Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 667–671
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Evaluation of the bioavailability of flurbiprofen and its β-cyclodextrin inclusion complex in four different doses upon oral administration to rats

Atsushi Muraoka, Tadakazu Tokumura*, Yoshiharu Machida

Department of Drug Delivery Research, Hoshi University, Shinagawa, Tokyo, Japan

Received 6 January 2004; accepted in revised form 5 March 2004

Available online 7 June 2004

Abstract

The dissolution profiles of flurbiprofen (Flu) and its β-cyclodextrin inclusion complex (Flu/β-CD) in buffer solutions at various pH values were examined. The percent dissolved at 15 min for Flu and Flu/β-CD was almost 100% at pH 6.8 and 8.0 but the dissolution rate of Flu was extremely reduced at pH 1.2 and 4.0. In these lower pH conditions, Flu/β-CD improved the dissolution rate of Flu. The percent dissolved at 1 h for Flu/β-CD at pH 1.2 and 4.0 were 33.4 and 41.3%, respectively, and about 10 times larger than those for Flu. The oral bioavailability of Flu from Flu or Flu/β-CD at doses of 1, 3, 10, and 30 mg/kg (as Flu) was examined in rats. An apparent linear relationship between doses and C_{max}, and AUC was observed after administration of Flu and Flu/β-CD. The Flu C_{max} and AUC values 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 gap was quite small. The results suggest that the absorption of Flu in rats was saturated at 10 mg/kg, and that the enhanced dissolution rate of Flu/β-CD increased the saturation dose to 30 mg/kg.

Keywords: Flurbiprofen; β-Cyclodextrin; Dissolution rate; Bioavailability; Dose; Inclusion complex; Absorption; Rats

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]. In these studies, the dissolution rate of Flu from cyclodextrin inclusion complexes in water and the oral bioavailability of Flu after the oral administration of these inclusion complexes at a dose of 50 mg/kg as Flu to rabbits were examined. However, the dissolution profiles of Flu from the inclusion complexes in buffer solutions at various pH values have not been reported, and the relationship between oral doses and the bioavailability parameters has not been made clear. In order to elucidate the usefulness of the inclusion complexes for clinical use, studies were conducted on the dissolution and the effect of doses on the oral bioavailability of Flu and its inclusion complex with β-cyclodextrin (Flu/β-CD).

Previously, a simple high-performance liquid chromatographic (HPLC) procedure for determining the concentration of Flu in rat plasma was reported, as well as the linear pharmacokinetics of Flu in rats after intravenous administration at doses of 1, 3, and 10 mg/kg [4].

In this report, the effect of the pH of the dissolution test medium on the dissolution rate of Flu from intact Flu and the Flu/β-CD, and the effect of the dose on the oral bioavailability after the oral administration of Flu alone and Flu/β-CD are described.

2. Materials and methods

2.1. Materials

Flurbiprofen and β-cyclodextrin were purchased from Sigma Chemical Company (St. Louis, MO, USA) and Wako Pure Chemical Industries, Ltd (Osaka, Japan), respectively. Other chemicals were of reagent or HPLC grade.

* Corresponding author. Department of Drug Delivery Research, Hoshi University, Ebara 2-4-41, Shinagawa, Tokyo 142-8501, Japan.
E-mail address: tokumura@pep.ne.jp (T. Tokumura).

0939-6411/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2004.03.030

An inclusion complex of Flu with β-CD was prepared by the coprecipitation method reported by Otagiri et al. [3]. The content of Flu in the inclusion complex was 17.0%, which indicated that the stoichiometry of the complex was 1:1 (Flu:β-CD). This value agreed well with the value reported [5].

2.2. Procedure for dissolution studies

The paddle method of The Japanese Pharmacopoeia Fourteenth Edition (JP14) was used in pH 1.2, 4.0, 6.8, and 8.0. The buffer solutions at pH 1.2 and 6.8 used the 1st fluid (pH 1.2) and 2nd fluid (pH 6.8) of JP14. The solutions at pH 4.0 and 8.0 were made up of the 1st and 2nd fluids, and the addition of NaOH to the 2nd fluid, respectively. A certain amount of each sample corresponding to 40 mg of Flu (passing a 850 μm sieve) was placed in a beaker containing the medium (900 ml) equilibrated to 37 °C. Samples were taken at appropriate time intervals and assayed by HPLC, which was similar to the method for the plasma assay. Fresh medium was added to replace the sample taken.

2.3. 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 ID, particle diameter 5 μm) obtained 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.

2.4. 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 0.33, 1, 3.3 and 10 mg/ml as Flu. 0.3 ml/kg of each suspension was administered orally. The doses of Flu and Flu/β-CD were 1, 3, 10, and 30 mg/kg as Flu. Blood samples (0.3 ml) were withdrawn from the jugular vein of the rats which were lightly anesthetized with diethylether. 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, Hoshi University.'

Statistical comparisons of the pharmacokinetic parameters and mean plasma concentrations were made using the unpaired *t*-test.

3. Results and discussion

3.1. Dissolution behavior of Flu and Flu/β-CD

The dissolution rate of Flu from Flu alone and Flu/β-CD in water at 25 °C has been previously reported [2]. In this report, dissolution tests were carried out at pH 1.2–8.0 to investigate the influence of solubility on bioavailability.

Fig. 1 shows the results of the dissolution tests of intact Flu. The dissolution of intact Flu at pH 8.0 and 6.8 was very rapid but largely decreased at pH 4.0 and 1.2. Fig. 2 shows the dissolution behavior of Flu/β-CD. Satisfactory dissolution was also observed at pH 8.0 and 6.8, and Flu/β-CD dissolved even at pH 4.0 and 1.2. All values of the dissolution tests at pH 6.8 and 8.0 were almost 100% at 15 min, and rapid dissolution was observed from both Flu and Flu/β-CD. There were no appreciable differences between Flu and Flu/β-CD at the higher pH values. At pH 1.2 and 4.0, however, the difference in dissolution between Flu and Flu/β-CD was quite obvious. One hour from the start of the dissolution test, the values of dissolved Flu were 3.4 and 4.6%, respectively, but those for Flu/β-CD were 33.4 and 41.3%. The dissolution values of Flu/β-CD at the lower pH values were about 10 times larger than the corresponding values for Flu. It was confirmed from these results that the dissolution rate of Flu was enhanced by the inclusion complexation, which agreed with the results of Otagiri et al. [2]. Flu rapidly dissolves at pH 6.8 and above, from which it is considered to be absorbed from the lower small intestine and upper colon and not absorbed from the upper gastro-intestinal (GI) tract due to poor solubility in an acidic environment. Flu/β-CD showed an enhanced dissolution rate in the acidic media and should improve the absorption of Flu in the upper GI tract, especially the duodenum. This improvement will cause the rapid appearance of Flu in the blood after oral administration, and increased absorption at higher doses as a result of a spreading of the absorption window.

3.2. Comparison of the bioavailability of Flu between the oral dosing of Flu and Flu/β-CD at four different doses

Fig. 3 shows the mean plasma levels of Flu after the oral administration of Flu and Flu/β-CD to rats at doses of 1, 3, 10, and 30 mg/kg as Flu. The bioavailability

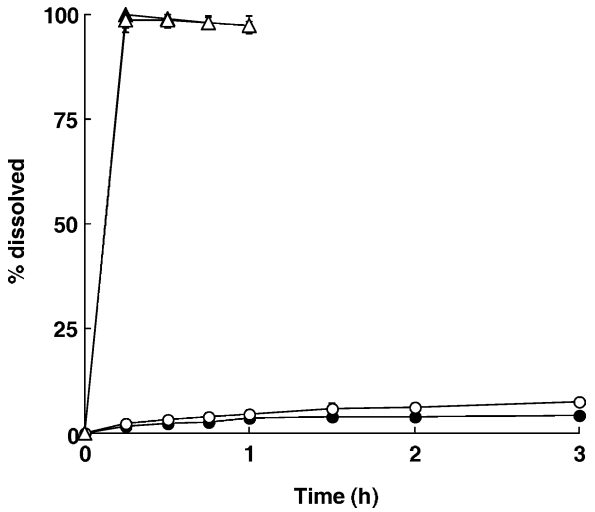

Fig. 1. Dissolution profiles of Flu in buffer solutions of various pH values. (●) pH 1.2, (○) pH 4.0, (▲) pH 6.8, (△) pH 8.0. Each point is the mean and SD of three determinations.

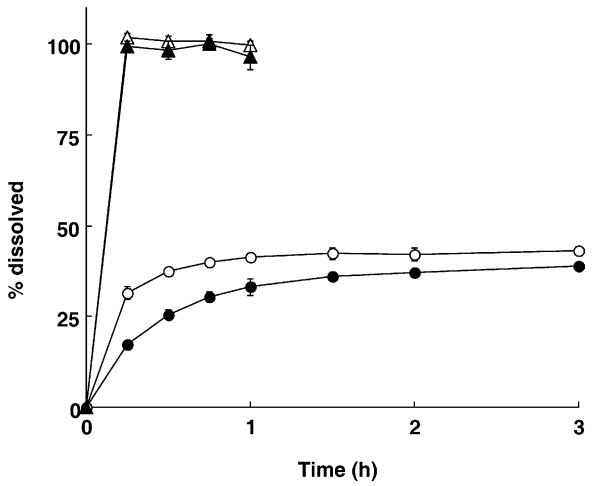

Fig. 2. Dissolution profiles of Flu/β-CD in buffer solutions of various pH values. (●) pH 1.2, (○) pH 4.0, (▲) pH 6.8, (△) pH 8.0. Each point is the mean and SD of three determinations.

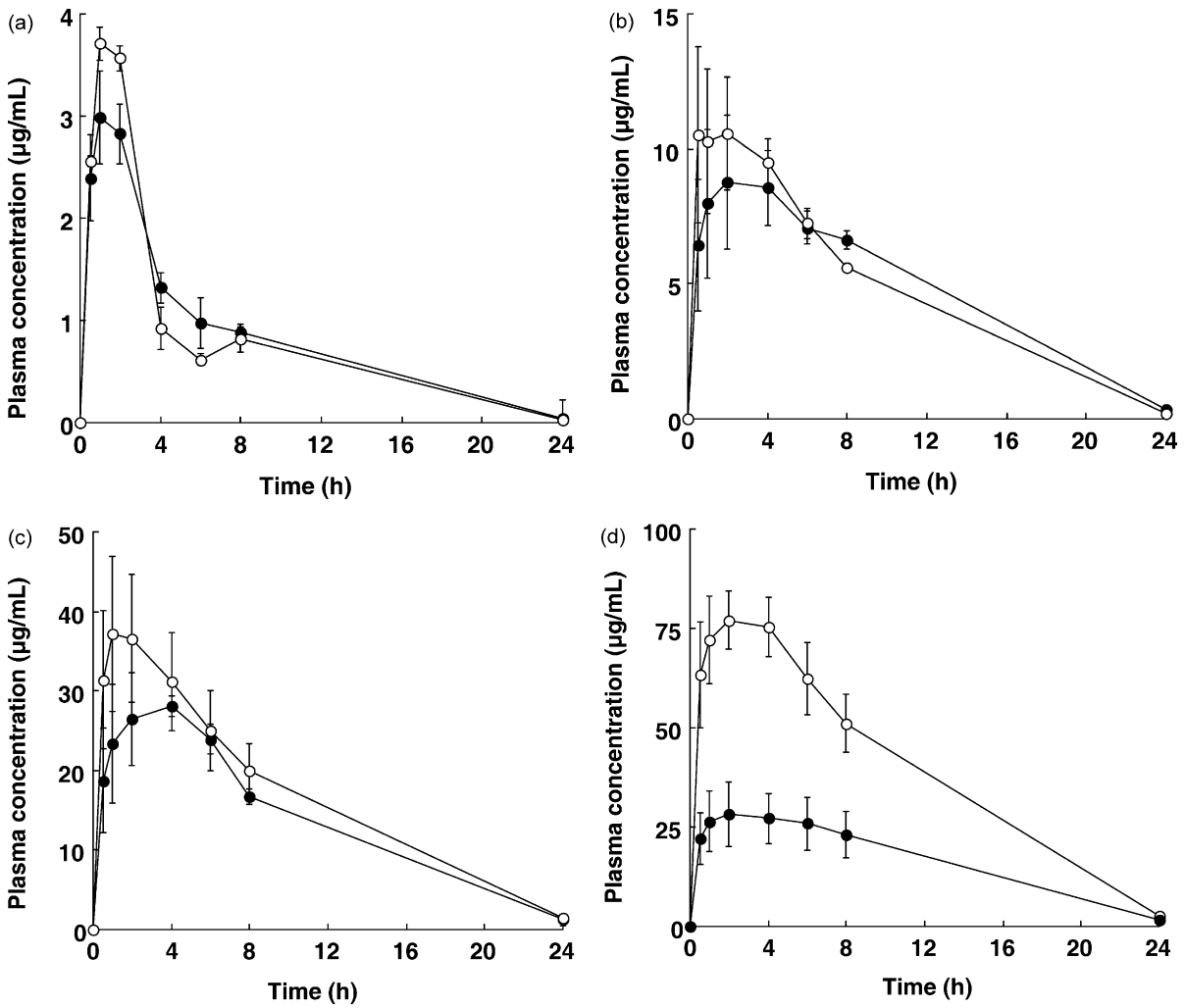

Fig. 3. Time course of plasma Flu concentration in rats after oral administration of Flu (●) and Flu/β-CD (○). (a) 1, (b) 3, (c) 10, and (d) 30 mg/kg. Each point represents the mean ± SE of three rats.

Table 1
Bioavailability parameters of Flu after oral administration of Flu alone and Flu/β-CD at doses of 1, 3, 10, and 30 mg/kg to rats

	Flu	Flu/β-CD
Dose, 1 mg/kg		
C_{max} (μg/ml)	2.36 ± 0.34*	3.71 ± 0.16*
T_{max} (h)	1.67 ± 0.33	1.00 ± 0.00
AUC (μg h/ml)	20.16 ± 0.40	20.08 ± 2.00
MRT (h)	7.27 ± 1.21	4.77 ± 0.32
Dose, 3 mg/kg		
C_{max} (μg/ml)	9.89 ± 1.40	11.55 ± 2.79
T_{max} (h)	3.67 ± 2.19	2.83 ± 1.17
AUC (μg h/ml)	117.60 ± 8.87	115.18 ± 9.67
MRT (h)	6.67 ± 0.48	5.91 ± 0.35
Dose, 10 mg/kg		
C_{max} (μg/ml)	31.66 ± 0.88	38.64 ± 9.17
T_{max} (h)	2.67 ± 0.67	1.67 ± 0.33
AUC (μg h/ml)	340.37 ± 16.17	412.80 ± 66.90
MRT (h)	6.85 ± 0.71	6.84 ± 0.85
Dose, 30 mg/kg		
C_{max} (μg/ml)	35.28 ± 3.51*	85.41 ± 3.78*
T_{max} (h)	4.00 ± 2.00	3.00 ± 1.00
AUC (μg h/ml)	411.53 ± 49.27*	971.59 ± 74.52*
MRT (h)	7.19 ± 0.45	6.42 ± 0.19

Each value represents the mean ± SE of three rats. *$P < 0.05$.

parameters from these treatments are listed in Table 1. At a dose of 1 mg/kg, the mean plasma concentrations for Flu/β-CD at 1 and 2 h, and C_{max} were significantly larger than those of Flu, but no difference was observed between the AUC values. The mean plasma concentrations of Flu/β-CD at 0.5, 1, 2, and 4 h were larger than those for Flu, but there was also no significant difference at doses of 3 and 10 mg/kg. The bioavailability parameters at 3 and 10 mg/kg were almost identical between Flu and Flu/β-CD except for AUC at 10 mg/kg. The mean AUC value of Flu/β-CD at 10 mg/kg was larger than that of Flu, but no significant difference was observed due to the large scatter of the Flu/β-CD data. The mean plasma concentrations for Flu/β-CD at 30 mg/kg were significantly larger than those for Flu except at 24 h. The C_{max} and AUC values of Flu/β-CD were also larger than those of Flu and significantly different.

A tendency for the Flu plasma concentrations after Flu/β-CD administration at doses of 1, 3, and 10 mg/kg to be larger than those after Flu administration was observed. This resulted from the rapid dissolution rate of Flu/β-CD under acidic conditions. The largest absorption difference between Flu and Flu/β-CD was observed at 30 mg/kg.

The reason for this clear difference at 30 mg/kg is considered to be as follows. The absorption of Flu from the GI tract is saturated at 10 mg/kg due to poor solubility. At 30 mg/kg, the absorption after the oral

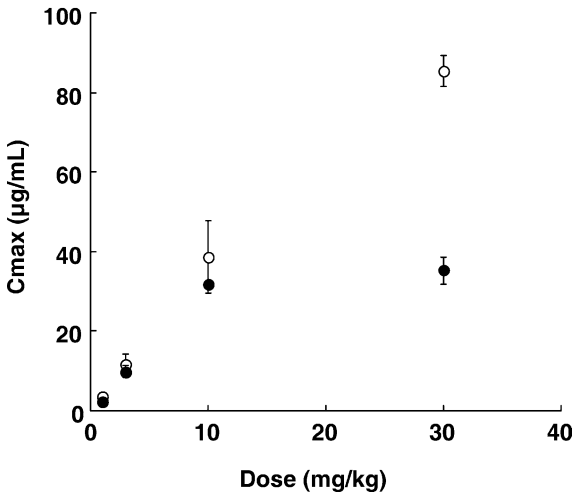

Fig. 4. Relationship between dose and C_{max} after oral administration of Flu (●) and Flu/β-CD (○). Each point represents the mean ± SE of three rats.

administration of Flu was not sufficient by saturation, and there was little increase in plasma concentration. On the other hand, the absorption of Flu after the oral administration of Flu/β-CD was not saturated because of a wider absorption window due to enhancement of the dissolution rate at low pH.

Figs. 4 and 5 show the relationship between doses and C_{max}, and AUC for Flu and Flu/β-CD dosing. These figures clearly illustrate the difference between Flu and Flu/β-CD. At doses from 1 to 10 mg/kg, C_{max} and AUC increase with dose and the relationships were almost linear for both Flu and Flu/β-CD dosing. The C_{max} and AUC values after 30 mg/kg Flu doses were much lower than predicted from the 1 to 10 mg/kg results. The corresponding values upon Flu/β-CD dosing were closer to the predicted value. This further illustrates the difference between Flu and Flu/β-CD. The difference

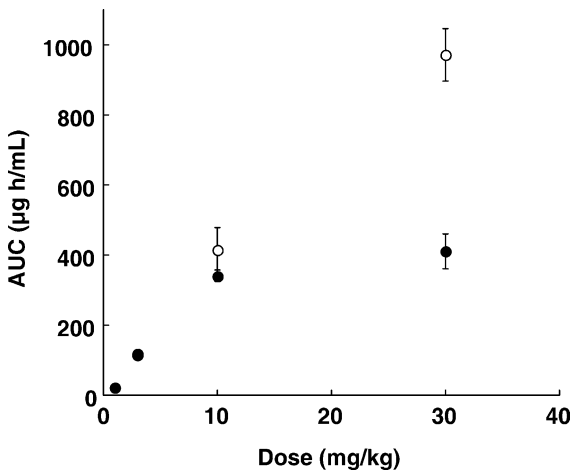

Fig. 5. Relationship between dose and AUC after oral administration of Flu (●) and Flu/β-CD (○). Each point represents the mean ± SE of three rats.

described above was caused by the different dissolution rates. These results suggest that the amount of Flu absorption from the GI tract in rats was saturated at 10 mg/kg, and the enhanced dissolution rate of Flu/β-CD increased the saturation dose to 30 mg/kg or possibly higher.

The in vivo evaluation of pharmaceutical drug preparations with poor solubility with an improved dissolution rate was performed for a single dose including Flu/β-CD. However, the dissolution ability of the GI tract for poorly soluble drugs differs among animal species. In animal studies, a comparative study of bioavailability at a single dose may detect the effect of an improved dissolution rate on in vivo absorption, but a study using different animal species at the same dose may give different results due to the different dissolving abilities. It seemed important to evaluate the effect of an improved dissolution rate on oral bioavailability on the basis of the relationship between doses and bioavailability parameters, such as C_{max} and AUC. The collection of these data is expected to clarify the difference among animal species of the absorption of drugs with poor solubility. Further investigations using dose dependence as an index for drugs with poor solubility is recommended.

Acknowledgements

The authors are very grateful to Ms Azusa Mikami for her assistance in the experimental work.

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] K. Vekama, Pharmaceutical applications of cyclodextrin complexations, Yakagaku Zasshi 101 (1981) 857–873.