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Inclusion Mode of Flurbiprofen with β-Cyclodextrin and Heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin, and Improvements of Some Pharmaceutical Properties of Flurbiprofen by Complexation

TERUKO IMAI,[a] MASAKI OTAGIRI,[a] HAZIME SAITÔ[b] and KANETO UEKAMA*,[a]

Faculty of Pharmaceutical Sciences, Kumamoto University,[a] 5–1, Oe-honmachi, Kumamoto 862, Japan and Biophysics Division, National Cancer Research Institute,[b] Tsukiji, Chuo-ku, Tokyo 104, Japan

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The inclusion behavior of flurbiprofen (FP) with heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin (TM-β-CyD) in solution and in the solid state was compared with that of FP with β-cyclodextrin (β-CyD) by microcalorimetry and solid-state nuclear magnetic resonance (NMR) measurements. Furthermore, solid complexes of FP with β-CyD and TM-β-CyD were obtained in the molar ratio of 1 : 1, and their dissolution behavior, permeation through a silicone membrane and *in vivo* absorption behavior were examined, in comparison with those of FP alone. The data suggest that the inclusion behavior of FP with TM-β-CyD is somewhat different from that with β-CyD. The apparent rates of dissolution, permeation and the bioavailability of FP were significantly increased by the inclusion complex formation. However, no differences between the pharmacokinetic parameters were found for the two complexes. It is concluded that the enhanced bioavailability of FP after oral administration may be due to the fast dissolution of the complexes.

Keywords——inclusion mode; flurbiprofen; β-cyclodextrin; heptakis(2,3,6-tri-*O*-methyl)-β-cyclodextrin; complexation; bioavailability

Recently, chemically modified cyclodextrins (CyDs) have received considerable attention in many fields,[1-8] because their physicochemical properties are different from those of natural CyD. For example, selectively or completely methylated CyD, heptakis(2,6-di-*O*-methyl)-β-CyD or heptakis(2,3,6-tri-*O*-methyl)-β-CyD (TM-β-CyD), is much more soluble in both water and organic solvents as compared with the parent CyD. In addition, the methylated CyD ring is remarkably distorted from the regular symmetry of CyD owing to steric hindrance involving the methyl groups.[4] We have previously reported inclusion complex formation of flurbiprofen (FP) with β-CyD or methylated β-CyDs in aqueous solution and in the solid state.[5] Moreover, improvements of some pharmaceutical properties of FP, such as dissolution rate and release from suppository bases, by means of inclusion complexation, were demonstrated at our laboratories.[7] Thus, as a continuation of those investigations, we report further on the inclusion mode of FP with β-CyD or TM-β-CyD. In addition, the effects of β-CyD and TM-β-CyD on the dissolution, permeation and absorption characteristics of FP were examined.

Experimental

Materials——FP was kindly supplied by Mitsubishi Chemical Industries Ltd. (Tokyo, Japan) and used without further purification. β-CyD was purchased from Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan), and recrystallized twice from water. TM-β-CyD was synthesized according to the method of Hakomori[9] at our laboratory. The 1 : 1

complexes of FP with β-CyD or TM-β-CyD were prepared according to the method described previously.[7] All other materials and solvents were of analytical reagent grade. Deionized and double-distilled water was used throughout the study.

Instruments——Microcalorimetric measurements were performed by using an LKB flow microcalorimeter, model 10700-1 (Bromma, Sweden), modified by the replacement of the water-bath with a Tronac water-bath, model 1005, provided with a PTC 40 temperature controller (Tronac Inc., Orem, Utha). This enabled the temperature to be controlled at 25 ± 0.0002°C. The calibration constant was approximately $0.060\,V \cdot W^{-1}$. Flow rates (usually about $3.2\,\mu l \cdot s^{-1}$) were measured at frequent intervals. All solutions were prepared in 0.1 M phosphate buffer. Heat flux was measured as a function of CyD concentration with the FP concentration fixed at 5×10^{-4} M. Full details of the experimental procedure were given elsewhere.[10] Circular dichroism (CD) and cross polarization/magic angle spinning ^{13}C-nuclear magnetic resonance (CP/MAS ^{13}C-NMR) spectra were recorded with a Jasco J-50A recording spectropolarimeter (Tokyo, Japan) and a Bruker CXP-300 spectrometer (Silber-Streifen, West Germany) operated at 75.46 MHz. The sample was placed in an Andrew–Beams-type unit machined from perdeuterated poly(methyl methacrylate) and spun as fast as 3—4 kHz. The contact time was chosen as 1 ms and repetition time was 2 s. The spectral width and data points were 30 kHz and 4K, respectively. ^{13}C-Chemical shifts were calibrated indirectly through the use of external benzene (128.5 ppm from tetramethylsilane).

Dissolution Studies——The dissolution behavior of FP and its complexes in water was examined according to the dispersed amount method.[11] An amount equivalent to 30 mg of FP as a 100 mesh powder was weighed and put in a dissolution cell. The dissolution medium (75 ml of water) was maintained at 37 °C and stirred at 57 rpm. At appropriate intervals, 0.5 ml of solution was taken with a pipet equipped with a cotton plug, diluted with water, and assayed, taking account of cumulative dilution caused by replacing the samples with equal volumes of the original medium.

Membrane Permeation Studies——The membrane permeation apparatus described previously[12] was used for the measurements of permeation behavior through a silicone membrane. The silicone membrane was distilled and washed with distilled water before use. In the permeation cell, 50 ml of FP (2.0×10^{-4} M) in the absence and presence of β-CyD and TM-β-CyD (2.0×10^{-4}M) was put into a donor compartment, while the same volume of water was put into a receptor cell. In the case of a suspension, the test powder (< 100 mesh) of FP (70 mg) or its CyD complexes (equivalent to 70 mg FP) was put into 50 ml of water in the donor cell. The permeation cell was kept at 37 °C in the thermostated water-bath, and stirred by a magnetic bar at 57 rpm. At predetermined intervals, a 1 ml sample was pipetted off from the receptor solution and the concentration of FP which had permeated from the donor cell was measured spectrophotometrically.

***In Vivo* Absorption Studies**——Five rabbits weighing 2.4—2.7 kg were used at intervals of more than one week. They were fasted for 24 h prior to drug administration. FP or its CyD complex was administered orally (30 mg/kg as equivalent of FP) as a suspension in 70 ml of water, using a stomach catheter. Blood samples (1 ml) taken from the ear vein were centrifuged (10000 rpm, 5 min) and the serum was stored in the refrigerator until assayed. Assay of FP in serum was carried out as described previously.[13]

Results and Discussion

Inclusion Mode in Aqueous Solutions and in the Solid State

Inclusion complexation of FP with β-CyD or TM-β-CyD in solution and in the solid state has been assessed by using spectroscopy (ultraviolet (UV), fluorescence, infrared (IR), NMR) and X-ray diffractometry, as reported previously.[5,7] In this study, microcalorimetry, CD spectroscopy and CP/MAS ^{13}C-NMR were employed to further gain insight into the inclusion mode.

Figure 1 shows the heat flux generated following the interaction of FP with β-CyD or TM-β-CyD. The heat flux is assumed to be proportional to the amount of the drug bound to the host molecules.[10] If only one binding site contributes to the heat flux, then the data can be readily interpreted in terms of the thermodynamic parameters, ΔG, ΔH and ΔS, for binding to the site. Based upon the data of Fig. 1 the binding constants can be estimated, assuming a 1 : 1 interaction. The binding constants and the derived thermodynamic parameters are shown in Table I. Large negative enthalpies were obtained for both interactions. This negative enthalpy suggests strong involvement of dipoles in the complex formation. The large differences in entropy can be accounted for the changes in the behavior of the solvent, water, after the binding process. The differences in thermodynamic parameters found with the two systems may indicate different binding modes. This hypothesis is supported by spectroscopic data.

TABLE I. Stability Constants and Thermodynamic Parameters[a] for Interaction of FP with CyDs in 0.1 M Phosphate Buffer of pH 7.0 at 25 °C

System	Stability constant (M^{-1})		ΔG	ΔH	ΔS
	25 °C	37 °C[b]			
β-CyD	3950	3670	−20520	−11300	31.1
TM-β-CyD	1380	480	−17870	−20900	−10.2

a) ΔG and ΔH are in J/mol and ΔS is in J/mol·K. b) According to ref. 7.

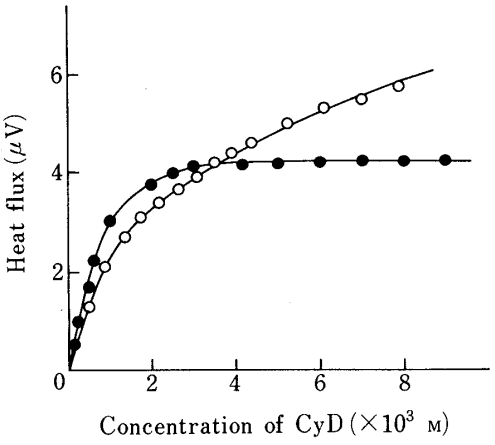

Fig. 1. Heat Fluxes as a Function of CyD Concentration with the FP Concentration Fixed at 5×10^{-4} M

●, β-CyD system; ○, TM-β-CyD system.

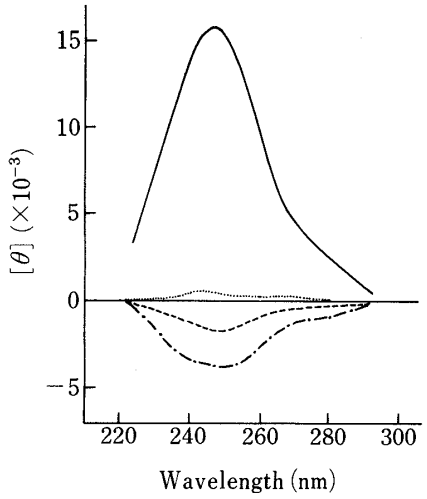

Fig. 2. Induced CD Spectra of FP in the Presence of CyDs in 0.1 M Phosphate Buffer (pH 7.0) at 25 °C

——, *RS*(±)-, *R*(−)-, *S*(+)-FP with β-CyD; ------, *RS*(±)-FP with TM-β-CyD; ·····, *R*(−)-FP with TM-β-CyD; —·—, *S*(+)-FP with TM-β-CyD.

That is, as can be seen in Fig. 2, there are differences in the magnitude of the induced Cotton effects between *R*(−) and *S*(+)-FP bound to TM-β-CyD, whereas the induced ellipticities of enantiomers are almost the same for β-CyD. This may be due to the interaction between TM-β-CyD and the propionate portion of FP, having a chiral center. The NMR and X-ray crystal structure analysis[5,14,15] also indicated differences in the inclusion mode between the two CyDs; the phenyl group of FP is located at the entrance to the TM-β-CyD cavity, while the biphenyl portion of FP penetrates further into the β-CyD cavity. Therefore, the thermodynamic parameters observed in the TM-β-CyD system may reflect the interaction within the CyD cavity as well as outside the CyD cavity. Anyhow, more insight into the mechanism of the binding mode can be gained by measuring enthalpy changes as a function of temperature; such measurements can be made with the flow microcalorimeter.

Figure 3 shows the CP/MAS ^{13}C-NMR spectra of β-CyD and TM-β-CyD and their inclusion complexes with FP. In these investigations, CP/MAS ^{13}C-NMR was used for the first time to study drug–TM-β-CyD complex. The assignments of TM-β-CyD resonances were based on those in solution. In the case of the complex, no appreciable signals from guest molecule were observed under these experimental conditions. The ^{13}C-signals from guest molecules are generally lost or obscured because the amount of the guest molecule is low compared with that of the glucose residues. It can be clearly seen that complex formation

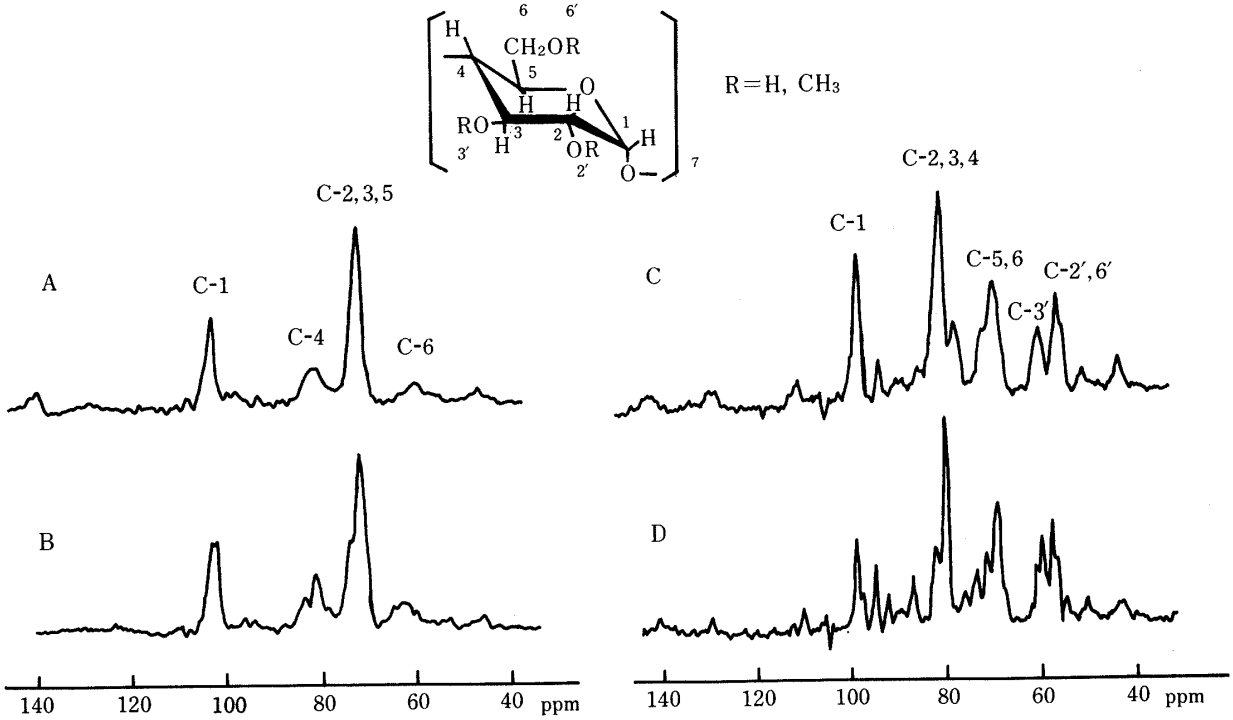

Fig. 3. CP/MAS ^{13}C-NMR Spectra of FP–CyD Systems

A, FP–β-CyD complex; B, β-CyD alone; C, FP–TM-β-CyD complex; D, TM-β-CyD alone.

resulted in a change in the line-shape of the glucose signals, especially at C-1, C-4, C-6. In particular, the chemical shifts of C-1 and C-4 reflect the rotation state about the glycosidic linkage, which is specified by the dihedral angles.[16,17] Therefore, splitting of signals in β-CyD and TM-β-CyD can be explained in terms of superposition of displaced signals from glucose residues whose dihedral angles are distributed to some extent because of the distorted macrocyclic conformation. The relatively large changes in C-1 and C-4 resonances on formation of the FP–TM-β-CyD complex suggest that the conformation of the TM-β-CyD macrocycle is significantly perturbed by FP compared to the conformation of β-CyD having a round macrocycle.[18] In contrast to TM-β-CyD complex, the macrocyclic conformations of β-CyD before and after the inclusion of FP are not so different from each other, as found by X-ray crystal analysis.[14] Thus, the above results indicate that the inclusion mode of FP with TM-β-CyD is somewhat different from that with β-CyD in solution and in the solid state.

Dissolution and Permeation Behavior

Figure 4 shows the dissolution profiles of β-CyD and TM-β-CyD complexes in water at 37 °C, compared with that of FP itself. It is evident that the dissolution rate of FP was significantly improved by complexation. The enhanced dissolution rate of FP may be due to the increase in solubility and wettability along with the decrease in crystallinity of the drug by inclusion complexation. It should be noted that the dissolution profile of TM-β-CyD complex exhibited greater curvature than the β-CyD complex with the passage of time, although the initial rate of the former was greater than that of the latter. This may be explained by the fact that the TM-β-CyD complex is dissociated rather quickly in the dissolution medium because of its smaller stability constant (see Table I).

Figure 5 shows the permeation profiles of FP from the donor solution through a silicone membrane in the absence and presence of β-CyD and TM-β-CyD. It was clear that the greater the stability constant of the complex, the lesser the permeation of the drug. This may be due to the poor permeability of the complex, because the permeation through a silicone membrane is

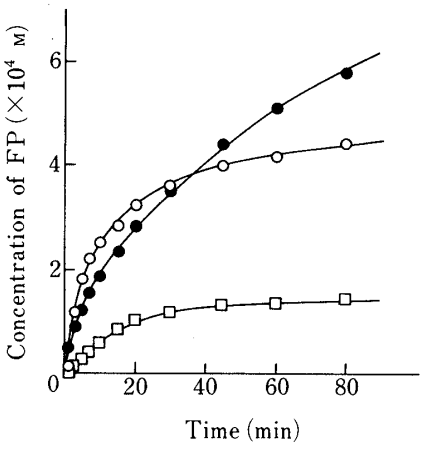

Fig. 4. Dissolution Profiles of FP and Its CyD Complexes in Water at 37 °C, Measured by the Dispersed Amount Method

□, FP alone; ●, β-CyD complex; ○, TM-β-CyD complex.

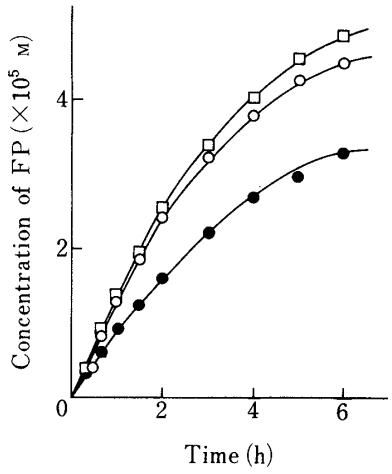

Fig. 5. Permeation Profiles of FP in the Absence and Presence of CyDs, through a Silicone Membrane in Aqueous Solution at 37 °C

□, without CyDs; ●, with β-CyD; ○, with TM-β-CyD.

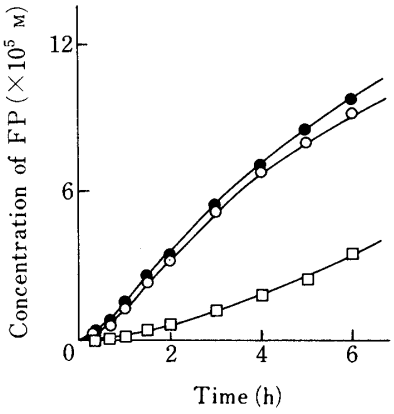

Fig. 6. Permeation Profiles of FP and Its CyD Complexes through a Silicone Membrane in Aqueous Suspension at 37 °C

□, FP alone; ●, β-CyD complex; ○, TM-β-CyD complex.

determined by the pore-size and the partition into the membrane.[19] In fact, both CyDs scarcely permeated through a silicone membrane, although TM-β-CyD is highly lipophilic.[20] On the other hand, when the test powders were suspended in the donor cell, an increase in permeation of FP after CyD complexation was observed, as shown in Fig. 6. In this case, the enhanced permeation of FP from the complex can be explained on the basis of the permeation and dissolution characteristics of the test sample. That is, rapid dissolution of the complex more than cancels out the negative effect due to the poor permeability of the complex itself and produces a net increase in permeation of the drug.

In Vivo Absorption Study

β-CyD and TM-β-CyD complexes of FP were expected to have good bioavailability after oral administration, because the dissolution rates of the complexes were superior to those of the drug alone. Figure 7 shows the mean serum levels of FP following the oral administration of FP or its CyD complexes. There was a significant difference in the peak concentrations between FP and its CyD complexes. The maximum serum level (C_{max}) was 14.2 μg/ml after administration of FP, while β-CyD and TM-β-CyD complexes resulted in the rapid appearance of FP in the serum, showing C_{max} values of 37.6 and 43.0 μg/ml, respectively. The mean residence time (*MRT*), which may represent the rate of bioavailability, was improved by inclusion complexation, as shown in Table II. The areas under the serum concentration time

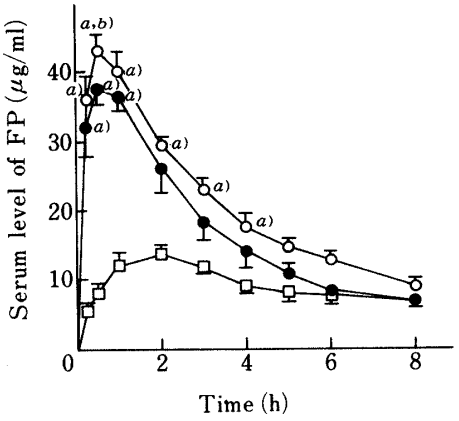

Fig. 7. Serum Levels of FP Following the Oral Administration of FP and Its CyD Complexes to Rabbits

□, FP alone; ●, β-CyD complex; ○, TM-β-CyD complex.
Values represent the mean ± S.E. of 5 rabbits.
a) $p<0.01$ *vs.* FP alone. *b*) $p<0.01$ *vs.* β-CyD complex.

TABLE II. Pharmacokinetic Parameters of FP and Its CyD Complexes following Oral Administration to Rabbits

Compound	AUC_{0-8} (μg·h/ml)	*MRT* (h)	*VRT* (h^2)
FP	73.9± 8.4	3.67±0.15	4.78±0.14
β-CyD complex	136.7±12.6[a)]	2.75±0.11[a)]	4.44±0.19
TM-β-CyD complex	160.0± 7.4[b)]	2.83±0.07[b)]	4.51±0.12

a) $p<0.02$ *vs.* FP alone. *b*) $p<0.005$ *vs.* FP alone.
VRT: variance of residence time.

curves (*AUC*) of β-CyD and TM-β-CyD complexes up to 8 h post administration were about twice that of FP alone. However, no significant difference between the pharmacokinetic parameters of the two complexes was observed after oral administration. This may be due to the dissociation of the complex following the rapid dissolution in the medium of the gastrointestinal tract rather than modification of drug disposition by β-CyD or TM-β-CyD. Since TM-β-CyD itself possibly alters the lipid barrier of the absorption site, further study on the enhancing mechanism of methylated β-CyD is under way at this laboratory.

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