Journal of Inclusion Phenomena and Macrocyclic Chemistry **44:** 93–96, 2002.

Model Analysis for Oral Absorption of a Drug/Cyclodextrin Complex Involving Competitive Inclusion Complexes

NAOMI ONO[1,2], FUMITOSHI HIRAYAMA[2], HIDETOSHI ARIMA[2], KANETO UEKAMA[2] and J. HOWARD RYTTING[1]*

[1]*Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66047-2504, USA;* [2]*Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862-0973, Japan*

(Received: 7 May 2002; in final form: 1 October 2002)

Key words: bioavailability, competitive inclusion, competitor, intestinal absorption, permeation rate, phenacetin

Abstract

The bioavailability parameters of a drug after oral administration of a preparation containing drug/CyD complexes may be modified by formation of competitive inclusion complexes. In this study, we examined the effects of competitors on drug permeation from its CyD complex through *in-vitro* artificial membranes and *in-situ* recirculation conditions, for comparison with the results under *in-vivo* conditions in the bile duct of cannulated rats. Phenacetin, an antipyretic, was used as a model drug, natural CyDs and maltosyl-β-CyD as host molecules, and benzoic acid derivatives, sodium taurocholate and acetaminophen as competitors. The *in-vitro* cellophane membrane permeation rate and the *in-situ* absorption rate of phenacetin were quantitatively predicted by theoretical calculation using the stability constants of drug/CyD and competitor/CyD complexes when CyD weakly interacts with membrane components in lower CyD concentrations (generally below 10 mM). The *in-vivo* absorption behavior was only qualitatively reproducible by the theoretical calculation, probably because of various physicochemical and physiological factors affecting the absorption. The present results may be useful not only for prediction of intestinal absorption of drugs from CyD preparations, but also for formulation design of CyD preparations containing multi-components.

Introduction

The rate and extent of bioavailability of a poorly water-soluble drug from its cyclodextrin (CyD) complex can be optimized by adjusting various factors affecting the dissociation equilibrium of the complex in both the formulation and the biophase in which the complex is administered [1]. Figure 1 shows model drug absorption from competitive inclusion complexes in the gastrointestinal tract. Only the free form of the drug, which is in equilibrium with the complexed form in solution, is capable of penetrating lipophilic barriers consisting of either mucosal epithelia or stratified cell layers and eventually entering the systemic circulation. In general, nonprescription drugs, such as antipyretics, are formulated with several pharmaceutical additives, and administered concomitantly with other pharmacologically active drugs. In that case, the bioavailability parameters of the drug after oral administration of the preparation containing drug/CyD complexes may be modified by a competitive inclusion [2, 3]. However, it is not clear under what conditions the competing inclusions occur.

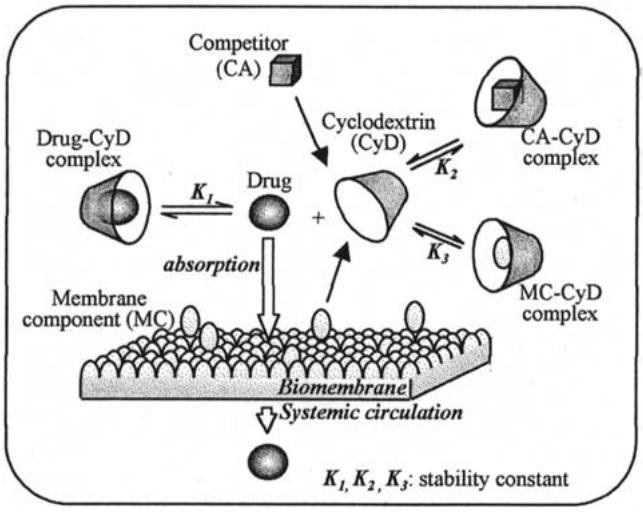

Figure 1. Scheme showing drug absorption from an inclusion complex involving competitive inclusion complexation in the gastroinstestinal tract.

In this study, we examined the effects of competitors on drug permeation from its CyD complex through *in-vitro* artificial membranes and *in-situ* recirculation conditions, for comparison with the results under *in-vivo* conditions in the bile duct of cannulated rats. Phenacetin, an antipyretic, was used as a model drug, natural CyDs and maltosyl-β-CyD as host molecules, and benzoic acid derivatives, sodium taurocholate and acetaminophen as competitors.

* Author for correspondence. E-mail: rytting@ku.edu

Experimental

Materials

Natural CyDs were supplied from Nihon Shokuhin Kako Co. (Tokyo, Japan). Maltosyl-β-CyD (G2-β-CyD) was supplied from Bio Research Corporation of Yokohama (Yokohama, Japan). The following chemicals were used after recrystallization from methanol/water: phenacetin (Sigma-Aldrich, USA), and *m*-bromobenzoic acid (Nakalai Tesqe, Tokyo, Japan). Sodium taurocholate and acetaminophen were purchased from Nakalai Tesqe (Tokyo, Japan). All other chemicals and solvents were of analytical reagent grade, and deionized double-distilled water was used throughout the study.

In-vitro *permeation studies*

Permeation behavior of phenacetin and benzoic acids through a cellophane membrane (Spectra/Por® MWCO 500, SPECTRUM, Houston, USA) was examined using a side-by-side type permeation cell apparatus as reported previously [4]. A cellophane membrane was selected because of its impermeability for CyD under the present experimental conditions (permeation of CyD for 6 h: $< 0.04\%$ through Spectra/Por® MWCO). The sample solution in 10 mM phosphate buffer (pH 7.4, 55 mL) was put into the donor cell compartment while the same volume of the phosphate buffer solution without samples was placed in the acceptor cell compartment. Both solutions in the permeation cells were stirred with a stainless steel bar at 100 rpm at 37 °C. At appropriate intervals an aliquot (0.2 ml) was withdrawn from the donor and acceptor cell compartments and the drug concentrations were measured by HPLC. An equal volume of the phosphate buffer was added to maintain a constant volume of the medium.

In-situ *recirculation studies*

Male Wistar rats, weighing 200 to 220 g, were fasted 24 h before experiments. The absorption of drug from the small intestine (jejunum 10 cm) was measured in bile duct ligated rats. The sample solution in isotonic phosphate buffer (pH 6.5, 50 mL) was perfused through the intestine at a rate of 5 mL/min [5]. At given intervals for 10 min, an aliquot (0.2 mL) of perfused sample solution was taken from the donor phase, and the drug concentrations were measured by HPLC. An equal volume of the phosphate buffer was added to maintain a constant volume of the medium.

In-vivo *absorption studies*

Male Wistar rats, weighing 200 to 220 g, were treated to eliminate bile acid under anesthetization with ether, and fasted for 24 h prior to drug administration, while water was allowed ad libitum. The samples were administered orally to the rats. Blood samples (about 0.8 ml, Group A; 5, 15, 40, 60 min, Group B; 10, 20, 90, 120 min) were taken periodically from the jugular vein, and centrifuged at 3000 rpm for 10 min at 4 °C. The plasma phenacetin was extracted with ethylacetate and assayed by HPLC under the following conditions [6]: a L-7100 pump and a L-7400 UV monitor (Hitachi, Tokyo, Japan), a YMC Pack column AM-303-S-5 (4.6 × 250 mm, Tokyo, Japan), a mobile phase of methanol/0.01 M ammonium carbonate (4:7 v/v), a flow rate of 0.6 mL/min, and detection at 254 nm.

Results and discussion

In-vitro *permeation studies*

We selected a cellophane membrane for the *in-vitro* permeation study because the permeation of β-CyDs was negligible under the experimental conditions. Figure 2 shows the permeation of phenacetin in the presence of β-CyD and a competing agent, *m*-bromobenzoic acid. The permeation rate of phenacetin decreased with the addition of β-CyD since complex formation occurs only in the donor phase and only free guest molecules transfer into the acceptor phase. The permeation rate of phenacetin was restored by the addition of a competing agent in a concentration-dependent manner, because of an increase in the fraction of free phenacetin available. The permeation of *m*-bromobenzoic acid was also restored. The solid lines represent theoretical curves calculated according to the Runge–Kutta–Gill method. The data points fit well on the theoretical curves calculated using the stability constants of drug/CyD and competitor/CyD complexes ($K_1 = 182\ \mathrm{M}^{-1}$ and $K_2 = 312\ \mathrm{M}^{-1}$, see Figure 1), and the permeation rate constant of phenacetin (k) [7]. Therefore, the permeation behavior of the drug through the cellophane membrane in the presence of β-CyD and competing agent can be reasonably estimated using these parameters.

In-situ *recirculation studies*

In the *in-situ* recirculation studies, all experiments were performed in bile-ligated Male Wistar rats to avoid the effects of bile acids on the absorption [8]. The absorption of β-CyD was negligible under the experimental conditions. Figure 3a shows the remaining phenacetin in the presence of β-CyD and a competing agent, *m*-bromobenzoic acid. The absorption rate of phenacetin through a rat intestinal segment decreased upon the addition of β-CyD, and was restored by the addition of *m*-bromobenzoic acid in a concentration-dependent manner. These results are similar to those found in the *in-vitro* artificial membrane permeation study described above. The solid lines are theoretical curves that were calculated using stability constants ($K_1 = 182\ \mathrm{M}^{-1}$ and $K_2 = 312\ \mathrm{M}^{-1}$, see Figure 1) and rate constants. The data fit well on the theoretical curves calculated using the K_1 and K_2 values, in spite of the slight release of some membrane components from the intestinal tract. These results suggest that the permeation behavior of phenacetin through rat intestinal membrane is predictable by calculation using stability constants and permeation rate constants of the drug and competing agents. Figure 3b shows the effect of sodium

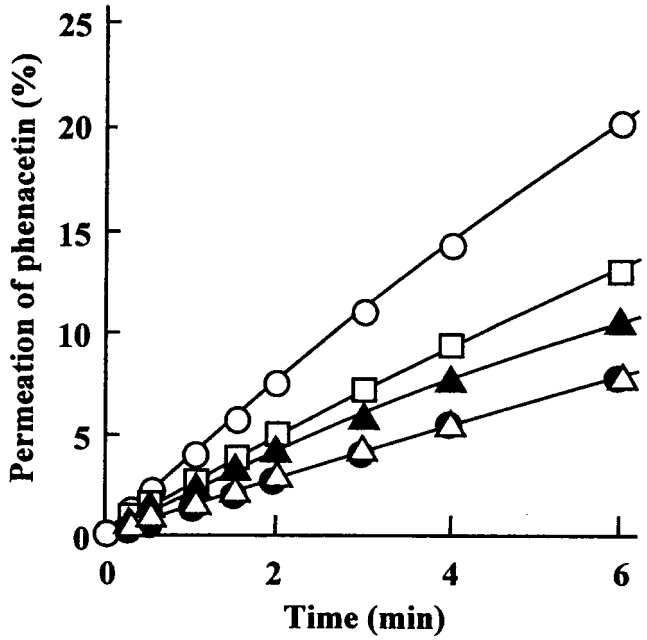

Figure 2. Effect of *m*-bromobenzoic acid on the permeation of phenacetin (0.1 mM) with or without *m*-bromobenzoic acid and β-CyD through a cellophane membrane (MWCO 500) in isotonic phosphate buffer at 37 °C. The solid lines were theoretical curves calculated by the Runge–Kutta–Gill method. ○: phenacetin alone, ●: phenacetin/*m*-bromobenzoic acid/β-CyD = 1/0/100 (molar ratio), △: 1/10/100, ▲: 1/50/100, □: 1/100/100.

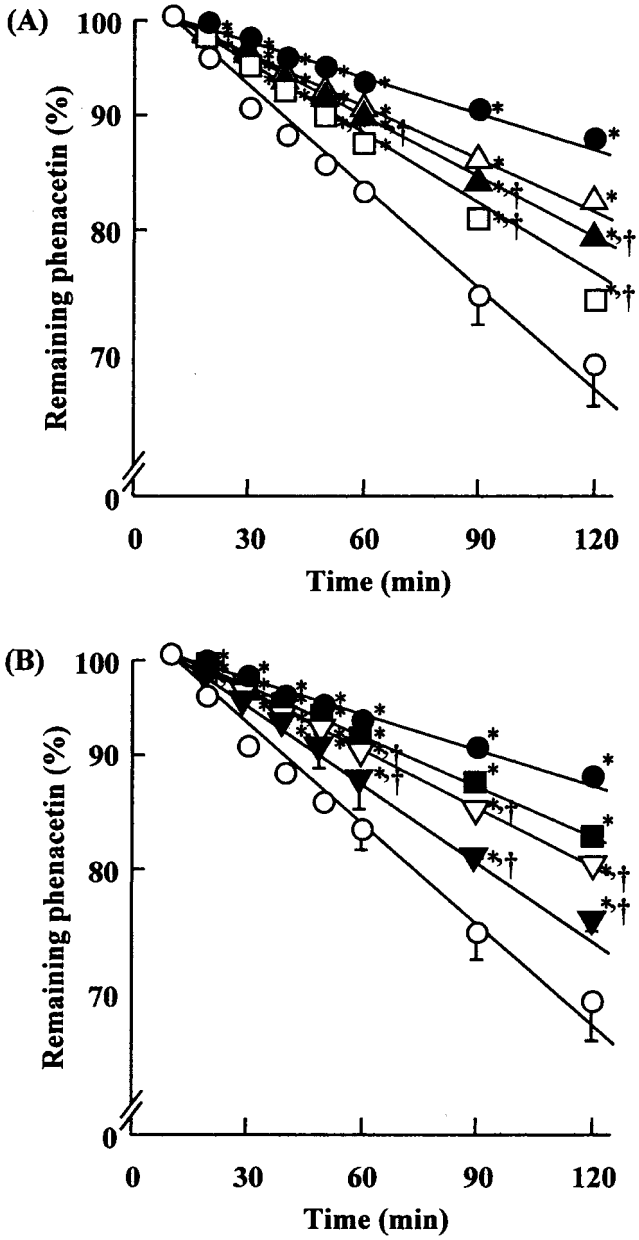

Figure 3. Effect of competitors on the disappearance of phenacetin (0.01 mM) with or without competitors and β-CyD from a rat intestinal segment (jejunum) in isotonic phosphate buffer (pH 6.5) at 37 °C with the under bile duct ligated. (A) Competitor is *m*-bromobenzoic acid. (B) Competitor is sodium taurocholate. The solid lines were theoretical curves calculated by the Runge–Kutta–Gill method. *: $P < 0.01$ vs. phenacetin alone, †: $P < 0.01$ vs. phenacetin/β-CyD = 1/500. ○: phenacetin alone, ●: phenacetin/β-CyD = 1/500 (molar ratio), △: phenacetin/*m*-bromobenzoic acid/β-CyD = 1/500/500 (molar ratio), ▲: 1/600/500, □: 1/800/500, ■: phenacetin/sodium taurocholate/β-CyD = 1/50/500 (molar ratio), ▽: 1/100/500, ▼: 1/500/500.

taurocholate as a competitor on the absorption of phenacetin. Sodium taurocholate was not absorbed under the experimental conditions. The absorption rate of phenacetin was restored by the addition of sodium taurocholate, which was itself poorly absorbable in a concentration-dependent manner. Restoration of the absorption rate of phenacetin was due to an increase in the fraction of free drug molecule. These results for sodium taurocholate are similar to those for the absorbable competitor, *m*-bromobenzoic acid, as described above.

Thus, in the permeation of phenacetin through an intestinal membrane under *in-situ* recirculation conditions, the permeation behavior of the drug was predictable by calculation using the stability constants and the permeation rate constants when CyDs weakly or negligibly interact with membrane components (β-CyD < 10 mM). However, alkaline phosphatase and LDH were released from the intestinal membrane at higher β-CyD concentrations (> 10 mM, data not shown) suggesting some perturbations in membrane integrity and absorption.

In-vivo *absorption studies*

The *in-vivo* absorption behavior of phenacetin after oral administration was investigated using bile-ligated Male Wistar rats. We could not use β-CyD at high concentrations because of its limited solubility. Instead, we used a branched chain CyD, G2-β-CyD, having a higher aqueous solubility. The stabilities of phenacetin/G2-β-CyD ($K_1 = 215\ M^{-1}$) and sodium taurochorate/G2-β-CyD ($K_2 = 630\ M^{-1}$) were similar to both of the guests with β-CyD complexes. Figure 4 shows the plasma levels of phenacetin with time after oral administration of solutions containing phenacetin along with G2-β-CyD, sodium taurocholate or both compounds, to rats. The absorption rate of phenacetin decreased upon the addition of G2-β-CyD, since only free guest molecules are absorbed from the small intestine. The absorption rate and drug plasma concentration of phenacetin were restored by the addition of the competing agent, because of an increase in the fraction of free drug molecule. There was no effect of sodium taurocholate on phenacetin absorption. Thus, these

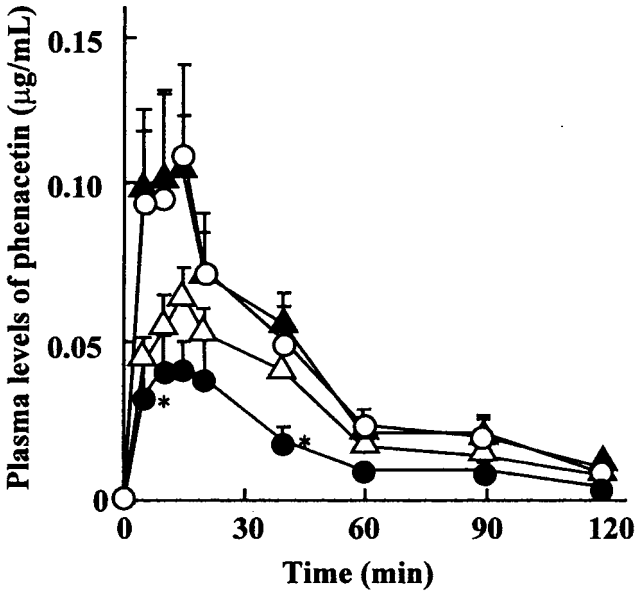

Figure 4. Plasma levels of phenacetin after oral administration of phenacetin (1 mg/kg) with or without G2-β-CyD and sodium taurocholate in solution to rats under bile duct ligated conditions. *: $P < 0.01$ vs. phenacetin alone. ○: phenacetin alone, ●: phenacetin/sodium taurocholate/G2-β-CyD = 1/0/100 (molar ratio), △: 1/1/100, ▲: 1/1/0.

results show that the absorption behavior of the drug form the gastrointestinal tract can be altered by the addition of CyD and a competing agent.

Conclusion

The *in-vitro* cellophane membrane permeation rate and the *in-situ* absorption rate of phenacetin were quantitatively predictable by theoretical calculation using the stability constants of drug/CyD and competitor/CyD complexes when CyD weakly interacts with membrane components in lower CyD concentrations (generally below 10 mM). The *in-vivo* absorption behavior was only qualitatively reproducible by the theoretical calculation probably because of various physicochemical and physiological factors affecting the absorption. The present results may be useful not only for the prediction of intestinal absorption of drugs from CyD preparations but also for formulation design of CyD preparations containing multi-components.

References

1. K. Uekama, F. Hirayama, and T. Irie: *Chem. Rev.* **98**, 2045 (1998).
2. V.J. Stella, V.M. Rao, E.A. Zannou, and V. Zia: *Adv. Drug Deliv. Rev.* **36**, 3 (1999).
3. T. Tokumura, M. Nanba, Y. Tsushima, K. Tatsuishi, M. Kayano, Y. Machida, and T. Nagai: *J. Pharm. Sci.* **75**, 391 (1986).
4. A. Ohki, M. Takagi, and K. Ueno: *Chem. Lett.*, 1591 (1980).
5. K. Hosoya, H. Kubo, H. Natsume, K. Sugibayashi, Y. Morimoto, and S. Yamashita: *Biopharm. Drug Disp.* **14**, 685 (1993).
6. G.G. Duggin: *J. Chromatogr.* **121**, 156 (1976).
7. N. Ono, H. Arima, F. Hirayama, and K. Uekama: *Eur. J. Pharm. Sci.* **8**, 133 (1999).
8. K. Nakanishi, M. Masada, T. Nadai, and K. Miyajima: *Chem. Pharm. Bull.* **37**, 211 (1989).