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Improvement of oral bioavailability of flurbiprofen from flurbiprofen/β-cyclodextrin inclusion complex by action of cinnarizine

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ABSTRACT

Improvement of the oral bioavailability of flurbiprofen (Flu) after oral administration of flurbiprofen/β-cyclodextrin inclusion complex (Flu/β-CD) by the action of cinnarizine (CN) was investigated. Flu and Flu/β-CD were administered orally to fasted rats at a dose of 20 mg/kg as Flu. Thirty minutes after drug administration, CN dissolved in pH 4.0 buffer solution or pH 4.0 buffer solution alone was administered to the rats. The dose of CN was 0.17 mg/kg. Blood samples were taken from rats and Flu concentrations in plasma samples were determined by HPLC. It was found from the comparison of Flu and Flu with CN (Flu + CN) that CN had no effect on plasma concentrations of Flu after oral administration of Flu. The mean plasma levels after oral administration of Flu/β-CD with CN (Flu/β-CD + CN) were larger not only than those of Flu and Flu + CN but also than those of Flu/β-CD. The value of C_{max} in Flu/β-CD + CN was significantly larger than that of Flu/β-CD. This is considered to be caused by the action of CN as a competing agent. This mechanism was supported by the result of solubility study in which Flu solubility in β-CD solution decreased with the addition of CN. It was found from these results that CN had strong ability as a competing agent in vivo.

1. Introduction

Flurbiprofen, 2-(2-fluoro-4-biphenyl) propionic acid (Flu), is an anti-inflammatory drug that is effective and safe in the treatment of rheumatoid arthritis [1]; however, because of its low water solubility, poor absorption characteristics of Flu have been reported [2]. To improve the dissolution rate and oral bioavailability, several investigations were made using inclusion complexes of the drug with cyclodextrins [2,3]. However, the dissolution profiles of Flu from inclusion complexes in buffer solutions at various pH values have not been reported, and the relationship between oral doses and bioavailability parameters has not been clarified. Therefore, a bioavailability study for Flu was started, and the following results were reported: (1) A simple high-performance liquid chromatographic (HPLC) procedure for determining the concentration of Flu in rat plasma was developed [4]. (2) The pharmacokinetics of Flu in rats after intravenous administration at doses of 1, 3, and 10 mg/kg were linear [4]. (3) In the dissolution profiles of Flu and its β-cyclodextrin inclusion complex (Flu/β-CD) in buffer solutions at various pH values, the dissolution of Flu from Flu and Flu/β-CD was almost complete within 15 min at pH 6.8 and 8.0 but was extremely reduced at pH 1.2 and 4.0, at which the rates of Flu/β-CD were about 10 times higher than those for Flu [5]. (4) An apparent linear relationship between doses and C_{max}, and AUC was observed after oral administration of Flu and Flu/β-CD at a dose range of 1–10 mg/kg in rats. The values of C_{max} and AUC for Flu at 30 mg/kg, however, were much lower than would have been predicted from doses of 1–10 mg/kg. Those of Flu/β-CD were also lower than the predicted values, but the difference was slight [5].

On the other hand, cinnarizine (CN) and β-CD formed an inclusion complex with a molar ratio of 1:2 (CN:β-CD). The apparent stability constant was 6200 M^{-1} [6]. It was confirmed from a membrane permeation study that CN acted as a competing agent [7] and that its action was much stronger than that of DL-phenylalanine previously reported as a competing agent [8].

The AUC of Flu after intravenous administration at 10 mg/kg was 417.08 μg h/ml in rats [5]. The absolute bioavailability after oral administration of Flu and Flu/β-CD at 10 mg/kg was 81.6% and 99.0%, respectively. At a dose of 10 mg/kg, no significant difference was observed between the oral bioavailability parameters of Flu and Flu/β-CD. It is reasonable that no significant difference was observed in the AUC value due to Flu showing a rapid dissolution rate at neutral pH; however, C_{max} and T_{max} should differ because the dissolution rate of Flu/β-CD was much faster at acidic pH. This phenomenon was considered to be caused by the large stability constant of Flu/β-CD, which was reported to be 5080 M^{-1} [9]. Therefore, we tried to improve C_{max} and T_{max} after oral administration of Flu/β-CD by the action of CN as a competing agent. The dose of this experiment, 20 mg/kg as Flu, was chosen for the following two reasons: (1) the dose was considered to be in the

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range of linearity in the relationship between the dose of Flu/β-CD and AUC; and (2) when CN achieves an improvement, higher doses of Flu/β-CD can represent the stronger action of CN. In this report, we describe the improvement of Flu bioavailability with oral administration of Flu/β-CD by the action of CN. In addition, the action of CN as a competing agent was confirmed by the solubility study because it had been found that membrane permeation studies for Flu/β-CD and CN are impossible due to the adsorption characteristics of Flu and CN [7].

2. Materials and methods

2.1. Materials

β-Cyclodextrin (β-CD) was purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan), and flurbiprofen (Flu) and cinnarizine (CN) were from Sigma Chemical Company (St. Louis, MO, USA). Other chemicals were of reagent or HPLC grade.

2.2. Preparation of Flu/β-CD inclusion complex

The inclusion complex of Flu with β-CD (Flu/β-CD) [3] was prepared by the coprecipitation method, according to the previously reported method. The content of the drug in Flu/β-CD was 17.04%. This value agreed with the previously reported values [9].

2.3. Solubility study

Five-hundred milligram of Flu to 50 ml of 0.5 mmol/l (0.5 M) β-CD solution was added, which was stirred for 48 h at room temperature (23–25 °C). This suspension was filtered through a 0.45 μm membrane filter. The filtrate of which the initial 5 ml was discarded was collected. Then 0.99 ml of the filtrate was added to a test tube. Ten microliters of dimethylsulfoxide (DMSO) solutions containing 0, 10, 30, 50 and 100 μg of CN was added to the tubes. The final concentrations of CN in the tubes were 0, 10, 30, 50, and 100 μg/ml. After the tubes were well stirred and stored for 24 h at 23–25 °C, they were centrifuged at 3500 rpm for 20 min. The concentration of Flu in the supernatant was determined by HPLC. The solubility of Flu under the same conditions was also determined.

2.4. Determination of Flu in rat plasma by HPLC

The concentration of Flu in plasma was determined with an HPLC assay [4] consisting of a Model LC-9A pump, equipped with a Model SCL-6B system controller, a Model SPD-6A UV spectrophotometric detector, a Model CTO-6A column oven, a Model C-R4AX Chromatopac, and a Model SIL-6B autoinjector, all from Shimadzu (Kyoto, Japan). The mobile phase was acetonitrile–water–perchloric acid (60%)–sodium perchlorate monohydrate (520:480:1:5, v/v/v/w). The chromatographic column was a YMC Pack AM312 ODS (150 mm × 6 mm I.D., particle diameter 5 μm) from YMC Co., Ltd. (Kyoto, Japan). The flow rate, wavelength for determination, and temperature of the column were 1 ml/min, 254 nm and 40 °C, respectively. Methanol (200 μl) containing 0.2% perchloric acid was added to 100 μl of plasma cooled in an ice-bath. The mixture was stirred on a vortex mixer for 1 min and centrifuged at 3000 rpm for 5 min; 50 μl of the supernatant was injected into the chromatograph.

2.5. Animal study

Male Sprague-Dawley rats were used. All rats (272–285 g body weight) were allowed free access to water but were fasted for 18 h before drug administration and 8 h after drug administration. Flu and Flu/β-CD were dispersed in 0.5% methylcellulose solution to make suspensions with concentrations of 6.7 mg/ml as Flu; 3 ml/kg of each suspension was administered orally. The doses of Flu and Flu/β-CD were 20 mg/kg as Flu. Thirty minutes after drug administration, a solution or a pH 4 buffer solution of CN, competing agents, was administered orally at 3 ml/kg. The concentration of CN in CN solution was 56.4 μg/ml. CN was dissolved in phosphate buffer at pH 4. Buffer solution containing no CN was also administered. The dose of CN was 0.17 mg /kg. Blood samples (0.3 ml) were withdrawn from the jugular vein of rats which were lightly anesthetized with diethylether at 1, 1.5, 2, 3, 5, 7, and 24 h after dosing. The samples were centrifuged at 3000 rpm for 15 min to obtain plasma (0.12–0.15 ml), which was subjected to HPLC to determine the Flu concentration on the same day, according to the method described above. All animal experiments were carried out according to the Guiding Principles for the Care and Use of Laboratory Animals of Hoshi University.

2.6. Data analysis

Pharmacokinetic analysis of plasma concentration data was performed using model independent methods. The area under the plasma concentration–time curve (AUC) and the mean residence time (MRT) were calculated by a previously described method [10]. The peak plasma concentration (C_{max}) and the time taken for attaining the peak concentration (T_{max}) were determined from individual plasma concentration–time curves.

Statistical comparisons of pharmacokinetic parameters were made using one-way analysis of variance, and when significant differences were found, Scheffe's F test was applied.

3. Results and discussion

Fig. 1 shows the mean plasma levels of Flu after the oral administration of Flu and Flu/β-CD with or without CN to rats at a dose of 20 mg/kg as Flu. The bioavailability parameters of these treatments are listed in Table 1.

The mean plasma levels after oral administration of Flu and Flu with CN (Flu + CN) were almost the same, as shown in Fig. 1, and no difference was observed between these bioavailability parameters in Table 1. This result indicates that CN does not affect the absorption of Flu. The values of C_{max} and AUC at 10 mg/kg, which were previously reported, were 31.66 μg/ml and 340.37 μg h/ml, respectively [5]. The AUC value at 20 mg/kg was two times higher

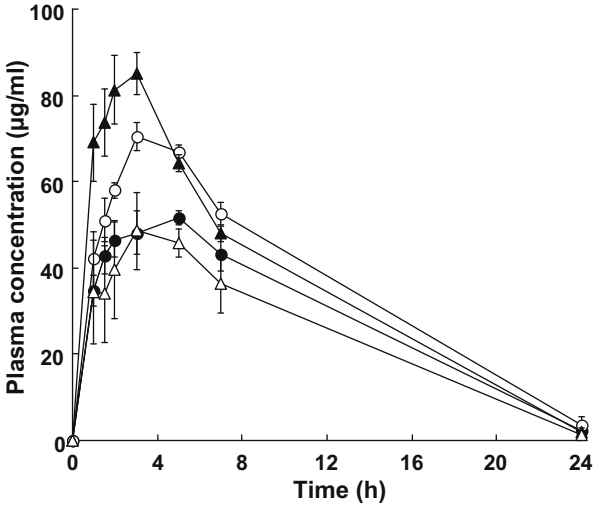

Fig. 1. Effect of CN on plasma Flu concentration profiles after oral administration of Flu or Flu/β-CD to rats at a dose of 20 mg/kg as Flu. (●) Flu, (○) Flu/β-CD, (▲) Flu/β-CD + CN, (△) Flu + CN. Each point represents the mean ± SE of four rats.

Table 1
Effect of CN as a competing agent on bioavailability parameters of Flu after oral administration to rats at a dose of 20 mg/kg.

Samples administered	C_{max} (μg/ml)	T_{max} (h)	AUC (μg h/ml)	MRT (h)
Flu	51.92 ± 2.88	5.00 ± 0.82	694.15 ± 72.72	7.33 ± 0.49
Flu/β-CD	71.27 ± 2.55[b]	3.50 ± 0.50	870.11 ± 75.36	7.32 ± 0.80
Flu/β-CD + CN	87.99 ± 5.09[a]	2.75 ± 0.25	868.78 ± 72.27	7.22 ± 0.73
Flu + CN	56.62 ± 2.49	4.00 ± 1.00	568.96 ± 80.37	7.00 ± 0.57

Each value represents the mean and SE of four rats.
[a] C_{max} value is significantly larger than the values of Flu, Flu/β-CD, and Flu + CN ($p < 0.05$).
[b] C_{max} value is significantly larger than the values of Flu, and Flu + CN ($p < 0.05$).
Flu and Flu/β-CD were administered orally as suspensions of 0.5% methylcellulose solution. CN was administered orally 30 min after drug administration. CN was dissolved in pH 4 buffer solution. When CN was not administered, pH 4 buffer solution without CN was administered.

than that at 10 mg/kg, which indicates that the dose of 20 mg/kg is in the range of linearity and that the assumption at the start of the experiment was correct. The C_{max} at 20 mg/kg was lower than the value calculated from that at 10 mg/kg. This was considered to be caused by the poor solubility of Flu at acidic pH.

The C_{max} and AUC values of Flu/β-CD at 20 mg/kg were 71.27 ± 2.55 μg/ml and 870.11 ± 75.36 μg h/ml, respectively, two times higher than those at 10 mg/kg reported previously [5]. The mean plasma concentrations after Flu/β-CD dosing were larger than those of Flu dosing (Flu and Flu + CN). Significant difference was observed at 5 h and in C_{max} values. The AUC of Flu/β-CD was larger than those of Flu and Flu + CN, but no significant difference was observed. These results agree well with the previously reported data at doses of 1, 3, 10, and 30 mg/kg [5].

The mean plasma levels after oral administration of Flu/β-CD with CN (Flu/β-CD + CN) were higher not only than those of Flu and Flu + CN but also higher than those of Flu/β-CD, as shown in Fig. 1. Significant difference was observed at 2, 3, and 5 h between Flu/β-CD + CN and Flu and Flu + CN. No significant difference was observed between Flu/β-CD + CN and Flu/β-CD at each sampling point; however, C_{max} of Flu/β-CD + CN was larger than that of Flu/β-CD with significant difference. The AUC of Flu/β-CD + CN and Flu/β-CD was the same, and a tendency for the T_{max} of Flu/β-CD + CN to be smaller than that of Flu/β-CD was observed. This tendency showed no significant difference. These results indicate that the improvement of Flu bioavailability, especially C_{max}, was caused by the following mechanism. Flu/β-CD was dissolved at acidic pH in the stomach, but the inclusion formation inhibited the rapid absorption of Flu from the upper intestine. With Flu/β-CD + CN, the existence of CN increased free Flu concentration in the upper intestine; as a result, a rapid absorption of Flu was observed.

This mechanism was supported by the following result of the solubility study. The concentration of Flu in the 0.5 mM β-CD solution added to the DMSO solution without CN was 50.1 ± 0.3 (mean ± SD, $n = 3$) μg/ml. When CN was added, the Flu concentration was decreased with the amount of CN added. Each concentration of Flu was 41.4 ± 0.8, 37.9 ± 0.4, 35.4 ± 1.1 and 31.1 ± 0.3 μg/ml for the CN concentrations, 10, 30, 50, and 100 μg/ml, respectively. The Flu concentration in the solutions is formed by two kinds of Flu, free Flu and the inclusion complex with β-CD. When CN was added to the solutions, a part of β-CD in the solution was considered to be taken out of the inclusion complex of Flu and form a new inclusion complex with CN. In fact, immediately after the addition of CN, the solution became turbid due to poor solubility of CN in water, but the turbidity disappeared within 15 min. In the case of the solution not containing β-CD, for example water and a Flu solution, the turbidity did not disappear. The addition of CN was considered to cause the increase in free Flu concentration according to the amount of CN added, and the free Flu which was over the solubility was precipitated. The decrease in Flu concentrations were considered to be caused by this precipitation, indicating that CN worked as a competing agent.

Therefore, CN acts as a competing agent to Flu/β-CD in the gastrointestinal tract of rats. As a reason for the lack of difference in AUC, it is considered that the absolute bioavailability of Flu/β-CD at 20 mg/kg was almost 100%.

As described above, the action of CN increased free drug concentration at the absorption site, which improved C_{max}, a bioavailability parameter with regard to velocity. For Flu, an anti-inflammatory drug, the increase in the absorption rate is very important from the viewpoint of its rapid action on pain. Moreover, the action of CN as a competing agent was achieved by much lower dose compared with the dose of Flu in Flu/β-CD. This indicates that CN is a strong competing agent. Our present study showed the importance of competing agents in developing a drug delivery system with inclusion complexes and that CN is a useful and strong competing agent. In addition, when Flu/β-CD and CN are used clinically for treatment, the absorption of Flu from Flu/β-CD will be modified as described above, but the absorption of CN will not be changed because the bioavailability of CN and CN/β-CD inclusion complex was the same in beagle dogs [8]. This shows that CN has a possibility to be a useful competing agent in clinical studies.

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References

[1] A. Fukuhara, T. Imai, K. Inoue, M. Otagiri, Effect of oral multiple-dose administration of anti-inflammatory flurbiprofen chimera drug on gastric lesion, other toxicities and disposition kinetics, Biol. Pharm. Bull. 18 (1995) 140–147.
[2] M. Otagiri, T. Imai, N. Matumoto, K. Uekama, Improvements to some pharmaceutical properties of flurbiprofen by β- and γ-cyclodextrin complexations, Acta Pharm. Suec. 20 (1983) 1–10.
[3] M. Otagiri, T. Imai, F. Hirayama, K. Uekama, M. Yamasaki, Inclusion complex formations of the anti-inflammatory drug flurbiprofen with cyclodextrins in aqueous solution and in solid state, Acta Pharm. Suec. 20 (1983) 11–20.
[4] T. Tokumura, A. Muraoka, Y. Machida, A simple determination method for flurbiprofen by high-performance liquid chromatography and its pharmacokinetics on intravenous administration in rats, STP Pharma Sci. 13 (2003) 415–418.
[5] A. Muraoka, T. Tokumura, Y. Machida, Evaluation of the bioavailability of flurbiprofen and its β-cyclodextrin inclusion complex in four different doses upon oral administration to rats, Eur. J. Pharm. Biopharm. 58 (2004) 607–671.
[6] T. Tokumura, H. Ueda, Y. Tsushima, M. Kasai, M. Kayano, I. Amada, T. Nagai, Inclusion complexes of cinnarizine with β-cyclodextrin in aqueous solution and in the solid state, Chem. Pharm. Bull. 32 (1984) 4179–4184.
[7] A. Muraoka, T. Tokumura, Y. Machida, In vitro evaluation of cinnarizine as a competing agent to β-cyclodextrin inclusion complexes: effect of cinnarizine on the membrane permeation rate of progesterone from its β-cyclodextrin inclusion complex, Yakugaku Zasshi 128 (2008) 89–95.
[8] T. Tokumura, M. Nanba, Y. Tsushima, K. Tatsuishi, M. Kayano, Y. Machida, T. Nagai, Enhancement of bioavailability of cinnarizine from its β-cyclodextrin complex on oral administration with DL-phenylalanine as a competing agent, J. Pharm. Sci. 75 (1986) 391–394.
[9] K. Uekama, Pharmaceutical application of cyclodextrin complexation, Yakugaku Zasshi 101 (1981) 857–873.
[10] R. Machida, T. Tokumura, Y. Tsuchiya, A. Sasaki, K. Abe, Pharmacokinetics of novel hexapeptides with neurotensin activity in rats, Biol. Pharm. Bull. 16 (1993) 43–47.