Effect of the Interaction of Drug–β-Cyclodextrin Complex with Bile Salts on the Drug Absorption from Rat Small Intestinal Lumen

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This investigation was concerned with the change of the bioavailability of a drug owing to the interaction of the drug–β-cyclodextrin complex with bile salts in rat intestinal lumen. The absorption of sulfamethizole (SMZ) from rat intestinal lumen after administration of SMZ–β-cyclodextrin complex was determined by a closed-loop method in the presence or absence of bile. The blood level of SMZ after administration of SMZ–β-cyclodextrin complex was significantly decreased in comparison with that after administration of SMZ alone in bile duct-ligated rats. On the other hand, the blood level of SMZ after SMZ–β-cyclodextrin administration in intact rats (bile duct non-ligated) or on the addition of sodium cholate was similar to the level in the case of SMZ alone. Thus, bile salts were found to act as a competing agent in the gastrointestinal tract.

Keywords bioavailability; intestinal absorption; β-cyclodextrin; sulfamethizole; bile salt; sodium cholate; rat

Introduction

β-Cyclodextrin(β-CyD), a cyclic oligosaccharide consisting of 7α-1,4-linked glucose units, forms inclusion complexes with various hydrophobic drugs, resulting in the improvement of solubility,[1,2] dissolution rate,[3] and bioavailability of the drugs.[4] However, as the formation constant of a drug with β-CyD is large, the absorption of a drug administered as a drug–β-CyD complex may not be so effective as that of the drug alone, since only free drug is absorbed. The administration of cinnarizine–β-CyD complex did not improve the bioavailability of the drug, but the bioavailability was improved by the coadministration of DL-phenylalanine.[5]

It is well known that bile salts form inclusion complexes with β-CyD in pH 7.2 phosphate buffer solution.[6] If an exchange reaction between drug molecule and bile salt molecule takes place in the gastrointestinal tract, the release of free drug molecules will lead to improved bioavailability. From this viewpoint, we investigated the effects of endogenous compounds such as bile salts and exogenous compounds such as sodium cholate on the intestinal absorption of a drug administered as the drug–β-CyD complex. The model drug used in this study was sulfamethizole (SMZ), which was shown to have a lower formation constant with β-CyD than the formation constants of bile salts–β-CyD.[6]

Methods

Materials SMZ, sodium cholate and β-CyD were obtained from Sigma Chemical Company and Wako Pure Chemicals, respectively. All other chemicals were of analytical grade.

Solubility Studies Solubility measurements were carried out according to the method of Higuchi and Connors.[7] An excess amount of the drug was placed in pH 6.5 phosphate buffer solution and shaken at 37±0.5 °C. After equilibration (2 d), the equilibrated mixture was filtered with Dismic-3cp (pore size 0.5 μm; Toyo). The filtrate was diluted with water and the SMZ content was determined spectrophotometrically. The apparent formation constant was calculated from the initial slope of the solubility curve.

Absorption Experiment Male Wistar rats, weighing 200—230 g were used in all experiments.

Method 1: The *in situ* single-perfusion method: The absorption of SMZ from the small intestine was measured by an *in situ* single-perfusion method in bile duct-ligated rats. A solution of SMZ (1.9 mM) and β-CyD (0—10 mM) in pH 6.5 isotonic phosphate buffer was perfused through the intestine at a rate of 2 ml/min with or without sodium cholate. At given intervals for 1 h, 0.3 ml of blood was taken from the jugular vein and the blood concentration of SMZ was determined.

Method 2: The *in situ* closed-loop method: The effect of endogenous bile salts on the absorption of SMZ was examined by the *in situ* closed-loop method. The rat small intestine was exposed, then a segment (about 15 cm from the duodenal end) was cut and two pieces of silicone tubing were inserted into the two ends. Isotonic saline solution warmed to 37 °C was slowly passed through the lumen and expelled from the lumen with air. One milliliter of SMZ–β-CyD complex solution (each 10 mM) in pH 6.5 isotonic phosphate buffer was introduced into the lumen of bile duct-ligated or non-ligated rats. At various intervals for 3 h, blood samples were taken from the jugular vein. The remaining solution was carefully expelled from the lumen and the lumen washed with isotonic saline, then the solution was analyzed to determine the unabsorbed amounts of SMZ and β-CyD.

Analytical Methods SMZ was determined spectrophotometrically as described in our previous paper.[8] β-CyD was determined by high-performance liquid chromatography (HPLC) by the method of Koizumi *et al.*[9] An HPLC apparatus (Shimadzu LC-3A, Japan) equipped with a variable-wavelength ultraviolet (UV) detector (SPD-6A, Shimadzu) was used in a reversed-phase mode with a stationary phase of Nucleosil $5C_{18}$ packed in 4.6 mm i.d. × 150 mm tubing (Gasukuro Kogyo Inc., Japan) and operated at ambient temperature. A mixture of acetonitrile–water (92 : 8, v/v) was used as the mobile phase at a flow rate of 1 ml/min. The effluent was monitored by measurement of the UV absorption at 231 nm. The β-CyD concentration was determined from the peak height using a calibration curve. Bile salts in bile were determined by using a Total Bile Acid-Test Kit (Wako Pure Chemicals). The amount of total bile salts was calculated as sodium cholate.

Data Analysis The area under the blood concentration–time curves (*AUC*) was calculated by 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. The level of free SMZ after SMZ–β-CyD complex administration with or without sodium cholate was calculated by the use of Eqs. 1 and 2.

Results and Discussion

The effect of complexation of SMZ with β-CyD on the drug solubility in pH 6.5 phosphate buffer was studied. The phase-solubility diagram shown in Fig. 1 can be classified as type A_L according to Higuchi and Connors.[7] The solubility of SMZ increased linearly with β-CyD concentration. The apparent formation constant calculated from the linear portion was approximately 208 M^{-1} in pH 6.5 phosphate buffer solution at 37 °C. The molar ratio of the complex precipitated from the solution was found to be 1 : 1, calculated according to the method of Koizumi *et al.*[10]

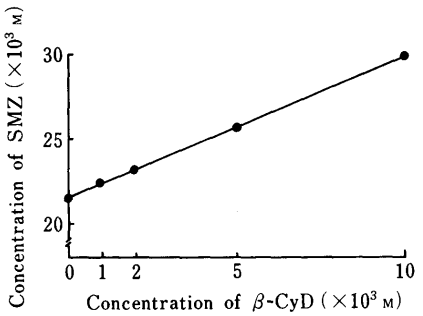

Fig. 1. Phase Solubility Diagram of SMZ–β-CyD System in pH 6.5 Phosphate Buffer at 37 °C

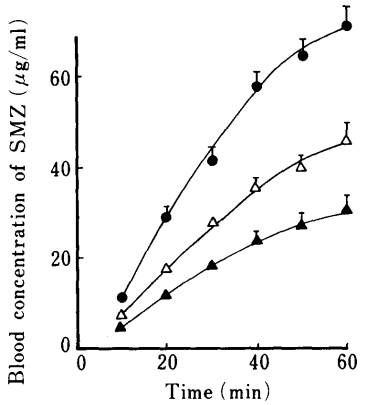

Fig. 2. Effect of β-CyD on the Absorption of SMZ Determined by the *in Situ* Single-Perfusion Method

—●—, control (1.9 mM SMZ alone); —△—, 2 mM β-CyD; —▲—, 10 mM β-CyD. Each point represents the mean value of 4 rats with the standard error shown as a bar.

Figure 2 shows the blood concentration–time curve of SMZ in the presence of β-CyD obtained by the *in situ* single-perfusion method. As is evident from the figure, the absorption of SMZ was significantly decreased in the presence of β-CyD. The decrease of SMZ absorption was dependent on the perfusate concentration of β-CyD. This result is thought to reflect the formation of SMZ–β-CyD complex, which is not absorbed from the small intestine.

The effect of exogenous bile salts on the absorption of SMZ was studied. The formation constants of bile salts such as sodium cholate, sodium deoxycholate, sodium glycocholate, and sodium taurocholate with β-CyD are 1100, 2670, 410 and 406 M^{-1}, in pH 7.2 phosphate buffer solution, respectively.[6] These values are larger than that of SMZ (208 M^{-1}). Therefore, to clarify whether sodium cholate acts as a competing agent in the intestinal lumen or not, we investigated the absorption of SMZ during the perfusion of SMZ–β-CyD complex solution with the coadministration of exogenous bile salts. Figure 3 shows the area under the blood concentration–time curve up to 60 min (AUC_{0-60}) for SMZ after the perfusion of SMZ alone and SMZ–β-CyD complex (β-CyD, 2 mM) in bile duct-ligated rats by an *in situ* single-perfusion method. On the perfusion of SMZ as SMZ–β-CyD complex, the value of AUC_{0-60} was 1500 μg·min/ml, which is two-thirds of that of SMZ alone ($p<0.05$). On the perfusion of SMZ–β-CyD complex with 2 mM sodium cholate, the AUC_{0-60} value was slightly increased as compared to that of the β-CyD complex alone. However, the perfusion of SMZ–β-CyD complex with 4 mM

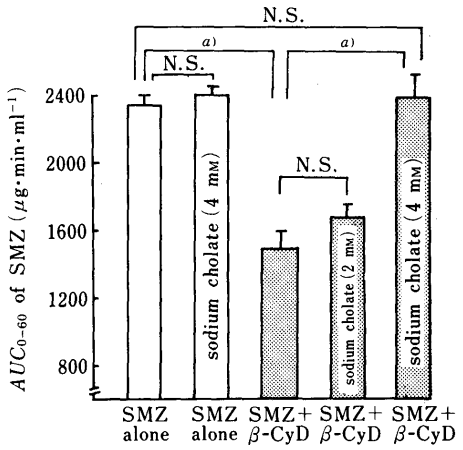

Fig. 3. Effect of Sodium Cholate on the Absorption of SMZ alone (□) or with β-CyD (▨) in Bile Duct-Ligated Rats Determined by the *in Situ* Single-Perfusion Method

Each bar represents the mean ± S.E.M. of 3—4 rats. Statistical significance: *a*) $p<0.05$. N.S. = not significant.

sodium cholate brought about a significant increase in the AUC_{0-60} value, which was at the same level as that in the case of SMZ alone. The enhancement of AUC_{0-60} value by the addition of sodium cholate may be due to the increase of free SMZ level. Therefore, the levels of free SMZ in buffer solution containing 2 mM β-CyD with or without sodium cholate were calculated by using the following equations.

$$[CyD]_T=[CyD]_f+\frac{K_s[SMZ]_t[CyD]_f}{1-K_s[CyD]_f}+\frac{K_c[cholate]_t[CyD]_f}{1-K_c[CyD]_f} \quad (1)$$

$$[SMZ]_f=\frac{[SMZ]_t}{1-K_s[CyD]_f} \quad (2)$$

These equation are derived on the assumption that SMZ and cholate form a 1 : 1 complex in molar ratio. In Eq. 1, $[CyD]_t$ and $[CyD]_f$ are the concentrations of total and free β-CyD. K_s and K_c are the formation constants of SMZ–β-CyD and cholate–β-CyD complexes. $[SMZ]_t$ and $[SMZ]_f$ are the concentrations of total and free SMZ, respectively. $(Cholate)_t$ is the total concentration of sodium cholate. Experimental values of K_s and K_c are 208 and 1100 M^{-1}, respectively. The free concentration of β-CyD was calculated by the Newton–Raphson method. The values of $(SMZ)_f$ were calculated by means of Eq. 2 using the $(CyD)_f$ obtained from Eq. 1. The level of free SMZ was 75% in buffer solution containing 2 mM β-CyD. This corresponds to the relative value of AUC_{0-60} after administration of SMZ–β-CyD complex (about 67%). Moreover, on the addition of sodium cholate (4 mM) to SMZ–β-CyD solution, the level of free SMZ was markedly increased from 75 to 92%. A good correlation was obtained between the level of free SMZ obtained from calculation and the value of AUC_{0-60} obtained in the *in situ* absorption study. This indicates that sodium cholate acts as a competing agent.

The absorption of SMZ without β-CyD was found to be the same in the presence or absence of sodium cholate (4 mM). Thus, no effect of micelles of sodium cholate on the absorption of SMZ was observed in this study.

The effect of endogenous bile salts following administration of SMZ–β-CyD complex was studied. Figure 4

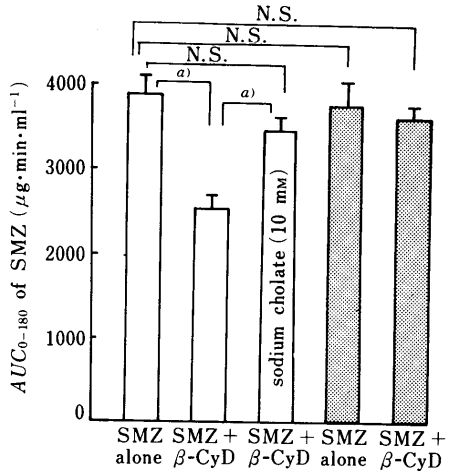

Fig. 4. Absorption of SMZ after Duodenal Administration of SMZ and Its β-CyD Complex to Bile Duct-Ligated (⊓) or Non-ligated (▣) Rats Determined by the *in Situ* Closed Loop Method

Each bar represents the mean ± S.E.M. of 4—5 rats. Statistical significance: *a*) $p < 0.05$. N.S. = not significant.

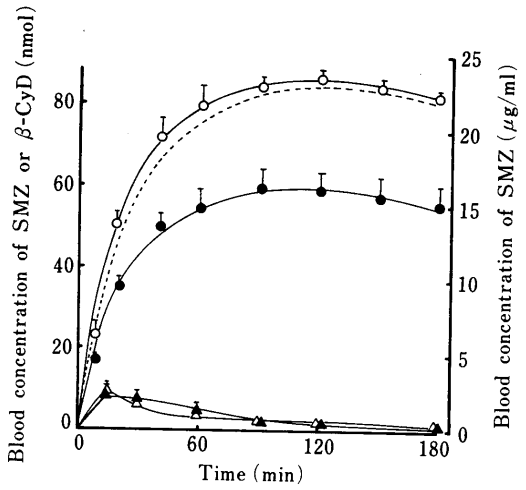

Fig. 5. Blood Concentrations of SMZ and β-CyD after Duodenal Administration of SMZ and β-CyD Complex with or without Bile by the *in Situ* Closed Loop Method

—●—, SMZ without bile; —○—, SMZ with bile; —▲—, β-CyD without bile; —△—, β-CyD with bile; ----, net blood level of SMZ calculated as the difference between blood level of total SMZ and blood level of SMZ complexed with β-CyD. Each bar represents the mean ± S.E.M. of 4—5 rats.

shows the AUC_{0-180} value of SMZ after duodenal administration of SMZ–β-CyD complex to bile duct-ligated or non-ligated rats by a closed-loop method. There was no statistically significant difference in AUC_{0-180} value after the administration of SMZ alone in the bile duct-ligated or non-ligated rats. In bile duct-ligated rats, the AUC_{0-180} value of SMZ–β-CyD complex administration was decreased in comparison with that of SMZ alone ($p < 0.05$). On the other hand, an increase of AUC_{0-180} value of SMZ–β-CyD complex administration was caused by influx of bile or addition of sodium cholate in the rat intestinal loop. The amount of bile salt influx into the intestinal loop was determined. The amount of total bile salts was about 11 mg (about 30 mM) as sodium cholate in 1 h. Under the present experimental conditions, this amount of bile acids was more than the added amount of sodium cholate in Fig. 4. Consequently, the observed enhancement of the bioavailability of SMZ–β-CyD complex after duodenal administration in intact rat may be attributed to the endogenous bile salts acting as a competing agent.

Recently, it was reported that the absorption of β-CyD from the small intestine can be induced by the presence of sodium cholate and ethylenediaminetetraacetic acid (EDTA).[11] On the other hand, Szejtli *et al.* reported that the absorption of ^{14}C-labeled β-CyD did not take place from the stomach and small intestine, but only from the colon.[12] Also, Koizumi *et al.* reported that β-CyD was slightly absorbed (2% in 20 min) from the small intestinal lumen (about 10 cm at the ileal end) after administration of phenobarbital–β-CyD complex.[9] To examine whether the absorption of β-CyD occurred under our experimental conditions, we determined β-CyD itself in blood and in perfusate. Figure 5 shows the blood concentrations of SMZ and β-CyD after duodenal administration of the SMZ–β-CyD complex by an *in situ* closed-loop method with or without bile. The absorption of β-CyD itself under these experimental condition was a low with or without bile. Therefore, the amount of SMZ absorbed as the β-CyD complex is considered to be negligible. Similarly, the disappearance of β-CyD from the lumen in intact rats (with bile) was slight (8.9 ± 0.9% in 3.0 h), and β-CyD remained almost wholly in the complex form with bile salts or biological compounds in the intestinal lumen. Consequently, the enhancement of bioavailability of SMZ after the β-CyD complex administration is based not on the absorption of β-CyD complex but on the increase of free SMZ, since β-CyD was only slightly absorbed in the intact form from the small intestine under the experimental conditions used.

In general, the enhancement of drug bioavailability by β-CyD complex administration is attributed to increased dissolution rate and solubility of the drug. In the case of cinnarizine, which has a large formation constant with β-CyD (6300 M^{-1}), the enhancement of the dissolution rate of the complex did not affect the bioavailability.[12] Pharmaceutically, it is desirable to use drug–β-CyD complexes whose formation constants are between 100 and 1000 M^{-1}.[13] Drugs with a small formation constant form unstable complexes, while those with a large formation constant are too stable for exchange with bile salt to occur. If the formation constant of an orally administered drug–β-CyD complex is within the above range, competition between the drug molecules and bile salts for complex formation would take place in the duodenum. Consequently, drug molecules are excluded from the cavity of β-CyD by bile salt molecules, and the free drug molecules thus produced are absorbed from the intestinal lumen.

In conclusion, the interaction of β-CyD complex with bile salts in the intestinal lumen plays an important role in the drug absorption from orally administered drug–β-CyD complexes.

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