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Improvement of oral and rectal bioavailabilities of carmofur by methylated β-cyclodextrin complexations

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Summary

Inclusion complex formation of carmofur (1-hexylcarbamoyl-5-fluorouracil; HCFU) with β-CyD, heptakis(2,6-di-O-methyl)-β-cyclodextrin (DM-β-CyD) and heptakis(2,3,6-tri-O-methyl)-β-cyclodextrin (TM-β-CyD) were assessed by solubility method, thermal analysis and powder X-ray diffractometry. The hydrolysis of HCFU in aqueous solution was decelerated by three β-CyDs, depending on the magnitude of the stability constant of the inclusion complex (TM-β-CyD < β-CyD < DM-β-CyD). The solid state stability of HCFU in methylated β-CyD complexes was superior to that in the parent β-CyD complex because of their low hygroscopicity. The greater stability of HCFU in methylated β-CyD complexes was retained also in Witepsol H-15 suppository base. The complexes dissolved more rapidly than HCFU itself, and attained a faster release of HCFU from the suppository base (DM-β-CyD complex > β-CyD complex > TM-β-CyD complex > HCFU alone). Higher plasma levels of HCFU were observed after oral and rectal administrations of the complexes, particularly for the DM-β-CyD complex in rabbits. All the data suggested that DM-β-CyD may have more utility over parent β-CyD and TM-β-CyD for the improvement of the pharmaceutical properties of HCFU.

Introduction

It is well known that physicochemical properties and inclusion behavior of methylated cyclodextrins are significantly different from those of natural cyclodextrins (CyDs) (Szejtli, 1983; Uekama, 1985). For example, heptakis(2,6-di-O-methyl)-β-CyD (DM-β-CyD) and heptakis-(2,3,6-tri-O-methyl)-β-CyD (TM-β-CyD) are extremely soluble in water as well as in organic solvents, less hygroscopic, and highly surface active, compared to the parent β-CyD.

Carmofur (1-hexylcarbamoyl-5-fluorouracil, HCFU) is one of the masked forms of 5-fluorouracil (5-FU), and has been widely used to treat carcinomas of breast and gastrointestinal tract (Koyama and Koyama, 1980; Taguchi, 1980). However, its low solubility and chemical instability have hampered the development of new dosage forms, presenting a challenge to pharmaceutical scientists. From biopharmaceutical standpoint, HCFU is desirable to be absorbed in as intact form as possible, because of its higher therapeutic efficacy and wider antitumor spectrum together with the lower irritation against gastrointestinal

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tract, compared to parent 5-FU (Iigo et al., 1978a and b; Tokuzen et al., 1980). In a previous paper (Kikuchi et al., 1984), we have reported that solubility, dissolution rate, chemical instability and in vivo absorption characteristics of HCFU were improved by complexation with α-, β- and γ-CyDs. In this study, effects of DM- and TM-β-CyDs on some pharmaceutical properties of HCFU were studied in the hope of achieving greater improvements in oral and rectal bioavailabilities over those of the parent β-CyD complex.

Materials and Methods

Materials

HCFU was donated by Mitsui Pharmaceutical Co. (Tokyo, Japan). β-CyD and DM-β-CyD were supplied by Nihon Shokuhin Kako Co. (Tokyo, Japan) and Toshin Chemical Co. (Tokyo, Japan), respectively, and used after recrystallization from water. TM-β-CyD was prepared according to the reported method (Hakomori, 1964). All other materials and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Solubility studies

Solubility measurement and the analytical method for HCFU were essentially the same as those reported previously (Kikuchi et al., 1984), except for the temperature (37 °C) and pH of the aqueous phase. In this study, acidic medium (phosphate buffer, pH 2.5) was used as an aqueous phase to prevent the degradation of HCFU (Kikuchi et al., 1984). The apparent 1 : 1 stability constant (K_c) of inclusion complexes in aqueous solution was calculated from the slope and intercept of the initial linear portion of phase solubility diagrams according to Higuchi and Connors (1965).

Preparation of solid complexes

HCFU-β-CyD complexes in a 1 : 1 molar ratio were prepared according to the kneading method (Tsuruoka et al., 1981). For example, the semi-crystalline mixture of HCFU (1 g) and DM-β-CyD (5.2 g) was triturated with a small amount of pH 3.0 phosphate buffer (about 2 ml). The slurry was further kneaded thoroughly for about 40 min, and then dried under a reduced pressure at room temperature for 3 days. Under these experimental conditions, no appreciable degradation of HCFU was observed. Differential (DTA) and gravimetric (TG) thermograms and powder X-ray diffraction patterns of the complexes were taken under the similar conditions to those described previously (Kikuchi et al., 1984; Uekama et al., 1985).

Stability studies

In aqueous solution. The hydrolysis rate of HCFU was spectrophotometrically monitored by measuring the decrease in absorbance at 241 nm. The kinetic conditions were the same as those reported previously (Kikuchi et al., 1984).

In the solid state. HCFU-β-CyD complexes (50 mg, 100 mesh) in open tubes were placed in a Tabai Platinous Rainbow PR-1G incubator (Tokyo, Japan) which was kept at constant temperature and constant relative humidity (R.H.). At appropriate intervals, a test sample was dissolved in 50 v/v% methanol–phosphate buffer (pH 3.0), and subjected to high-performance liquid chromatographic analysis (HPLC) for simultaneous determination of HCFU and its degradation product, 5-FU. HPLC conditions were as follows: pump and detector, Hitachi 635A type with 638-41 UV monitor (Tokyo, Japan); column, LiChrosorb-RP-18 (10 μm, 4φ × 250 mm, Merck, F.R.G.); mobile phase, methanol–water (4 : 1 v/v%); flow rate, 0.8 ml/min; detection, 261 nm. Components were quantitated by measuring peak height using an internal standard, hexyl *p*-hydroxybenzoate.

In the suppository base. The suppository (2 g) was prepared by suspending HCFU or its complexes in melted Witepsol H-15 base (Dynamit Nobel Chemicals, Troisdorf-Oberlar, F.R.G.) to yield a drug concentration of 0.015% (w/w). The melt was then poured into a suppository mold (Erwaka G.m.b.H., Frankfurt, F.R.G.) and allowed to cool at room temperature. The stability studies were conducted under conditions of 25 °C and 75% R.H. At appropriate intervals, HCFU and 5-FU in the suppository were extracted by agitating with methanol–0.1 M phosphoric acid (19 : 1 v/v) for 10 min at 45 °C. The methanol

phase was separated by centrifugation, and then placed in a refrigerator for one day to solidify small amounts of the suppository base in the methanol phase. After centrifugation ($700 \times g$, 10 min), the methanol phase was analyzed by HPLC: mobile phase, methanol – 0.07 M acetic acid (18 : 7 v/v); flow rate, 1.0 ml/min. Other HPLC conditions were the same as those described above.

Moisture sorption studies

The sample powders (500 mg, 100 mesh) in a weighing bottle were placed in the incubator of various R.H.s and constant temperature, and at appropriate intervals the weight of the sample were measured by a Shimadzu Libror AEL-160 electric reading balance. Water content of the complex was estimated from the increase in weight after the moisture sorption equilibrium was attained (about 2 days).

Dissolution and suppository release studies

The dissolution rate was measured by the method of Nogami et al. (1969). The experimental conditions were as follows: *sample,* HCFU (46 mg, 100 mesh) or its equivalent amount of the complex (100 mesh), *dissolution medium*, 25 ml of Japanese Pharmacopoeia (JP XI) 1st fluid (2.0 g of NaCl and 24 ml of 10% HCl in 1000 ml of water, pH about 1.2), *temperature,* 37 °C, *stirring speed,* 91 rpm. The concentration of HCFU dissolved in the medium was determined spectrophotometrically at 261 nm.

The release rate of HCFU from Witepsol H-15 suppositories was measured using a suppository release apparatus (Toyama Sangyo Co., Osaka, Japan) according to the method of Muranishi et al. (1979). The release phase (300 ml of phosphate buffer, pH 7.4) was separated by a membrane filter (pore size, 3.0 μm; Millipore Co., Bedford, MA), and stirred with a magnetic stirrer at 100 rpm at 37 °C. The rotation rate of the steel rod in the suppository chamber was 25 rpm. At appropriate intervals, 1 ml of sample was withdrawn from the release phase and assayed for HCFU and 5-FU using HPLC. HPLC conditions were the same as those used in the solid-state stability study.

In vivo absorption studies

Five male albino rabbits, weighing 2.0–3.0 kg, were fasted for 1 day prior to drug administration. Intervals of at least 2 weeks were taken in a cross-over matrix to minimize the cumulative effect of the preceding dose. A test powder (15 mg/kg of body weight as HCFU, 100 mesh) was orally administered as a suspension in 60 ml water using a stomach catheter. The suppository (10 mg/kg of body weight as HCFU) was inserted into the rectum and the anus was then closed with a clip to prevent leakage. A 1.0 ml blood sample was taken from the marginal ear vein at predetermined times, added to 3.8% trisodium citrate (0.1 ml) and centrifuged ($1400 \times g$) for 10 min at 4 °C. After 1.0 ml of 0.1 M phosphoric acid was added to 0.3 ml of the citrated plasma, HCFU was extracted with 6.0 ml of chloroform containing 1-octylcarbamoyl-5-FU (internal standard). Five ml of the organic phase was evaporated under reduced pressure and the residue was dissolved in 200 μl of methanol–0.07 M acetic acid (18 : 7 v/v), 30 μl of which was analyzed by HPLC. HPLC conditions were the same as those described in the suppository stability study.

Results and Discussion

Inclusion complex formation

Fig. 1 shows the phase solubility diagrams for the HCFU-β-CyD systems in phosphate buffer (pH 2.5) at 37 °C. Under the concentration ranges of β-CyDs studied, the solubility of HCFU increased as a function of host concentration and the solubility curve can be classified as type A_L (Higuchi and Connors, 1965). In the higher temperature ranges (45 °C), however, the methylated β-CyD systems showed the B_s type solubility curves, precipitating the 1 : 1 crystalline (guest : host molar ratio) complexes. We have previously reported (Kikuchi et al., 1984) that the solubility curve of HCFU-β-CyD system at 25 °C was B_s type and the stoichiometry of the complex was found to be 1 : 1. Similarly, the change in the type of solubility curve with a temperature was observed for flurbiprofen-β-CyD complexes (Uekama et al., 1985). Therefore, the stability

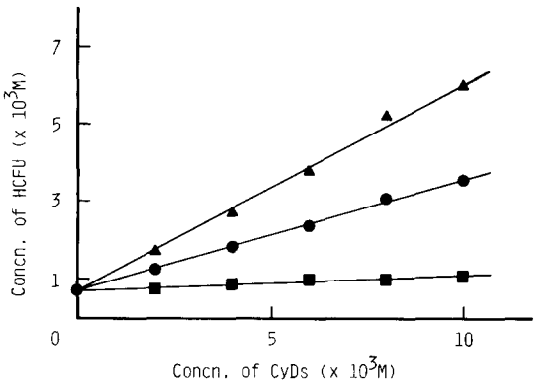


Fig. 1. Phase solubility diagrams of HCFU-β-CyD systems in phosphate buffer (pH 2.5) at 37°C. ●, β-CyD system; ▲, DM-β-CyD system; ■, TM-β-CyD system.

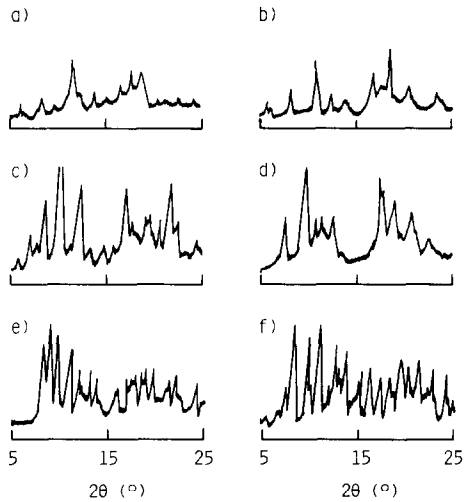


Fig. 3. Powder X-ray diffraction patterns of HCFU-β-CyD systems. a = physical mixture of HCFU and β-CyD; b = β-CyD complex; c = physical mixture of HCFU and DM-β-CyD; d = DM-β-CyD complex; e = physical mixture of HCFU and TM-β-CyD; f = TM-β-CyD complex.

constants (K_c) of the present systems were also calculated, assuming a 1 : 1 complexation. The K_c values were obtained for the DM-β-CyD complex (1500 M^{-1}), β-CyD complex (530 M^{-1}) and TM-β-CyD complex (50 M^{-1}), indicating that HCFU interacts most strongly with DM-β-CyD among the three β-CyDs.

The complexes of HCFU with three β-CyDs in a molar ratio of 1 : 1 were prepared according to the kneading method and their interactions in the solid state were examined by thermal analysis and X-ray diffractometry and compared with the corresponding physical mixtures. Fig. 2 shows the DTA and TG thermograms of HCFU-β-CyD complexes. In the case of HCFU alone, three endothermic peaks due to the melting and degradation of HCFU and degradation of the resulting 5-FU were observed around 112, 152 and 280°C, respectively. In sharp contrast, the complexes showed no appreciable peaks for HCFU within the melting and/or degradation temperature range of β-CyDs. Furthermore, the weight

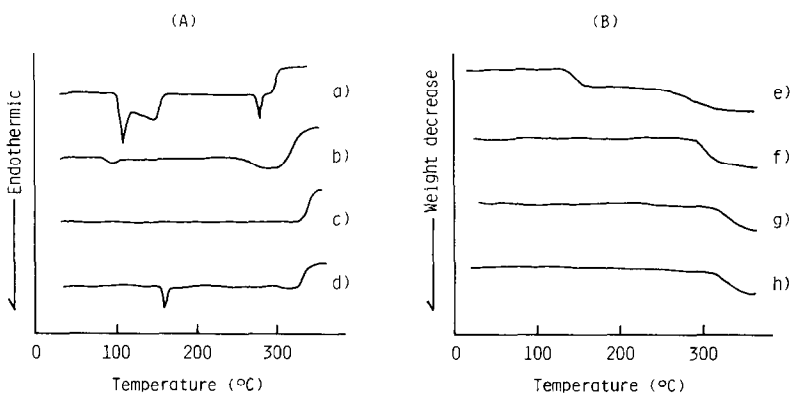


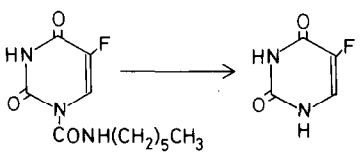
Fig. 2. DTA (A) and TG (B) thermograms of HCFU-β-CyD systems. a and e = HCFU alone; b and f = β-CyD complex; c and g = DM-β-CyD complex; d and h = TM-β-CyD complex.

decrease of HCFU at its degradation temperature disappeared when complexed, as is apparent from the TG thermograms.

Fig. 3 shows the powder X-ray diffraction patterns of the complexes and physical mixtures. The diffraction patterns of physical mixtures were simply a superposition of each component, while those of the complexes were apparently different from those of the physical mixtures, suggesting the formation of new solid phases. These data clearly indicate that the HCFU-β-CyD complexes exist in the solid state.

Stability studies

HCFU is known to be susceptible to hydrolysis to 5-FU in aqueous solution (Scheme 1) (Buur and



Scheme 1. Hydrolysis of HCFU to 5-FU.

Bundgaard, 1985), which may decrease the therapeutic efficiency as well as oral bioavailability. Fig. 4 shows the influence of three β-CyDs on the hydrolysis rate of HCFU over the pH range of 5.0–12.5, where the k is first-order hydrolysis rate constant. It is apparent that the hydrolysis was decelerated about 1.6, 3.0 and 1.1 times by the addition of β-CyD, DM-β-CyD and TM-β-CyD, respectively, over the pH range studied. This order is well correlated with the magnitude of the stability constants of the inclusion complexes.

Fig. 5 shows the time course of the degradation of HCFU and β-CyD complexes in the solid state under the condition of 75% R.H. and 70°C. The main degradation product of HCFU was found to be 5-FU and no appreciable side reactions such as ring opening of the uracil moiety were observed. It is apparent that the degradation rate of HCFU in the β-CyD complex was extremely fast, compared with that in methylated β-CyD complexes. We have recently reported (Kikuchi et al., 1987) that the degradation rate of HCFU in natural β-CyD complex was significantly influenced by the hygroscopic nature of the host molecules. Thus, the moisture sorption behavior of HCFU-methylated β-CyD complexes was investigated under various R.H. conditions and compared with the β-CyD complex. As is shown in Fig. 6, the water content increased with R.H. in the order of: HCFU alone < TM-β-CyD complex < DM-β-CyD complex < β-CyD complex, which order is inversely proportional to that of the stability of HCFU in the complexes. These results suggest that the greater stability of HCFU in the methylated β-CyD com-

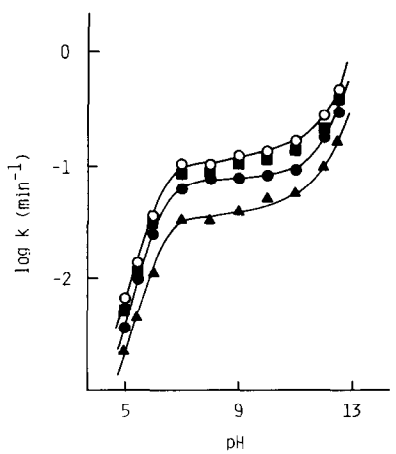


Fig. 4. pH profiles for the hydrolysis rate of HCFU in the absence and presence of β-CyDs (1.0×10^{-2} M) in phosphate buffer at 37°C. ○, HCFU alone; ●, β-CyD system; ▲, DM-β-CyD system; ■, TM-β-CyD system.

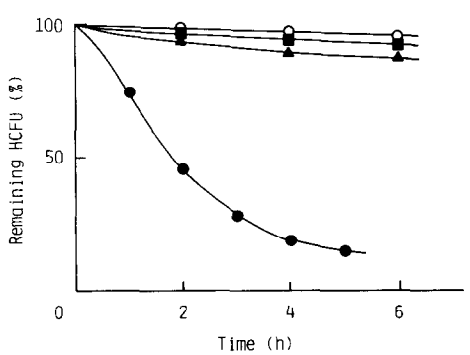


Fig. 5. Time courses of decomposition of HCFU and its β-CyD complexes in the solid state (70°C, 75% R.H.). ○, HCFU alone; ●, β-CyDcomplex; ▲, DM-β-CyD complex; ■, TM-β-CyD complex.

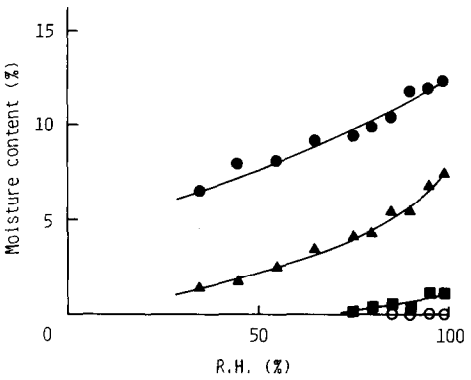


Fig. 6. Moisture sorption curves of HCFU-β-CyD complexes at 25°C. ○, HCFU alone; ●, β-CyD complex; ▲, DM-β-CyD complex; ■, TM-β-CyD complex.

plexes than in the β-CyD complex may be mainly due to their lower hygroscopicity, in addition to the stabilizing effect observed in aqueous solution.

Fig. 7 shows the time course of the degradation of HCFU and its β-CyD complexes in Witepsol H-15 suppository base at 75% R.H. and 25°C. It is apparent that the degradation rate of HCFU in the suppository base was slowed in methylated β-CyD complexes, compared to the β-CyD complex and even to HCFU, itself. Since the chemical stability of HCFU is greater in solid state than in solution, one of the decelerating factors for the methylated β-CyD complexes may be their low solubility in the base (20.0, 2.2, 15.0 and 14.8 mg/g as HCFU at 45°C for the drug alone,

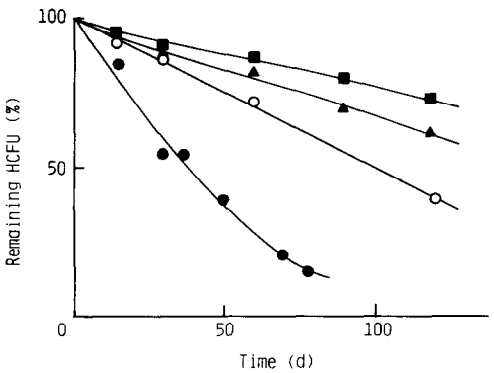


Fig. 7. Time courses of decomposition of HCFU and its β-CyD complexes in Witepsol H-15 suppository base (25°C, 75% R.H.). ○, HCFU alone; ●, β-CyD complex; ▲, DM-β-CyD complex; ■, TM-β-CyD complex.

β-CyD complex, DM-β-CyD complex and TM-β-CyD complex, respectively) (Takahashi et al., 1986). In the case of the β-CyD complex, this deceleration effect may be more than cancelled out by its higher hygroscopicity and/or water content. Of course, other factors such as miscibility of adsorbed water and the suppository base, partition of HCFU between them, and participation of hydroxyl group of the base in the reaction should be considered for the deceleration and acceleration effects of β-CyDs. Further studies are now in progress to elucidate the detailed mechanisms, including single-crystal X-ray analysis of the complexes because the difference in inclusion mode of HCFU in the complexes should also influence the degradation rate.

Dissolution and release from suppository base

Fig. 8 shows the dissolution profiles of HCFU and its β-CyD complexes in JP XI 1st fluid (pH about 1.2). Fig. 9 shows the release profiles of the drug from Witepsol H-15 suppository base into phosphate buffer (pH 7.4), including the hydrolysis of HCFU. It is apparent that both dissolution and release rates of HCFU was significantly improved by β-CyD complexations, in the order of: DM-β-CyD > β-CyD > TM-β-CyD > HCFU alone. The observed rate increases may be due to the increase in solubility, as expected from Fig. 1. In the case of suppository release, the decrease in concentration of HCFU after a peak (about 20 min) is due to the hydrolysis of HCFU to 5-FU in the release phase, as is apparent from Fig. 4.

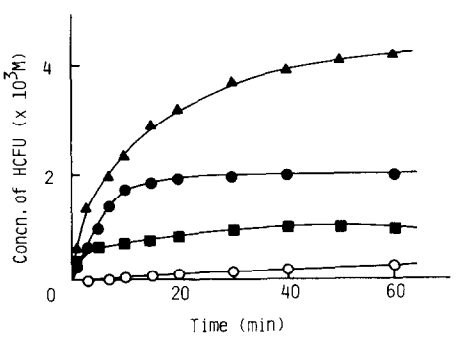


Fig. 8. Dissolution profiles of HCFU and its β-CyD complexes in JP XI 1st fluid at 37°C. ○, HCFU alone; ●, β-CyD complex; ▲, DM-β-CyD complex; ■, TM-β-CyD complex.

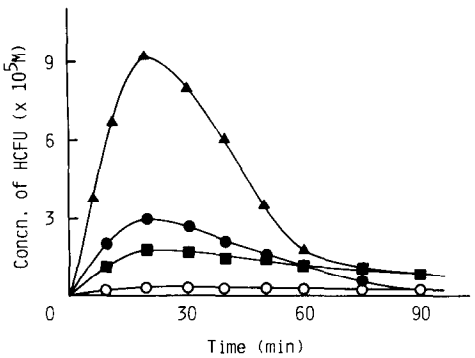


Fig. 9. Release profiles of HCFU and its β-CyD complexes from Witepsol H-15 suppository base into phosphate buffer (pH 7.4) at 37°C. ○, HCFU alone; ●, β-CyD complex; ▲, DM-β-CyD complex; ■, TM-β-CyD complex.

In vivo absorption behavior

HCFU and its three β-CyD complexes were administered orally or rectally to rabbits to evaluate their absorption characteristics, since the above data suggested that the complexes might have better bioavailability. Fig. 10 shows the mean plasma levels of HCFU following the oral administration of HCFU or its β-CyD complexes (15 mg/kg). The plasma levels of HCFU during the initial 2 h period were much higher when the drug was administered as the DM-β-CyD complexed form. In the case of HCFU alone, the maximum plasma levels (C_{max}) of 0.74 ± 0.28 μg/ml was observed at 0.18 ± 0.03 h. On the other hand, the β-CyD, DM-β-CyD and TM-β-CyD complexes showed C_{max} of 5.83 ± 1.13 μg/ml at 0.17 ± 0.03 h, 7.15 ± 1.99 μg/ml at 0.20 ± 0.04 h, and 4.38 ± 1.48 μg/ml at 0.21 ± 0.04 h, respectively. Furthermore, the area under plasma concentration–time curves (AUC) of β-CyD, DM-β-CyD and TM-β-CyD complexes up to 6 h post-administration were about 3.0, 7.5 and 3.0 times as much as that of HCFU alone, respectively.

Fig. 11 shows the mean plasma levels of HCFU following the rectal administration of HCFU or its β-CyD complexes (10 mg/kg) as suppositories. The higher plasma levels of HCFU were observed in the rectal administration, compared to the oral administration. The C_{max} and the T_{max} (time to reach the maximum plasma concentration) in the rectal administration were as follows: 6.19 ± 1.65 μg/ml at 0.35 ± 0.05 h, 7.54 ± 0.77 μg/ml at 0.40 ± 0.05 h, 14.72 ± 3.48 μg/ml at 0.65 ± 0.07 h, and 5.64 ± 0.56 μg/ml at 0.55 ± 0.08 h for HCFU alone, β-CyD complex, DM-β-CyD complex and TM-β-CyD complex, respectively. The AUC of HCFU was markedly enhanced by administration of inclusion complexes as follows: the AUC of β-CyD, DM-β-CyD and TM-β-CyD complexes up to 6 h post-administration were about 1.2, 3.3 and 1.4 times greater than that of HCFU alone, respectively.

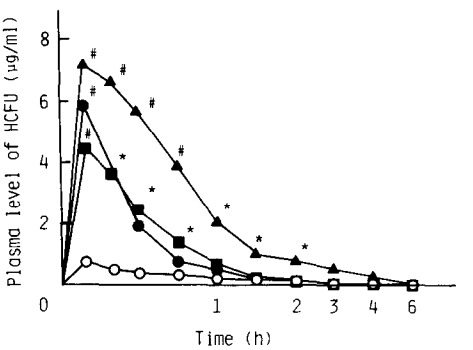


Fig. 10. Plasma concentrations of HCFU following oral administration of HCFU or its β-CyD complexes (equivalent to 15 mg/kg HCFU) to rabbits. ○, HCFU alone; ●, β-CyD complex; ▲, DM-β-CyD complex; ■, TM-β-CyD complex. * $P < 0.05$ in the complex vs HCFU; # $P < 0.01$ in the complex vs HCFU.

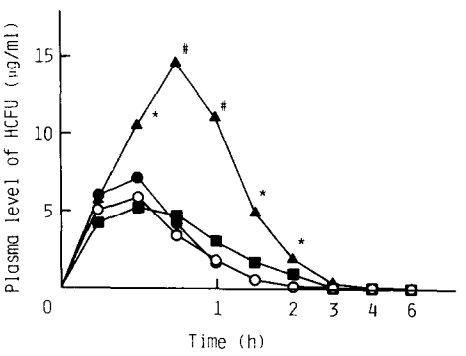


Fig. 11. Plasma concentrations of HCFU following rectal administration of HCFU or its β-CyD complexes (equivalent to 10 mg/kg HCFU) as Witepsol H-15 suppository to rabbits. ○, HCFU alone; ●, β-CyD complex; ▲, DM-β-CyD complex; ■, TM-β-CyD complex. * $P < 0.05$ in the complex vs HCFU; # $P < 0.01$ in the complex vs HCFU.

The above data clearly indicated that the methylated β-CyDs, particularly DM-β-CyD, seemed to have more utility over the parent β-CyD, from the viewpoint of bioavailability as well as quality assurance of HCFU.

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