Effect of Low-Molecular-Weight β-Cyclodextrin Polymer on Release of Drugs from Mucoadhesive Buccal Film Dosage Forms

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We investigated the effect of low-molecular-weight β-cyclodextrin (β-CyD) polymer on *in vitro* release of two drugs with different lipophilicities (*i.e.*, lidocaine and ketoprofen) from mucoadhesive buccal film dosage forms. When β-CyD polymer was added to hydroxypropylcellulose (HPC) or polyvinylalcohol (PVA) film dosage forms, the release of lidocaine into artificial saliva (pH 5.7) was reduced by 40% of the control. In contrast, the release of ketoprofen from the polymer film was enhanced by addition of β-CyD polymer to the vehicle. When lidocaine and ketoprofen was incubated with β-CyD polymer in the artificial saliva, concentration of free lidocaine molecules decreased in a β-CyD polymer concentration-dependent manner. The association constant with β-CyD polymer was 6.9±0.6 and 520±90 M^{-1} for lidocaine and ketoprofen, respectively. Retarded release of the hydrophilic lidocaine by β-CyD polymer might be due to the decrease in thermodynamic activity by inclusion complex formation, whereas enhanced release of the lipophilic ketoprofen by the β-CyD polymer might be due to prevention of recrystallization occurring after contacting the film with aqueous solution. Thus, effects of low-molecular-weight β-CyD polymer to the drug release rate from film dosage forms would vary according to the strength of interaction with and the solubility of active ingredient.

Key words β-cyclodextrin polymer; buccal delivery; mucoadehive film dosage form; lidocaine; ketoprofen; hydroxypropylcellulose

Buccal drug delivery systems have been attracting much attention as drug formulations intended for anti-inflammatory and analgesic therapies in the oral cavity. These formulations are expected to increase therapeutic effects and reduce systemic adverse reactions by concentrating the drug in the target tissue. One particular problem associated with the treatment of oral cavity diseases is short duration of therapeutic efficacy due to rapid dilution and washout from the oral cavity by saliva.[1] The use of bioadhesive polymers that can interact with biological membranes is a way of retarding the residence time of drug in oral cavity.[2—6]

Among bioadhesive mucosal dosage forms developed, buccal film are preferable over adhesive tablets in terms of flexibility and comfort, and can circumvent relatively short residence time of oral gels.[7] In order to attain better therapeutic effect, it would be expected to regulate the release rate of drug from the polymer film. Assuming that formation of the inclusion complex might change the release rate of drug, we intended to investigate the effect of β-CyD incorporated in bioadhesive polymer film dosage forms. However, our preliminary experiments indicated that the film containing β-CyD was not elastic enough to be used practically. In the present study, therefore, we investigated the feasibility of low-molecular-weight β-CyD polymer for controlled release of drugs from the film dosage forms. It is known that β-CyD can be readily cross-linked through their hydroxyl groups with epicholohydrin. So far, high-molecular-weight β-CyD polymer has been investigated as a tablet disintegrating agent because of its rapid and high swelling capacity.[8,9] In this study, we selected two drugs varying in lipophilicity (*i.e.*, lidocaine and ketoprofen) as model drugs, prepared the film dosage forms containing water-soluble, low-molecular-weight β-CyD polymer, and evaluated *in vitro* release of lidocaine in artificial saliva. In addition, we investigated interaction of these drugs with β-CyD polymer by solubility measurement following ultra-filtration and competitive inclusion complexation experiment using a fluorescent probe.

MATERIALS AND METHODS

Materials Lidocaine was obtained from Nacalai Tesque Inc., (Tokyo, Japan). Ketoprofen, hydroxypropylcellulose (HPC) and polyvinylalcohol (PVA) were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan). Low-molecular-weight β-CyD polymer was kindly supplied from Nihon Syokuhin Kako Co., Ltd., (Fuji, Shizuoka, Japan). The molecular weight of the β-CyD polymer used was approximately 5000, indicating that four or five units of β-CyD are crosslinked with epichlorohydrin (Fig. 1). 6-(*p*-Toluidino)-2-naphthalenesulfonic acid sodium salt (TNS) was purchased from Sigma Co. (St. Louis, MO, U.S.A.). The other reagents used were of analytical grade.

Preparation of Film Dosage Forms Lidocaine or ketoprofen and β-CyD polymer was mixed at a weight ratio of 1 : 4.85 or 1 : 4.46 (corresponding to guest/host molar ratio of 1 : 1) and kneaded with a little of water. Then, water was added to yield a final lidocaine concentration of 0.5 w/v%,

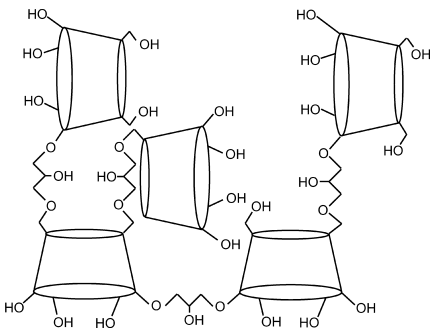

Fig. 1. Structure of Water-Soluble Low-Molecular-Weight β-Cyclodextrin (β-CyD) Polymer

Four or five CyDs were crosslinked by epichlorohydrin.

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together with addition of 5 w/v% HPC or PVA. One hundred and twenty-five microliters of the aqueous solution was placed on a Teflon cell (0.79 cm^2) and put in a desiccator with silica gel at room temperature overnight. Additional 125 μl drug solution was overlaid to the film and dried in the desiccator with silica gel at room temperature completely. The reason why two-step drying was conducted was to minimize the formation of the film edge.

Dissolution Test *In vitro* release of drugs was tested using a modified JP XIV dissolution apparatus. The film dosage form prepared according to the above-mentioned method was attached to the rotatory shaft with an adhesive tape. The shaft was rotated at 50 rpm in 50 ml of artificial saliva in a 50 ml glass beaker thermostated at 37 °C. The artificial saliva was composed of 14.4 mM NaCl, 16.1 mM KCl, 1.31 mM $CaCl_2 \cdot 2H_2O$, 0.545 mM $MgCl_2 \cdot 6H_2O$, and 1.96 mM K_2HPO_4, and the pH was adjusted to 5.7 with HCl. Two hundred and fifty microliters were taken at 1, 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, and 120 min and subjected to further analysis. To keep the volume of dissolution medium constant, an equivalent volume of fresh artificial saliva was added after sampling.

Determination of Lidocaine The lidocaine concentrations in the samples obtained from dissolution test were determined using an HPLC system (LC-6A, Shimadzu, Kyoto). A mobile phase composed of water, methanol, and phosphoric acid (79 : 20 : 1) was flowed through an Inersil ODS-2 column (5 mm, 4.6×150 mm, Nacalai Tesque Inc., Tokyo) at the rate of 1.2 ml/min at 36 °C. The detection wavelength for lidocaine was 220 nm.

Determination of Ketoprofen The ketoprofen concentrations in the samples obtained from dissolution test were determined using an HPLC system (LC-10Avp, Shimadzu, Kyoto). A mobile phase composed of 0.06 M KH_2PO_4, acetonitrile, and triethylamine (65 : 35 : 0.1) was flowed through an Inersil ODS-2 column (5 mm, 4.6×150 mm, Nacalai Tesque Inc., Tokyo) at the rate of 1.0 ml/min. The detection wavelength for ketoprofen was 275 nm.

Differential Scanning Calorimetry (DSC) Analysis DSC thermograms of pure drugs, film dosage forms containing drug and β-CyD polymer, and the control films containing drug alone were recorded by DSC system (DSC3200, Mac Science Co,. Tokyo, Japan). All samples containing 1 mg drug were placed in aluminium pans and heated lineally at a scanning rate of 10 °C/min from ambient temperature to 200 °C. Aluminium oxide was used to calibrate the apparatus.

Competitive Inclusion Complexation Assay TNS (20 μM) and β-CyD polymer (β-CyD unit concentration of 200 μM) were dissolved in pH 7.4 phosphate buffered saline (PBS). One milliliter of the mixture and 5 ml of varying drug concentrations in PBS were mixed and adjusted to 10 ml. The final concentrations of TNS and β-CyD units of the polymer were 2 μM and 20 μM, respectively. After the mixture was incubated at 25 °C for 60 min, the fluorescence intensity associated was determined by spectrofluorophotometer (RF-540, Shimadzu, Kyoto), where the excitation and emission wavelengths were 324 and 437 nm, respectively.

Binding Experiment Four hundred milligrams per milliliter lidocaine or 40 mg/ml ketoprofen was incubated with varying amounts of β-CyD polymer in the artificial saliva at 25 °C. Approximately 2 ml aliquot of the incubation medium was subjected to ultra-filtration with a polyethersulfone membrane filter (Vivaspin 2 (5000 MWCO), Vivascience AG, Goettingen, Germany). Five hundred microliters aliquot of the filtrate was taken and diluted by 10 times. The drug concentration in the diluted filtrate was determined by the HPLC method. Assuming that the drug and β-CyD untis of the polymer form a 1 : 1 complex and that the concentration of complex was negligible as compared to the concentration of β-CyD, the association constant (K) was calculated using the following equation:

$$\frac{[\mathrm{Durg}]_{\mathrm{total}}}{[\mathrm{Drug}]_{\mathrm{free}}} - 1 = K \cdot [\beta\text{-CyD}]_{\mathrm{total}} \quad (1)$$

where $[\mathrm{Drug}]_{\mathrm{total}}$, $[\mathrm{Drug}]_{\mathrm{free}}$, and $[\beta\text{-CyD}]_{\mathrm{total}}$ were concentrations of free drug, total drug, and total β-CyD, respectively. The K values and their computer-calcualated standard errors were estimated by linear regression method.

RESULTS

Dissolution of Lidocaine and Ketoprofen from Film Dosage Forms Lidocaine film dosage forms were prepared, using HPC or PVA as a film base. Figure 2 shows the dissolution profiles of lidocaine from the films. When HPC was used as a film base, more than 80% of applied lidocaine was released within 15 min. When β-CyD polymer was added to the film, it took approximately 30 min to release 80% of applied lidocaine. Similar retardation of lidocaine release associated with addition of β-CyD polymer was observed with the PVA base, although the lidocaine release tended to be slower for PVA as compared with HPC. Initial dissolution rates were calculated from the linear portion in early time phase (Table 1). Addition of β-CyD polymer reduced an initial dissolution rate by 40% for both HPC and PVA bases.

Film dosage forms of ketoprofen were also prepared using HPC as a film base. Figure 3 shows the dissolution profiles of ketoprofen from the films. As compared to lidocaine, the release rate of ketoprofen was slower, where it took more than 75 min for 80% of the amount to be released. When β-

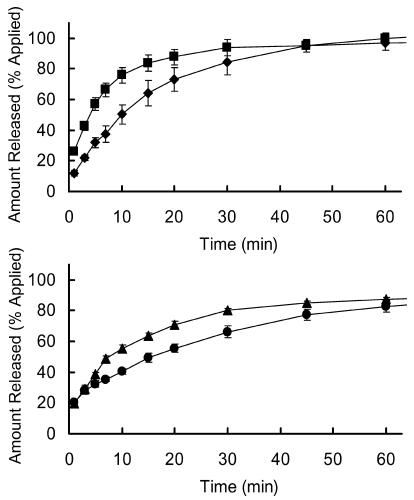

Fig. 2. Dissolution Profiles of Lidocaine from Film Dosage Forms

Key: HPC (■); HPC/β-CyD polymer (◆); PVA (▲); PVA/β-CyD polymer (●). Each data point expresses mean±S.E. of four experiments.

Table 1. Initial Dissolution Rate of Lidocaine from Film Dosage Forms

Formulations	Dissolution rate (%/h)
HPC	6.51±0.13
HPC/β-CyD polymer	3.69±0.03[a)]
PVA	4.17±0.06
PVA/β-CyD polymer	2.55±0.03[b)]

a) $p<0.05$ *vs.* HPC, *b*) $p<0.01$ *vs.* PVA.

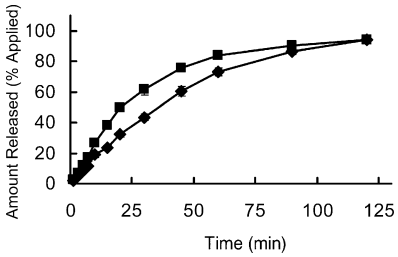

Fig. 3. Dissolution Profiles of Ketoprofen from Film Dosage Forms

Key: HPC (◆); HPC/β-CyD polymer (■). Each data point expresses mean±S.E. of four experiments.

Table 2. Initial Dissolution Rate of Ketoprofen from Film Dosage Forms

Formulations	Dissolution rate (%/h)
HPC	1.47±0.03
HPC/β-CyD polymer	2.12±0.02[a)]

a) $p<0.05$ *vs.* HPC.

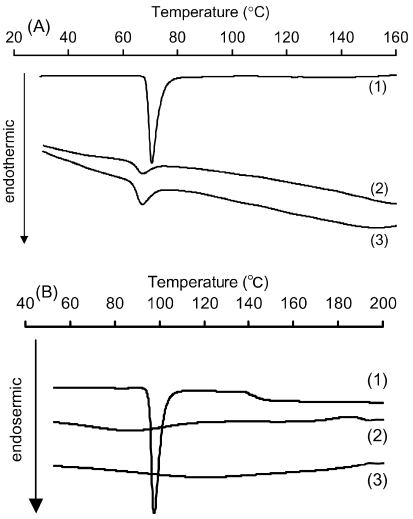

Fig. 4. Differential Scanning Calorimetory Analysis of (A) Lidocaine and (B) Ketoprofen Formulations

(1) Pure drugs, (2) drugs in HPC film, (3) drugs in HPC/β-CyD polymer.

CyD polymer was added to the film, the release rate of ketoprofen was increased, in contrast to the case of lidocaine. The initial dissolution rate was increased by 1.4-folds by addition of β-CyD polymer in the polymer film (Table 2).

DSC Analysis of Lidocaine and Ketoprofen Film Dosage Forms Figure 4 shows the DSC thermograms of pure lidocaine or ketoprofen, the HPC film, and the HPC/β-CyD polymer film. In the case of lidocaine, a small melting peak of lidocaine was observed at 68—69 °C for HPC and HPC/β-CyD polymer film. The endothermic peak for

Table 3. Data of Differential Scanning Calorimetory Analysis of Lidocaine or Ketoprofen and These Film Dosage Forms

	Melting point (°C)	Peak area (cal/g)
Lidocaine	70.5	4.15
Lidocaine/HPC	67.3	0.154
Lidocaine/HPC/β-CyD	67.0	0.268
Ketoprofen	95.0	7.10
Ketoprofen/HPC	—	—
Ketoprofen/HPC/β-CyD	—	—

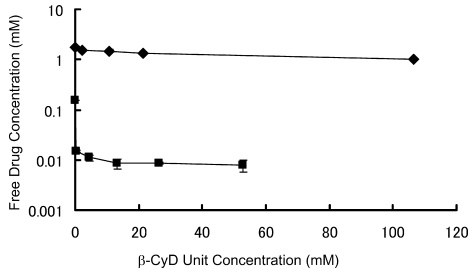

Fig. 5. Effect of β-CyD Polymer on Concentration of Free Lidocaine (◆) or Ketoprofen (■)

Polymer concentration was calculated with their β-CyD units of the polymer. Each data point expresses mean±S.E. of four experiments.

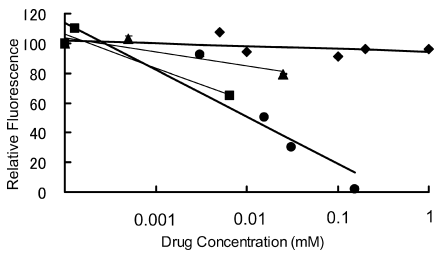

Fig. 6. Effect of Drugs on Fluoresence Associated with TNS–β-CyD Polymer Inclusion Complex

Key: lidocaine (◆); ketoprofen (●); progesterone (■); quercetin (▲).

HPC/β-CyD polymer film was slightly larger than that for the HPC film, indicating that crystallinity of lidocaine would be slightly increased by the addition of β-CyD polymer.

In contrast, the film dosage forms of ketoprofen did not exhibit any endothermic peak irrespectively of the addition of β-CyD polymer film. Thus, it was indicated that ketoprofen be in the amorphous state in the polymer films.

Confirmation of Drug-β-CyD Polymer Complexation by Ultrafiltration Method To confirm the interaction of drugs with β-CyD polymer, we performed binding experiments using ultra-filtration method. Figure 6 shows the relationship between free drug concentration and the concentration of β-CyD polymer. The concentration of free drug molecules in the artificial saliva decreased significantly in a β-CyD polymer concentration-dependent manner, while the effect of β-CyD polymer was much more significant with ketoprofen. The association constants calculated assuming that the β-CyD forms 1 : 1 complex with the guest molecule were 6.9±0.6 and 520±90 M^{-1} for lidocaine and ketoprofen, respectively. Here, it should be noted that the association constant was estimated with the concentration of β-CyD monomer units.

Detection of Lidocaine-β-CyD Polymer Inclusion Com-

plexes Using a Fluorescent Probe As one of convenient methods to investigate formation of inclusion complex, competitive experiments using a fluorescent probe such as TNS have been proposed.[9,10] The fluorescence associated with TNS increases markedly in a non-polar environment. When TNS forms an inclusion complex with β-CyD, the compound emits strong fluorescence due to hydrophobic environment in the cavity of β-CyD and restricted molecular motion. By using the fluorescent probe, we investigated whether or not lidocaine forms an inclusion complex with β-CyD polymer (Fig. 5). When TNS was incubated with β-CyD polymer, strong fluorescence was observed as reported with monomeric β-CyD.

Ketoprofen significantly reduced fluorescence associated with TNS-β-CyD polymer complex in a concentration-dependent manner, where relative fluorescence was decreased up to approximately 2% when 20 times higher concentration of ketoprofen than that of β-CyD unit of the polymer was added. Competitive inhibition effect of ketoprofen was comparable to that of progesterone[11] known to be strongly associated with β-CyD. In contrast, no effect of lidocaine was observed even when its concentration was increased up to 1 mM, being 50 times as high as that of β-CyD unit.

DISCUSSION

In the present study, β-CyD polymer retarded the release of lidocaine from mucoadhesive polymer films. Miyoshi *et al.*[12] reported that β-CyD forms an inclusion complex with lidocaine and improves solubility and stability of the drug. Dollo *et al.*[13] revealed in their NMR studies that significant changes of chemical shifts assigned to inner protons of β-CyD (H3 and H5), as well as proton near the cavity (H6 on the rim of the torus), due to the addition of lidocaine were observed whereas no appreciable shifts detected for protons located outside the cavity of β-CyD. Taking together with the results of DSC and infrared spectroscopy analyses, they concluded that lidocaine molecule may interact with the cavity of β-CyD.[13] Although lidocaine was not associated with β-CyD polymer as strongly as to inhibit the formation of TNS–β-CyD complex (Fig. 6), free concentration of the drug was significantly decreased in β-CyD polymer-concentration dependent manner (Fig. 5). Therefore, it is likely that decreased thermodynamic activity by complex formation might be one of the reasons why β-CyD polymer retarded the release of lidocaine from the film dosage form. Based on the relationship between β-CyD polymer concentration and free concentration of lidocaine, the association constant for lidocaine–β-CyD polymer complex was estimated to be $6.9 \pm 0.6\ \mathrm{M}^{-1}$. Taking into account that the association constant for binding of progesterone and quercetin with monomeric β-CyD was 5000[11] and 130[14] M^{-1}, respectively, it was indicated that the binding between lidocaine and β-CyD polymer was very weak.

The endothermic peak for HPC/β-CyD polymer film was slightly larger than that for the HPC film, indicating that crystallinity of lidocaine would be increased by the addition of β-CyD polymer. Since the amount of β-CyD polymer was almost a half of HPC in the film dosage form, it is not surprising that the structure of polymer network might be different between β-CyD polymer-containing film and the control. Thus, crystallinity of lidocaine in the films might be changed by the addition of β-CyD polymer. It cannot be ruled out that such effect might alter the release rate from the film dosage forms.

Since ketoprofen has a much larger association constant with β-CyD polymer than lidocaine, it was likely that retardation of drug release by β-CyD polymer be more significant with ketoprofen. Nevertheless, the release of ketoprofen from mucoadhesive polymer films was rather increased by addition of β-CyD polymer. There have also been controversial reports regarding the effect of β-CyD on dissolution of drugs in tablet formulations.[15—19] Horiuchi *et al.*[15] demonstrated that dissolution rate of diltiazem from the tablets was decreased by the addition of diethylated and triethylated β-CyD. In addition, ethylated β-CyD was reported to retard *in vitro* release of buserelin acetate from oily suspensions.[16] On the contrary, increased dissolution by inclusion complexation with β-CyD or its derivatives has been observed for nifedipine,[17] naproxen,[18] and famotidine.[19] Enhanced dissolution by β-CyD tends to be observed with water-insoluble drugs, presumably since dissolution of water-insoluble drugs in the crystal form would be slower than that from inclusion complexes. Although ketoprofen was in the amorphous state in the polymer films, recrystallization would occur easily after contacting with aqueous solution. When an inclusion complex with β-CyD polymer was being formed, however, the phase transition to water-insoluble crystal forms would not occur. Hence, the release rate of ketoprofen from β-CyD polymer-containing film dosage forms would be higher than that from the control film.

In conclusion, effect of low-molecular-weight β-CyD polymer to the drug release rate from film dosage forms appears to vary according to the strength of interaction with and the solubility of active ingredient. However, this study clearly demonstrated that addition of low-molecular-weight β-CyD polymer to film dosage forms could control *in vitro* dissolution rate of the drug.

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