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Prolonged Plasma Levels of Ketoprofen after Oral Administration of Its α-Cyclodextrin Conjugate/Ethylcellulose Dispersion in Rats

FUMITOSHI HIRAYAMA, MAKOTO KAMADA, HIDEKI YANO, KOICHI UDO, HIDETOSHI ARIMA and KANETO UEKAMA*
Faculty of Pharmaceutical Sciences, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan

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Abstract

6^A-*O*-[2-(3-benzoylphenyl)propinoyl]-α-cyclodextrin (KP/α-CyD conjugate), in which an anti-inflammatory drug, ketoprofen (KP), is covalently bound to one of the primary hydroxyl groups of α-cyclodextrin, was prepared, and the CyD conjugate-based prolonged-release system was designed by combining the KP/α-CyD conjugate (used as a delayed-release fraction) with the KP/ethylcellulose (EC) solid dispersion (used as a slow-release fraction). The conjugate showed a typical delayed-release pattern after oral administration to rats, i.e., plasma levels of KP increased after a lag time of about 3 h and reached a maximum concentration at about 9 h. The co-administration of the conjugate and the EC solid dispersion gave a sustained-release pattern of KP, i.e., a constant plasma KP level was maintained for at least 24 h. The long circulating release patterns in plasma KP levels after oral administration were reflected in the anti-inflammatory effect using with carrageenan-induced acute edema in rat paw.

Introduction

Controlled release systems can give optimized efficacy, safety and convenience of drugs because they can be designed to deliver a drug at a specified rate, for a specific period of time and even at a desired location [1, 2]. Sustained-release systems are often preferred over the multi-administration of drugs from a viewpoint of patient compliance. In previous papers [3–8], we reported that drug/CyD conjugates, in which a drug is covalently bound to hydroxyl groups of CyDs, work as colon-specific delivery prodrugs, because they produce the drug site-specifically in the cecum and colon. This release property can be classified as a delayed release. In this study, 6^A-*O*-[2-(3-benzoylphenyl)propinoyl]-α-CyD in which an anti-inflammatory drug, ketoprofen (KP), is covalently bound to α-CyD, was prepared, and the CyD-conjugate-based prolonged-release system was designed by combining the conjugate with the KP/ethylcellulose (EC) solid dispersion.

Experimental

Materials

KP was donated from Hisamitsu Pharmaceutical Co. (Tsukuba, Japan). α-CyD and ethylcellulose (EC, 10 cps) were from Nihon Shokuhin Kako Co. (Tokyo, Japan) and Katayama Chemical Co. (Tokyo, Japan), respectively. The KP/α-CyD conjugate was prepared according to the method reported previously [3, 4]. The solid dispersion of KP with EC was prepared by the spray-drying method under the following conditions: a weight ratio of 1:5.56 (KP/EC), a solvent of ethanol/methylenechloride (1:1 v/v), an air flow rate of 0.4 m^3/min, an air pressure of 1.0 kgf/cm^2, inlet and outlet temperatures of 85 °C and 50 °C, respectively. The solid dispersion was in amorphous state because it gave halo-pattern in powder X-ray diffractogram. Other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

Absorption of KP after oral administration to rat

Male Wistar rats weighing 175–185 g were fasted for 16 h prior to drug administration, while water was allowed *ad libitum*. The samples were filled in gelatin capsules (about 0.025 cm^3), and administered orally to the rats. Blood samples (about 0.2 ml) were taken periodically from the jugular vein, and centrifuged at 10000 rpm for 3 min at 4 °C. The plasma was assayed for KP by HPLC under the following conditions: a 655A-11 pump and a L-7400 UV monitor (Hitachi, Tokyo, Japan), a Dicel CHIRACEL® OJ column (4.6 × 250 mm, Tokyo, Japan), a flow rate of 1.0 ml/min, a detection at 248 nm.

* Author for correspondence: E-mail: uekama@gpo.kumamoto-u.ac.jp

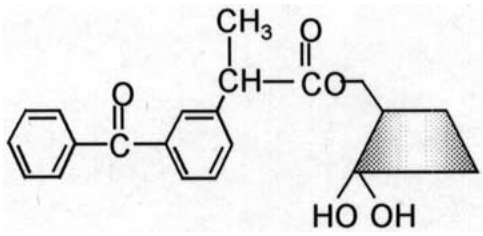

Figure 1. Chemical structure of KP/α-CyD conjugate.

Antiinflammatory responses

Male Wistar rats weighing 175–185 g were fasted for 16 h prior to drug administration, while water was allowed *ad libitum*. The paw swelling of rats (about 180 g) was volumetrically determined using a water plethysmometer. A 1%(w/v) carrageenan (Code 073-50, Lot M6P3336, Nacalai Tesque, Tokyo, Japan) in saline (0.1 ml) was injected subcutaneously into the right hind paw at various times after the samples had been administered orally. The time intervals between the drug administration and the carrageenan injection were 0.5 h, 3 h, 6 h and 12 h. Paw volume was measured 0.5 h, 1 h, 2 h, 3 h and 4 h after the carrageenan injection, and the results of the swelling were expressed as a percentage compared with the initial hind paw volume.

Results and discussion

Characterization of KP/α-CyD conjugate

The KP/α-CyD conjugate (Figure 1) was prepared by tosylating one of the primary hydroxyl groups of α-CyD using *p*-toluenesulfonyl chloride, followed the replacement of the sulfonyl group by KP. α-CyD was chosen as a pro-moiety for the conjugation, because the aqueous solubility of the α-CyD conjugate is higher than that of the β-CyD conjugate. NMR and mass spectroscopic studies indicated that the carboxyl group of KP is covalently bound to one of the primary hydroxyl groups of α-CyD through an ester bond. Further, HPLC analyses indicated that the conjugate consists of the mixture of the (R)-KP and (S)-KP conjugates in an equimolecular quantity, suggesting a negligible enantioselective preparation under the present conditions.

The drug release behavior of the KP/α-CyD conjugate in rat biological fluids was investigated (data not shown). The conjugate released no KP for at least 24 h at 37 °C in aqueous solution (pH 6.8) under the experimental conditions. Further, there were negligible releases of KP in the small (33%w/v, pH 7.4) and large (33%w/v, pH 6.8) intestinal homogenates without contents and in the blood (50%v/v, pH 7.4). In the cecum contents (10%w/v, pH 6.8), on the other hand, the conjugate released KP rapidly, the release being 100% within 2 h. This drug release behavior of KP/α-CyD conjugate was in accord with the results reported previously for the CyD conjugates with biphenylylacetic acid [3–5] and prednisolone [6–8]. The KP released from the α-CyD conjugate consisted of almost equimolecular amounts of (R)-KP and (S)-KP, suggesting negligible enantioselective hydrolysis. These results suggest that KP/α-CyD conjugate is first subjected to the ring-opening of CyD to give the KP/small saccharide conjugates, followed by the ester hydrolysis giving KP, by the action of enzymes produced by microflora in rat cecum and large intestine contents, as reported previously [4, 8].

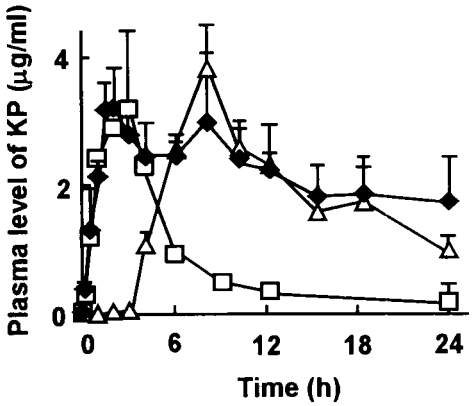

Figure 2. Plasma levels of KP after oral administrations of KP/EC solid dispersion (□, equivalent to 6 mg/kg KP), KP/α-CyD conjugate (△, equivalent to 5 mg/kg KP) and the combined preparation (♦, EC solid dispersion + conjugate, containing the equivalent amounts of KP). The drugs were administered as powder filled in capsules. Each point represents the mean ± S.E. of 3–4 experiments.

Co-administration of KP/α-CyD conjugate and KP/EC solid dispersion

Figure 2 shows plasma levels of KP after oral administrations of the capsules containing the KP/α-CyD conjugate alone, the KP/EC solid dispersion alone or a combination of the KP/α-CyD conjugate (delayed-release fraction) and the KP/EC solid dispersion (slow-release fraction) to rats. In the case of the conjugate alone, the plasma levels of KP increased after a lag time of about 3 h and reached a maximum level (C_{max}) after about 9 h, indicating that the conjugate releases KP site-specifically in the cecum and colon, therefore working as a delayed-release prodrug. The KP/EC solid dispersion gave a maximum KP level at about 3 h. In the case of the combination of the conjugate and the KP/EC solid dispersion, the plasma KP profile was a superposition of those of each component. Therefore, the combination of the conjugate and the solid dispersion maintained a constant KP level (2–3 μg/mL) for at least 24 h under the present experimental conditions, showing a long circulating pattern. These results indicate that the combined system of the CyD conjugate with the EC solid dispersion can work as a prolonged-release preparation of KP.

Anti-inflammatory effects

The anti-inflammatory effect of the combined system after oral administration was evaluated using the model rats of carrageenan-induced acute edema. Figure 3 shows the swelling percentages of the rat paw 0.5, 1, 2, 3 and 4 h after the carrageenan injection into the rat paw. The conjugate was orally administered at various intervals before the carrageenan injection. The dose was double those used in the plasma level measurements (the conjugate 10 mg and the EC solid

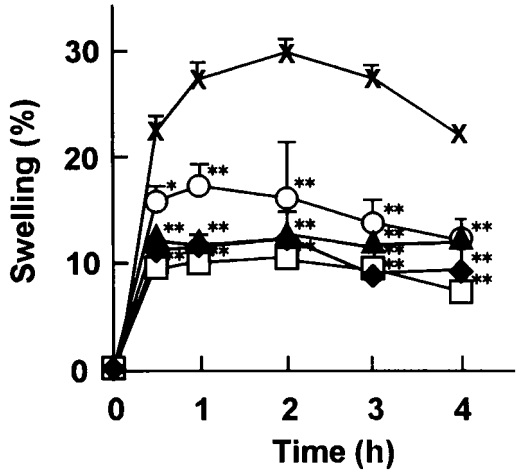

Figure 3. Anti-inflammatory effects of the combined preparation of KP/EC solid dispersion (equivalent to 12 mg/kg KP) and KP/α-CyD conjugate (equivalent to 10 mg/kg KP) on the carrageenan-induced acute edema in rat paw. The drugs were orally administered at various times (× control (without the drugs); ○, 0.5 h; ▲, 3 h; □, 6 h; ♦, 12 h) before the 1%w/v carrageenan injection (0.1 ml). Each point represents the mean ± S.E. of 3–4 experiments. **: $p < 0.01$ versus control.

dispersion 12 mg), because of a weak anti-inflammatory response at smaller doses. The KP alone system showed an inhibitory effect only when it was administered 0.5 h before the carrageenan injection. The anti-inflammatory response of the conjugate alone was significantly delayed, i.e., the response was higher when it was administered 6 and 12 h before the carrageenan injection, whereas the response was negligible or smaller at 0.5 h and 3 h, reflecting its delayed-releasing property. These anti-inflammatory effects of KP alone and the conjugate alone were similar to those observed for the biphenylylacetic acid/CyD conjugates, as reported previously [4, 5]. On the other hand, the combination of the conjugate and the EC solid dispersion showed high anti-inflammatory effects at all times investigated as shown in Figure 3, indicating a prolonged-release *in vivo*.

Conclusion

The present results suggest that the KP/α-CyD conjugate can work as a delayed-release type prodrug for colon-specific delivery. Further, the combined system of the conjugate with a slow-release fraction such as the EC solid dispersion can provide the prolonged-release preparation of KP. This CyD conjugate-based approach for the release control may be useful, because of the simple mixing of both components without employing highly skilful techniques such as film-coatings.

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