Effect of Cyclodextrins on Biological Membrane. I. Effect of Cyclodextrins on the Absorption of a Non-absorbable Drug from Rat Small Intestine and Rectum

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The effects of three kinds of cyclodextrins (CyDs), α-, β- and γ-CyD on biological membranes were investigated by changes in absorption of a non-absorbable drug, sulfanilic acid (SA), from the rat small intestine and rectum using an *in situ* perfusion technique. The absorption of SA from the intestine was slight and was not affected by the addition of CyDs. After pretreatment with a mucolytic agent, *N*-acetyl-L-cysteine (N-Ac), the absorption of SA was increased compared with SA alone in the presence of only β-CyD. Similar treatment with sodium deoxycholate (SDC) and sodium lauryl sulfate (SLS) to gastro-intestinal membrane showed the enhanced absorption of SA by the addition of β-CyD. The mucin layer on the surface of the gastro-intestinal membrane may play an important role in the absorption of drugs. On the other hand, enhanced absorption of SA from the rat rectum was not induced by β-CyD with or without pretreatment with *N*-Ac, SDC or SLS.

Simultaneously, the release of neutral sugars in the perfusate after treatment with adjuvants was also observed with N-Ac, SDC and SLS. These results indicate that the mucin layer works as a barrier to the increased absorption of SA by β-CyD.

Keywords cyclodextrin; absorption; non-absorbable drug; sulfanilic acid; enhancing effect; intestine; rectum; *N*-acetyl-L-cysteine; adjuvant; mucin

Introduction

Cyclodextrins (CyDs) form inclusion complexes with various hydrophobic drugs, resulting in the improvement of solubility,[1] dissolution rate[2] and bioavailability.[3,4] The enhancement of bioavailability is generally attributed to the increased solubility and dissolution rate of the drug complexed with CyDs. Moreover, we have revealed that the enhanced bioavailability of such drugs is due to the interaction between bile acids and inclusion drug complexes in the small intestinal tract.[5]

CyD-membrane interactions have also been reported: CyDs induce a morphological change and a hemolytic activity in human erythrocytes.[6] CyD complex administration as a nasal spray provided much greater absorption of insulin than the administration of solution without α-CyD.[7] CyDs also enhance the leakage of calcein from phosphatidylcholine or phosphatidylcholine–cholesterol liposomes.[8] Phosphatidylcholine–cholesterol (molar ratio 1 : 1) liposomes coated with polysaccharides with hydrophobic anchor, such as pullulan, are more resistant to β-CyD-induced leakage.[9] This resistance may be ascribed to the contact between polysaccharides and CyDs molecules on the liposome surface. Although the effects of CyDs on artifical or red cell membranes are thought to be due to interactions with membrane lipids,[6,8] the direct action of CyDs on biological membranes at the absorption site of the drug is not yet fully understood. Moreover, it is well known that mucin on the surface of intestinal membrane protects the underlying epithelial cells from acids and enzymes, and acts as a barrier in the process of drug absorption from the gastro-intestinal tract.[10]

From this viewpoint, we investigated the effect of CyDs on drug absorption through the intestinal and rectal tracts. Intestinal and rectal absorption of drugs with or without CyDs was examined by an *in situ* single perfusion method in rats. Further, effects of depletion of the mucin layer on the CyD-induced absorption of drug were examined using various adjuvants.

Experimental

Materials Sulfanilic acid (SA), α-, β-, γ-CyD, *N*-acetyl-L-cysteine (N-Ac), sodium deoxycholate (SDC), sodium lauryl sulfate (SLS), trypsin, disodium ethylenediaminetetraacetate (EDTA), sodium alginate and pectic acid were purchased from either Wako Pure Chemicals Co. or Nacalai Tesque, Inc. Other chemicals were of reagent grade.

Absorption Experiments Male Wistar rats (200—230 g) were used in all experiments. The absorption of SA from the whole small intestine and rectum was carried out by an *in situ* single perfusion technique in bile duct-ligated rats as described previously.[11] SA (final concentration, 2 mg/ml) and 10 mM CyDs were dissolved in a pH 6.5 isotonic phosphate buffer solution and then perfused through the whole small intestine and rectum at a rate of 2 ml/min.

Pretreatment Experiment with Adjuvants The small intestine or rectum was pretreated with various adjuvants (1% N-Ac for 10 min, 0.5 mM SDC for 10 min, 0.5 mM SLS for 10 min, 1% trypsin for 30 min, 5 mM EDTA for 30 min, 0.25% sodium alginate for 30 min, and 0.5% pectic acid for 30 min). Immediately afterwards a SA solution with or without CyD was perfused and then blood samples were taken just before the start of perfusion and at intervals of 10 min thereafter. The SA concentration in the blood was determined spectrophotometrically.

Measurement of Mucin in Perfusate After washout of the intestinal contents with 50 ml of a physiological saline at 37 °C, the rats were allowed to stand for 30 min. The small intestinal lumen was perfused with a pH 6.5 phosphate buffer solution at 37 °C with or without adjuvants, and mucin in the perfusate was determined spectrophotometrically.

Analytical Method SA was determined spectrophotometrically as described in our previous paper.[11] Neutral sugar in perfusate was measured using D-glucose as a standard by the method of Roe.[12]

Data Analysis The area under the blood concentration–time curve (*AUC*) was calculated using the trapezoidal rule. All mean values of the data are presented with their standard error (S.E.). Student's *t*-test was utilized to determine the significance of differences.

Results and Discussion

The bioavailability of an orally administered drug–CyD complex depends on several factors, such as its solubility and absorption rate. SA was scarcely formed inclusion complex with β-CyD in pH 6.5 phosphate buffer solution, because the solubility in the presence of β-CyD at pH 6.5 was little changed.[13] SA is completely ionized at pH 6.5.

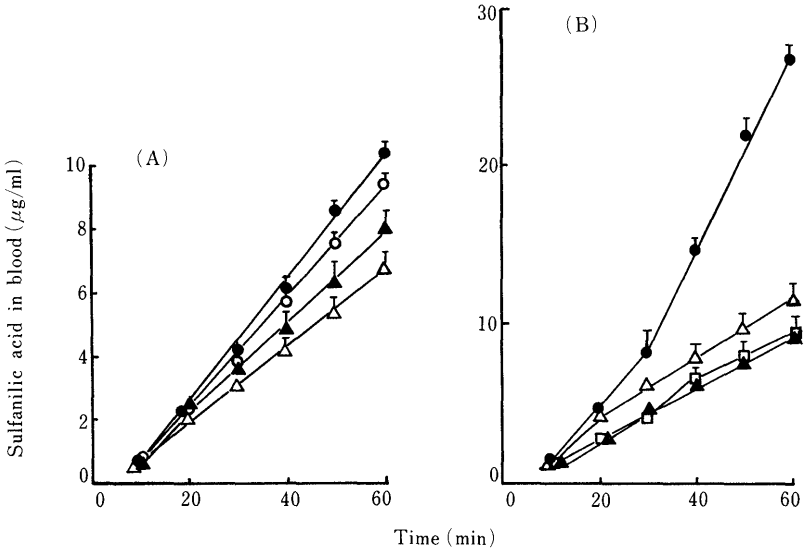

Fig. 1. Effect of CyDs on the Intestinal Absorption of SA after Pretreatment without (A) and with N-Ac (B)
—○—, control; —□—, N-Ac treat. alone; —△—, α-CyD; —●—, β-CyD; —▲—, γ-CyD. Each point represents the mean ± S.E.

These properties are quite advantageous for the investigation of membrane-β-CyD-interactions through permeability experiments.

Effect of CyDs on SA Absorption from Intestine Figure 1A shows the blood concentration–time curve of SA in the presence of CyDs. The blood level of SA was 9 μg/ml at 60 min with the perfusion of SA alone (control), and the presence of 10 mM CyDs did not significantly increase the blood level of SA.

Reportedly, leakage from liposomes by CyDs is reduced in polysaccharide-coated liposomes.[9] Similarly, the surface of the intestines is covered with mucin and a carbohydrate-rich glycocalyx consisting of glycoproteins.[14] CyDs cannot enhance the absorption of SA in the small intestine under normal conditions; this may be due to the presence of a mucin layer covering the intestinal membrane. Therefore, we measured the absorption of SA after washing off the mucin layer with N-Ac. N-Ac and dithiothreitol as disulfide bond breaking agents, and trypsin, papain and chymotrypsin as proteolytic enzymes have been used as mucotropic and mucokinetic agents.[15]

The effect of N-Ac pretreatment on the absorption of SA in the presence of CyDs is presented in Fig. 1B. After pretreatment with 1% N-Ac for 10 min, the blood level of SA in the absence of CyDs was the same as that of isotonic phosphate buffer (control), and N-Ac itself did not influence SA absorption. On the perfusion with SA and CyDs after the pretreatment with N-Ac, the absorption of SA in the presence of α-CyD or γ-CyD was unchanged. However, the blood level of SA in the presence of β-CyD was significantly increased compared with the control. In summary, SA absorption from the intestine is not significantly influenced by the presence of any kind of CyD, but after pretreatment with N-Ac the absorption of SA was significantly enhanced by β-CyD.

In order to elucidate the role of the mucin layer on the intestinal membrane, we examined SA absorption after pretreatment with various adjuvants. Tables I and II show the *AUC* values up to 60 min for SA perfusion with or without β-CyD after pretreatment with adjuvants in the intestine and rectum. The adjuvants used were surfactants (SDC and SLS),[16] a chelating agent (EDTA),[17] and sodium alginate, pectic acid[18] and trypsin.[15] These compounds are known to affect membrane permeability to drugs in the small intestine and rectum. The concentration of adjuvant in perfusate was selected to be below the concentration which affected membrane permeability. After pretreatment with the adjuvants, *AUC* of SA perfusion with β-CyD was significantly increased in the cases of trypsin, SLS and SDC, but not with EDTA, sodium alginate or pectic acid. The enhancing effect on the *AUC*

Table I. Effect of β-CyD on the Intestinal Absorption of SA after Pretreatment with Adjuvants

Pretreatment	Conc.	SA alone[a]	With β-CyD[a]	Ratio
Control	—	253.4± 8.0	271.4±13.4	1.07
N-Ac	1.0 (%)	272.5±20.0	665.8±10.9[b]	2.44
SDC	0.5 (mM)	386.1±21.7	881.2±51.6[b]	2.28
SLS	0.5 (mM)	330.8±12.1	590.4±50.5[b]	1.78
Trypsin	0.1 (mg/ml)	320.2±12.0	422.6±19.7[b]	1.32
EDTA	2.0 (mM)	328.7±20.0	339.4±14.4	1.03
Sodium alginate	0.25 (%)	275.6±15.9	277.4±22.9	1.01
Pectic acid	0.5 (%)	221.3±23.1	249.7± 8.0	1.13

Each value represents the mean ± S.E. *a*) *AUC* (μg·min·ml^{-1}). *b*) Significant increase of absorption different from SA alone with each treatment, $p<0.05$. Ratio: *AUC* with β-CyD/*AUC* without β-CyD.

Table II. Effect of β-CyD on the Rectal Absorption of SA after Pretreatment with Adjuvants

Pretreatment	Conc.	SA alone[a]	With β-CyD[a]	Ratio
Control	—	90.0±14.3	98.0± 3.1	1.08
N-Ac	1.0 (%)	95.6±11.3	93.8± 9.3	0.98
	2.0 (%)	108.6±21.8	110.3± 6.6	1.02
SDC	0.5 (mM)	215.6±37.5	205.9±10.4	0.96
	1.0 (mM)	903.6±79.5	966.1±66.8	1.07
SLS	0.5 (mM)	190.3±22.9	178.0± 7.7	0.94
	1.0 (mM)	789.6±32.3	723.3±19.6	0.92

Each value represents the mean ± S.E. *a*) *AUC* (μg·min·ml^{-1}). Ratio; *AUC* with β-CyD/*AUC* without β-CyD.

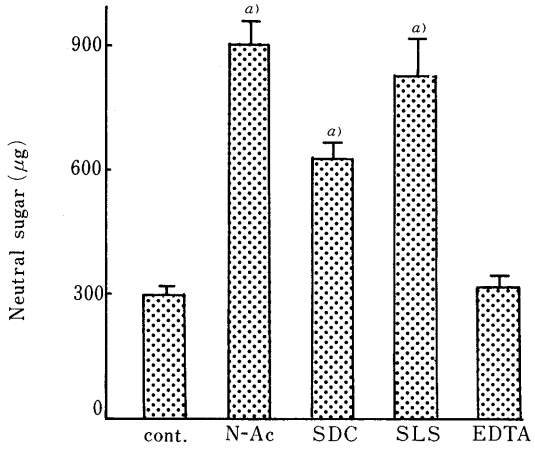

Fig. 2. Effect of Adjuvants on the Release of Mucin from Intestine
Each bar represents the mean ± S.E. *a*) Significantly different from control, $p < 0.05$.

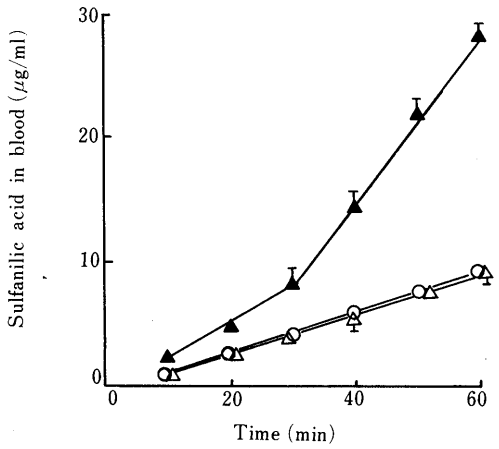

Fig. 3. Recovery from Enhanced Intestinal Absorption of SA with β-CyD after N-Ac Pretreatment
—○—, control; —▲—, immediately after N-Ac pretreatment; —△—, 30 min after N—Ac pretreatment. Each point represents the mean ± S.E.

of SA lies in the order of N-Ac > SDC > SLS > trypsin.

The absorption of SA from rat rectum was investigated after pretreatment with N-Ac, SDC and SLS in the rectum under the same conditions as those for the small intestine. The adjuvant pretreatment did not change the *AUC* values of SA in the presence of β-CyD. Further, absorption of SA from the rectum was not influenced even when the rectum was treated with higher concentrations of adjuvants.

These results indicate that there are differences in the small intestine and rectum in the enhancing effect of β-CyD. It is well known that the potency of absorption promoter is greater in rectum than in intestine.[19] The enhancing effect of β-CyD after pretreatment with adjuvants was only found in intestine. Therefore, we are unable to conclude at present whether or not this different action of β-CyD is ascribable to the tissue structure between the intestine and rectum.

Release of Mucin in Perfusate Mucin adheres to the luminal surface of the gastro-intestinal tract as a gel or a highly viscous solution. The effect of adjuvants on the release of mucin from the intestinal membrane surface was examined (Fig. 2). The released neutral sugar in phosphate buffer (control) was about 300 μg and that in N-Ac solution was three-fold higher. SDC and SLS also increased the release of a significant amount of the neutral sugar; however, no release was observed with the addition of EDTA. (Trypsin and sodium alginate were not measured by this method.)

Absorption of SA after pretreatment with these adjuvants was significantly enhanced by N-Ac, SDC, SLS ann trypsin, while EDTA, sodium alginate and pectic acid showed no effect. SA absorption was also found to be enhanced in the mucin liberated from intestine by the adjuvants. These results indicate that mucin may play an important role in the absorption of non-absorbable drugs by β-CyD.

Recovery Experiments Recovery from the enhanced permeability of the intestinal membrane after N-Ac pretreatment was evaluated on the basis of the blood levels of SA. These levels were determined immediately and 30 min after the N-Ac pretreatment. (Fig. 3) At 30 min, no increase was shown the blood level, indicating that the effect of β-CyD had disappeared by that time.

Nakamura *et al.* reported that the recovery of intestinal mucus begins 15 min after the treatment.[20] In this experiment, recovery was completed within 30 min. This recovery may be the result of the secretion of mucin from goblet cells of the small intestine.

Miyajima *et al.* revealed that the effect of CyDs on artifical lipid membrane is influenced by the presence of cholesterol. The leakage of calcein lies in the order of α- > β- > γ-CyD for egg yolk lecithin liposomes, while it is in the order of β- > α- > γ-CyD for liposomes composed of an equimolar mixture of lecithin and cholesterol.[21] The effect of α-CyD was reduced with increasing cholesterol content. Similarly, Irie *et al.* reported that CyDs cause hemolysis of human erythrocytes in the order of β- > α- > γ-CyD,[6] which are composed of an approximately equimolar mixture of lecithins and cholesterol.[22] The molar ratio of cholesterol to phospholipid in rat small intestinal brush border membrane of weaned and juvenile rats is 0.9—1.2.[23] The absorption of non-absorbable drugs was significantly increased by β-CyD. This fact is consistent with the release experiment from liposomes composed of an approximately equimolar ratio of cholesterol and the phospholipid.[21]

It was found that β-CyD enhanced the absorption of non-absorbable drugs after pretreatment with adjuvants, but little affected the absorption in the presence of mucin on the surface of intestinal membrane. If the mucin layer from intestine is removed by adjuvants, the interaction of CyDs with lipids will take place on the surface of the mucosal membrane. Then, CyD may be withdrawn the lipid molecules by formation of the inclusion complex, resulting in the enhancement of membrane permeability. This phenomenon is in line with findings of the release experiment of polysaccharide coated liposomes by CyDs.[9]

The physiological role of mucin is considered to be protecting the underlying delicate epithelial cells from acid and enzyme attack, mechanical damage, and pathogenic organisms.[24] Mucus depletion in ulcerative colitis and changes of the mucus content in Crohn's disease have been reported.[25] If the barrier function of intestinal membranes is reduced after washing-off of the mucin layer with adjuvants or in cases of ulcerative colitis or Crohn's disease, β-CyD may enhance the absorption of non-absorbable drugs through the small intestine.

Additional studies are in progress to clarify the

mechanism of alteration of membrane premeability by CyD.

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