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In-vivo and In-vitro Correlation for Delayed-release Behaviour of a Molsidomine/*O*-carboxymethyl-*O*-ethyl-β-cyclodextrin Complex in Gastric Acidity-controlled Dogs

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Abstract

The in-vivo absorption behaviour of molsidomine from the delayed-release tablets of an *O*-carboxymethyl-*O*-ethyl-β-cyclodextrin complex was investigated using gastric acidity-controlled dogs under fasted and non-fasted conditions.

The in-vitro release profiles were generated by changing the pH of the dissolution medium at different rotation paddle speeds. The absorptivity of molsidomine in the high acidity dog was correlated with the pH-changed release profile (pH 1·2 to 7·0 after 2 h), whereas that in the low acidity dog was correlated with the release profile at a constant pH of 7·0. The absorption in fasted dogs was well correlated with the in-vitro release at the low-rotation paddle speed (< 5 rev min^{-1}), whereas that in the non-fasted dogs was correlated with that of high rotation (100 rev min^{-1}).

The present results suggested that the in-vivo delayed-release behaviour of the complex is predictable from the in-vitro release profiles generated using pH-variable dissolution testing apparatus at different rotation speeds of the paddle.

Cyclodextrins (CyDs) are useful for the improvement of desirable properties, such as solubility, stability and bioavailability, of drugs, making them candidates for novel drug carriers (Uekama & Otagiri 1987; Szejtli 1988). Recently, many kinds of hydrophilic and hydrophobic CyD derivatives have been prepared to improve the physicochemical and pharmaceutical properties of the administered molecules (Müller & Brauns 1985; Pitha & Pitha 1985; Duchêne 1991; Uekama et al 1991). *O*-Carboxymethyl-*O*-ethyl-β-CyD (CME-β-CyD) has unique properties as a drug carrier; for example, it can stabilize chemically labile drugs, such as prostaglandin E_1 (Adachi et al 1992) and carmofur (Horiuchi et al 1991b), in neutral and alkaline regions, because of its inclusion and pH-adjusting abilities. Furthermore, the release rate of water-soluble drugs, such as diltiazem hydrochloride, from CME-β-CyD complexes can be suppressed at the low pH region in the stomach, and increased at the intestinal pH due to the ionization of the carboxyl group of the drug molecule (pK_a ca 4) (Uekama et al 1989, 1993; Horiuchi et al 1991a). Thus, CME-β-CyD may serve as a delayed-release type carrier for water-soluble drugs which are unstable in the stomach and are absorbed mainly from the intestinal tract. However, the release rate of drugs from a CME-β-CyD complex may be affected by gastric pH, gastric emptying rate and the presence of food. In this study, the absorption behaviour of a water-soluble drug, molsidomine, from tablets of a CME-β-CyD complex was investigated using gastric acidity-controlled dogs under fasted and non-fasted conditions. The in-vivo absorption behaviour was compared with the in-vitro release profiles generated by a pH-adjustable dissolution testing apparatus at different rotation speeds of the paddle. Molsidomine is a peripheral vasodilator which is soluble in water (2·5 g L^{-1} at 25°C) and has a short biological half-life (2·1–2·7 h) in man (Yashiki et al 1985).

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Materials and Methods

Materials

CME-β-CyD (degree of substitution: ethyl group 15·7, carboxymethyl group 2·7) was donated by Wako Pure Chemical Industries (Osaka, Japan) and molsidomine was a gift from Takeda Chemical Ind. Ltd (Osaka, Japan). Omeprazole, an H^+, K^+-adenosine triphosphatase inhibitor, was donated by Yoshitomi Pharmaceutical Ind. Ltd (Fukuoka, Japan) and tetragastrin was purchased from Mecto Co. (Tokyo, Japan). Other chemicals and solvents were of analytical reagent grade, and de-ionized double-distilled water was used throughout the study.

Release studies

The solid complex of molsidomine with CME-β-CyD in a molar ratio of 1 : 1 was prepared by the kneading method, as reported previously (Uekama et al 1993). The complexation of molsidomine with CME-β-CyD and resulting phase change in the solid state were confirmed by X-ray diffractometry and thermal analysis (Adachi et al 1992). Plain tablets (diam. 5 mm) were prepared by compressing the complex (equivalent to 10 mg molsidomine, 100 mesh) for 30 s under a pressure of 1000 kg cm^{-2} in a hydraulic press (Riken Seiki Model 16-B, Tokyo, Japan). The release rate of molsidomine from the tablets was measured, using a pH-changeable dissolution apparatus, equipped with a pH controlling system (Uekama et al 1990). The dissolution test was essentially performed according to the paddle method in

the Japanese Pharmacopoeia (JP) XII, where the rotation speed of the paddle was 0–100 rev min^{-1}. The dissolution medium was a mixed buffer solution (500 mL) of 0·05 M hydrochloric acid, 0·05 M acetic acid and 0·05 M phosphoric acid, initially adjusted to pH 1·2 and maintained at 37°C. The volume of the dissolution medium employed was sufficient to maintain sink conditions in the pH ranges tested. A tablet was placed in the bottom of the reservoir and the dissolution medium was continuously circulated through a glass filter (porosity 20–30 μm) at a flow rate of 3·0 mL min^{-1}. After 2 h, the pH of the dissolution medium was increased from 1·2 to 7·0, by the addition of 2·0 M NaOH with the titration rates of 0·20 mL min^{-1} for 0–115 min and 0·12 mL min^{-1} for 115–120 min through an Autoburette (Hiranuma UCB 9000RS, Tokyo, Japan), controlled by a personal computer (NEC 9801, Tokyo, Japan). The amount of molsidomine released in the medium was continuously measured using a UV monitor at 313 nm. The release rates of the drug as a function of pH were computed with the personal computer. Three replicate values agreed to within less than 3% of the mean.

Absorption studies

Male beagle dogs, 10–11 kg, having a high gastric acidity (less than pH 2·0) or a low gastric acidity (higher than pH 6·0) were prepared by treatments with tetragastrin (4 μg kg^{-1}, i.m., × 2) or omeprazole (1 mg kg^{-1}, i.v., × 3), respectively, as reported previously (Yamada et al 1989; Uekama et al 1993). The fasted dogs received no food, but had free access to water, for 24 h before drug administration. A tablet (5 mm diam.) of the CME-β-CyD complex (equivalent to 10 mg molsidomine) was administered orally with water (50 mL) to the gastric pH-controlled dogs under the fasted and non-fasted conditions. The dose (10 mg) of molsidomine was confirmed to be within the range showing a linear dose-dependence of area under plasma concentration-time curve (up to 12 h, AUC). Blood samples (2·0 mL) were withdrawn from the cephalic vein and centrifuged (1700 *g*) for 10 min, and the plasma was analysed for molsidomine by high-performance liquid chromatography (HPLC) using a Hitachi 655A-11 chromatograph (Tokyo, Japan) and a YMC AQ-312 ODS column (Kyoto, Japan), with a mobile phase of 0·01 M sodium acetate buffer/acetonitrile (4 : 1 v/v), detection at 313 nm and a flow rate of 1·0 mL min^{-1}.

Results and Discussion

In-vivo absorption

We have recently reported that the gastric acidity of beagle dogs can be maintained at a pH of about 7·3 for at least 4 h, by omeprazole intravenously injected at a dose of 1 mg kg^{-1} at 48, 24 and 1 h before drug administration (Uekama et al 1993). On the other hand, when tetragastrin is intramuscularly injected at a dose of 4 μg kg^{-1} at 15 min before and 45 min after drug administration, the gastric acidity is maintained at a pH of about 1·5 for at least 2 h. In this study, therefore, tetragastrin or omeprazole was administered to the fasted and non-fasted dogs according to the above time schedules. Fig. 1 shows the plasma level-time curves of molsidomine after oral administration of the tablet containing the CME-β-CyD complex. The pharmacokinetic parameters are summarized in Table 1. In the case of the high gastric acidity dogs, the absorption of molsidomine was significantly retarded, compared with the low gastric acidity dogs, indicating delayed-release characteristics. The delayed-release pattern was more clearly reflected in the fasted dogs, as a longer lag time, because the gastric motility and emptying rate of fasted dogs are lower than those of non-fasted dogs. The AUC of molsidomine was almost the same in the high and low gastric acidity dogs under the fasted and non-fasted conditions.

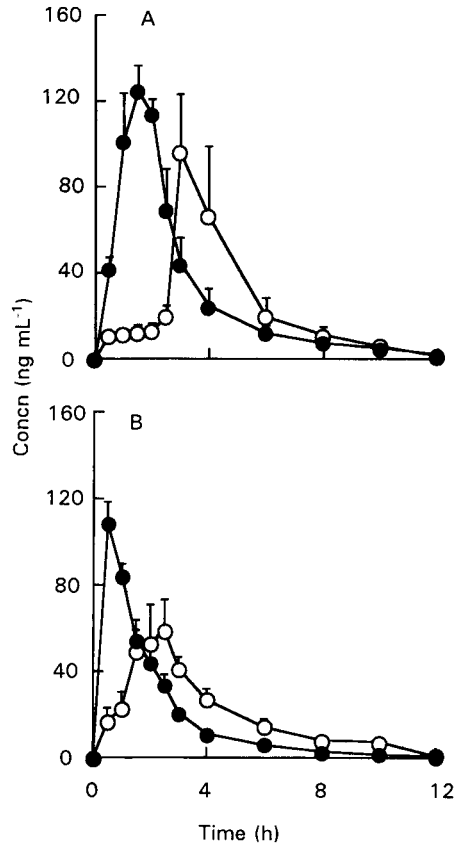

FIG. 1. Plasma levels of molsidomine after oral administration of tablets containing its CME-β-CyD complex (equivalent to 10 mg molsidomine) in gastric acidity-controlled dogs under fasted (A) and non-fasted (B) conditions. ● Low gastric-acidity dogs, ○ high gastric-acidity dogs.

In-vitro release

Fig. 2 shows the release profiles of molsidomine from the tablet containing the CME-β-CyD complex in dissolution media at different pH values (JP XII first fluid (pH 1·2), sodium acetate buffer (pH 4·0), and JP XII second fluid (pH 6·8)). The release rate of molsidomine was suppressed at low pH, while it was increased with increase in pH, showing a typical delayed-release pattern. This can be ascribed to the higher solubility and dissolution rate of CME-β-CyD at higher pH regions, due to the ionization of the carboxyl group. Fig. 3 shows the release profiles of molsidomine from the tablets containing the CME-β-CyD complex, where the pH of the dissolution medium was fixed at 7·0 from the beginning or shifted from 1·2 to 7·0 after 2 h, corresponding

Table 1. Pharmacokinetic parameters of molsidomine after oral administration of its CME-β-CyD complex (equivalent to 10 mg molsidomine) in gastric acidity-controlled dogs (n = 4).

System	C_{max} (ng mL^{-1})	t_{max} (h)	AUC (ng h mL^{-1})	MRT (h)
In fasted dogs				
High gastric acidity	105·6 ± 30·6	3·3 ± 0·3	281·0 ± 91·9	4·3 ± 0·2
Low gastric acidity	135·1 ± 9·6	1·5 ± 0·2	348·4 ± 47·7	2·9 ± 0·1
In non-fasted dogs				
High gastric acidity	73·6 ± 13·0	2·2 ± 0·3	233·7 ± 26·7	3·9 ± 0·2
Low gastric acidity	108·7 ± 10·3	0·5 ± 0·0	217·6 ± 17·9	2·2 ± 0·1

Values are mean ± s.e.m.

to the low and high gastric-acidity dogs, respectively. The paddle of the dissolution apparatus was rotated at various speeds (0–100 rev min^{-1}), taking into consideration the gastric motility due to the food effects in-vivo. At a constant pH of 7·0, the CME-β-CyD complex showed first-order release of molsidomine, except for the release at < 5 rev min^{-1}, where a sigmoidal curve was observed. A pH-dependent release was observed when the pH was changed from 1·2 to 7·0 after 2 h, and the change of rotation speed was clearly reflected in the release rate and lag time.

In-vivo/in-vitro correlation

Recently, development of an in-vitro/in-vivo testing and correlation for oral controlled/modified release dosage forms, including delayed-release preparations, has been conducted (Skelly et al 1990). In this approach, correlation levels A–C were proposed in descending order of quality, evaluating alternative procedures until a 'correlation' can be established to an acceptable degree. According to the definition, a level A correlation is categorized as the most confident, where the in-vitro release curve is superimposable upon the in-vivo release curve, demonstrating a 1 : 1 correlation between them.

In this study, therefore, an attempt was made to examine the possible level A correlation between the in-vitro and in-vivo release profiles for the delayed-release tablets containing a molsidomine/CME-β-CyD complex. The in-vivo absorptivities of molsidomine were calculated on the basis of the data of Fig. 1 and the intravenous administration of the drug (5 mg), by using the deconvolution method (Tett et al 1992). Fig. 4 shows the plots of the in-vivo absorptivities vs the in-vitro release from Fig. 3, where good point-to-point relationships were observed for absorptivity vs the pH 7·0 release at 100 rev min^{-1} for the low gastric-acidity dog under the non-fasted condition, absorptivity vs the pH 7·0 release at 0–5 rev min^{-1} for the low gastric-acidity dog under the fasted condition, absorptivity vs the pH-changed

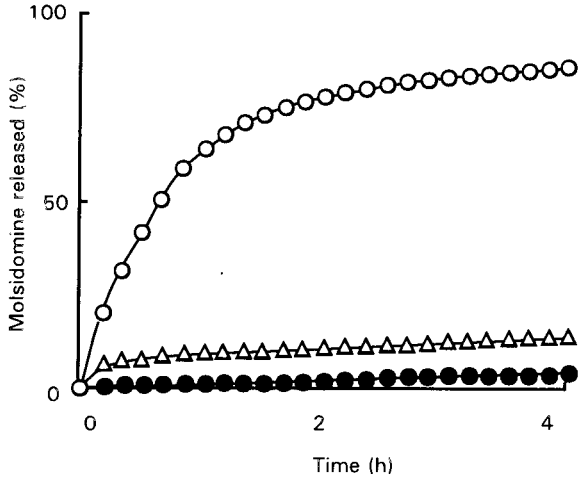

FIG. 2. Release profiles of molsidomine from tablets containing its CME-β-CyD complex (equivalent to 10 mg molsidomine) in dissolution media of different pH values at 37°C. ● JP XII 1st fluid (pH 1·2), △ sodium acetate buffer (pH 4·0), ○ JP XII 2nd fluid (pH 6·8).

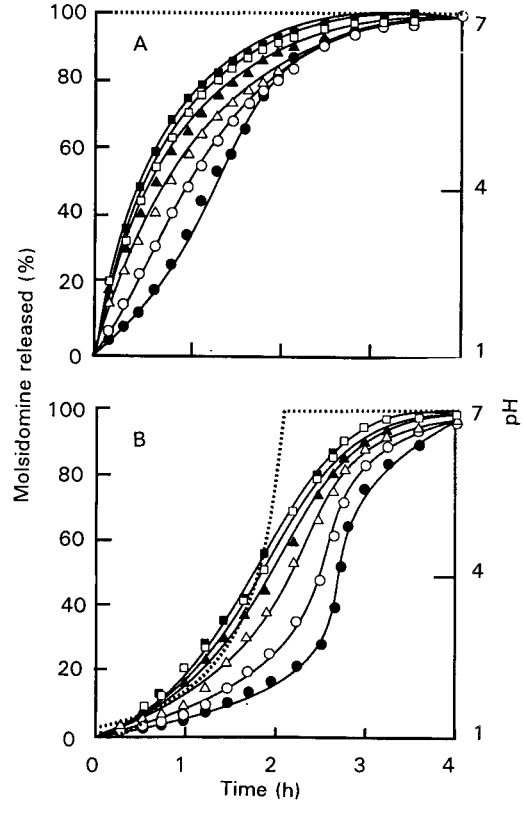

FIG. 3. Release profiles of molsidomine from tablets containing its CME-β-CyD complex (equivalent to 10 mg molsidomine) at 37°C, as a function of pH (dotted line) of dissolution media at various rotation speeds of paddle. A, pH was fixed at 7·0 from the beginning; B, pH was shifted from 1·2 to 7·0 after 2 h (see text). ● 0, ○ 5, △ 10, ▲ 25, □ 50, and ■ 100 rev min^{-1}.

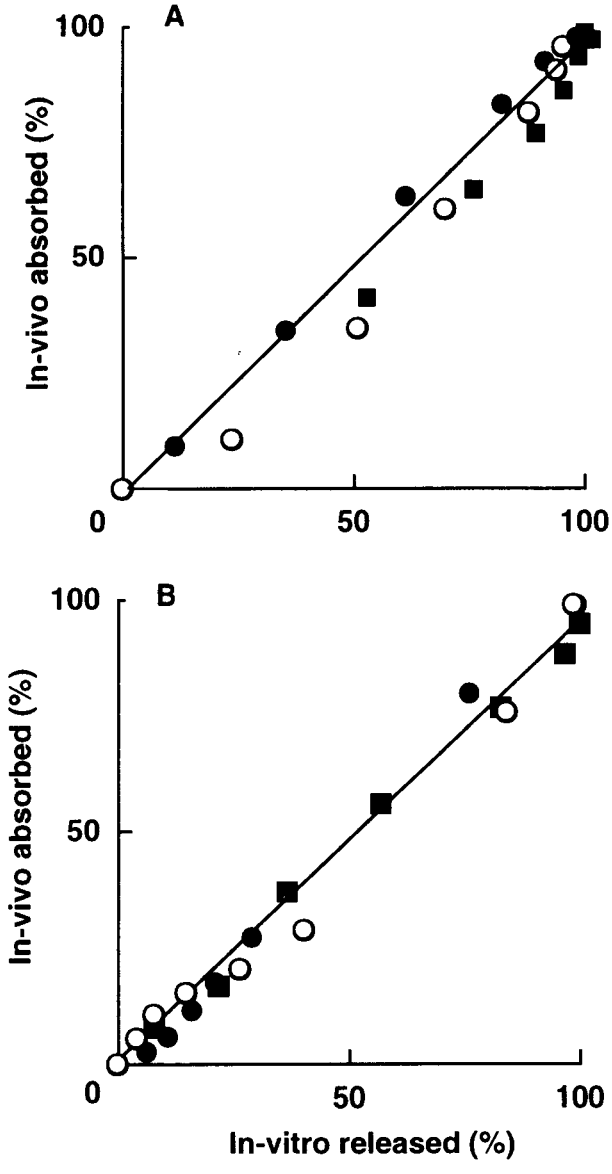

FIG. 4. Relationship between in-vivo absorptivity of molsidomine in gastric acidity-controlled dogs under fasted and non-fasted conditions. A, Low gastric acidity dogs; B, high gastric acidity dogs. ● 0, ○ 5, and ■ 100 rev min^{-1}.

release at 100 rev min^{-1} for the high gastric-acidity dog under the non-fasted condition, and absorptivity vs the pH-changed release at 0–5 rev min^{-1} for the high gastric-acidity dog under the fasted condition. These findings indicate that the absorptivity of the low gastric-acidity dogs correlated with the in-vitro release at a constant pH of 7·0, whereas that of the high gastric-acidity dogs correlated with the pH-changed release. Furthermore, the absorptivity of the fasted dogs correlated well with the in-vitro release at 0–5 rev min^{-1}, whereas that of the non-fasted dogs correlated with the release at 100 rev min^{-1}. All these in-vivo/in-vitro correlations gave a straight line, passing through the origin, with slopes of unity and correlation coefficients of 0·99. This indicates that the in-vivo release curve is directly superimposable upon the in-vitro release curve (1 : 1 in-vivo/in-vitro relationship), defined as a level A correlation.

In conclusion, the in-vivo absorption profiles of molsidomine in the gastric acidity-controlled dogs correlated well with the in-vitro release profiles generated by the pH-adjustable dissolution testing apparatus. Furthermore, the food effect on the absorption behaviour of molsidomine can be predicted by the change of the rotation speed of the paddle (< 5 or 100 rev min^{-1}). The present results clearly suggest that CME-β-CyD can serve as a delayed-release-type carrier for molsidomine and the in-vivo/in-vitro procedures described here may offer a method for evaluating delayed-release formulations of other water-soluble drugs.

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