PHARMACOKINETIC AND PHARMACODYNAMIC EVALUATION OF GELATIN MICROCAPSULE CONTAINING PIRETANIDE IN BEAGLE DOGS*

TSUYOSHI TSUIJYAMA,** SHIGERU GOTO, MASAKAZU KAWATA*** AND NOBUO SUZUKI**

Research and Development Laboratories, Hoechst Japan Limited,** Minamidai 1-3-2, Kawagoe-shi, Saitama, 350, Japan and Faculty of Pharmaceutical Sciences, Kyushu University,*** Maidashi 3-1-1, Higashi-ku, Fukuoka, 812, Japan

(Received August 20, 1985)

A sustained-release property of gelatin microcapsules of piretanide was evaluated by pharmacodynamic parameters. The gelatin microcapsules of piretandide showed long-lasting, slow-release properties, when compared with its ordinary tablet form in the plasma piretandide level, piretandide excretion rate, urine excretion rate and urinary electrolyte (Na⁺, K⁺, Cl⁻) excretion rate without any loss in diuretic and saluretic activities, which fully satisfied the criteria for a sustained-release preparation.  

Keywords — gelatin microcapsule; piretandide; pharmacokinetic comparison; pharmacodynamic comparison; sustained-release formulation

INTRODUCTION

Piretandide, 4-phenoxy-3-(1-pyrrolidinyl)-5-sulamoylbenzoic acid, has similar pharmacological effects to those of other loopdiuretics such as furosemide and bumetanide. The diuretic action of piretandide is 6 or 7 times as potent as furosemide1) and the excreted potassium concentration is lower than that caused by bumetanide.2) Furthermore, piretandide has been on the European market as an antihypertensive agent because of its effect against hypertension. However, there are cases where piretandide hinders drug therapy owing to pollakisurae caused by its marked diuretic action in the early stage of treatment.3) For effective hypotensive action, a device was needed to maintain a constant and long-lasting level of piretandide in blood, which suggested the use of a long-acting slow-release formulation. Previously, we prepared gelatin microcapsules containing piretandide, evaluated their dissolution property and the plasma piretandide level after oral administrations to beagle dogs and proved that gelatin microcapsules of piretandide thus prepared have an excellent sustained-release property.4) The aim of the present study is to evaluate a sustained-release property of gelatin microcapsules containing piretandide pharmacodynamically with pharmacokinetic evaluation so that its usefulness as a slow-release preparation may be further ensured.

MATERIALS AND METHODS

Materials — Piretandide 3 mg tablets with score (Arelix® Tablet) and piretandide were supplied from Hoechst Japan Limited and piretandide was passed through a 100-mesh sieve prior to microencapsulation. Gelatin (Art. 4072, Gelatin Weiss, Merck) and bumetanide (Lunetron Injection, Sankyo Co.) were commercially available products. The other reagents were of analytical grade.

Preparation of Gelatin Microcapsules — Gelatin microcapsules were prepared in a manner similar to that reported previously.4) This microencapsulation method5) aims at preparing diffusion/dissolution-controlled microcapsules consisting of dispersed solid drug particles in a gelatin matrix, and is schematized in Chart 1. The particle size of the microcapsules used in the present study ranged from 250 to 1000 μm.

Animals — Male beagle dogs weighing 12–15 kg were individually maintained in metabolic cages in an animal room kept at 23 °C and 55% relative humidity. The animals were daily given 250 g of dog chow (DS, Oriental Yeast Co.). They were fasted for 18 h prior to the experiment.

Oral Administration — After urine was completely removed through a urethral catheter, beagle dogs were orally loaded with 300 ml of physiological saline and, thereafter, with 60 ml

* This paper forms part XIII of a series entitled “Evaluation of Microcapsule.”
at 1 h intervals for 8 h. The volume of urine excreted from 1 to 2 h after the first loading was designated pre-dosing value. The gelatin microcapsules containing piretanide and ordinary piretanide tablet were orally administered in a dose of 0.5 or 0.1 mg/kg at the time of the third loading of physiological saline. Then, the excreted urine volume was measured at the 1 h intervals for 6 h after the drug administration, and blood was collected through the cephalic vein 30 min post-dosing and, thereafter, at the same interval as the urine collection for 6 h. Animals treated in the same manner without the test drug served as control.

**Measurement of Piretanide in Plasma and Urine** — The concentration of piretanide in plasma and urine samples was determined by the high performance liquid chromatographic (HPLC) method described by Kawano *et al.* 8 with some modifications. To 0.5 ml of plasma, 0.5 ml of internal standard (bupetamide solution, 1 μg/ml), 1 ml of 0.5 N hydrochloric acid, and 5 ml of dichloromethane were added and mixed by shaking for 15 min. After centrifugation at 3000 rpm for 20 min, the dichloromethane layer was passed through a silica gel cartridge (Seppak, Waters Associates Inc.). The cartridge, which had adsorbed piretanide, was washed with 10 ml of dichloromethane and piretanide was eluted with 5 ml of dichloromethane containing 4% acetic acid. The eluate was evaporated to dryness at 50 °C under a stream of nitrogen gas, and the residue was dissolved in 2 ml of the chromatographic mobile phase. One hundred microliters of the solution were injected into the chromatograph.

The collected urine was diluted 1- to 5-fold with water. To 0.5 ml of the diluted urine, 0.5 ml of the internal standard was added and treated in the same manner as for the determination of the plasma piretanide level.

The HPLC consisted of a solvent pump (TRI ROTAR V, Jasco), an autoinjector (710B, Waters), a 5-μm particle size reversed-phase octadecyl column (4.6 mm ×150 mm, Nucleosil C18, Machery & Nagel), a precolumn (Bondapack C18/Corsasil, Waters), a fluorescence detector (350 nm excitation wavelength, 430 nm emission fluorescence wavelength, Jasco), and an integrator (peak height mode, 3390 A, 353)

---

**CHART 1. Preparation of Gelatin Microcapsules**

- 3.1 g of piretanide (less than 149 μm in diameter)
- add 26.3 ml of H₂O to 8 g of gelatin, allow to swell (55 – 60 °C), cool, and cut into cubes
- dissolve (55 – 60 °C)
- stir (55 – 60 °C, stirrer with 4 propellers, 200 rpm, 20 min, under reduced pressure)
- pour into 88.2 ml of liquid paraffin previously heated at 50 – 60 °C
- stir (55 – 60 °C, stirrer with 4 propellers, 300 rpm, 5 min)
- cool (5 °C, stirrer with 4 propellers, 450 rpm, 90 min)
- add 49.2 ml of isopropanol previously cooled to 5 °C
- stir (5 °C, stirrer with 4 propellers, 450 rpm, 30 min)
- filter
- wash with 30 ml of isopropanol (3 times)
- immerse 1 g of microcapsules in 10 ml of 10% formalin-isopropanol at 5 °C for 24 h
- filter
- dry
Hewlett-Packard). The mobile phase was 45% acetonitrile in 1/30 M phosphate buffer (pH 6.5). The flow rate was 1.0 ml/min.

Cumulative Urine Measurement — The excreted urine volume in the metabolic cages and that collected through a catheter in course of time were combined as the cumulative urine volume.

Measurement of Urinary Na⁺, K⁺ and Cl⁻ Levels — The urinary Na⁺, K⁺ and Cl⁻ levels were measured by the ion selective electrode method with an automated Na⁺, K⁺ and Cl⁻ analyzer (Sera 300A, Horiba).

Statistical Analysis — Data on the cumulative urine volume and cumulative urinary electrolyte amounts were analyzed by Student's t-test to evaluate differences among the microcapsule-dosed group, tablet-dosed group and control group.

RESULTS

Plasma Piretanide Level

The time-coursed plasma piretanide levels after oral administration of the gelatin microcapsules containing piretanide and ordinary piretanide tablet to beagle dogs in a dose of 0.5 mg/kg are shown in Fig. 1. A sustained plasma piretanide level was observed after the microcapsule dosing, unlike the pattern obtained with the ordinary tablet. The area under the concentration-time curve of the plasma piretanide level for 1–6 h (AUC₀⁻⁶) was 0.70 (µg/ml·h) for the gelatin microcapsule and 0.83 (µg/ml·h) for the ordinary tablet; that is, the AUC₀⁻₆ of the ordinary tablet was 1.19-fold that of the gelatin microcapsule. At a dose level of 0.1 mg/kg, complete plasma piretanide level-time curves could not be drawn because the plasma piretanide level was under the detection limit in some samples.

Piretanide Excretion Rate

Figures 2 and 3 show the time-coursed urinary excretion rate of piretanide after the oral administration of 0.5 and 0.1 mg/kg of the gel-
tin microcapsule and ordinary tablet. The piretanide excretion rate-time curve after gelatin microcapsule administration indicated a slow excretion of piretanide for a long period, which was different from that of the ordinary tablet. Their patterns were similar to that of the plasma piretanide level-time curves shown in Fig. 1.

**Urine Excretion Rate and Cumulative Urine Volume**

Figure 4 shows the time-coursed urine excretion rate after the oral administration of 0.5 and 0.1 mg/kg of the gelatin microcapsule and ordinary tablet to beagle dogs. At a dose of 0.5 mg/kg, the urine excretion rate was maximum between 0 and 1 h for both preparations and was lower with the gelatin microcapsule than with

**FIG. 4. Fractionally Excreted Urine Volume after Oral Administration of Microcapsule (●) Containing 20% (w/w) Piretanide, Tablet (〇) Containing 3 mg Piretanide per Tablet, and Control (□) in Beagle Dogs**

*Each point represents the mean value and each vertical line, the standard error.*

**FIG. 5. Urinary Sodium Excretion after Oral Administration of Microcapsule (●) Containing 20% (w/w) Piretanide, Tablet (〇) Containing 3 mg Piretanide per Tablet, and Control (□) in Beagle Dogs**

*Each point represents the mean value and each vertical line, the standard error.*
the ordinary tablet by ca. 27%. Between 1 and 6 h, however, the excretion rate was higher with the gelatin microcapsule than with the ordinary tablet. At 0.1 mg/kg, the maximum urine excretion rate for the ordinary tablet was observed between 0 and 1 h as in the case of 0.5 mg/kg, whereas that for the gelatin microcapsule shifted between 1 and 2 h and was 52% of that of the ordinary tablet observed between 0 and 1 h.

These results suggested that the sustained-release property was more favorable at a dose level of 0.1 mg/kg than 0.5 mg/kg.

**Urinary Electrolyte Excretion Rate and Cumulative Urinary Electrolytes (Na⁺, K⁺, Cl⁻)**

The urinary electrolyte (Na⁺, K⁺, Cl⁻) excretion rates after the oral administration of 0.5 and 0.1 mg/kg of the gelatin microcapsule and ordinary tablet to beagle dogs are summarized in

**FIG. 6. Urinary Potassium Excretion after Oral Administration of Microcapsule (●) Containing 20% (w/w) Piretanide, Tablet (○) Containing 3 mg Piretanide per Tablet, and Control (□) in Beagle Dogs**

Each point represents the mean value and each vertical line, the standard error.

**FIG. 7. Urinary Chloride Excretion after Oral Administration of Microcapsule (●) Containing 20% (w/w) Piretanide, Tablet (○) Containing 3 mg Piretanide per Tablet, and Control (□) in Beagle Dogs**

Each point represents the mean value and each vertical line, the standard error.
TABLE I. Cumulative Urine Volume and Urine Electrolytes during 6 h after Oral Administration of Microcapsule (0.5, 0.1 mg/kg) Containing 20% (w/w) Piretanide and Tablet (0.5, 0.1 mg/kg) Containing 2 mg Piretanide per Tablet in Beagle Dogs

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Dosage form</th>
<th>Urine volume (ml)</th>
<th>Na⁺ (meq)</th>
<th>K⁺ (meq)</th>
<th>Cl⁻ (meq)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>Control ( (n=4) )</td>
<td>310 ± 35</td>
<td>55 ± 5</td>
<td>7.4 ± 0.8</td>
<td>53 ± 5</td>
</tr>
<tr>
<td>Tablet ( b) \ (n=4)</td>
<td>703 ± 44</td>
<td>102 ± 6</td>
<td>18 ± 2</td>
<td>116 ± 7</td>
<td></td>
</tr>
<tr>
<td>Microcapsule ( b) \ (n=4)</td>
<td>744 ± 42</td>
<td>108 ± 5</td>
<td>19 ± 2</td>
<td>119 ± 6</td>
<td></td>
</tr>
<tr>
<td>0.1</td>
<td>Control ( (n=3) )</td>
<td>290 ± 33</td>
<td>52 ± 6</td>
<td>8.4 ± 2.3</td>
<td>52 ± 7</td>
</tr>
<tr>
<td>Tablet ( b) \ (n=3)</td>
<td>474 ± 20</td>
<td>75 ± 4</td>
<td>10 ± 1</td>
<td>77 ± 4</td>
<td></td>
</tr>
<tr>
<td>Microcapsule ( b) \ (n=3)</td>
<td>446 ± 14</td>
<td>80 ± 5</td>
<td>9.1 ± 1.5</td>
<td>81 ± 4</td>
<td></td>
</tr>
</tbody>
</table>

\( a) \) Results are expressed as mean ± S.E. \( b) \) No significant difference between tablet and microcapsule \( (p > 0.05) \). \( c) \) Significantly different from control \( (p < 0.05) \).

Figs. 5, 6 and 7. A similar pattern was observed between the urinary electrolyte excretion rate and the urine excretion rate (Fig. 4), except that the patterns of the K⁺ excretion rate after the administration of 0.1 mg/kg of the gelatin microcapsule and ordinary tablet showed almost no difference from that of the control.

Table I summarizes the pharmacodynamic data, i.e., the cumulative urine volume and cumulative urinary electrolyte amounts (Na⁺, K⁺, Cl⁻), for 6 h after the oral administration of both preparations to beagle dogs and those of control dogs. No significant difference was observed between the gelatin microcapsule and ordinary tablet in the cumulative urine volume and cumulative urinary electrolyte amounts \( (p > 0.05) \). However, a significant difference from the control was observed in all the parameters except the urinary K⁺ amount at 0.1 mg/kg \( (p < 0.05) \).

**DISCUSSION**

In a previous study, we obtained a favorable linear relationship between the AUC and dose level after the oral administration of 2–6 mg/kg of the piretanide powder, gelatin microcapsule and ordinary tablet, and concluded that there was little possibility of a saturation phenomenon occurring in the oral doses from 0 to 6 mg/kg. Furthermore, the time-coursed plasma piretanide levels had not changed in the pattern itself at different dose levels. These results suggested that the oral administration of 0.1 mg/kg would show a pattern of the plasma piretanide level-time curve similar to that after the oral administration of 0.5 mg/kg; however, piretanide could not be detected in some plasma samples at 0.1 mg/kg as stated in the Plasma piretanide level section.

Although we have not established the criterion to evaluate the sustained-release property of pharmaceutical preparations, the gelatin microcapsule of piretanide had been estimated in the previous study to have a favorable sustained-release property as compared with the ordinary preparation at the same oral doses from the following results: (1) the time to reach the maximum plasma piretanide concentration \( (T_{max}) \) increased more after the administration of the microcapsule than the ordinary tablet; (2) the dissolution/absorption rate constant for piretanide from the microcapsule became smaller than that from the ordinary tablet; (3) the maximum plasma piretanide concentration \( (C_{max}) \) after microcapsule dosing was lower than that of the ordinary tablet; and (4) regardless of the above-mentioned results, the AUC's of the microcapsule and the ordinary tablet were almost the same.

In the present pharmacodynamic evaluation, the urine excretion rate and urinary electrolyte excretion rate, after the oral administration of the gelatin microcapsule, tended to become smaller in the maximum value and was sustained for a longer period than the ordinary tablet. Furthermore, no difference was observed between the two dosage forms in the cumulative urine volume and cumulative urinary electrolyte excretion for 6 h after the administration. These results may also indicate the prolongation of the release of piretanide from the gelatin microcapsules.
CONCLUSION

Piretanide, which is expected to be used frequently as a hypotensive diuretic, was microencapsulated with gelatin. This procedure was applied to piretanide to sustain or prolong the antihypertensive action of piretanide. The prepared gelatin microcapsules containing piretanide were effective in exhibiting a satisfactory prolongation property without any loss of various cumulative pharmacodynamic activities, as compared with the ordinary tablet used as the standard. Furthermore, the microencapsulation obviously reduced a side effect, acute diuresis (rate of pharmacodynamic activity), caused by the administration of the ordinary tablet. Unfortunately, we could not evaluate the prolongation of the antihypertensive effect itself in this study, because no suitable method has been established to measure successive changes of the blood pressure in beagle dogs. However, we are convinced that because of the physicochemical properties and in vivo pharmacodynamic activities of piretanide it is the compound that is favorable for formulation as a sustained-release preparation.

REFERENCES