treatments II and III. The variances of the inter-individual variations and the intra-individual variations are assumed to be the same among the treatments. Table II presents the results of MULTI(ELS). Model 3 gives the minimum AIC, and this result agrees with that of ANOVA. Sheiner et al. proposed the $\chi^2$ test for the selection of a population model.\(^6\) The difference of Ob between models 1 and 2 is 17.9, which is greater than the critical value of 10.6 ($p < 0.005$, degree of freedom 2). The difference of Ob between models 2 and 3 is 22.0, which is greater than the critical value of 7.88 ($p < 0.005$, degree of freedom 1). The difference of Ob between models 3 and 4 is 0.5, which is less than the critical value. Therefore, the $\chi^2$ test selects model 3 as the best model, which coincides with the result of the AIC method. The estimated population

<table>
<thead>
<tr>
<th>Model</th>
<th>Ob</th>
<th>AIC</th>
<th>Number of parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model 1</td>
<td>697.4</td>
<td>713.4</td>
<td>8</td>
</tr>
<tr>
<td>Model 2</td>
<td>679.5</td>
<td>699.5</td>
<td>10</td>
</tr>
<tr>
<td>Model 3</td>
<td>657.5</td>
<td>679.4</td>
<td>11</td>
</tr>
<tr>
<td>Model 4</td>
<td>657.0</td>
<td>681.0</td>
<td>12</td>
</tr>
</tbody>
</table>
It is concluded that probenecid exclusively inhibits the excretion of cefpiramide into the bile of rats. Probenecid is believed to inhibit the active transport of organic acids through the renal tubular membrane.\textsuperscript{5) Therefore, the present result raises the possibility that the excretion of cefpiramide into the bile includes an active transport process in the liver. The volume of distribution and the urinary excretion rate of cefpiramide are not affected by the presence of probenecid.

Biliary data could not be obtained for some rats in the present report. The ordinary least-squares method cannot estimate the parameters for biliary excretion in this case. However, population pharmacokinetics can be used to evaluate groups of time courses where some data are deficient.

<table>
<thead>
<tr>
<th>Table III. The Estimated Population Mean Parameters and Variances of Inter- and Intra-individual Variations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_a = 312$ (ml/kg), $\omega^2 (V_a) = 654$ (ml$^2$/kg$^2$)</td>
</tr>
<tr>
<td>$k_{e} (I) = 0.0247$ (/min), $k_{e} (II) = k_{e} (III) = 0.0156$ (/min)</td>
</tr>
<tr>
<td>$\omega^2 (k_{e}) = 4.98 \times 10^{-5}$ (/min$^2$)</td>
</tr>
<tr>
<td>$F_{b}^{e-1} (I) = 57.6$ ($%$), $F_{b}^{e-1} (II) = 26.3$ ($%$), $F_{b}^{e-1} (III) = 14.8$ ($%$)</td>
</tr>
<tr>
<td>$\omega^2 (F_{b}^{e-1}) = 8.55$ ($%^2$)</td>
</tr>
<tr>
<td>$\sigma^2 (C_{p}) = 293$ (µg/ml$^2$)</td>
</tr>
<tr>
<td>$\sigma^2 (F_{b}) = 1.70$ ($%^2$)</td>
</tr>
</tbody>
</table>

I, II and III specify treatments I, II and III, respectively. $\omega^2$ and $\sigma^2$ specify the variances of inter- and intra-individual variations, respectively.

References