Evaluation of a Pharmaceutical Preparation of 2-[4-(*p*-Fluorobenzoyl)-piperidin-1-yl]-2'-acetonephthone Hydrochloride with Enhanced Dissolution Rate

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A granule of 2-[4-(*p*-fluorobenzoyl)-piperidin-1-yl]-2'-acetonephthone hydrochloride (E2001) with an enhanced dissolution rate (preparation A) prepared by a method previously reported (T. Tokumura, T. Tanaka, *Yakuzaigaku*, accepted) was evaluated by the dissolution test and its bioavailability in beagle dogs was determined.

The dissolution rate of E2001 from preparation A at pH 6.0–6.8 was increased significantly in comparison with that from preparation B (a physical mixture of the same formula). There was no significant difference in $C_{\max}$, area under the concentration–time curve (AUC_{0-6h}), or $T_{\max}$ between preparations A and B when a dose equivalent to 20 mg of E2001 was administered to the beagle dogs. However, a significant difference was observed between the AUC_{0-6h} values after oral administration of the two preparations with NaHCO_3 . These findings suggest that the bioavailability of E2001 from the oral administration preparation A will be higher than that of preparation B.

Keywords anti-ischemic agent; dissolution rate; bioavailability; beagle dog; pH

2-[4-(*p*-Fluorobenzoyl)-piperidin-1-yl]-2'-acetonephthone hydrochloride, E2001, is a novel anti-ischemic agent with an inhibitory effect on glutamate release, and is expected to be effective in the treatment of memory impairment and neurological deficits in cerebrovascular disease.¹⁾

We have studied the physicochemical properties and oral bioavailability of E2001. Its solubility around a neutral pH was extremely poor (0.1 $\mu\text{g}/\text{ml}$) and its oral bioavailability was decreased by administration with NaHCO_3 in beagle dogs.²⁾ Methods to enhance the dissolution rate of E2001 around a neutral pH have been sought, since the oral bioavailability in persons with achlorhydria and anacidity may decrease, and many aged persons have these conditions.³⁾

The dissolution rate of E2001 at pH 6.8 was enhanced by ground mixtures and coprecipitates. In accordance with the result of the stability study, the ground mixture of E2001 with lactose was employed to enhance the rate of dissolution. Powders obtained by grinding usually have low fluidity and high dustability and adhesion, and these properties are generally improved by granulation. A granule with an enhanced dissolution rate was achieved by granulating the ground mixture with ethanol and hydroxypropylcellulose-L (HPC-L) as solvent and binder, respectively. Furthermore, a tablet with an enhanced dissolution rate was prepared from the granule. In a comparison of the granule and the tablet, the granule had more rapid dissolution rate.⁴⁾

In the previous study,²⁾ to examine the bioavailability of E2001 a capsule containing 20 mg of E2001, 280 mg of lactose and 15 mg of HPC-L was administered orally to beagle dogs. The dissolution rate of E2001 from the content of the capsule (corresponding to preparation B in this paper) can be enhanced by preparing and granulating a ground mixture.⁴⁾ Therefore, we prepared a granule (preparation A), the formula of which was the same as that of the capsule (preparation B) administered to dogs, with the enhanced dissolution rate. The dissolution

behavior of E2001 from this enhanced rate granule (corresponding to preparation A in this paper) at pH 6.8 was reported previously.⁴⁾ In this report, we determined the dissolution rates of E2001 from the granule in buffer solutions with various pH values and the oral bioavailability of the capsule with the granule in beagle dogs.

MATERIALS AND METHODS

Materials E2001 was synthesized in our laboratories.⁵⁾ All other reagents and chemicals used were of analytical grade or of the grade listed in the Pharmacopoeia of Japan.

Procedure for Dissolution Study The paddle method described in the Pharmacopoeia of Japan, edition XII (JP XII), was used. Buffer solutions of pH 1.2, 4.0, 5.0, 6.0 and 6.8 were prepared by mixing the 1st fluid and 2nd fluid of JP XII. A certain amount of each sample corresponding to 5 mg of E2001 was placed in a beaker containing the medium (500 ml) equilibrated to 37 °C. The paddle was rotated at 100 rpm, and 3 ml of the sample solution was taken through a disposable filter (Acrodisc, 0.2 μm , German Co., Ltd.) at appropriate intervals. The concentrations of E2001 were determined by the UV absorption method at 250 nm.

Preparation of Sample for Dissolution Test Preparation A: 1 g of E2001 and 14 g of lactose were ground in a labo-mill (model UT-21 from Yamato, Tokyo, Japan) for 2 h. After the ground mixture (14.1 g) and 0.72 g of HPC-L were mixed in a mortar, the mixture was granulated by addition of 5 ml of ethanol, and then the granule was dried for 2 h at 40 °C. Granules retained on a 150 mesh sieve and passing a 60 mesh sieve were used as preparation A.

Preparation B: E2001 (0.6 g), 8.4 g of lactose and 0.45 g of HPC-L were mixed in a mortar, and the mixture was used as preparation B. The formula of preparation B is the same as that of the content in the capsule administered to beagle dogs previously.²⁾

Absorption Study Three male beagle dogs were used after fasting for 18 h. The intervals between drug ad-

ministrations were more than one week. Preparation A corresponding to 20 mg of E2001 was packed into a gelatin capsule, which was administered alone or together with 400 mg of NaHCO_3 in gelatin capsules with 30 ml of water. At given intervals, a 1.5 ml blood sample was taken from the forefoot vein. The blood samples were centrifuged for 10 min at 3000 rpm. The plasma layer was removed and frozen until analysis. E2001 in the plasma was determined according to the method reported previously.²⁾

RESULTS AND DISCUSSION

Dissolution Behavior Figure 1 shows the dissolution behavior of E2001 from preparation A. Good dissolution was observed at pH 1.2–6.0; at pH 6.8 it was lower, but the percent dissolved was over 60%. Figure 2 shows the results of the tests with preparation B. At pH 1.2–5.0 dissolution was very rapid but it decreased at a pH above 5.0. The solubility of E2001 at pH 5–6.8 was about $0.1 \mu\text{g/ml}$.²⁾ The percent dissolved in the dissolution test

corresponding to the solubility was about 1%. These test results for both preparations at pH 5–6.8 indicate supersaturation. The supersaturation of preparation B may be caused by the addition of HPL-C. The higher supersaturation of preparation A is considered to be caused by its more rapid dissolution rate. Figure 3 shows the percent dissolved after 20 min in the dissolution tests of the two preparations, and indicate clearly that the dissolution of preparation A is faster than that of preparation B at pH 6.0 and 6.8. These findings suggest that oral administration of preparation A will result in sufficient dissolution of E2001 even in achlorhydric patients.

Absorption Study Figure 4 shows the mean plasma levels of E2001 after oral administration of preparation A to the dogs, and the previous reported data of preparation B.²⁾ When a dose equivalent to 20 mg of E2001 was administered to dogs, preparation A gave a plasma level of $114.6 \pm 13.5 \text{ ng/ml}$ (mean $\pm$ S.E.) at 0.33 h, which is 2.6 times that obtained with preparation B. This initial increase in drug absorption might be due to the higher

Fig. 1. Dissolution Rate Curves of E2001 from Preparation A in Buffer Solutions of Various pH Values

●, pH 1.2; ○, pH 4.0; □, pH 5.0; ■, pH 6.0; △, pH 6.8. Each point is the mean $\pm$ S.D. of three determinations.

Fig. 2. Dissolution Rate Curves of E2001 from Preparation B in Buffer Solutions of Various pH Values

●, pH 1.2; ○, pH 4.0; □, pH 5.0; ■, pH 6.0; △, pH 6.8. Each point is the mean $\pm$ S.D. of three determinations.

Fig. 3. Percent of E2001 Dissolved in Preparations A (■) and B (□) at 20 min after the Start of the Dissolution Test

Each bar represents the mean $\pm$ S.D. of three determinations.

Fig. 4. Plasma Concentration of E2001 after Oral Administration of Preparations A (●) and B (○)^{a)}

a) Data from ref. 2. The dose of E2001 is 20 mg. Each point represents the mean $\pm$ S.E. of 3 dogs.

TABLE I. Bioavailability Parameters of E2001 for Oral Administration of Preparations A and B

Administered sample	$C_{\max}$ (ng/ml)	$T_{\max}$ (h)	AUC_{0-6h} (ng h/ml)
Preparation A	189.5 ± 37.8	0.56 ± 0.11	165.8 ± 34.1
Preparation B ^{a)}	175.1 ± 75.4	0.67 ± 0.19	172.6 ± 52.5
Preparation A + NaHCO ₃	146.8 ± 41.5	0.56 ± 0.22	124.5 ± 18.2 ^{b)}
Preparation B + NaHCO ₃ ^{a)}	53.0 ± 7.9	0.56 ± 0.11	61.2 ± 1.8 ^{b)}

Each value represents the mean $\pm$ S.E. of 3 dogs. a) Data from ref. 2. b) $p < 0.05$ by t -test.

Fig. 5. Plasma Concentration of E2001 after Oral Administration of Preparations A (●) and B (○)^{a)} with NaHCO₃ (400 mg)

a) Data from ref. 2. The dose of E2001 is 20 mg. Each point represents the mean $\pm$ S.E. of 3 dogs.

dissolution rate of preparation A compared with that of preparation B as mentioned above. However, there was no significant difference in the maximum plasma level of E2001 ($C_{\max}$), the area under the concentration-time curve (AUC_{0-6h}) or the value of the time to reach $C_{\max}$ ($T_{\max}$) between the two preparations, as Table I shows. This indicates that enhancement of the dissolution rate of

preparation A does not affect the bioavailability of E2001. The absence of this enhancement of bioavailability is probably due to the acidity of the stomach. The gastric pH of a beagle dog under fasting conditions was reported to show a large inter-individual variation (pH range; 2–7).⁶⁾ However, the pH beyond 6.0 is considered to be a phenomenon that appears only rarely. Accordingly, the enhanced dissolution rate of preparation A at pH 6.0–6.8 does not affect the bioavailability.

Figure 5 shows the effect of NaHCO₃ on the absorption of E2001 from preparations A and B. When preparation B was administered to dogs with NaHCO₃, the plasma levels of E2001 remained extremely low.²⁾ However, in the case of preparation A, the plasma levels and other bioavailability parameters were unaffected by simultaneous administration of NaHCO₃ as Table I shows. A significant difference was observed between the AUC_{0-6h} values after oral administration of both preparations with NaHCO₃. These findings reflect the difference in the dissolution rate of E2001 around the neutral pH as shown in Fig. 3, because the acid in the stomach is neutralized by NaHCO₃. The condition of the stomach after administration of NaHCO₃ can be considered similar to that in patients with achlorhydria. In conclusion, oral administration of preparation A is expected to provide higher absorption of E2001 than that of preparation B in achlorhydric patients.

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