PHARMACOKINETIC AND PHARMACODYNAMIC STUDIES OF PIRETANIDE IN RABBITS. I. EFFECT OF DIFFERENT HYDRATED CONDITIONS

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The pharmacokinetics and pharmacodynamics of piretanide in rabbits were investigated. After intravenous administration, the plasma concentrations and the urinary excretion rates of piretanide, as well as the pharmacologic effects, were measured under two different hydrated conditions. In one experiment, the amount of the body fluid which was lost during diuresis was replaced by infusing Ringer's solution at exactly the same rate with the urine flow rate (treatment I). In another experiment, no compensatory infusion was made (treatment II). There was no appreciable difference between treatment I and II, as far as the plasma concentrations and urinary excretion rate were concerned. In spite of the similarities in the pharmacokinetic properties, the pharmacologic effects of piretanide were influenced considerably by the hydrated conditions of the body. The diuretic effect expressed as the excretion rate of the sum of urinary sodium and potassium was plotted against the corresponding urinary excretion rate of unchanged piretanide. The shape of the each graph showed a similar sigmoid like curve; however, the curve in treatment I was significantly shifted to the right compared to that in treatment II. This fact indicated that the pharmacologic effect of piretanide seemed to be modified by the contents of water and electrolytes in the body. The plasma concentrations and urinary excretion of piretanide were reasonably described by a linear three compartment open model. Although the diuretic effect of piretanide was related to the hypothetical biophase compartment using the conventional Hill's equation, the difference of pharmacologic intensity between the treatments could not be explained. Then, we introduced a feedback mechanism representing the depletion of body fluid into the Hill's equation and applied this equation to the pharmacologic response of piretanide. The results indicated that the diuretic effects of piretanide in treatment II (in the progressive hydropenic state) as well as in treatment I (in the hydrated state) were well explained.

Keywords — piretanide; diuretic; fluid replacement; pharmacokinetics; pharmacodynamics; hydration; hydropenia

INTRODUCTION

Piretanide, a substituted sulfamoylbenzoic acid derivative, is very closely related in chemical structure to the loop diuretics, such as furosemide and bumetanide, and has similar pharmacokinetic and pharmacodynamic properties. Studies in humans have shown that the absorption of piretanide is very rapid and the apparent volume of distribution is relatively small because of its high plasma protein binding. The decline of piretanide from plasma could be described by a mono-exponential equation; however, a number of instances have shown that a bi-exponential equation fits the data effectively. Piretanide is removed from plasma by the proximal tubular cells through the nonspecific organic acid secretory pathway. It has been reported that the metabolism of piretanide is not extensive and that about half of the administered dose is recovered in urine as the unchanged form during the first few hours. Like other loop diuretics, the principal site of action of piretanide is in the thick ascending limb of the loop of Henle where it exerts a diuretic effect by inhibiting sodium and chloride reabsorption. In comparative studies, it has generally been found that the diuretic potency of piretanide is approximately 6 times greater than furosemide and is only one-tenth of bumetanide, on a molecular weight basis. In spite of the differences in their potency, the ratios of sodium excretion rate to the urine flow rate and of chloride excretion rate to urine flow rate are almost identical among these three diuretics. After oral or intravenous administration of piretanide, both water and electrolyte excretion are increased rapidly but the
duration of the effect is extremely short.\textsuperscript{7)}

Recently, the relationship between the state of hydration and diuretic potency has been reported. Piretanide in the hydropenic subjects showed fundamentally different diuretic effects compared with those in the hydrated subjects.\textsuperscript{8,9)} The purpose of this study was to characterize the pharmacokinetic and pharmacodynamic relationship of piretanide in two different hydrated conditions in rabbits.

MATERIALS AND METHODS

Chemicals — Piretanide (Hoechst Japan Ltd., Tokyo), and bumetanide (Sankyo Co., Tokyo) were obtained commercially and were used as received. All other reagents used in the experiments were reagent grade and were used without further purification.

Animal Experiments — Unanesthetized adult male albino rabbits (Shizuoka Laboratory Animal Center, Hamamatsu) weighing 3 to 4 kg were used in the experiment. Fixing on the back, the rabbit was catheterized into the bladder (#3 Nelaton catheter, Tokyo) to take urine samples. Ringer’s solution (NaCl 147.0 mM, KCl 4.0 mM, CaCl\textsubscript{2} 6.0 mM) containing inulin (200 mg/dl) was infused from the marginal auricular vein at the rate of 100 ml/h. After reaching a steady-state urine output, piretanide was administered via the same vein within 30 s. After the administration of the drug, the experiments were carried out under two different conditions. One experimental condition was that the amount of water and electrolytes which were lost from the animal body during the diuresis were completely replaced by the additional infusion of Ringer’s solution (treatment I). The rate of the infusion was adjusted according to the urine flow rate, using an extra infusion pump. Another experimental condition was that no compensatory infusion was made (treatment II).

Urine samples were collected just prior to piretanide dosing (blank) and at 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, 180, 240, 300 and 360 min after dosing. Blood samples were drawn from the marginal ear vein with a heparinized syringe at the midpoint between each two urine sample collection periods.

Determinations of Piretanide in Plasma and in Urine — The measurement of piretanide was carried out by the high performance liquid chromatography (LC-6A, Shimadzu, Kyoto) with a fluorescence detector (RF-530, Shimadzu, Kyoto). The column (150 × 3.5 mm i.d.) was packed with Lichrosorb RP-18 (5 μm particle size; E. Merck, Darmstadt). The mobile phase was 50% methanol in 0.005 M tetra-\textit{n}-butylammonium phosphate ion-pair buffer and the flow rate was fixed at 1.0 ml/min. Piretanide and bumetanide, the internal standard, were monitored at an emission wavelength at 455 nm and at an excitation wavelength of 280 nm.

Heparinized blood samples were centrifuged immediately after collection at 10000 rpm for 2 min and 0.1 ml of plasma was separated. After adding 0.5 ml of methanol and 0.1 ml of bumetanide methanol solution (2 μg/ml), the plasma sample was centrifuged again and the deproteinized sample was obtained. The solvent was evaporated under reduced pressure at room temperature, the residue was dissolved in 0.2 ml of the chromatographic mobile phase and 10 μl of the solution were subjected to chromatography.

Urine samples were diluted 10-fold with distilled water and 0.1 ml each of the diluted samples was mixed with an equal volume of 2 μg/ml bumetanide methanol solution. After centrifugation, 10 μl of the solution were submitted to analysis. Recovery by this analytical method was greater than 95% (data not shown).

Measurement of Glomerular Filtration Rate — The glomerular filtration rate (GFR) was obtained by determining the renal clearance of inulin. The plasma concentration and urinary excretion of inulin were determined according to the method of Dische \textit{et al.} \textsuperscript{10)}

Measurement of Sodium and Potassium in Plasma and Urine — The sodium and potassium concentrations in plasma and urine were assayed by flame photometry (model 139–0400, Hitachi Ltd., Tokyo) at 589 and 768 nm wavelengths, respectively.

Data Analysis — All of the calculations were carried out by using the nonlinear least squares method with the aid of a digital computer (PDP-11/34, Digital Equipment Corp., Maynard, Mass.).

The statistical significance (\textit{p} < 0.05) was evaluated by the Student’s \textit{t}-test.

RESULTS AND DISCUSSION

Figure 1 shows the semilogarithmic plots of the time course of the plasma concentration and urinary excretion rate, after an intravenous
bolus injection of piretanide (1.5 and 15 mg/kg). The plots are shown as the normalized dose data. Figure 1A was obtained under treatment I and Fig. 1B under treatment II. There seems to be no difference between treatment I and II as far as the disposition of piretanide are concerned. There was also no evidence for the existence of dose dependent pharmacokinetics, between doses. Accordingly, the data of plasma concentrations and urinary excretion rates of piretanide were simultaneously fitted to the following three compartment open model.

\[ C_p(t) = P_1 \cdot \exp(-\lambda_1 \cdot t) + P_2 \cdot \exp(-\lambda_2 \cdot t) + P_3 \cdot \exp(-\lambda_3 \cdot t) \]  

\[ \frac{dX_u}{dt} = CL_r \cdot C_p \]

Where \( C_p \) is the plasma concentration, \( dX_u/dt \) is the urinary excretion rate and \( CL_r \) is the renal clearance of piretanide. \( P_i \) and \( \lambda_i (i = 1 \text{ to } 3) \) are coefficients and rate constants of the linear compartment model, respectively. The solid lines in Fig. 1 are the theoretical values using

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment I (^b)) mean ± S.D.</th>
<th>Treatment II (^c)) mean ± S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_1 (\mu g \cdot ml^{-1}) )</td>
<td>4.598 ± 1.582</td>
<td>7.338 ± 2.157</td>
</tr>
<tr>
<td>( P_2 (\mu g \cdot ml^{-1}) )</td>
<td>0.5671 ± 0.2538</td>
<td>0.5617 ± 0.2468</td>
</tr>
<tr>
<td>( P_3 (\mu g \cdot ml^{-1}) )</td>
<td>0.03140 ± 0.01944</td>
<td>0.06574 ± 0.04268</td>
</tr>
<tr>
<td>( \lambda_1 (min^{-1}) )</td>
<td>0.1404 ± 0.0493</td>
<td>0.1384 ± 0.0285</td>
</tr>
<tr>
<td>( \lambda_2 (min^{-1}) )</td>
<td>0.03129 ± 0.00830</td>
<td>0.02860 ± 0.00901</td>
</tr>
<tr>
<td>( \lambda_3 (min^{-1}) )</td>
<td>0.004852 ± 0.002168</td>
<td>0.007048 ± 0.002131</td>
</tr>
<tr>
<td>( CL_r (ml \cdot min^{-1}) )</td>
<td>14.55 ± 1.41</td>
<td>14.98 ± 1.18</td>
</tr>
</tbody>
</table>

\(^a)\) Data in piretanide plasma concentration and urinary excretion rate in 1.5 and 15 mg/kg dose were normalized to 1.0 mg/kg. \(^b)\) n = 9. \(^c)\) n = 17.
Eqs. 1 and 2. The estimated parameters are listed in Table I.

The CLr values of both treatment I and II were very close to the basal GFR value of the rabbits (about 16.7 ml/min). Considering that piretanide bound plasma protein extensively (it was more than 95% in rabbit, based on our data) and that only the free fraction of the drug could be filtered at the glomerulus, the active secretion in the tubule plays a very important role in the renal clearance of piretanide. In Table I, no statistical significant difference was observed between the treatments. This fact indicates that the pharmacokinetics of piretanide are not influenced by the hydration state of animals. These results are also consistent with the recent report of Li et al.\textsuperscript{11}

It was reported that piretanide is able not only to stimulate natriuresis but also to induce kaliuresis.\textsuperscript{2b} The mechanism may be explained in part by the moderate action of piretanide on the proximal tubule\textsuperscript{19} and, in part, by the secondary action of natriuresis which increases passage of sodium ion through the distal tubule, stimulating the sodium–potassium exchange reaction. Accordingly, it is the sum of the sodium and potassium excretion rate that reflects the net effect of piretanide. The relationship between the urine flow rate and the sum of sodium and potassium excretion rates in the treatment I is shown in Fig. 2. The ratio of the urine flow rate to the sum of sodium and potassium excretion rates is approximately constant throughout the experiment, irrespective of high dosage (15 mg/kg) or low dosage (1.5 mg/kg). This indicates that the urine flow rate is dependent upon the sum of sodium and potassium excretions. The value of the ratio of the sum of the electrolytes excretion rate to the urine flow rate suggested that the urine was nearly isotonic to the plasma.

Based on the above evidence, we used the sum of the sodium and potassium excretion rate as the index of diuretic response of piretanide.

Figure 3 shows a similar relationship between the urine flow rate and the sum of sodium and potassium excretion rates, in treatment II. A good linear relation was also observed. The slope is slightly smaller than in the case of treatment I. This indicates that urine osmolality was reduced, even though the large amount of water loss may

![Fig. 2. The Relationship between Urine Flow Rate and the Sum of the Sodium and Potassium Excretion Rates under Treatment I](image)

- \(\bigcirc\), 1.5 mg/kg \((n = 3)\); \(\square\), 15 mg/kg \((n = 6)\). The correlation coefficient and slope were 0.9963 and 0.1388 in the 1.5 mg/kg dose study, and were 0.9963 and 0.1389 in the 15 mg/kg study, respectively. Each point is shown as the mean ± S.E.

![Fig. 3. The Relationship between Urine Flow Rate and the Sum of the Sodium and Potassium Excretion Rates under Treatment II](image)

- \(\bigcirc\), 1.5 mg/kg \((n = 8)\); \(\square\), 15 mg/kg \((n = 9)\). The correlation coefficient and the slope were 0.9986 and 0.1121, in the 1.5 mg/kg dose study, and were 0.9985 and 0.1223 in the 15 mg/kg study, respectively. Each point is shown as the mean ± S.E.
stimulate antidiuretic hormone secretion. Similar phenomena were also reported in human studies.8,9

Figure 4 shows the effect of piretanide on GFR under treatment I. The GFR values were not changed by piretanide administration and the values were maintained at about 1000 ml/h. On the contrary, the GFR values were significantly reduced by piretanide i.v. injection under treatment II (Fig. 5). In treatment II, the profile

FIG. 4. Effect of Piretanide on GFR under Treatment I in Rabbits.
The dose of piretanide was 1.5 mg/kg, and each point is shown as the mean ± S.E. (n = 3).

FIG. 5. Effect of Piretanide on GFR under the Treatment II in Rabbits.
○, 1.5 mg/kg (n = 5); □, 15 mg/kg (n = 6). Each point is shown as the mean ± S.E.

FIG. 6. Relationship between the Urinary Excretion Rate of Piretanide and the Sum of Sodium and Potassium Excretion Rate.
A: treatment I (n = 9), B: treatment II (n = 17). ○, 1.5 mg/kg; □, 15 mg/kg. Each point is shown as the mean ± S.E. The solid lines and dotted lines in the figures are theoretical values using a PK-PD model described in the text.
of the time course of the GFR reduction after piretanide injection was different from the corresponding diuretic effect. It took a much longer time for both to attain the maximum effect and to return to the control level. It appears that this phenomenon is the result of the reduction of the effective glomerular filtration pressure, which might be caused by the antihypertensive activity of piretanide. However, this was not the case. The GFR values were not influenced by the piretanide administration in the treatment I, even though the blood pressure might have been lowered.

Piretanide is one of the most potent diuretics and its site and the mechanisms of action have been basically clarified. Since more than 70% of administered piretanide is recovered in urine as the unchanged form during the first 6 h, it is reasonable to relate the pharmacologic response with the tubule levels of piretanide, which were directly expressed by the urinary excretion rate. Figure 6 shows the semilogarithmic plots of the urinary excretion rate of piretanide versus diuretic effects, which are expressed as the sum of the sodium and potassium excretion rates. The data of Fig. 6A were obtained by treatment I and data of Fig. 6B by treatment II. A potent diuresis appeared immediately after intravenous administration and the maximum responses were almost the same for both treatments. The diuretic response curve in the higher dose (15 mg/kg) seemed to shift to the right, compared to that in the lower dose (1.5 mg/kg), in both treatments. It is also observed that the diuretic response curves obtained in treatment I was always at the left side, compared to the corresponding curve obtained in treatment II. These facts indicate that the diuretic response intensity was dependent on both drug levels at the active site and the hydration state in the body.

In order to clarify the concentration–effect relationship of piretanide in detail, a pharmacokinetic(PK)–pharmacodynamic(PD) model was constructed under the following assumptions. 1) The relationship between the diuretic response and the concentration at the active site (the biophase concentration) can be described by the Hill's equation. 2) The biophase compartment is directly connected to the central compartment (plasma compartment) by a first-order process, according to the method of Sheiner et al. These relationships are also expressed by Eqs. 3 and 4.

\[
E = \frac{E_{\text{max}} \cdot D'}{D_{50}' + D'} 
\]

\[
D = \frac{K_{\text{eo}}}{V_c} \sum_{i=1}^{3} P_i \cdot \frac{(\exp(-\lambda_i \cdot t) - \exp(-K_{\text{eo}} \cdot t))}{(K_{\text{eo}} - \lambda_i)} \]

where \( E \) is the diuretic effect of piretanide, expressed by the sum of the sodium and potassium excretion rates, \( E_{\text{max}} \) is the maximum effect, \( D_{50}' \) is a hypothetical biophase concentration at 50% of maximum effect, \( r \) is a Hill's constant, \( D \) is the hypothetical biophase concentration of piretanide, \( K_{\text{eo}} \) is the elimination constant from the biophase compartment and \( V_c \) is the distribution volume of the central compartment. \( P_i \) and \( \lambda_i \) were defined previously. Although simultaneous data fitting was carried out using Eqs. 1 through 4 and diuretic data obtained in treatments I and II, none of the fittings showed convergency. As shown in Fig. 6, the diuretic responses in the treatment II are always lower than those in the treatment I. The difference is considered to be the effect of the feedback system which was activated by the loss of water and electrolytes from the body during diuresis. This fact indicates that the PD model of PK-PD relationship should be modified in order to respond to the effect of the feedback system. Accordingly, we have introduced a new equation to explain the relationship between the piretanide disposition and the diuretic response.

\[
E = f \cdot \frac{E_{\text{max}} \cdot D'}{D_{50}' + D'} 
\]

\[
f = 1 - \frac{E_{\text{max}}' \cdot \Sigma E'}{D_{50}' + \Sigma E'} 
\]

where \( f \) is the modification factor of Eq. 3, which was defined in Eq. 6 as a function of the accumulated amount of the body fluid loss. \( E_{\text{max}}' \) is the maximum ratio of the body fluid decrease, \( D_{50}' \) is the sum of sodium and potassium at 50% of the \( E_{\text{max}}' \), and \( \Sigma E' \) is the body fluid decrease that is also expressed as the sum of the sodium and potassium excretion. The second term of Eq. 6 reflects the activity of the feedback
TABLE II. Pharmacodynamic Parameters of Piretanide on Intravenous Administration

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Parameter estimate</th>
<th>S.D.</th>
</tr>
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<tbody>
<tr>
<td>$K_{co}$ (min$^{-1}$)</td>
<td>0.1151</td>
<td>0.0383</td>
</tr>
<tr>
<td>$E_{max}$ (meq·min$^{-1}$)</td>
<td>0.6838</td>
<td>0.0687</td>
</tr>
<tr>
<td>$r$</td>
<td>0.9593</td>
<td>0.2253</td>
</tr>
<tr>
<td>$D_{\text{max}}$ (µg·ml$^{-1}$)</td>
<td>0.0008330</td>
<td>0.0001476</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>0.7629</td>
<td>0.1455</td>
</tr>
<tr>
<td>$D_{\text{so}}$ (meq)</td>
<td>25.00</td>
<td>10.50</td>
</tr>
</tbody>
</table>

system. According to Eqs. 5 and 6, the diuretic effect will diminish with time, unless replacement of water and electrolytes is made, even though the concentration of the drug remains constant. Simultaneous data fitting was carried out again, using Eqs. 1, 2, 4, 5 and 6 and the pharmacologic data of diuresis. In treatment I, the $f$ value is assumed to be unity. The pharmacokinetic parameters listed in the Table I are also used to calculate the hypothetical biophase concentration $D$. The convergency of the fitting was determined by a function of the weighed sum of the squares. In the present work, a weight value of 1 was used. The solid line in Fig. 6 show the results of the fitting, and the estimated parameters are listed in Table II.

The diuretic effects of piretanide (1.5 and 15 mg/kg) in treatment II were well explained by the model. Although the data of diuresis at the higher dose in treatment I also fitted the model, the diuretic effect at the lower dose of piretanide could not be described by the present PK-PD model. This fact indicates that other factors, in addition to the feedback system stimulated by the water and electrolyte depletion, may contribute to the diuretic effect of piretanide.

Although the importance of fluid or electrolyte replacement during diuretic drug therapy has been recognized, quantitative investigations of the effect of fluid replacement on the PK-PD relationship have not been fully elucidated. In the present study of piretanide, the development of acute tolerance in diuretic effect was observed, unless water and electrolyte replacement was made (treatment II). The pharmacokinetic and pharmacodynamic model, including a new PD model, can reasonably and quantitatively describe the effect of piretanide as well as the acute tolerance in diuresis. As we have indicated, the PD model used in this study may be useful in assessing the pharmacologic effect of other diuretics, such as bumetanide or furosemide.

CONCLUSION

The disposition of piretanide in rabbits after intravenous administration was described by a linear three compartment open model. Although the disposition of piretanide was less affected by the state of hydration, the diuretic effect was significantly influenced by the water and electrolyte contents in the body. The diuretic response curves were also altered under different doses of piretanide. Simultaneous modeling of pharmacokinetics and pharmacodynamics using a modified Hill’s equation and a conventional link model was effective for characterizing the diuretic effect of piretanide.

REFERENCES


