Design and In Vitro Evaluation of Slow-Release Dosage Form of Piretanide: Utility of β-Cyclodextrin:Cellulose Derivative Combination as a Modified-Release Drug Carrier

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Abstract To modify the release rate of piretanide, a potent loop diuretic, a double-layer tablet was designed, and in vitro release was evaluated. For a rapidly releasing portion, hydrophilic β-cyclodextrin derivatives were employed to form a water-soluble complex with piretanide. For a sustained-release portion, cellulose derivatives were used to provide a prolonged release behavior. The release rate of piretanide in the pH range 1.2–6.8 was automatically monitored by a pH-changeable dissolution testing apparatus. The solubility of piretanide in an acidic medium was significantly improved by complexations with dimethyl-β-cyclodextrin (DM-β-CyD) and hydroxypropyl-β-cyclodextrin (HP-β-CyD). The pH-independent slow release was attained by use of hydroxypropylcellulose (HPC):ethylcellulose (EC) matrices. Then, an optimal formulation of a double-layer tablet was obtained by the combination of each fraction. For example, the tablet consisting of the [DM-β-CyD(HPC:EC)] system in the weight ratio [1:3(3:1)] provided a sufficiently slow release of the drug over a period of 8 h in a wide pH region following an initial rapid dissolution.

Many attempts have been made to design slow-release oral preparations of loop diuretics such as furosemide.1–3 These efforts were based on the clinical need to prevent the high peak concentrations of diuretics normally seen after dosing with a regular tablet.4 Piretanide, a candidate as an antihypertensive diuretic, has also been known to cause pollakiuria due to its remarkable diuretic action.4 To obtain an effective hypotensive effect with piretanide, maintenance of a suitable blood level for a long period of time with minimum frequency of administration is required.5 Since the stomach is expected to be an important absorption site of piretanide,6 absorption has to start there and no lag time should be allowed for drug release from the dosage form. This led us to design a double-layer tablet formulation consisting of a pH-independent slow-release matrix together with a rapid-dissolving fraction. In the present study, water-soluble β-cyclodextrin derivatives such as 2-hydroxypropyl-β-cyclodextrin (HP-β-CyD)7 and 2,6-dimethyl-β-cyclodextrin (DM-β-CyD)8 were used for the fast-dissolving fraction to improve the low solubility of piretanide in an acidic medium. For the slow-release fraction, a combination of ethylcellulose (EC) and hydroxypropylcellulose (HPC) or hydroxypropylmethylcellulose (HPMC) was used to elicit an appropriate hydrophobicity. The in vitro release behavior of piretanide from the tablets was evaluated by a pH-changeable dissolution testing apparatus in the pH 1.2 to 6.8 region.

Experimental Section

Materials—Piretanide was supplied by Hoechst Japan (Kawagoe, Japan). β-Cyclodextrin, 2-hydroxypropyl-β-cyclodextrin (degree of substitution, 5.8),7 and 2,6-dimethyl-β-cyclodextrin were supplied by Nihon Syokuhin Kako (Tokyo, Japan). Ethylcellulose (Hercules, Wilmington, DE), hydroxypropylcellulose-M (mol wt = 130,000; Nihon Soda, Tokyo, Japan), hydroxypropylmethylcellulose (type-2910, 3 cps, mol wt = 13,000; Shin-Etsu Chemical, Tokyo, Japan), and potato starch (Wako Pure Chemical Industries, Osaka, Japan) were used as supplied. Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Solubility Studies—Solubility measurements were carried out according to Higuchi and Connors.9 A 10-mg amount of piretanide was added to 3.0 mL of the first fluid of disintegration test in the Japanese Pharmacopoeia XI (JP XI) (2.0 g of NaCl and 24 mL of 10% HCl in 1000 mL of water, pH 1.2), containing various amounts of β-CyD derivatives (1.0–50.0 × 10−3 M) in glass-stoppered test tubes. The tubes were sealed and then shaken in a thermostated water bath at 37°C. In the case of β-CyD, the concentration range 1.0–20.0 × 10−3 M was employed because of the limited solubility in water (−2%, w/v, at 25°C). After equilibrium was attained (−3 days), an aliquot was centrifuged and pipetted through a cotton plug. The 0.5 mL of filtrate was diluted (7–50 fold) with 0.5 M NaOH solution and analyzed spectrophotometrically at a wavelength of 275 nm. An apparent 1:1 stability constant (K) was calculated from the initial linear portion of the phase solubility diagram (see Figure 2) in the same manner as reported previously.9

Preparation of Tablets—All the same powders were prepared according to the kneading method.10 For example, piretanide (1.0 g) and DM-β-CyD (4.0 g) were triturated with a small amount of water (−5 mL), and the slurry was further kneaded thoroughly for −80 min. The paste thus obtained was dried under reduced pressure at room temperature for 2 days. Differential scanning calorimetry (DSC) and X-ray diffraction patterns of the sample powders were taken to examine a phase change of piretanide in the solid state under conditions similar to those reported previously.10 The plain tablets of 5-mm diameter were prepared by compressing −50 mg of sample powder (content of piretanide, 10 mg) in various combination ratios under a pressure of 1000 kg/cm2 in a hydraulic press (model P-16B; Hattori, Japan). In the case of double-layer tablets, the slow-release fraction was lightly compressed, and then the fast-dissolving fraction was directly added onto the tablet and prepared in the same manner as the plain tablets. A total mass of the double-layer tablet was adjusted to give a net content of 10 mg of piretanide on the basis of the fractional amount of the drug in each constituent.

Dissolution Studies—A pH-changeable dissolution testing apparatus was made to evaluate the drug release from the controlled-release type dosage forms. As illustrated in Figure 1, this apparatus basically consisted of a commercially available dissolution testing apparatus (Toyama Sangyo, Magistr-Bath, Tokyo, Japan), a pH stat (Toaenden, Tokyo, Japan), a pumping unit (Hitachi 655A-11, Tokyo, Japan), a UV monitor (Hitachi L-4000, Tokyo, Japan), and a personal computer system (NEC PC-9801, Tokyo, Japan). All the sections were securely connected with Teflon tubes and stainless steel materials to provide a closed system. The dissolution medium employed was a mixed buffer solution (1000 mL) of 0.05 M hydrochloric acid, 0.05 M acetic acid, and 0.05 M phosphoric acid, which was initially adjusted to pH 1.2 and maintained at 37°C. The volume of the dissolution medium employed was sufficient to maintain a sink condition in the pH range 1.2–6.8. The dissolution test was essentially performed...
Figure 1—pH-Changeable dissolution testing apparatus.

According to the paddle method in JP XI, where the rotation speed of paddle was fixed at 100 rpm unless otherwise stated. A tablet was placed in the bottom of the reservoir using a stainless steel sinker to avoid flotation of the tablet, and the dissolution medium was continuously circulated through a glass filter (33; Ø 20–30 μm) at a flow rate of 3 mL/min. The lag time from the reservoir to the UV monitor was ~0.5 min. At specified time points, the pH of the dissolution medium was shifted from 1.2 to 4.0 and then fixed at 6.8 by the addition of 0.1 M NaOH solution directly to the original dissolution medium. The amount of piretanide dissolved in the medium was automatically measured by UV monitor at a wavelength of 276 nm, where the linearity of concentration versus absorption was ascertained. The release rates of the drug as a function of pH were computed with a personal computer. All samples were run in duplicate and agreed to within <3% of the mean.

Results and Discussion

Fast-Dissolving Form of Piretanide—To improve the poor dissolution characteristic of piretanide in acidic medium, inclusion complexation with β-CyD and its water-soluble derivatives was employed. Figure 2 shows the phase solubility diagrams of piretanide:β-CyDs systems in acidic medium (pH 1.2). The solubility of piretanide (2.30 × 10⁻⁴ M at pH 1.2) increased in a linear fashion as a function of β-CyDs concentration, and the resulting solubility curves can be classified as a type A. The apparent stability constants (K') were estimated on the basis of the assumption that a 1:1 complex is formed. The K' values obtained for β-CyD, HP-β-CyD, and DM-β-CyD complexes were of 175, 247, and 634 M⁻¹, respectively. The phase changes of piretanide in the solid state through the inclusion complexations were examined by powder X-ray diffractometry and DSC, comparing with the corresponding physical mixtures in the same molar ratio. As shown in Figure 3, the diffraction pattern of the physical mixture of piretanide:HP-β-CyD system, as a typical example, was simply the superposition of each component, while that of the complex was somewhat different from each constituent and constitutes a new solid phase. Other complexes also gave a diffuse diffraction pattern, suggesting that they are much less crystalline than the physical mixtures. It was difficult to determine the crystal packing of the complexes because the diffraction patterns were too complicated to be reliably indexed by the powder method. As shown in Figure 4, the DSC thermograms also suggested the less crystalline nature of piretanide in the form of HP-β-CyD complex [i.e., the endothermic peak (230 °C) due to the melting of piretanide decreased significantly in the complex].

Figure 5 shows the release profiles of piretanide from the tablets containing piretanide and its β-CyD complexes as a function of pH of the medium. The poor dissolution characteristic of the un-ionized form of piretanide (pKₐ for COOH group: 3.8) in the acidic pH region was significantly improved by inclusion complexation with β-CyDs, while no remarkable rate change was observed for starch granulation, as reported previously. From inspection of the data in Figures 2 and 5, both hydrophilic β-CyD derivatives were more effective than the parent β-CyD in increasing the dissolution rate of piretanide, probably due to the limited solubility of β-CyD in water. Therefore, DM-β-CyD and HP-β-CyD were selected as the drug carriers of the fast-dissolving fraction in the following studies.

Slow-Release Fraction of Piretanide—To obtain a suitable slow-release fraction, ethylcellulose (EC), a typical hydrophobic cellulose derivative, was used in combination with hydrophilic cellulose derivatives such as HPC or HPMC. Figure 6 shows the release profiles of piretanide from the tablets consisting of three different cellulose derivatives as a function of the pH of the medium. The release rate was significantly suppressed by the use of EC and HPC compared with HPMC, showing a pH-independent pseudo zero-order release in the pH range 1.2–6.8.

Then, the effect of the mixing ratio of EC and HPC or HPMC on the release rate of piretanide was further surveyed. As shown in Figure 7A, the decrease in rate appeared to be

Figure 2—Phase solubility diagrams of the piretanide:β-cycloexdextrin systems in aqueous solution (pH 1.2) at 37 °C. Key: [C] β-CyD; [●] HP-β-CyD; [△] DM-β-CyD.

Figure 3—Powder X-ray diffraction patterns of the piretanide:hydroxypropyl-β-cycloextrin system in a 1:1 molar ratio. Key: [a] piretanide alone; [b] HP-β-CyD alone; [c] physical mixture of piretanide and HP-β-CyD; and [d] piretanide:HP-β-CyD complex.
The DSC thermograms of the piretanide:hydroxypropyl-β-cyclodextrin system in a 1:1 molar ratio. Key: (a) piretanide alone; (b) HP-β-CyD alone; (c) physical mixture of piretanide and HP-β-CyD; and (d) piretanide:HP-β-CyD complex.

Figure 5—Release profiles of piretanide from plain tablets containing piretanide (10 mg) in starch or β-cyclodextrin complexes at 37 °C as a function of the pH of the medium. Key: (○) starch mixture; (●) β-CyD complex; (■) HP-β-CyD complex; (▲) DM-β-CyD complex. The broken line shows the pH of the medium.

Figure 6—Release profiles of piretanide from plain tablets containing piretanide (10 mg) in various cellulose derivatives at 37 °C as a function of the pH of the medium. Key: (○) HPC; (●) HPMC; and (▲) EC. The broken line shows the pH of the medium.

Release of Piretanide from Double-Layer Tablet—The next step was an optimization of the release rate of piretanide from double-layer tablets. For the in vitro evaluation of the slow-release formulation, the following desirable attributes were sought: (1) a release rate should be at least 90% within 6–8 h, which may be the average passage time of a tablet in the gastrointestinal tract after oral administration; (2) a sufficient release of drug (at least 50% for total content) in the stomach should be necessary to offer a more balanced bioavailability because the stomach is an important absorption site as well as one of the high metabolic organs for piretanide (in analogy with furosemide); (3) a release rate should not be remarkably affected by changes in the pH of the dissolution medium and rotation speed of the paddle to minimize the effects of gastrointestinal pH and motility, and the effect of foods in the controlled-release type oral preparations. On the basis of these goals, the amount of each component in the double-layer tablets was determined from the survey of the release rates as a function of pH in the dissolution medium and rotation speed of the paddle.

Figure 8 shows the release profiles of piretanide from the double-layer tablets consisting of the DM-β-CyD or HP-β-CyD complex as the fast-dissolving fraction and the HPC/EC (1:3) matrix as the slow-release fraction in 1:1, 1:3, and 1:5 weight ratios, respectively (the rotation speed of the paddle was fixed at 100 rpm). It is apparent that the initial release rate of the drug increased with increasing amount of the fast-dissolving fractions (1:5 < 1:3 < 1:1), where the
release behavior of the DM-β-CyD system (Figure 8A) and the HP-β-CyD system (Figure 8B) was nearly identical, as expected from the dissolution profiles of the corresponding complex (Figure 5). From inspection of the release profiles, the tablets consisting of the [DM-β-CyD(HPC:EC)] system (Figure 8A) and the [HP-β-CyD(HPC:EC)] system (Figure 8B) in the weight ratio [1:3:1] elicited a sufficient slow release for a long period of time in a wide pH region following an initial rapid dissolution (>50% drug release at stomach pH), where almost 90% of drug release was attained after 8 h. As shown in Figure 9, however, the release rate from the DM-β-CyD system (Figure 9A) was little affected by the rotation speed of the paddle with the HP-β-CyD system (Figure 9B). This may be due to the appropriate binding of the powders between DM-β-CyD and cellulose derivatives to make up the double-layer tablet, owing to the highly surface active nature of DM-β-CyD. Therefore, the DM-β-CyD system may be preferable to the slow-release type oral preparation of piretanide. From the practical point of view, however, HP-β-CyD cannot be excluded because of the superior properties (e.g., highly water soluble, amorphous powder with no detectable oral toxicity) as a pharmaceutical additive.\textsuperscript{7,16}

Conclusions

From the above mentioned data it is concluded that the combination of DM-β-CyD and cellulose derivatives (EC, HPC) may be useful as the release-modifying additives of piretanide. In particular, the tablet consisting of the [DM-β-CyD(HPC:EC)] system in the weight ratio [1:3:1] could almost satisfy the goals made for in vitro conditions [i.e., (I) an initial rapid dissolution (>50% drug release) at stomach pH; (II) a slow-release for a long period of time (>90% within 8 h); and (III) a little alteration of the release rate by pH of the dissolution medium and rotation speed of the paddle]. This kind of knowledge would allow the release control of oral preparations to attain an immediate and efficient bioavailability of acidic drugs. In addition, the pH-changeable dissolution testing apparatus used here may be extended to evaluate the release behaviors of enteric-coating type or delayed-release type dosage forms which are much more sensitive to in vivo physiological pH changes in the gastrointestinal fluid.\textsuperscript{14} Anyhow, in vitro vivo correlation studies will serve to clarify the above-mentioned subjects.

References and Notes

11. The details of the programming and procedure are available from the authors on request.

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