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Intestinal Absorption of Drugs II. The Effect of Inclusion in Cyclodextrins on the Absorption of Dantrolene

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Abstract. The inclusion of dantrolene sodium, a muscle relaxant with poor water-solubility, in α-, β- and γ-cyclodextrins was determined. Subsequently, the influence of the cyclodextrins on the absorption of the drug from aqueous solutions was investigated in a chronically isolated internal loop in the small intestine of the rat.

A good correlation was found between the inclusion of dantrolene in the various cyclodextrins and the decrease in the absorption rate resulting from a reduction of the thermodynamically active dantrolene according to the phase-separation model. It was concluded that the cyclodextrins do not have a significant influence on the passage of dantrolene across the absorption barrier. Furthermore, experimental evidence was collected to support the fact that β-cyclodextrin was absorbed to a limited, if not negligible, extent in the small intestine of the rat.

Key words. Cyclodextrins, solubility method, phase-separation, isolated intestinal loop, dantrolene.

1. Introduction

The bioavailability (rate and extent of absorption) of a drug administered orally in a solid dosage form is controlled by: (1) the dissolution rate of the drug in the gastrointestinal (GI) fluid and (2) the transfer of the dissolved compound across the absorption barrier (a combination of stagnant water layer, mucus and cellular membranes of the intestinal wall) to the blood. In many instances the dissolution of poorly water soluble lipophilic drugs in the fluid at the absorption site is the slowest and rate-limiting step in the absorption process. The limited volume of GI fluid (partly ingested orally, partly secreted by the GI tract and associated glands, daily 8–10 liter [1]), the reabsorption of water and the restricted transit time in the intestine (6–10 hours [1]) limits the fraction of dissolved – and subsequently absorbed – drug after administration. Besides dissolution kinetics the transfer of the dissolved compound across the absorption barrier might also be an important limitation in the absorption process.

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Especially for poorly water soluble drugs the inclusion of the compound in cyclodextrins (CD) has been proved to increase the bioavailability [2, 3, 4, 5]. For more detailed information on CD and the inclusion of drugs in CD the reader is referred to the work of Szejtli [6], Uekama [2] and Pitha [7]. The increase in bioavailability due to CD is generally ascribed to the higher apparent solubility and, therefore, to the increased dissolution rate of poorly water soluble drugs in the presence of CD.

This study was performed, because in many reports it is not clear whether the increased bioavailability of drugs in the presence of CD is only the result of an increased dissolution rate, or whether CD also affects the transfer of the dissolved compound across the absorption barrier. In the present study the influence of CD on the absorption process of a drug was investigated in such a way that the effect of CD on the dissolution rate of the drug was eliminated. For this purpose perfusion experiments were performed with a solution of the drug to which CDs were added. For this study the poorly water soluble lipophilic drug dantrolene sodium (Da) was selected as a model compound. The influence of α-, β- and γ-CD on the apparent solubility of Da was studied *in vitro*. The influence of CD on the absorption of Da in an aqueous solution from the small intestine of the rat was studied *in situ* in a chronically isolated internal loop in the rat.

2. Materials and Methods

2.1. DANTROLENE SODIUM

Dantrolene sodium (Da) was a gift from Norwich Eaton Pharmaceuticals Inc., NY, U.S.A. and the cyclodextrins were a gift from AVEBE, Foxhol, NL. Da, a poorly water soluble lipophilic drug with muscle relaxing and anti-hyperthermic properties, is administered in doses up to 400 mg daily [8] and shows a low and varying bioavailability [9].

The solubility of Da ($pK_a \approx 7.5$ [11]) in phosphate-buffered saline (66 mM sodium phosphate, 88 mM sodium chloride, pH = 7.4) containing an amount of 0, 2, 4, 6, 8 and 10×10^{-3} M α-, β- or γ-CD was determined by shaking 10 mL of each solution with approximately 2 mg Da for 24 hours at 37°C. After centrifugation at 4000 rpm for 15 min the concentration of Da in the supernatant liquid was determined spectrophotometrically at the isosbestic point of the cyclodextrin-Da

Table I. Isosbestic points of the Da/CD complexes (λ), maximum solubility of Da in buffer and in 10 mM solutions of CD, calculated apparent binding constant K_{app} and percentage of Da free in solution [% free]; the ratio of the first-order disappearance rate constant [k_{dis}] from the perfusate with and without CD is given as $r = k_{dis+}/k_{dis-}$.

	λ (nm)	max sol ($n = 3$) (10^{-5} M) ±SD	K_{app} (M^{-1}) ±SD	% free ±SD	r ($n = 4$–6) ±SD
buffer	–	1.8 ± 0.1	–	–	–
α-CD	409	3.6 ± 0.2	100 ± 4	50 ± 2	0.56 ± 0.09
β-CD	405	6.9 ± 0.2	286 ± 19	26 ± 2	0.35 ± 0.06
γ-CD	387	2.5 ± 0.1	39 ± 2	72 ± 4	0.76 ± 0.07

complex (see Table I). The measured apparent concentration includes the Da complexed with CD and the Da free in solution.

2.2. CHRONICALLY ISOLATED INTERNAL LOOP

Male Wistar albino rats (230–290 g) were used in all experiments. An ileal segment of approximately 8 cm (15–20 cm proximal to the ileo-ceacal valve) with intact blood supply was chronically isolated inside the peritoneal cavity of the rat. The two open ends of the loop were connected to the perfusion system by two Delrin® cannulas attached to the abdominal wall and the loop was returned into the peritoneal cavity. The head-tail connection of the remaining intestine was restored by end-to-end anastomosis. After surgery the rat was placed in a restraining cage and after recovery from the operation (2–4 days) the rat was ready for use in perfusion experiments. Full details of the procedure are given elsewhere [11]. The absorption characteristics of Da in the isolated loop remained stable during at least two weeks, as has been reported earlier [11].

2.2.1. *Perfusions with Da/CD Solutions* in situ

The absorption of Da was studied in a cross-over experimental scheme in 4–6 rats. The perfusion solution was pumped through a heat exchange device to bring the solution to body temperature just before entering the loop. The isolated internal loop was perfused at a rate of 1.0 mL/min with a solution of Da ($\approx 1.5 \times 10^{-5}$ M) in phosphate-buffered saline (66 mM sodium phosphate, 88 mM sodium chloride, pH = 7.4) both with and without 10 mM cyclodextrins. A perfusate concentration of 10 mM CD was chosen because it is close to the maximal solubility of β-CD and to ensure maximal inclusion of Da in CD.

All perfusions were performed in a recirculating mode; the total volume of the Da solution used was 60 mL. During the perfusion experiments the pH never decreased more than 0.2 unit. The absorption of Da was evaluated by measuring the disappearance of Da from the perfusate. For this, the concentration of Da in the perfusate was directly determined at regular intervals by a HPLC-method [12]: Column: LiChrosorb RP8 (Merck, F.R.G.), eluent: acetonitrile/phosphate buffer, pH = 6.8, 45/55 v/v, wavelength of detection: 405 nm.

Since Da is passively absorbed, a first-order decay of the concentration in the perfusate will be observed: the absorption process is characterized by the first-order disappearance rate constant k_{dis}, which equals the slope of the plot of ln[fraction remaining] versus time. Since disappearance rate constants are determined in a cross-over experimental scheme, the effect of CD on the absorption of Da can be described by a ratio: $r = k_{\text{dis with CD}}/k_{\text{dis without CD}}$.

2.2.2. *Absorption of β-cyclodextrins*

The absorption of β-CD was studied by measuring the change in concentration of β-CD in a phosphate-buffered saline solution (pH = 7.4) of 3.75×10^{-4} M β-CD during a recirculating perfusion (flow rate 1.0 mL/min). The concentration of β-CD was determined using the phenolphthalein-inclusion method [13].

3. Results and Discussion

The results of the solubility determinations are presented in Figure 1. In Table I a further analysis of the data is given: the apparent solubility of Da in 10 mM cyclodextrin, the apparent binding constants K_{app} (calculated as: $[Da_{bound}]/[Da_{free}] \cdot [CD] = \text{slope/intercept}$) and the calculated percentage of free Da in a solution with 10 mM cyclodextrin. The percentage of Da_{free} is calculated from the solubility data using Equations 1 and 2:

$$\%Da_{free} = (1 - p) \cdot 100\% \quad (1)$$

$$p = (C^+ - C^-)/C^+ \quad (2)$$

where C^+ is the solubility of Da in the 10 mM CD solution, and C^- is the solubility in the same medium without CD.

According to the phase separation model the fraction of Da_{free} is considered constant and independent of the concentration of Da for a particular concentration of CD. According to this model, the concentration of free Da is only 26% of its apparent concentration when 10 mM β-CD is added to the perfusion solution used in the *in situ* absorption experiments. For α-CD and γ-CD the fractions of free Da will be 50% and 72%, respectively (see Table I). If it is assumed that the absorption of Da complexed with CD is negligible and the CD concentration remains constant, the decreased concentration of free Da in the presence of CD will result in a proportional decrease of the absorption rate of Da.

In Figure 2 an example is given of the amount of Da remaining in solution versus time during one of the absorption experiments. The disappearance of Da from the solution can be described by a first order process, as can be seen from the straight line fitted through the logarithmic data vs. time. The disappearance of the drug from the solution with CD is lower, since the free concentration of Da is diminished

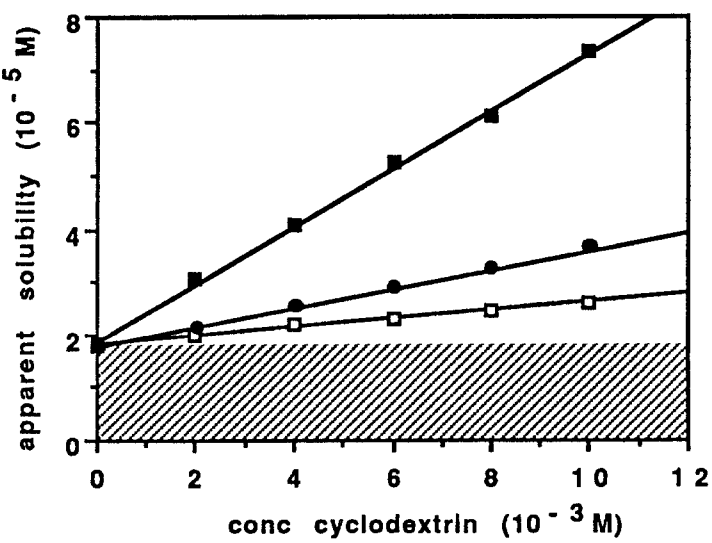

Fig. 1. Apparent solubility of dantrolene sodium in solutions with increasing concentrations of cyclodextrins. Open squares: γ-CD; closed circles: α-CD; closed squares: β-CD. The shaded area indicates the solubility of Da in phosphate-buffered saline at pH = 7.4, 37°C.

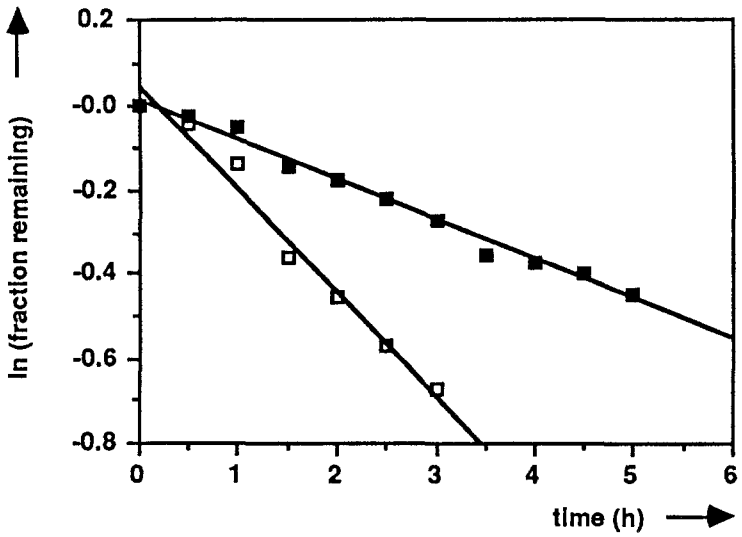

Fig. 2. A typical example of the fraction of Da remaining in the perfusate as a function of time. Open squares: Da in buffer pH 7.4; closed squares: with 10^{-2} M β-cyclodextrin.

partly by complexation with the excess of CD. Habon *et al.* [14] stressed this consequence for the absorption of complexable drugs which occurs at the end of an absorption process from a solid dosage form with CD. The mean ratio of the disappearance rate constants for all rats with and without CD [$r = k_{dis+}/k_{dis-}$] is given in Table I and plotted in Figure 3 with the mean calculated percentage of Da free in solution after addition of the various cyclodextrins (% Da_{free}).

The results depicted in Figure 3 show that the calculated percentage of free Da correlates well with the reduction in the disappearance rate constant of Da. The

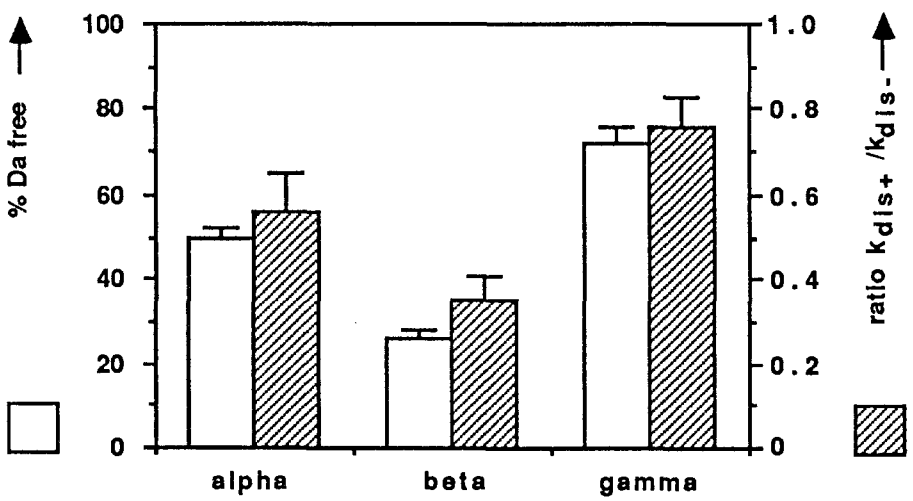

Fig. 3. The calculated percentage of Da free in solution (white bars, $\pm$SD, $n = 3$) and reduction (ratio k_{dis+}/k_{dis-}) in the disappearance rate constant k_{dis} (shaded bars, $\pm$SD, $n = 4$–6).

absorption of Da is controlled by passive diffusion through the absorption barrier (see Figure 2). According to Fick's law, passive diffusion is determined by the concentration of the free compound in solution and the absorption rate constant, which describes the characteristics of the absorption (diffusion) barrier: surface area, thickness and diffusion coefficient through the absorption barrier. Since the absorption of Da in the presence of CD is proportionally decreased with the concentration of free Da, cyclodextrins do not seem to affect the absorption rate constant, indicating that the characteristics of the absorption barrier were not changed.

The experimentally observed absorption data show a slight and consistent deviation from the model mentioned above. Several mechanisms can be formulated to explain these deviations. β-CD might disappear from the perfusate (e.g. by absorption from the loop): according to the results shown in Figure 1 this will result in an increasing fraction of free Da in solution, thus leading to a proportional increase in the disappearance rate. However, the concentration of β-CD during a recirculating perfusion with 10 mM β-CD was studied, and as is shown in Figure 4, the β-CD concentration in the perfusate remained fairly constant: the decrease in β-CD concentration was less than 2%. A 2% decrease of the β-CD concentration will result in a small shift of the equilibrium between the free Da and the complexed Da (see Figure 1) leading to an increase in Da_{free} of 0.4%. This increase in driving force cannot explain the deviation of 9%, that is observed with β-CD (see Table I).

The absorption experiments of β-CD were in agreement with studies by Szejtli and coworkers [15] who orally administered β-CD to rats and found an almost negligible absorption of β-CD. Another possible mechanism by which the fraction of free Da might increase is the inclusion of other compounds (for instance (phospho-) lipids and cholesterol originating from the cellular membranes or mucus) into CD. This

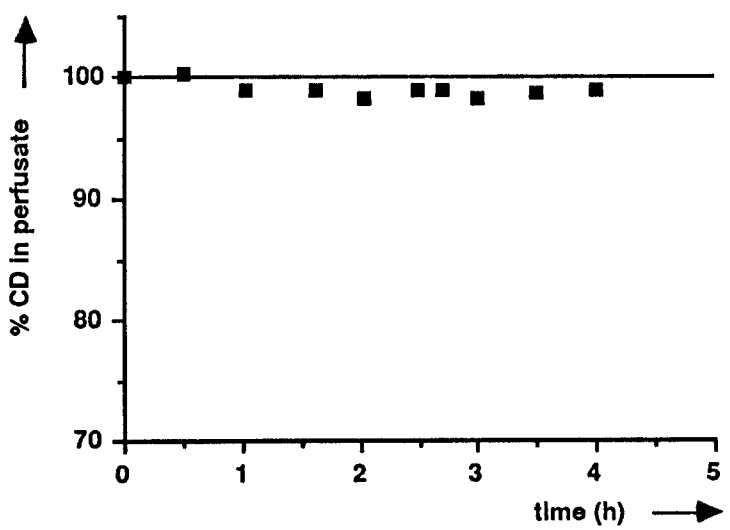

Fig. 4. A plot of the concentration of CD in the perfusate during a recirculating perfusion with 3.75×10^{-4} M β-CD. The straight line indicates the perfusate concentration at the start of the perfusion (100%).

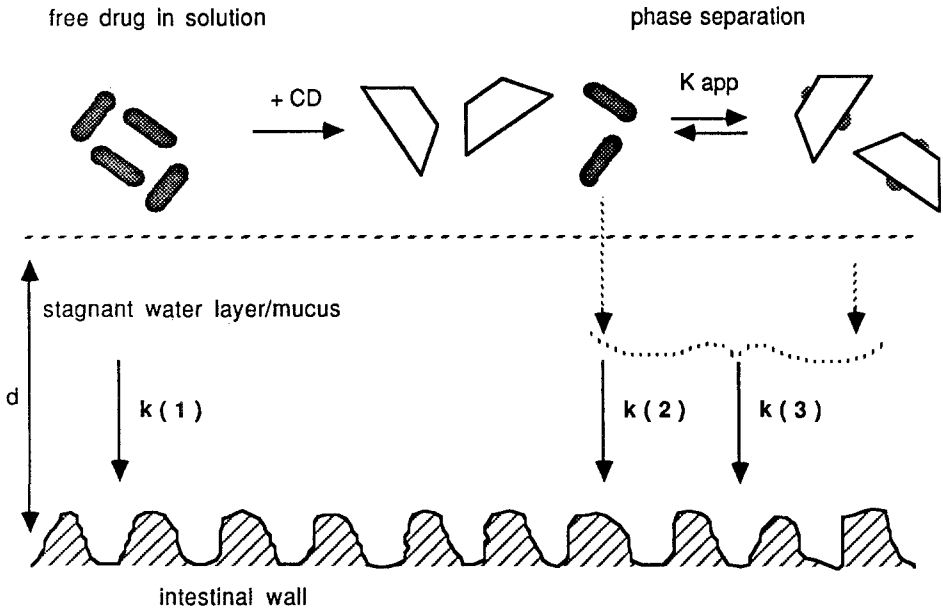

Fig. 5. In this diagram the transport of Da and its CD-complex through the stagnant water layer/mucus is illustrated schematically. Intestinal drug absorption without CD (absorption rate: k (1)) and with CD as a complex forming agent. Transport kinetics according to the phase-separation model: absorption rate; k (2) with only the free drug contributing to the transport process, or if the transport of the drug complexed with CD is also contributing to the mass transfer: absorption rate: k (3). d indicates the thickness of the absorption barrier.

concept of competing agents was studied by Tokumura and coworkers [16]. They found an increase in the bioavailability of cinnarizine administered orally as its β-CD complex upon co-administration of DL-phenylalanine as a competing agent. β-CD might influence the transport rate of Da across the absorption barrier in two ways: the fraction of Da complexed with CD might also contribute to the transport across the stagnant water layer, which is an important rate-determining diffusion barrier in the overall transport of lipophilic compounds [17] (see Figure 5). Secondly, CD might affect the surface of the intestinal wall, thus affecting its permeability [2]. However, no evidence for this hypothesis has been found until now.

From the data presented in this study it is impossible to indicate which of the above discussed mechanisms occur and the exact cause of the observed slight deviations of the expected absorption profiles remains unclear.

4. Conclusions

The solubility and thus the rate of dissolution of the poorly water soluble drug dantrolene can be improved by inclusion in cyclodextrins. This approach is often used to improve the bioavailability of poorly water soluble lipophilic drugs: the oral administration of a solid lipophilic drug-cyclodextrin-complex results in an enhanced dissolution rate and thereby an improved absorption. However, if cyclodextrins are added to an aqueous solution of a drug with which they can form

a complex, no effect of cyclodextrins on the dissolution rate can be expected, but the effect – if present – on the absorption process itself can be studied. The complexation of the drug with cyclodextrins causes a reduction in the thermodynamically active concentration of the drug, since the drug can be regarded as distributed over two phases: the aqueous phase and the drug–cyclodextrin complex. The absorption rate of dantrolene, a lipophilic drug which interacts with cyclodextrins, in a perfused intestinal segment of the rat was decreased, almost proportionally to the reduction of the concentration of free dantrolene by cyclodextrins. Apart from the reduction of free dantrolene by complex formation, no clear influence of cyclodextrins on the transport process of the drug across the pre-epithelial diffusion barrier or the intestinal wall could be seen or detected. It was shown that intestinal absorption of β-cyclodextrins occurred only in a very limited if not negligible extent.

Despite the fact that cyclodextrins do not improve the mass transfer kinetics of solubilized lipophilic drugs over the epithelial wall of the gastrointestinal tract, they have pharmaceutical potential as they promote the overall process of drug absorption by strongly enhancing the – in many cases rate limiting – dissolution rate.

Further research will deal with the competitive effect of intestinal membrane/mucus components, bile salts and fatty acids on the inclusion of dantrolene in cyclodextrins and the consequences for the absorption of dantrolene.

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