IMPROVEMENT OF SOME PHARMACEUTICAL PROPERTIES OF CARMOFUL BY CYCRODEXTRIN COMPLEXATION

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ABSTRACT. Inclusion complexation of carmoful (1-hexylcarbamoyl-5-fluorouracil, HCFU) with three cyclodextrins (α-, β- and γ-CyDs) were studied by solubility method and X-ray diffractometry. On the basis of the phase solubility diagrams, solid complexes of HCFU with α-, β- and γ-CyDs were obtained in the molar ratios (host:guest) of 2:1, 1:1 and 1:1, respectively. The dissolution rate of HCFU from the solid complexes was much greater than that of HCFU itself (α-CyD > complex > β-CyD complex > γ-CyD complex > HCFU alone). The hydrolysis of HCFU was suppressed by β-CyD, while no appreciable inhibition was observed by α- and γ-CyDs. The rapid dissolving form of HCFU-CyD complexes was found to increase significantly the serum levels of the drug after oral administration to rabbits.

1. INTRODUCTION

Carmoful (1-hexylcarbamoyl-5-fluorouracil, HCFU), one of the masked compounds of 5-fluorouracil, has been widely used in the treatment for the carcinomas of breast and gastrointestinal tract (1,2). However, its low solubility and chemical instability in water have limited the dosage form design and presented a substantial challenge to pharmaceutical scientists (3). Cyclodextrins (CyDs) have been successfully applied to improve the pharmaceutical properties of various drugs (4,5,6). In our preliminary study, it was found that HCFU forms solid complexes with α-, β- and γ-CyDs. Thus, the present study dealt with the inclusion complexation of HCFU with three CyDs in an attempt to obtain improved solubility, dissolution rate, chemical stability, and bioavailability of HCFU.

2. EXPERIMENTAL

2.1. Materials

HCFU was kindly donated from Mitsui Pharmaceutical Co., Ltd. CyDs were purchased from Nippon Shokuhin Kako Ltd., and recrystallized

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from water. All other chemicals and solvents were of analytical reagent grade and deionized double-distilled water was used throughout the study.

2.2. Solubility Studies

Excess amounts of HCFU were added to CyD solution and were shaken at 25°C. 0.1 M Phosphate buffer of pH 3.0 was used as solvent because of the facile hydrolysis of HCFU in neutral and alkaline conditions (3). After equilibration was attained (about 2 weeks), an aliquot was centrifuged and pipetted through a cotton plug. The filtrate was properly diluted and analyzed spectrophotometrically for the total HCFU at 261 nm. The apparent stability constant (K') was calculated from the initial straight line portion of the phase solubility diagram according to following equation (7).

$$K' = \frac{\text{slope}}{\text{intercept } (1 - \text{slope})} \qquad \text{Eq. 1}$$

2.3. Preparation of Solid Complexes

The solid complexes were obtained by mixing appropriate amounts of HCFU and CyDs in phosphate buffer (pH 3.0). Amounts were calculated from the descending portion of the phase solubility diagrams (see Fig. 1). For example, 400 mg of HCFU and 7.8 g α-CyD were added in 80 ml buffer, sealed in a flask and the mixture was agitated at 25°C for 10 days. The complex, which precipitated as a microcrystalline powder, was removed by filtration and dried under vacuum at room temperature for 3 days. This powder corresponded to a 1:2 HCFU-α-CyD complex which had a molecular weight of 2202. Other CyD complexes were prepared in a similar manner.

2.4. Dissolution Studies

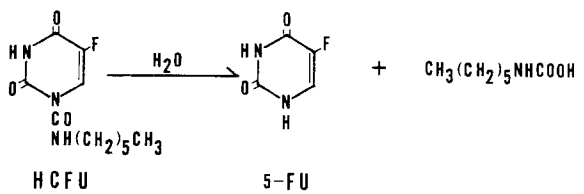
The dissolution rate was measured according to the dispersed amount method (8). The equivalent amount of 46 mg of HCFU as 100 mesh powder was weighed and put into a dissolution cell. The dissolution medium (25 ml of phosphate buffer, pH 3.0) was maintained at 37°C and stirred at 91 rpm. At appropriate intervals, 0.5 ml sample was removed from the flask, diluted with the buffer and assayed spectrophotometrically. Corrections were applied for cumulative dilution caused by replacing the sample by equal volumes of the original medium.

2.5. X-ray Diffractometry

The powder X-ray diffraction patterns were obtained on a Riguku Denki Geiger Flex 2012 diffractometer under the following conditions, X-ray: Ni-filtered Cu-Kα radiation, voltage: 30 kV, current: 20 mA, time constant: 2 s, scanning speed: 1 °/min.

2.6. Stability Studies

The hydrolysis rates of HCFU in aqueous solution (scheme I)(3) were followed by measuring the decrease in the absorbance at 261 nm. The reaction was initiated by addition of a stock solution of HCFU in MeOH into a quartz cell (1 cm pass-length) containing phosphate buffer at 37°C. The final concentrations of HCFU and MeOH were 6.5×10^{-5} M and 3 v/v %, respectively. The hydrolysis followed exactly the first-order kinetics. Under these experimental conditions, no appreciable side reactions such as ring-opening of the uracil moiety were observed.



Scheme I. Scheme for the hydrolysis of HCFU in aqueous solution

2.7. In Vivo Absorption Studies

Five male albino rabbits weighing 2.0-2.5 kg were used at intervals of more than two weeks. They were fasted for 1 day prior to drug administrations. HCFU or its CyD complexes as a 100 mesh powder were administered orally (15 mg/kg as equivalent of HCFU) as a suspension in 80 ml water, using a stomach catheter. Blood samples (1.5 ml) were taken from the ear vein at 0.25, 0.50, 1, 1.5, 2, 4, 6, and 8 hour after the oral administrations. The blood samples were then centrifuged (10000 rpm, 3 min), and the serum was stored in the refrigerator until assayed.

2.8. Assay of HCFU in Serum

The serum samples were assayed for 5-fluorouracil (5-FU) after the hydrolysis of HCFU, using high performance liquid chromatography (HPLC)(9). That is, to 0.5 ml serum was added 0.5 ml of 200 g/l aqueous Na_2SO_4 solution and 50 µl of 1.0 M acetate buffer (pH 4.8), and the serum was then extracted with 15 ml of n-propanol-ether (16:84). Fourteen ml of organic phase were agitated with 3 ml of 0.1 N NaOH solution for 1 hour to hydrolize HCFU, and then 5-FU was extracted to the aqueous phase. The aqueous phase (0.5 ml) was acidified by the addition of 1.0 M H_2SO_4 (10 µl) and subjected to HPLC analysis. The chromatograph was operated at a flow rate of 1.0 ml/min, and the eluent was spectrophotometrically monitored at 267 nm. The separation was achieved on a column of LiChrosorb RP-18 (10 µm, 4 ϕ x 250 mm) with potassium phosphate buffer (pH 3.0) as a mobile phase. Components were quantitated by measuring peak hights and comparing them with those of known amounts of an internal standard, cytosine.

3. RESULTS AND DISCUSSION

3.1. Inclusion Complex Formation of HCFU with Three CyDs

The complexations of HCFU with α-, β- and γ-CyDs in aqueous solution were studied by the solubility method. Figure 1 shows the phase solubility diagrams obtained for the three CyD-HCFU systems. In all cases, the plots show typical Bs-type solubility curves (7) with microcrystalline complexes precipitating at high CyD concentrations. The apparent stability constants (K') of the complexes were estimated from Eq. (1) based on the assumption that a 1:1 complex is initially formed (1200 M^{-1} for α-CyD complex, 670 M^{-1} for β-CyD complex and 180 M^{-1} for γ-CyD complex). The stoichiometries of the solid complexes were determined by the plateau region of the solubility diagrams and the chemical analysis of crystalline complexes. Two α-CyD molecules are available for the inclusion of one HCFU molecule because of the small cavity size. On the other hand, β- and γ-CyDs having larger cavity form 1:1 complexes with HCFU. Solubilities of β-CyD and γ-CyD complexes were estimated to be about 1.6×10^{-3} M and 1.0×10^{-3} M, respectively, from the initial straight line portion of the phase solubility diagrams, which were in good agreement with those estimated at high CyD concentration in the diagrams. On the other hand, the solubility of α-CyD complex estimated from the initial straight line portion is significantly different from that estimated at high α-CyD concentrations, indicating the higher order complex formation. Solubilities of the 1:1 and 1:2 (HCFU:α-CyD) complexes were then estimated to be about 4.0×10^{-3} M and 5.5×10^{-4} M, respectively, assuming that the initial increase in solubility of HCFU up to the plateau region is simply the sum of the solubilities of 1:1 and 1:2 complexes and the solubility of HCFU at high CyD concentration is that of 1:2 complex.

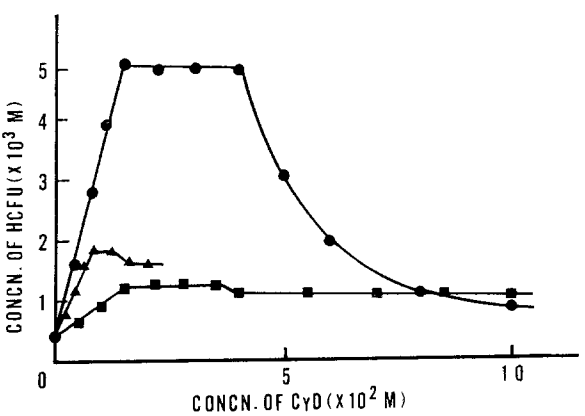


Figure 1. Phase solubility diagrams of HCFU-CyD systems in phosphate buffer (pH 3) at 25°C
● : α-CyD, ▲ : β-CyD, ■ : γ-CyD.

Figure 2 shows the powder X-ray diffraction patterns of HCFU-CyD complexes in comparison with the corresponding physical mixture at the same molar ratios. The diffraction patterns of the complexes were significantly different from those of physical mixtures which gave a simple superposition of diffraction peaks for each components. It was also found that the complexes gave the sharp diffraction peaks, suggesting a good crystallinity. Indeed, single crystals of these complexes were isolated from the aqueous solution containing both components. Inclusion complex formations of HCFU and three CyDs in the solid phase were further confirmed by IR spectroscopy and thermal analysis, and the details will be reported elsewhere.

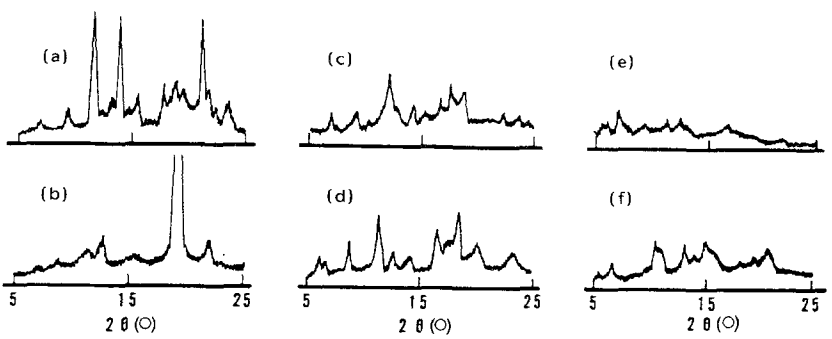


Figure 2. Powder X-ray diffraction patterns of HCFU-CyD systems
(a) : physical mixture of HCFU and α-CyD,
(b) : complex of HCFU with α-CyD,
(c) : physical mixture of HCFU and β-CyD,
(d) : complex of HCFU with β-CyD,
(e) : physical mixture of HCFU and γ-CyD,
(f) : complex of HCFU with γ-CyD.

3.2. Dissolution Behavior of the Complexes

The dissolution profiles of HCFU and its α-, β- and γ-CyD complexes were examined in phosphate buffer (pH 3.0), under which condition the hydrolysis of HCFU is negligible. As shown in Figure 3, the dissolution rate of HCFU was significantly improved by the CyD complexations, particularly by α-CyD. The observed increase in the rate may be due to the increase in solubility as expected from Figure 1, although other factors such as diffusion coefficient, wettability and the dissoci-

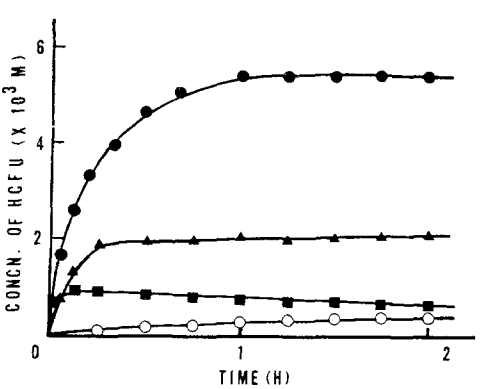


Figure 3. Dissolution profiles of HCFU and its CyD complexes in phosphate buffer (pH 3) at 37°C by dispersed amount method
○: HCFU, ●: α-CyD complex, ▲: β-CyD complex, ■: γ-CyD complex.

ation of the complex in the dissolution medium should be considered in the rate enhancement (10). In the case of γ-CyD complex, concentration of HCFU reached a peak at about 8 min and then gradually decreased with the passage of time. This may be due to the dissociation of the complex to its components after the dissolution, because the stability constant of γ-CyD complex is rather small compared to other complexes.

3.3. Effects of CyDs on the Hydrolysis of HCFU

HCFU is susceptible to hydrolysis in gastrointestinal fluid, and the therapeutic efficiency as well as oral bioavailability may decrease as a result. Thus the effects of CyDs on the hydrolysis of HCFU were studied in the hope of improving the chemical stability of HCFU in aqueous solution. Figure 4 shows log rate constant(K)-profiles for the hydrolysis in the absence and presence of β-CyD over the pH range of 5.0-12.5 at 37°C. The pH-profiles were found to be biphasic with a slope of +1 at lower and higher pH regions (pH < 7.0 and pH > 12.0), as a consequence of the protolytic dissociation of the imino group in the uracil moiety (pKa = 6.71)(3). Interestingly, β-CyD retarded the hydrolysis rate about 1.6 times over the pH range employed, while α- and γ-CyDs had no significant effect on the reaction rate. Therefore, it is reasonable to assume that the reactive amide moiety of HCFU is preferentially located in a β-CyD cavity to prevent the hydrolysis. Further studies are now under way to elucidate the inhibition mechanism of β-CyD.

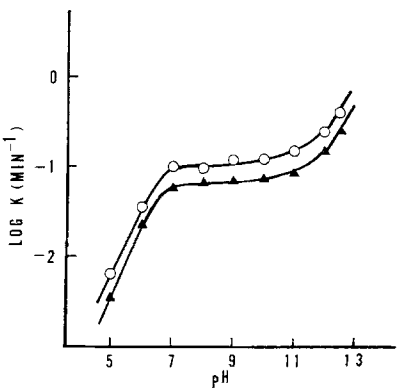


Figure 4. pH-profiles for hydrolysis of HCFU in the absence and presence of β-CyDs (1.0×10^{-2} M) at 37°C
○ : HCFU, ▲ : HCFU with β-CyD.

3.4. In Vivo Absorption Studies

The CyD complexes of HCFU were expected to have good bioavailability after oral administration, because the dissolution and chemical stability of the complexes were superior to those of HCFU alone. Consequently, HCFU and its three CyD complexes were administered orally to rabbits to evaluate their absorption characteristics. The mean serum levels of 5-FU following the oral administration of HCFU of its CyD complex are shown in Figure 5. The bioavailability parameters were calculated from the serum level-time curves up to 8 hours post administration, and the results are summarized in Table I. The maximum serum level (Cmax) and the area under serum concentration-time curve (AUC) can be representative of the extent of bioavaila-

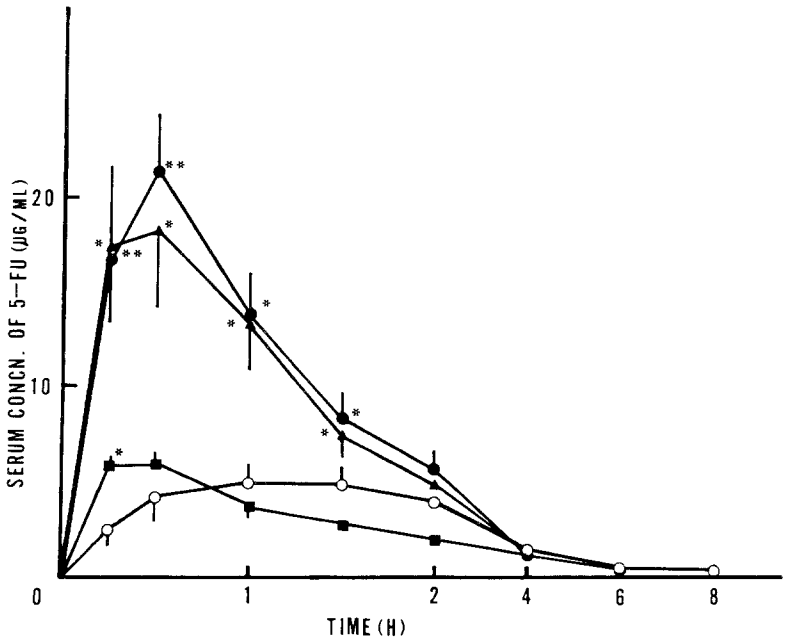


Figure 5. Serum concentrations of 5-FU following the oral administration of HCFU or its CyD complex (equivalent to 15 mg/kg HCFU) to rabbits
○ : HCFU, ● : α-CyD complex, ▲ : β-CyD complex, ■ : γ-CyD complex.
*) $p < 0.05$ in the complex versus HCFU.
**) $p < 0.01$ in the complex versus HCFU.

TABLE I. Bioavailability parameters of HCFU and its CyD complexes following the oral administration (equivalent to 15 mg/kg HCFU) to rabbits

Compound	C_{max} (μg/ml)	AUC (h·μg/ml)	T_{max} (h)	MRT (h)
HCFU	5.31 ± 1.09	16.39 ± 1.39	1.20 ± 0.20	2.44 ± 0.17
α-CyD complex	21.55 ± 3.25*	33.40 ± 4.18**	0.50 ± 0.14*	1.36 ± 0.15**
β-CyD complex	21.62 ± 4.85*	30.10 ± 4.23*	0.50 ± 0.14*	1.37 ± 0.14**
γ-CyD complex	6.32 ± 0.51	11.71 ± 0.93	0.35 ± 0.06*	1.99 ± 0.09*

Values represent the mean ± S.E. of 5 rabbits.
*) $p < 0.05$ in the complex versus HCFU.
**) $p < 0.01$ in the complex versus HCFU.

bility, and the peak concentration time (Tmax) and the mean residence time (MRT) may represent the rate of bioavailability. It is evident that both extent and rate of bioavailability of HCFU were significantly improved by inclusion complexations, particularly by α- and β-CyDs. It is reasonable to assume that the absorption rate of the complex is negligibly small compared with HCFU alone, owing to the poor membrane permeability and/or poor lipophilicity of the complex. Therefore, greatly enhanced dissolution rate and improved chemical stability of HCFU by CyDs more than may cancel out these negative effects and result in a net increase in the concentration of HCFU available for gastrointestinal absorption. Above results also suggest that the CyD complexes offer a decrease in dose in oral HCFU therapy.

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