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O-CARBOXYMETHYL-*O*-ETHYLCYCLOMALTOHEPTAOSE AS A DELAYED-RELEASE-TYPE DRUG CARRIER: IMPROVEMENT OF THE ORAL BIOAVAILABILITY OF DILTIAZEM IN THE DOG

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ABSTRACT

The utility of *O*-carboxymethyl-*O*-ethylcyclomaltoheptaose (carboxymethyl-ethyl-β-cyclodextrin, CME-βCD) as a delayed-release-type drug carrier was investigated *in vitro* and *in vivo*, using diltiazem hydrochloride as a model drug. The aqueous solubility of CME-βCD showed a marked dependency on pH, because of the ionization of the carboxyl group (pK_a 3.75). The formation of an inclusion complex between diltiazem and CME-βCD in aqueous solution and in the solid state was assessed by a solubility method and by X-ray diffractometry, respectively. The rate of release of the drug from the compressed tablet containing the complex was significantly retarded in solutions at low pH and increased with increase in pH, and this was reflected in the blood levels in the dog after the oral administration. The results suggested that the use of CME-βCD could improve the oral bioavailability of diltiazem and release the drug preferentially in the intestinal fluid but only slightly in the gastric fluid.

INTRODUCTION

Cyclomalto-hexaose, -heptaose, and -octaose [α-, β-, and γ-cyclodextrin (CD)] can alter various physical, chemical, and biological properties of drug molecules by the formation of inclusion complexes. This kind of molecular encapsulation offers the promise of new dosage forms and its importance in pharmaceutical formulations has been recognized[1–3]. β-Cyclodextrin (βCD) has been used widely to improve the pharmaceutical properties of drugs, but it has several unfavorable properties as a drug carrier[4–6]. βCD has been modified chemically for many different purposes[7–10]. Methylated[5,11], hydroxyalkylated[12–14], and branched[15,16] derivatives of βCD, which are hydrophilic, are useful for improving the solubility or the rates of dissolution of drugs that are poorly soluble in water because of the formation of inclusion complexes. In contrast, there is little in-

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formation concerning hydrophobic derivatives of βCD, which may be useful for controlling the rates of release of water-soluble drugs. When the hydroxyl groups of βCD were ethylated, the aqueous solubility decreased in proportion to the degree of substitution[17]. The complexes with ethylated derivatives of βCD released the drugs slowly in aqueous medium, thereby providing sustained-release after oral administration to rats[18]. Delayed-release in gastrointestinal fluids is advantageous for drugs that are unstable in acidic media and/or absorbable mainly from the intestinal tract. Therefore, the possible utility of *O*-carboxymethyl-*O*-ethyl derivatives of βCD (CME-βCD) as delayed-release-type drug carriers was investigated. These complexes would be expected to release drugs preferentially in the intestinal fluid after slow release in the gastric fluid, owing to the dissociation of the carboxymethyl group of the host molecule, and thus resemble the enteric-coating-type derivatives of cellulose, such as cellulose acetate phthalate (CAP) and carboxymethylethylcellulose (CMEC). Diltiazem hydrochloride, a potent calcium antagonist, was used as a model drug that is absorbed mainly from the jejunoileum and has a short biological half-life in humans (2.7–4.7 h)[19].

RESULTS AND DISCUSSION

Solubility of CME-βCD. — In order to release the drug preferentially in the intestinal fluid after oral administration of the CME-βCD complex, the host molecule should dissolve readily in the intestinal fluid (pH ~6.8), but only slightly in the gastric fluid (pH ~1.2). The solubility of CME-βCD was almost constant below pH ~2.5 (Fig. 1), increased steeply above pH ~4, and was freely soluble in water at pH >6. The pK_a value was obtained from the data (Fig. 1) by using equation *1*[20],

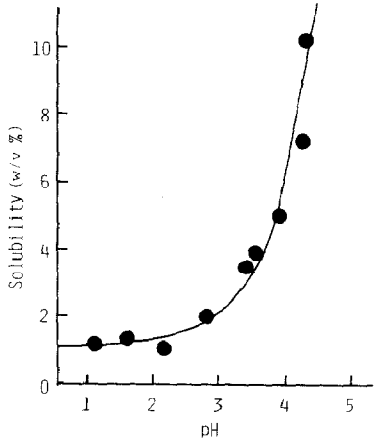

Fig. 1. Solubility profile of CME-βCD as a function of pH of the aqueous solution at 25°.

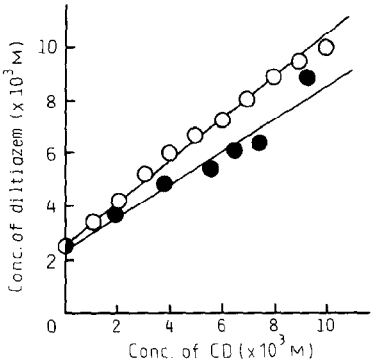

Fig. 2. Phase solubility diagrams in phosphate buffer (pH 7.0) at 25° of diltiazem–βCD (—○—) and diltiazem–CME-βCD (—●—).

$$S_t = S_o + S_i = S_o + \frac{K_a \cdot S_o}{[H^+]} \qquad 1$$

where S_o and S_i are the solubilities of unionized CME-βCD and its conjugate base, respectively, and S_t is the observed total solubility ($S_o + S_i$) at a given pH, and K_a is the apparent dissociation constant of CME-βCD. The values of pK_a and S_o of CME-βCD, refined by a non-linear least-squares method, were 3.75 and 1.2 g/dL, respectively. These results suggest that CME-βCD may have potential as a delayed-release-type drug carrier.

Formation of inclusion complexes. — Fig. 2 shows the phase solubility diagrams[21] for diltiazem with βCD and CME-βCD in phosphate buffer (pH 7.0). The solubility of the drug increased linearly as a function of concentration of βCD or CME-βCD, and the solubility curves are type A_L according to Higuchi and Connors[22], suggesting a 1:1 complex. Thus, the apparent 1:1 stability constants (K_c) of the complexes were calculated, in terms of equation 2[21,22].

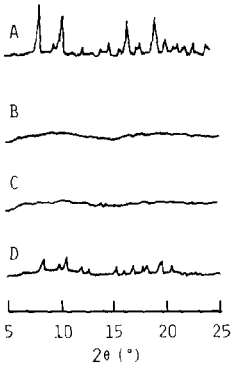

Fig. 3. Powder X-ray diffraction patterns of A, diltiazem; B, CME-βCD; C, diltiazem–CME-βCD complex; D, a mixture of diltiazem and CME-βCD.

$$K_c = \frac{\text{slope}}{\text{intercept}(1 - \text{slope})} \qquad 2$$

The K_a values were 750 M^{-1} for the CME-βCD complex and 1150 M^{-1} for the βCD complex. The substituents introduced into βCD seem to sterically hinder the inclusion of the drug.

Fig. 3 shows the powder X-ray diffraction pattern of the 1:1 diltiazem–CME-βCD complex, prepared according to the kneading method[23]. CME-βCD gave no diffraction peak in the 2θ range, suggesting an amorphous state. The diffraction pattern of the physical mixture was simply the superposition of those of each component, whereas, on binding to CME-βCD, the peaks of the drug almost completely disappeared and the complex was in a less crystalline or amorphous state. These data clearly indicate that the CME-βCD complex exists in the solid state.

Release of drug from the complex. — Fig. 4 shows the release of diltiazem from compressed tablets containing the drug (diluent starch) or its CME-βCD complex into the Japanese Pharmacopoeia XI (JP XI) first fluid (pH 1.2), JP XI second fluid (pH 6.8), and a solution of intermediate pH (4.0). The rate of release from the starch tablet was fast in each medium, due to the high aqueous solubility of the drug. The complex suppressed the release of the drug particularly at low pH and the rate of release increased with increase in pH, showing a typical delayed-release pattern *in vitro*. The pH-dependent release for the CME-βCD complex is attributable to its high aqueous solubility after the ionization of host molecule.

Absorption in vivo. — Fig. 5 shows the plasma levels of diltiazem following a single oral dose to dogs of the drug (diluent starch) or its CME-βCD complex in the compressed tablet. Table I summarizes the pharmacokinetic parameters obtained from the data in Fig. 5, where the mean residence time (MRT) in the systemic circulation, variance of residence time (VRT), and mean absorption time (MAT) were calculated by moment analysis[24]. The absorption of the drug from the starch tablet was rapid, with a short plasma half-life, whereas a delayed release was evident for the absorption of the drug from the tablet. The time (t_{max}) required to reach the maximum plasma concentration (C_{max}) was prolonged about four times

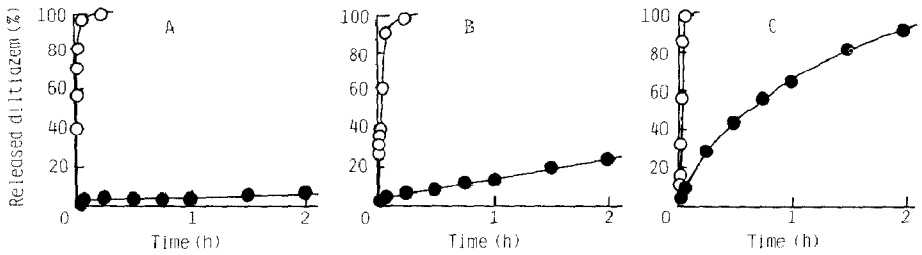

Fig. 4. Release of diltiazem from tablets containing diltiazem (diluent starch) (—○—) or the diltiazem–CME-βCD complex (equivalent to 6.0 mg of drug) (—●—) at 37°; A, JP XI first fluid (pH 1.2); B, acetate buffer (pH 4.0); C, JP XI second fluid (pH 6.8).

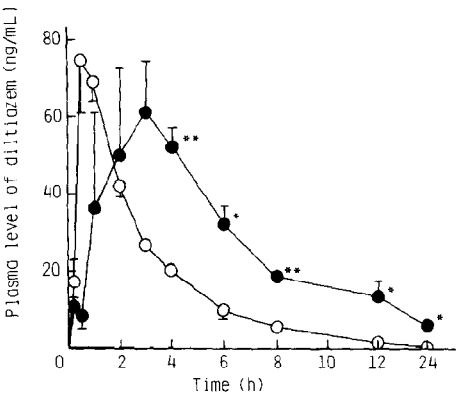

Fig. 5. Plasma levels of diltiazem after oral administration of tablets containing diltiazem (diluent starch) (—○—) or the diltiazem–CME-βCD complex (equivalent to 30 mg of drug) (—●—) to dogs. Each value represents the mean ±s.e. of 4 dogs. *, $p < 0.05$; **, $p < 0.01$ *versus* the drug alone.

after administration of the complex. In addition, the values of MRT and MAT, which are indices for maintenance of drug level in plasma or gastrointestinal tract[25], were significantly larger than those of the starch tablet. The rapid appearance of the drug in the plasma from the starch tablet reflects fast dissolution of the tablet even in the gastric fluid, as is apparent from Fig. 4. The complex probably released the drug preferentially in the small intestine, after passing through the stomach, which delays the drug absorption. The CME-βCD complex produced an ~2-fold increase in the area under the plasma concentration curve (AUC), up to 24 h post-administration, compared with the drug alone. Such an increase in the AUC may be responsible, at least in part, for the reduction of the first-pass effect, *i.e.*, the metabolism in the liver immediately after absorption is saturated due to the high concentration of the drug[24]. This effect may be due to rapid dissolution of the complex in the intestinal fluid, giving a high local concentration of the drug at the main site of absorption. Other factors, such as the influence of CME-βCD on the intestinal mucous membrane and stabilizational of the drug in gastrointestinal fluids, may also increase the AUC.

TABLE I

PHARMACOKINETIC PARAMETERS[a] OF DILTIAZEM AFTER ORAL ADMINISTRATION OF TABLETS CONTAINING THE DRUG OR ITS CME-βCD COMPLEX (EQUIVALENT TO 30.0 mg OF DILTIAZEM) TO DOGS

System	C_{max} *(ng/mL)*	t_{max} *(h)*	*AUC (h·ng/mL)*	*MRT (h)*	*VRT* (h^2)	*MAT (h)*
Diltiazem alone[b]	87.0 ±6.3	0.8 ±0.1	226.2 ±10.8	3.1 ±0.3	7.9 ±1.5	1.3 ±0.3
CME-βCD complex	78.7 ±13.0	3.0 ±0.7	490.6 ±61.3[c]	7.5 ±0.8[d]	32.6 ±3.7[d]	5.7 ±0.8[d]

[a]Each value represents the mean ±s.e. of 4 dogs. [b]Starch as diluent. [c]$p < 0.05$. [d]$p < 0.01$.

The above data indicate that CME-βCD may be a candidate for a delayed-release-type carrier for water-soluble drugs that are (*a*) unstable in the stomach, (*b*) absorbed mainly from the intestinal tract, and (*c*) irritate the gastric mucosa. The design of more suitable modified-release dosage forms, by varying the degree of substitution of the CME-βCD and/or combining CME-βCD with other hydrophilic or hydrophobic CD derivatives, is being studied.

EXPERIMENTAL

Materials. — Diltiazem hydrochloride was donated by Nisshin Flour Milling Co. (Tokyo), βCD was supplied by Nihon Shokuhin Kako Co. (Tokyo), and CME-βCD was kindly donated by Wako Pure Chemical Industries (Tokyo Research Division, Saitama). The degrees of substitution (d.s.) of the carboxymethyl and ethyl groups in CME-βCD were ~1.7 and ~10.5, determined by non-aqueous titration and iodometry of JP XI, respectively, and were in good agreement with those estimated by ^{1}H-n.m.r. spectroscopy. The preparation and characterization of CME-βCD will be reported elsewhere. Deionized, double-distilled water was used.

Solubility studies. — Excesses of CME-βCD were shaken with aqueous solutions of various pH values for 10 days at 25° and the concentration of CME-βCD in solution was determined polarimetrically. The solubility of diltiazem free-base in the presence of CME-βCD was determined according to Higuchi and Connors[22], *i.e.*, excess of the drug was shaken with 0.1M phosphate buffer (pH 7.0) containing various concentrations of CME-βCD and shaken at 25° for ~10 days. The concentration of the drug in solution was determined spectrophotometrically at 245 nm.

Preparation of the solid complex. — The 1:1 solid complex of diltiazem with CME-βCD was prepared by the kneading method[23], *i.e.*, the drug (1.0 g) and CME-βCD (3.4 g) were triturated with a small amount of ethanol–water (1:1, ~10 mL) and the slurry was kneaded thoroughly for ~40 min. The resulting paste was dried under reduced pressure at room temperature for 3 days.

Powder X-ray diffractometry. — A Rigaku Dennki Geiger Flex-2012 diffractometer was used with Ni-filtered Cu-$K\alpha$ radiation; voltage, 30 kV; current, 20 mA; time constant, 2 s; scanning speed, 1°/min; diffraction angle (2θ), 5–25°.

Release of the drug. — A sample (26 mg, <100 mesh) of powder that contained the drug (6.0 mg) and diluent (starch), or its equivalent amount of the complex, was compressed into a cylindrical tablet (diameter, 4 mm). A basket containing the tablet was immersed in the dissolution medium (25 mL) at 37° and rotated at 60 r.p.m. At intervals, an aliquot (0.3 mL) was filtered, diluted with the dissolution medium, and assayed spectrophotometrically at 245 nm. Dissolution media: JP XI first fluid (2.0 g of NaCl and 24 mL of aqueous 10% HCl in 1 L of water, pH 1.2), JP XI second fluid (250 mL of 0.2M KH_2PO_4 and 118 mL of 0.2M NaOH in 1 L of water, pH 6.8), and 0.05M acetate buffer (pH 4.0).

Absorption of the drug in vivo. — Four male beagle dogs (13–15 kg) were

fasted for ~24 h and a compressed tablet (132 mg; diameter, 7 mm) containing the drug (30 mg) or its CME-βCD complex (equivalent to 30.0 mg of drug) and a diluent (starch) was administered orally along with water (100 mL). Blood samples (3.0 mL) were collected at intervals using a citrated injection syringe, and centrifuged (1400*g*) for 10 min. Internal standard (chlorpromazine) and borate buffer (1.0 mL, pH 9.0) were added to the plasma (1.0 mL), the drug was extracted with hexane–2-propanol–28% ammonium hydroxide (4.0 mL, 100:2:0.1), and then 3.0 mL of the organic phase was concentrated under reduced pressure. The diltiazem was determined in a portion (30 μL) of a solution of the residue in hexane–dichloromethane–ethanol–28% ammonium hydroxide (0.1 mL, 70:20:10:0.1) by h.p.l.c. with a Jasco BIP-I pump equipped with a UVIDEC-100-V UV detector, a column (5 μm, 4.0 × 250 mm) of LiChrosorb Si-60 (Cica-Merck), and hexane–dichloromethane–ethanol–28% ammonium hydroxide (70:20:10:0.1) at 1.0 mL/min, and detection at 245 nm.

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REFERENCES

1 W. Saenger, *Angew. Chem. Int. Ed. Engl.*, 19 (1980) 344–362.
2 K. Uekama, *Yakugaku Zasshi*, 101 (1981) 857–873.
3 J. Szejtli, *Cyclodextrins and Their Inclusion Complexes*, Akadémiai Kiadó, Budapest, 1982.
4 J. Pitha, *Life Sci.*, 29 (1981) 307–311.
5 J. Szejtli, *J. Incl. Phenom.*, 1 (1983) 135–150.
6 K. Uekama, *Pharmacy Int.*, 6 (1985) 61–65.
7 A. P. Croft and R. A. Bartsch, *Tetrahedron*, 39 (1983) 1417–1474.
8 K. Uekama and M. Otagiri, in S. D. Bruck (Eds.), *Critical Reviews in Therapeutic Drug Carrier Systems*, Vol. 3, CRC Press, Boca Raton, 1987, pp. 1–40.
9 D. Duchêne, *Cyclodextrins and Their Industrial Uses*, Editions de Santé, Paris, 1987.
10 J. Szeman, H. Ueda, J. Szejtli, E. Fenyvesi, Y. Machida, and T. Nagai, *Chem. Pharm. Bull.*, 35 (1987) 282–288.
11 K. Uekama, in D. D. Breimer and P. Speiser (Eds.), *Topics in Pharmaceutical Sciences 1987*, Elsevier, Amsterdam, 1987, pp. 181–194.
12 B. W. Müller and U. Brauns, *Int. J. Pharm.*, 26 (1985) 77–88.
13 J. Pitha and J. Pitha, *J. Pharm. Sci.*, 74 (1985) 987–990.
14 A. Yoshida, H. Arima, K. Uekama, and J. Pitha, *Int. J. Pharm.*, 46 (1988) 217–222.
15 Y. Okada, Y. Kubota, K. Koizumi, S. Hizukuri, T. Ohfuji, and K. Ogata, *Chem. Pharm. Bull.*, 36 (1988) 2176–2185.
16 M. Yamamoto, A. Yoshida, F. Hirayama, and K. Uekama, *Int. J. Pharm.*, 49 (1989) 163–171.
17 K. Uekama, N. Hirashima, Y. Horiuchi, F. Hirayama, T. Ijitsu, and M. Ueno, *J. Pharm. Sci.*, 76 (1987) 660–661.
18 F. Hirayama, N. Hirashima, K. Abe, K. Uekama, T. Ijitsu, and M. Ueno, *J. Pharm. Sci.*, 77 (1988) 233–236.
19 J. P. Clozel, G. Caille, Y. Taeymans, P. Theroux, P. Biron, and J. G. Besner, *J. Pharm. Sci.*, 73 (1984) 207–209.
20 K. G. Mooney, M. A. Mintun, K. J. Himmelstein, and V. J. Stella, *J. Pharm. Sci.*, 70 (1981) 13–22.
21 T. Higuchi and J. L. Lach, *J. Am. Pharm. Assoc., Sci. Ed.*, 43 (1954) 349–354.
22 T. Higuchi and K. A. Connors, *Adv. Anal. Chem. Instrum.*, 4 (1965) 117–212.

23 M. TSURUOKA, T. HASHIMOTO, H. SEO, S. ICHIMASA, T. FUJINAGA, M. OTAGIRI, AND K. UEKAMA, *Yakugaku Zasshi*, 101 (1981) 360–367.
24 K. YAMAOKA, Y. TANIGAWARA, T. NAKAGAWA, AND T. UNO, *J. Pharmacobio-Dyn.*, 4 (1981) 879–885.
25 V. I. VASHI AND M. C. MEYER, *J. Pharm. Sci.*, 77 (1988) 760–764.
26 H. ECHIZEN AND M. EICHELBAUM, *Clin. Pharmacokinetics*, 11 (1986) 425–449.