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# Effect of bile on the intestinal absorption of α-cyclodextrin in rats

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## Summary

The effect of bile on the absorption of α-cyclodextrin from rat small intestine was examined using the in situ recirculating perfusion technique. Only very little intestinal absorption of α-cyclodextrin was observed when the bile duct was ligated, but some of it was absorbed from the intestine when bile was present; intact α-cyclodextrin then entered the systemic circulation. Sodium cholate and disodium ethylenediaminetetraacetate also promoted the absorption of α-cyclodextrin under the bile duct-ligated conditions and their promoting effects were completely inhibited by the addition of calcium choride. These results indicate that absorption of α-cyclodextrin from the intestine occurs through the paracellular pathway.

## Introduction

Molecular encapsulation of drugs with cyclodextrins (CyDs) has recently received much attention because of its potential application for drug formulation (Szejtli, 1982; Pitha et al., 1983). Improvement of oral bioavailability of poorly water-soluble drugs by cyclodextrins has been extensively studied (Szejtli, 1985; Uekama and Otagiri, 1987). It is generally recognized that when the drug–cyclodextrin complex is administered orally, free drug, which is in equilibrium with the complexed one, is available for intestinal absorption (i.e., cyclodextrins act only as carriers and help to transport the drug through an aqueous milieu to the lipophilic absorption site in the gastrointestinal tract). However, recent studies using the in vitro everted sac method (Koizumi and Kidera, 1977; Szabó et al., 1983) demonstrated that cyclodextrins were slowly absorbed by passive diffusion across intestinal membranes. Koizumi et al. (1985) also reported that when the phenobarbital-β-cyclodextrin complex was introduced into the intestinal lumen of in situ loop of rats, both the drug and β-cyclodextrin could be detected in the mesenteric vein. Thus, there are some discrepancies concerning intestinal absorption of cyclodextrins and their complexes. With the above observation in mind, we re-investigated the intestinal absorption of cyclodextrins using the in situ recirculating perfusion method in rats. In our preliminary studies cyclodextrins were found to be significantly absorbed from rat small intestine in

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the presence of bile. The present paper deals with the effect of bile on the intestinal absorption of α-cyclodextrin and the enhancing mechanism of bile is discussed. α-Cyclodextrin was chosen here because of its resistance to enzymatic degradation and its low irritancy to the mucosal membranes when compared with β-cyclodextrin (Uekama and Otagiri, 1987).

## Materials and Methods

α-Cyclodextrin was kindly donated by Nihon Shokuhin Kako Co., Ltd. (Tokyo, Japan) and recrystallized from water. All other materials and solvents were of analytical reagent grade. Intestinal absorption of α-cyclodextrin was examined by the in situ recirculating perfusion method of Schanker et al. (1958) with a slight modification. Male Wistar rats weighing 170–230 g were fasted for 24 h prior to surgery and were anesthetized with ethyl carbamate. The small intestine was exposed by midline abdominal incision and an intestinal segment (about 20 cm long and starting from the pylorus) was cannulated with or without ligation of the bile duct. After washing out the contents of the lumen, 100 ml of isotonic buffer solution (pH 6.4) containing α-cyclodextrin and other additives was perfused through the intestine at the rate of 5 ml/min at 37 °C. The solution was recirculated. The percentage of α-cyclodextrin absorbed was calculated on the basis of the concentration of α-cyclodextrin 10 min after perfusion. Blood samples were collected from the heart 2 h after perfusion. The concentration of α-cyclodextrin in the perfusate and the plasma was determined by high performance liquid chromatography according to the method of Koizumi et al. (1985).

## Results and Discussion

Fig. 1 shows the time course of disappearance of α-cyclodextrin from a loop of small intestine in rats when a bile duct was ligated or left intact. Under the bile duct-ligated conditions, the intestinal absorption of α-cyclodextrin was scarcely ob-

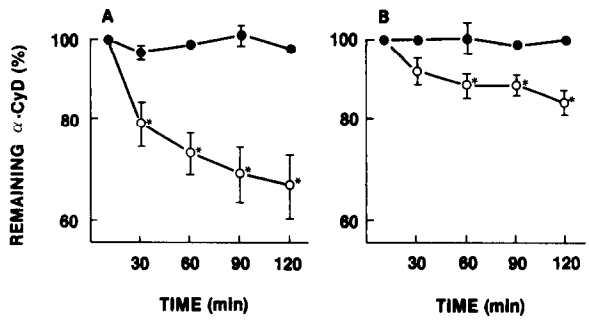


Fig. 1. Disappearance of α-cyclodextrin (α-CyD) from rat small intestine during in situ perfusion with (●) or without (○) ligation of the bile duct. The initial concentrations of α-cyclodextrin in A and B were 0.1 mM and 10 mM, respectively. Each value represents the mean ± S.E.M. of at least 4 rats. $*P < 0.05$ vs (●).

served, initial concentrations were either 0.1 mM or 10 mM. On the other hand, α-cyclodextrin was significantly absorbed from the intestine in the presence of bile, where the total concentration of bile acids secreted from the bile duct was 0.11 ± 0.01 mM in the perfusate 2 h after perfusion. It is evident from Fig. 1 that the absorption of α-cyclodextrin does not follow first order kinetics, and depends on the initial concentration of α-cyclodextrin. As shown in Fig. 2, α-cyclodextrin in an intact form could be detected in plasma 2 h after perfusion of 10 mM α-cyclodextrin in the

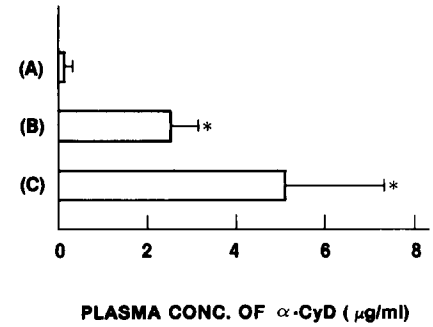


Fig. 2. Plasma concentrations of α-cyclodextrins (α-CyD) 2 h after the in situ perfusion through rat small intestine with or without ligation of the bile duct. A: with ligation of the bile duct; B: without ligation of the bile duct; C: in the presence of 0.1 mM sodium cholate with ligation of the bile duct. The initial concentration of α-cyclodextrin in the perfused solution was 10 mM (9720 μg/ml). Each value represents the mean ± S.E.M. of at least 5 rats. $*P < 0.05$ vs (A).

presence of bile, whereas the perfusion of α-cyclodextrin in the absence of bile gave a negligibly small plasma concentration of α-cyclodextrin.

To gain insight into the enhancing mechanism of bile, the effects of some additives on the intestinal absorption of α-cyclodextrin were examined under the bile duct-ligated conditions. It is well known that bile salts, which are the main components of bile, promote the intestinal absorption of poorly absorbable compounds (Kimura et al., 1985; Shiga et al., 1987). Generally, the enhanced absorption by bile salt can occur by two mechanisms: by the formation of a complex which is absorbed or by the direct action of bile on the mucous membranes. In this study, sodium cholate, a trihydroxy unconjugated bile salt, was used as a simple representative of bile salts. As shown in Fig. 2 and Table 1, the intestinal absorption of α-cyclodextrin was significantly promoted by the addition of 0.1 mM sodium cholate. Miyajima et al. (1986) recently reported that β-cyclodextrin forms stable inclusion complexes with several bile salts in aqueous solutions. Thus, the interaction between α-cyclodextrin and sodium cholate was assessed by solubility analysis and spectroscopy methods. All the results suggested that the ability of α-cyclodextrin to form a complex with sodium cholate is negligibly small in this case. The stability constant of sodium cholate with α-cyclodextrin was determined to be 190 $M^{-1}$ in isotonic phosphate buffer (pH 4.0) by solubility analysis. Under in situ experimental conditions (pH 6.4), cholate is partially ionized since its p$K$a is 6.5; this may further destabilize the complex with α-cyclodextrin (Uekama and Otagiri, 1987). Thus, it can be estimated that more than 98% of α-cyclodextrin exists in the free form in the perfused solutions, calculated on the basis of the magnitude of the stability constant of the complex. The micelle formation cannot be operative in this study, since sodium cholate is used well below its critical micelle concentration (Mills et al., 1986). Therefore, the enhanced absorption of α-cyclodextrin by sodium cholate cannot be explained by some interaction between these compounds.

It has been shown that bile salts increase the intestinal absorption of compounds by depleting endogenous calcium ion in the region of paracellular tight junctions (Murakami et al., 1984). To investigate whether calcium ion-depletion by sodium cholate participates in the observed enhancement of α-cyclodextrin absorption, the effects of disodium ethylenediaminetetraacetate and calcium chloride were studied. As shown in Table 1, ethylenediaminetetraacetate, which chelates calcium ion, also enhanced the absorption of α-cyclodextrin. Furthermore, the enhancement of α-cyclodextrin absorption obtained by sodium cholate could be completely inhibited by addition of calcium chloride. Bile salts were also reported to induce other direct actions on the mucous membranes such as the removal of membrane components and the structural changes of membranes (Martin et al., 1978). Under the present experimental conditions, small amounts of membrane components, such as proteins and lipids, could be released by the combined actions of bile, high concentration of α-cyclodextrin, and forced

TABLE 1

*Effects of some additives on the absorption of α-cyclodextrin (α-CyD) from rat small intestine with ligation of bile duct*

| Additives | % Absorbed in 110 min [a] | |
|---|---|---|
| | 0.1 mM α-CyD [b] | 10 mM α-CyD [b] |
| None | 3.12 ± 1.70 | 0.89 ± 2.45 |
| 0.1 mM sodium cholate | 19.28 ± 3.43 * | 15.55 ± 6.34 * |
| 0.1 mM disodium ethylenediaminetetraacetate | 24.31 ± 3.57 * | 16.57 ± 5.08 * |
| 0.1 mM sodium cholate with 0.1 mM calcium choride | 0.07 ± 2.95 | |

[a] Each value represents the mean ± S.E. of at least 4 rats.

[b] The initial concentration of α-cyclodextrin in the perfused solution.

* $P < 0.05$ versus the value without additives.

perfusion. However, there was no relationship between the extent of release of membrane components and the enhancement of the intestinal absorption of α-cyclodextrin and no observable morphological changes in the mucosal surface by light microscopy.

The present results indicate that α-cyclodextrin could be absorbed from rat intestine in the presence of endogenous absorption promoters such as bile salts. This absorption of α-cyclodextrin is affected by changes in the concentration of calcium ions in the same manner as those which occur through the disturbance of the paracellular, tight-junctional pathway; perhaps the same mechanism is involved. For this uptake to occur in vivo quite specific, but realizable, conditions have to be met. The present findings provide a basis for the effective and safe use of cyclodextrins even then.

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## References

Kimura, T., Inui, K. and Sezaki, H., Differences in effects on drug absorption between dihydroxy and trihydroxy bile salts. *J. Pharmacobio-Dyn.,* 8 (1985) 578–585.

Koizumi, K. and Kidera, Y., Effect of α- or β-cyclodextrin on gastrointestinal absorption of barbituric acid derivatives. *Yakugaku Zasshi,* 97 (1977) 705–711

Koizumi, K., Kubota, Y., Okada, Y. and Utamura, T., Micro-analysis of β-cyclodextrin in plasma by high-performance liquid chromatography. *J. Chromatogr.,* 341 (1985) 31–41.

Martin, G.P., Marriott, C. and Kellaway, I.W., Direct effect of bile salts and phospholipids on the physical properties of mucus. *Gut,* 19 (1978) 103–107.

Mills, C.O., Martin, G.H. and Elias, E., The effect of tyrosine conjugation on the critical micellar concentration of free and glycine-conjugated bile salts. *Biochim. Biophys. Acta,* 876 (1986) 677–683.

Miyajima, K., Yokoi, M., Komatsu, H. and Nakagaki, M., Interaction of β-cyclodextrin with bile salts in aqueous solutions. *Chem. Pharm. Bull.,* 34 (1986) 1395–1398.

Murakami, T., Sasaki, Y., Yamajo, R. and Yata, N., Effect of bile salts on the rectal absorption of sodium ampicillin in rats. *Chem. Pharm. Bull.,* 32 (1984) 1948–1955.

Pitha, J., Szente, L. and Szejtli, J., Molecular encapsulation of drugs by cyclodextrins and congeners. In Bruck, S.D. (Ed.), *Controlled Drug Delivery, Vol. I,* CRC, FL, 1983, pp. 125–148.

Schanker, L.S., Tocco, D.J., Brodie, B.B. and Hogben, C.A.M., Absorption of drugs from the rat small intestine. *J. Pharmacol. Expt. Ther.,* 123 (1958) 81–88.

Shiga, M., Hayashi, M., Horie, T. and Awazu, S., Differences in the promotion mechanism of the colonic absorption of antipyrine, phenol red and cefmetazole. *J. Pharm. Pharmacol.,* 39 (1987) 118–123.

Szabó, P., Ferenczy, T., Serfozo, J., Szejtli, J. and Lipták, A., Absorption and elimination of cyclodextrin derivatives by rabbits and rats. In Szejtli, J. (Ed.), *Proc. First Int. Symp. Cyclodextrins,* Reidel, The Netherlands and Akadémiai Kiadó, Hungary, 1983, pp. 115–122.

Szejtli, J., *Cyclodextrins and Their Inclusion Complexes,* Akadémiai Kiadó, Budapest, 1982.

Szejtli, J., Molecular entrapment and release properties of drugs by cyclodextrins. In Smolen, V.F. and Ball, L.A. (Eds), *Controlled Drug Bioavailability, Vol. 3,* Wiley, New York, 1985, pp. 365–420.

Uekama, K. and Otagiri, M., Cyclodextrins in drug carrier systems. *CRC Crit. Rev. Ther. Drug Carrier Systems,* 3 (1987) 1–40.