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Influence of hydroxypropyl-β-cyclodextrin and dimethyl-β-cyclodextrin on diphenhydramine intestinal absorption in a rat in situ model

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Abstract

The influence of complexation of diphenhydramine (DPHA) with hydroxypropyl-β-cyclodextrin (HPβCD) and dimethyl-β-cyclodextrin (DMβCD) on intestinal absorption of DPHA has been investigated on an in situ model in rats. The mean apparent stability constants of the complexes formed at 23°C between DPHA and the cyclodextrins DMβCD and HPβCD were 4988 and 1635 M^{-1}, respectively. At 37°C, the apparent stability constants were smaller: 895 and 494 M^{-1} for the complexes formed between DPHA and the cyclodextrins DMβCD and HPβCD, respectively. Complexation of DPHA with DMβCD led to a significant decrease (-36%) in the percentage of DPHA absorbed (30.6 ± 12.0 vs. $22.5 \pm 6.9\%$, $P = 0.018$). On the other hand, complexation of DPHA with HPβCD only slightly decreased (-8%) the extent of absorption (43.2 ± 9.0 vs. $40.0 \pm 7.7\%$, $P = 0.16$). These data suggest that the magnitude of the apparent stability constant of drug–cyclodextrin complexes should be considered when complexes are used to increase the oral absorption of drugs.

Keywords: Diphenhydramine; Inclusion complex; Hydroxypropyl-β-cyclodextrin; Dimethyl-β-cyclodextrin; Intestinal absorption; In situ model; Rats

1. Introduction

Among natural cyclodextrins (CDs), β-CD has been studied extensively despite a very low aqueous solubility but alkylated derivatives such as 2-hydroxypropyl-β-cyclodextrin, (2,6-di-*O*-methyl)-β-cyclodextrin and sulfobutyl-ether derivatives of β-CD have attracted growing interest due to greater water solubilities (Loftsson and Brewster, 1996; Rajewski and Stella, 1996), while toxicological issues and biological fate of CDs

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remain to be extensively investigated (Irie and Uekama, 1997).

One of the most interesting oral applications of drug–CD complexes is the improvement of drug bioavailability. The improvement in drug bioavailability may result from an increase in the rate at which the drug is available for absorption (i.e. when the rate-limiting step is drug dissolution) and/or from an increase in drug mucosal permeability.

Increase in drug absorption by means of interaction of CDs on the biological membranes has been investigated (Nakanishi et al., 1992). lndeed, in a rat intestinal model, liberation of membrane components has been shown and was different according to the type of CD. α-CD selectively released phospholipids while β-CD released mainly cholesterol from intestinal membrane. Although almost no morphological changes in the small intestine were observed, it was suggested that the interaction with membrane components may be in part responsible for the increased absorption of sulfanilic acid, a non-absorbable drug. Furthermore, electrophysiological experiments suggested that the increased permeability caused by β-CD occurred primarily in the transcellular pathway as a result of disorder induced in the cell membrane lipids. However, this increase was only evidenced in the presence of *N*-acetylcysteine, a mucolytic agent. The presence of the mucus layer was thought to disturb the direct interaction of CDs with the membrane lipids.

Thus, the choice of the CD candidate to be used is of paramount importance to reach the goal of improvement in bioavailability. Moreover, the magnitude of the interaction between the CD and the drug candidate should also be considered since the use of drug–CD complexes characterized by a high apparent stability constant may lead to an opposite (retarding) effect (Bekers et al., 1991; Szejtli, 1994). To investigate this phenomenon, we compared in a rat in situ model the absorption of diphenhydramine following administration of DPHA-HCl and DPHA complexed with HPβCD and DMβCD. DPHA was chosen as a model drug since it is a lipophilic drug (oily liquid) able to form complexes with CDs (Mwakibete et al., 1991; Tong et al., 1991). DPHA absorption from CD complex was studied in comparison with DPHA absorption from DPHA-HCl, the soluble form of the drug whose absorption is the rate-limiting step, i.e. avoiding any artifact from a dissolution–rate-limited absorption. We choose to study β-CD derivatives because, it has been shown that, in comparison to α-CD and γ-CD, β-CD forms the strongest interactions with amine drugs bearing the diphenylmethyl functionality (Mwakibete et al., 1991; Tong et al., 1991). HPβCD and DMβCD were chosen as CD candidates since, in comparison to HPβCD, DMβCD is known to lead to higher interaction with cholesterol and with the biological membranes as shown in an erythrocyte model (Irie and Uekama, 1997).

2. Materials and methods

2.1. Materials

HPβCD (Encapsin, Ref. 3022154, batch 02L-181/0, MS = 0.47, MW = 1300 g/mol) was purchased from Janssen Biotech (Olen, Belgium) and DMβCD (batch 03483/01, MW = 1331 g/mol) was purchased from Avebe (Veendam, The Netherlands). The water content was determined by the Karl Fischer method using a Methrom E408 Apparatus. The mean ($n = 3$) water content for DMβCD and HPβCD were 0.85 and 4.58%, respectively.

DPHA (Fig. 1), as an oily liquid, was prepared from DPHA hydrochloride (DPHA-HCl) (Ref. D-3630, batch 49F0379, Sigma Chemicals, France) by alkalinisation of a DPHA hydrochloride solution. The oily phase was rinsed with distilled water until neutral pH and dehydrated

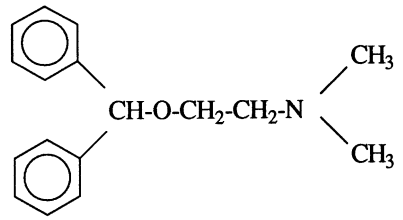

Fig. 1. Chemical structure of diphenhydramine.

with sodium sulfate. DPHA purity was checked by HPLC in comparison with DPHA hydrochloride.

All other reagents and solvents were of analytical grade.

2.2. Phase solubility study

The phase solubility study was achieved according to the method of Higuchi and Connors (1965). Excess amounts of DPHA (49 μg, 50 μl) were poured into 1-ml screwcap polypropylene tubes to which were added aqueous solutions containing various concentrations of CDs, ranging from 0 to 10% (w/v) for DMβCD and HPβCD. The emulsions formed were then rotated on a top to bottom shaker, at room temperature (23°C) and at 37 ± 0.1°C. After solubility equilibrium for 24 h, aliquots were filtered through a cotton filter, diluted with the mobile phase and total concentration of drug in the filtrate was analyzed by HPLC. The experiment was carried out in duplicate. The stability constant (K_s) was then calculated from the initial linear portion of the phase solubility diagrams, if a 1:1 stoichiometric ratio complex was formed at the initial step (slope smaller than 1 for all systems) according to the following equation $K_s = \text{slope}/S_o(1 - \text{slope})$, where S_o is the drug solubility in water (Higuchi and Connors, 1965).

2.3. Perfusion solutions

Perfusion solutions (40 ml) were obtained by dilution of 0.5 ml of DPHA (or DPHA complex) solution with 39.5 ml of a pH 7.50 isotonic phosphate buffer solution (PBS). PBS solution contained KH_2PO_4 (0.20 g), Na_2HPO_4 (1.80 g), KCl (0.20 g) and NaCl (8.0 g) dissolved in 1 l of distilled water.

2.4. Surgical preparation and perfusion technique

Male albino Sprague-Dawley rats (CERJ, Le Genest Saint Isle, France) were maintained in animal care facilities for at least 1 week before use. The animals, weighting 200–300 g, received food and water ad libitum and were fasted 12 h before each experiment. Anesthesia was induced by intraperitoneal urethane (1.5 mg/kg). A midventral incision extending 5–7 cm was made and the small intestine was exposed. The jejunum was located and an incision was made around 5 cm below the bile duct. A similar incision was made around 25 cm aboral to the first. The segment was gently flushed, avoiding excessive expansion of the intestine segment, with 40–50 ml of 0.9% NaCl solution at 37°C allowing a clear effluent. The ends of the segment were cannulated with a needle adaptor and secured with encircling surgical sutures. The two different perfusion methods, i.e. recirculating perfusion and single-pass perfusion are presented schematically in Fig. 2. The intestinal segment was perfused using a constant infusion pump (IPC, Ismatec, Zurich, Switzerland). The perfusate was maintained at 37 ± 1°C by a water bath. During the experiment the rat was kept on a slide warmer and the abdomen was covered with a saline-wetted gauze. The intestinal

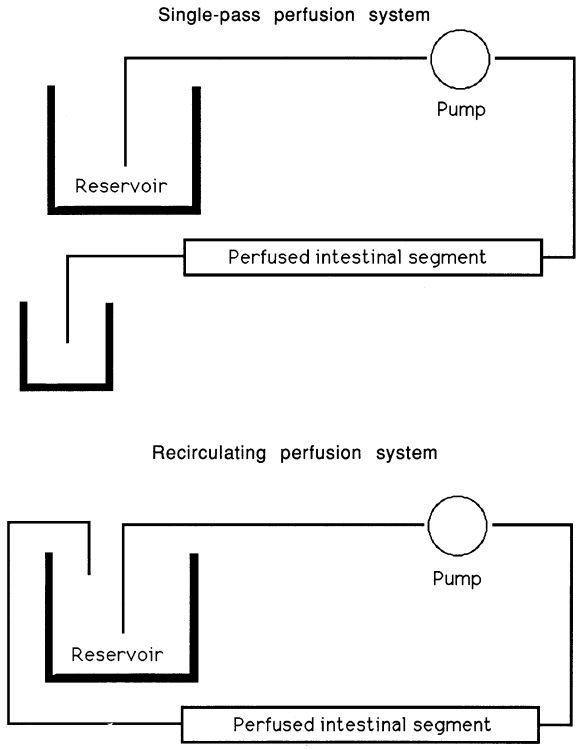

Fig. 2. Schematic drawings of the different perfusion systems: (i) single-pass perfusion system; and (ii) recirculating perfusion system.

segment was placed within the abdominal cavity carefully without crimping and kinking of the segment avoiding subsequent obstruction. The body temperature of the animal was maintained by a heating lamp. At the end of the experiment, the volume of the perfusion solution was measured (in the recirculating perfusion technique) and the length of the intestine segment was measured by placing a piece of string along the intestine and measuring the string with a ruler.

2.5. Study protocols

Single-pass perfusion experiments were performed in two animals to study the influence of the perfusion rate on DPHA absorption. In each animal, the flow rate was successively applied according to a decreasing and increasing scheme. The flow rates (0.25, 0.5, 1, 2 ml/min) were applied for a 20-min period and DPHA absorption was calculated at the end of the perfusion. The DPHA concentration in the perfusion was 0.125 mg/ml in PBS buffer. Each study period was preceded by a rinsing period corresponding to a volume of 5 ml. The flow rates applied to the first animal were from 0.25 to 2 ml/min and then from 2 to 0.25 ml/min. The flow rates applied to the second animal were from 2 to 0.25 ml/min and then from 0.25 to 2 ml/min.

Recirculating perfusion experiments were performed to study the influence of time, concentration and cyclodextrin complexation on DPHA absorption.

The influence of time on DPHA absorption was checked by three successive experiments achieved in two animals. Each experiment was achieved with a perfusion rate of 1 ml/min and lasted 2 h. The DPHA-HCl concentration in the perfusion solution was 0.125 mg/ml in pH 7.5 PBS buffer solution. DPHA concentrations were determined every 15 min.

The influence of DPHA-HCl concentration was checked by three successive experiments achieved in two animals. Each experiment was achieved with a perfusion rate of 1 ml/min and lasted 2 h. Three DPHA-HCl concentrations in the perfusion solution (0.025, 0.125 and 0.25 mg/ml in pH 7.5 PBS buffer solution) were studied successively. DPHA concentrations were determined every 15 min.

The influence of DMβCD and HPβCD on DPHA absorption was checked by comparison of DPHA absorption following a successive and cross-over perfusion of DPHA-HCl solution and either DPHA-DMβCD or DPHA-HPβCD solution. DPHA-HCl and DPHA-DMβCD were compared in a series of eight animals and DPHA-HCl and DPHA-HPβCD were compared in a series of six animals. The DPHA concentration in the perfusion was 0.125 mg/ml in PBS buffer solution. Each experiment was achieved with a perfusion rate of 1 ml/min and lasted 2 h. DPHA concentrations were determined every 15 min.

Two parameters were circulated to evaluate the intestinal absorption of DPHA, e.g. the amount absorbed (%) and the absorption rate (mg/h). The amount absorbed was calculated from the DPHA concentration in the perfusion medium at the end of the experiment. The absorption rate was calculated by linear regression from the amounts absorbed determined every 15 min throughout the experiment from the DPHA concentrations in the perfusion medium.

2.6. DPHA analysis

DPHA determination in the perfusion solution was performed by a high-performance liquid chromatographic (HPLC) method. The HPLC system consisted of a Waters (Milford, MA) Model 6000a pump equipped with a Waters model WISP 710 B automatic injector, an LDC Milton Roy (Riviera Beach, FL) Model Spectromonitor 3100 variable wavelength detector set at 205 nm and a Delsi (Suresnes, France) Model Enica 21 integrator. Analyses were performed with a Waters Model μBondapack C18 column maintained at 30°C. The mobile phase was a mixture of acetonitrile and 0.01 M potassium dihydrogenphosphate (40:60, v/v), pH 4.0, and was used at a flow rate of 1 ml/min.

2.7. Statistical analysis

To compare the absorption of DPHA alone or complexed, statistical analysis was performed us-

ing the student t-test (paired t-test for comparison within groups, t-test for comparison between periods). A P value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Phase solubility study

The solubility (S_o) of DPHA in water at 23 and 37°C was 0.25 mg/l (0.98 mM) and 1.04 mg/l (4.08 mM), respectively. DMβCD and HPβCD exhibited A_n type diagrams (Fig. 3). The mean apparent stability constant (K_s), at 23°C, of the complexes formed between DPHA and the cyclodextrins DMβCD and HPβCD were 4988 and 1635 M^{-1}, respectively. At 37°C, the apparent stability constants were smaller: 895 and 494 M^{-1} for the complexes formed between DPHA and the cyclodextrins DMβCD and HPβCD, respectively. At both temperatures, DMβCD formed complexes of higher stability than HPβCD. The influence of the temperature on the complexation showed the exothermic character of the complexation reaction.

3.2. Influence of flow rate

The choice of the flow rate has to be made with caution because it has been shown to influence the variability of absorption, a high variability (CV > 10%) being associated with fast (1 ml/min) and slow (0.25 ml/min) rates (Savina et al., 1981). In the perfusion flow rate range studied (0.25–2 ml/min), the variability was not apparently influenced by the flow rate and was rather small (lower than 4.5%). The choice of a flow rate of 1 ml/min was a compromise between technical and physiological aspects. Indeed, low flow rates led to long-lasting experiments and high flow rates (above 1.5 ml/min) have been considered non-physiological (Savina et al., 1981). Moreover, high perfusion rates should not allow subtle intralumenal influences normally affecting drug transport across the mucosal barrier.

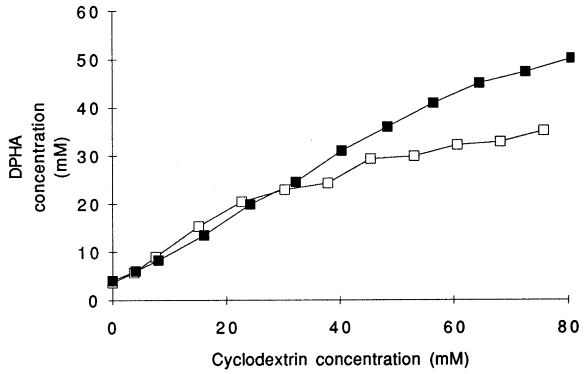

Fig. 3. Phase-solubility diagrams for DPHA in the presence of DMβCD (□) and HPβCD (■) (in water at 37°C).

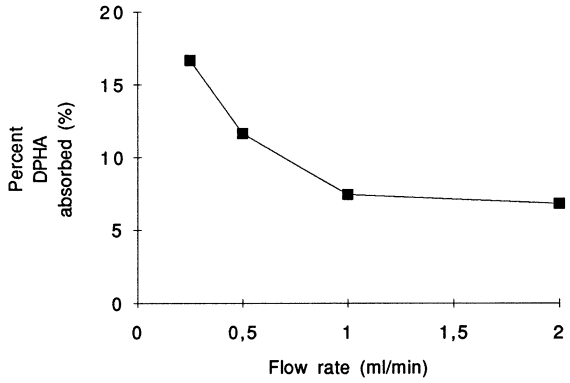

Fig. 4. Absorption of DPHA (%) following intestinal perfusion of DPHA-HCI as a function of the perfusion flow rate (ml/min) in the in situ intestinal model in rats.

Increase in flow rate significantly decreased the absorption (Fig. 4). The higher absorption with decreasing flow rates could be attributed to a higher residence time of drug molecules within the intestine segment, even though low flow rates are expected to increase the thickness and hence to decrease the permeability of the aqueous boundary layer (Amidon et al., 1981). Such increase in absorption with decreasing flow rates has been observed with progesterone, a lipophilic drug, suggesting that the mass transport of progesterone was aqueous boundary layer controlled in contrast to the membrane-controlled kinetics observed with hydrocortisone, a less lipophilic drug where in-

crease in flow rate decreased the absorption (Komiya et al., 1980). Considering the high lipophilicity of DPHA, a boundary layer-controlled absorption may be anticipated. Conversely, increased absorption with flow rate, shown for iopanoic acid, has been attributed to increased shear adjacent to the membrane according to the convective diffusion model (Savina et al., 1981).

3.3. Influence of time

Fig. 5 shows that the absorption of DPHA from 0 to 2 h and from 2 to 4 h was apparently superimposable, while a lowest absorption was noticed for the period from 4 to 6 h. On the basis of these data, the experiments investigating the influence of DMβCD and HPβCD on DPHA absorption were performed on a period lasting 4 h. In each study (DMβCD and HPβCD study), each animal received DPHA-HCl and DPHA complexes in successive periods lasting 2 h. In each study, DPHA-HCl and DPHA complexes were studied in a cross-over manner.

3.4. Influence of DPHA concentration

Fig. 6 shows that DPHA absorption was not influenced by the concentration in the range from 0.025 to 0.250 mg/ml. A DPHA concentration of 0.125 mg/ml was used for the further investigation of the influence of CD complexation.

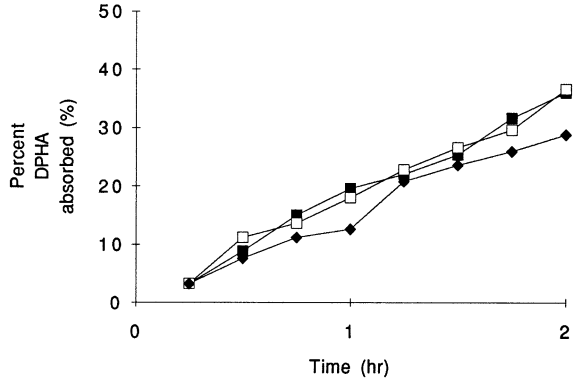

Fig. 5. Absorption of DPHA (%) following intestinal perfusion of DPHA-HCl as a function of time (□) from 0 to 2 h, (■) from 2 to 4 h and (◆) from 4 to 6 h in the in situ intestinal model in rats.

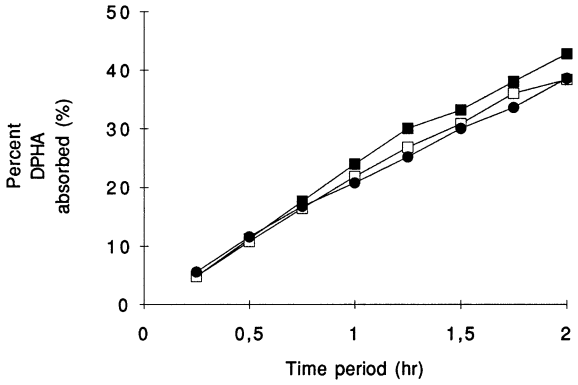

Fig. 6. Absorption of DPHA (%) following intestinal perfusion of DPHA-HCl at concentrations of 0.025 mg/ml (■), 0.125 mg/ml (□) and 0.250 mg/ml (●) in the in situ intestinal model in rats.

3.5. Influence of oligosaccharide

Since the presence of a cyclodextrin, as an oligosaccharide, may affect DPHA absorption, a control experiment with a low-molecular weight linear oligosaccharide that does not form a complex with DPHA was achieved in two animals. Dextran (MW 2000 Da) was used at a molar concentration identical to the cyclodextrin concentration. Fig. 7 shows that dextran did not apparently modify the absorption of DPHA.

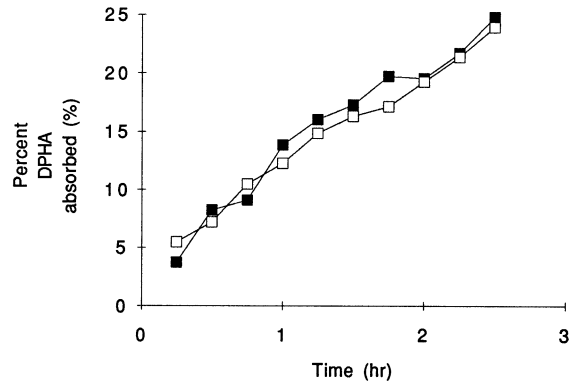

Fig. 7. Absorption of DPHA (%) following intestinal perfusion of DPHA-HCI in the presence (□) and in the absence (■) of dextran (MW 2000) in the in situ intestinal model in rats.

Table 1
Absorption of DPHA following intestinal perfusion of DPHA-HCl, and following intestinal perfusion of complexes between DMβCD, HPβCD and DPHA

	Study 1		Study 2	
	DPHA-HCl	DPHA-DM-β-CD	DPHA-HCl	DPHA-HP-β-CD
Animals	8		6	
Length of intestinal loop (cm)	22.3 ± 3.7*		28.0 ± 5.5	
Amount of water absorbed (ml)	5.0 ± 1.5[a]	5.4 ± 1.7	9.5 ± 2.7[a]	8.8 ± 2.5
Percent absorbed	30.6 ± 12.0**,***	22.5 ± 6.9	43.2 ± 9.0[a]	40.0 ± 7.7
Absorption rate (mg/h)	0.546 ± 0.190***	0.390 ± 0.155	0.799 ± 0.130[a]	0.739 ± 0.148

[a] NS between studies.
*$P<0.05$ between groups; **$P>0.05$ between groups; ***$P<0.05$ between studies.

3.6. Influence of complexation with cyclodextrins

Data in Table 1 report the influence of DMβCD and HPβCD on DPHA absorption. As a result of a difference in the intestinal segment length between the two studies (22.3 ± 3.7 vs. 28.0 ± 5.5 cm, $P = 0.034$), interstudy comparisons should be made with caution. Indeed, it is likely that the higher the intestinal segment length the higher the amount absorbed. This should explain in part why the amount of DPHA absorbed in the HPβCD-study ($43.2 \pm 9.0\%$) was higher than in the DMβCD-study ($30.6 \pm 12.0\%$) ($+41\%$, $P = 0.052$). However, the difference in DPHA absorption was highly reduced ($+14\%$), as well as less variable, when the amount of DPHA absorbed was expressed as a function of the intestinal length (1.35 ± 0.33 vs. $1.55 \pm 0.25\%$/cm, $P = 0.24$).

The amount of water absorbed in the study was not different between the study periods (DPHA-HCl alone or complexed DPHA) (Table 1). However, apparent differences in the amount of water absorbed appeared between the studies reflecting in part the difference in intestinal segment length.

Complexation of DPHA with DMβCD led to a significant decrease (-36%) in the percentage of DPHA absorbed (30.6 ± 12.0 vs. $22.5 \pm 6.9\%$, $P = 0.018$). On the other hand, complexation of DPHA with HPβCD only slightly decreased (-8%) the extent of absorption (43.2 ± 9.0 vs. $40.0 \pm 7.7\%$, $P = 0.16$). The expression of the extent of absorption of DPHA as a function of the intestinal length did not modify the statistical meaning of the above observations.

Since we did not add a mucolytic in our experiments, the interaction of the CDs with the cell membrane lipids should occur minimally. In comparison to HPβCD, DMβCD leads to higher interaction with cholesterol and with the erythrocyte membranes (Irie and Uekama, 1997). Thus, the interaction with the enterocyte membrane might also be higher and lead to a higher increased permeability. Hence, that DPHA absorption was decreased following DPHA-DMβCD administration and not following DPHA-HPβCD administration may confirm that no significant interaction with the intestine membrane occurred in our model. The influence of DMβCD on DPHA absorption should thus result from an interaction between DPHA and DMβCD in the perfusion solution and not at the membrane level, i.e. the CD acting only as a carrier agent transporting the drug through the aqueous milieu to the lipophilic absorption sites of the intestinal membrane. The affinity of DPHA for DMβCD, characterized by an apparent stability constant of 895 M^{-1}, may explain the decrease in the absorption rate of DPHA. The apparent stability constant of DPHA for HPβCD (494 M^{-1}) may be too small to lead to a decrease in the absorption rate of DPHA.

Our conclusions of the influence of complexation on drug absorption should not be extended to in vivo situations (i.e. bioavailability studies) and the impact of the complexation on DPHA

bioavailability remains to be investigated. lndeed, in a work on cinnarizine, Järvinen et al. (1995) showed an increase in bioavailability following complexation in comparison with the drug suspension, but there was no difference in the bioavailability of this drug following administration as complexes either with HPβCD or with a sulfobutylether derivative of BCD, despite differences in the magnitude of the apparent stability constants. However, the apparent stability constants were determined at 25°C and not at the body temperature. Moreover, as a result of a high apparent stability constant (Ueda et al., 1989), the bioavailability of cinnarizine following administration of the complex between BCD and cinnarizine was not different from that of the pure drug (Tokumura et al., 1986).

In conclusion, our data suggest that the magnitude of the apparent stability constant of drug–CD complexes should be considered when complexes are used to increase the oral absorption of drugs. indeed, if several CDs can be used to overcome the dissolution–rate-limiting absorption of a particular drug, the CD leading to the lowest apparent stability constant should be preferentially chosen.

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