International Journal of Pharmaceutics, 64 (1990) 195–205
Elsevier

IJP 02175

The effects of cyclodextrins on drug absorption

II. In vivo observations

H.W. Frijlink, A.C. Eissens, A.J.M. Schoonen and C.F. Lerk

Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen (The Netherlands)

(Received 9 March 1990)
(Accepted 21 April 1990)

Key words: Cyclodextrin; Complex; Bioavailability; Drug absorption; Micro-enema; Diazepam; Naproxen

Summary

Complex formation of diazepam and of naproxen with β-cyclodextrin results in increased aqueous solubility of the drug. The complex stability constants found were 179 and 2146 M^{-1}, respectively. To study the effect of complex formation of drugs with β-cyclodextrin in vivo, micro-enemas containing diazepam or naproxen with and without β-cyclodextrin were administered to human volunteers. Plasma levels of the drug were determined for the investigation of the absorption of the two drugs. The results demonstrated that absorption neither of cyclodextrin nor of the drug-cyclodextrin complex took place, and that a decrease in the rate of absorption of the drug did not occur when administered as the drug-cyclodextrin complex. The absence of absorption deceleration was explained by the fact that complexed drug is displaced from the complex by lipids in the mucus adjacent to the absorption membrane. The ability of mucus and bile samples to displace complexed drug was shown in vitro in displacement studies with phenolphthalein. Especially bile was shown to possess a high capacity for displacement. The micro-enemas in which all drug was dissolved through complexation showed much faster absorption as compared with those in which the drug was suspended.

Introduction

Cyclodextrins are cyclic oligosaccharides which are known to form inclusion complexes with many organic substances, including several drugs (Lach and Pauli, 1966). This complexation largely depends on both geometrical and electrochemical properties of the guest module (Bergeron, 1984; Saenger, 1984). Complexation often changes the physico-chemical characteristics of the guest molecule and can be of importance in the pharmaceutical field.

In the past decades the use of cyclodextrins as excipient in different drug dosage forms has received much attention. Upon complexation of the drug, advantages such as improved bioavailability, reduction in unwanted side effects or improved stability of the drug have often been claimed (Jones et al., 1984; Duchêne et al., 1987).

However, the improvement of bioavailability or increase in absorption rate has only been proved for solid dosage forms. The explanation given for the observed phenomena is usually limited to an increase in both the solubility and dissolution rate of the complexes as compared with the pure drugs.

Correspondence: H.W. Frijlink, Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Ant. Deusinglaan 2, 9713 AW Groningen, The Netherlands.

However, absorption of a drug from the gastrointestinal tract involves dissolution of the drug and subsequent membrane passage of the dissolved drug. Although solubility and dissolution certainly do play an important role in the drug absorption process, a good description should also be given of what happens with the drug or drug complexes after dissolution in the gastrointestinal fluid. This, however, is still lacking. The only available knowledge comes from a computer simulation study performed by Habon et al. (1984), and from two investigations in which an isolated internal loop in the rat was used (Jansen et al., 1988; Nakanishi et al., 1989). The importance of the process of membrane passage is further emphasized on realizing that the cyclodextrins as such are scarcely absorbed from the gastrointestinal tract (Gerloczy et al., 1985), and that dissociation of the complex must therefore occur before drug absorption.

In a previous paper (Frijlink et al., 1989), the effects of cyclodextrins on the in vitro phase transfer of dissolved lipophilic drugs from an aqueous to an organic phase were described. It was found that, in spite of the fact that both cyclodextrins and the complexes were unable to enter the organic layer, complexation barely decreased the rate of transport of the lipophilic drugs to the organic layer. This observation could be explained by displacement of the drug from the complex by organic solvent near the interface.

In this paper, the results of an in vivo study are described. Using human volunteers, micro-enemas containing diazepam or naproxen, with and without cyclodextrins, were administered. The drug plasma levels were measured and the analogy between drug absorption and the in vitro phase transfer is discussed.

Materials and Methods

Materials

β-Cyclodextrin was kindly supplied by AVEBE (Veendam, The Netherlands). Diazepam was obtained from Centrachemie (Etten-Leur, The Netherlands) and naproxen from Sigma (St. Louis, U.S.A.). The water used throughout the study was deionized and distilled. All other chemicals used were of analytical grade.

Instruments

The UV/Vis absorption measurements were performed on a Philips PU 8720 UV/VIS scanning spectrophotometer.

The HPLC system consisted of a Perkin Elmer Series 10 Liquid Chromatograph. A Promis autosampler was used for injection of the samples: sample loop, 100 μl; injection volume, 50 μl; flush volume, 50 μl. The detector was a Waters Associates Model 441 absorbance detector. For the β-cyclodextrin analysis a Waters 510 pump was used as pump for the post-column reagent.

Determination of the complex stability constants

The stability constants (K_c) between the drugs and β-cyclodextrin were determined using the phase solubility method of Higuchi and Connors (1965). An excess amount of drug was added to water containing increasing concentrations of β-cyclodextrin and shaken at 37 °C until equilibrium was reached. After removal of the solid particles by filtration (0.2 μm), the concentration of dissolved drug was determined spectrophotometrically (diazepam, 230 nm; naproxen, 272 nm). Complex stability constants were calculated from the linear portion of the phase solubility diagrams, using the equation:

$$K_c = \frac{\text{slope}}{S_0(1 - \text{slope})}$$

where S_0 is the solubility of the drug in pure water.

The initial free fractions of the drug in the micro-enemas were calculated using the stability constants as described previously (Frijlink et al., 1989).

Spectral displacement studies

The ability of different rectal mucus and bile samples to displace drugs from the complexes was studied by measuring the displacement of phenolphthalein from the β-cyclodextrin complex.

Rectal mucus samples were obtained by administering enemas of 50 ml water to human volun-

teers. The enema was then excreted after 4 min and stored frozen until analysis. The bile samples came from patients who had undergone gallbladder surgery; the samples were also frozen until analysis.

To measure the displacement, an aqueous solution of 5.33×10^{-5} M phenolphthalein buffered with 6.67×10^{-3} M sodium carbonate at pH 10.8 was prepared, and a similar solution which furthermore contained 2.67×10^{-5} M β-cyclodextrin. Next 3.0 ml of both solutions were mixed with 1.0 ml of the mucus or bile sample (where necessary, appropriately diluted with water). Water was used as a reference sample. The absorbance at 550 nm of the different mixtures was then measured, and the difference in absorption between the samples with and without β-cyclodextrin was determined.

Intrinsic dissolution rate measurements

Intrinsic dissolution rates of the pure drugs were measured using the rotating disc method described by Lagas (1980). Non-disintegrating tablets were prepared by compressing 400 mg powder with a force of 30 kN for 5 min. The dissolution medium was degassed before use and stored at 37 °C. Samples were taken manually and filtered through a 0.2 μm PTFE filter. The drug concentration was determined spectrophotometrically.

Bioanalysis

Diazepam. To 1.0 ml of plasma were added 3.0 ml dichloromethane and 1.0 ml internal standard solution (nitrazepam 25 ng/ml in a saturated borax solution). The mixture was shaken for 60 s and then centrifuged at 3000 rpm. The water layer was removed. The tubes were placed in liquid nitrogen for 15 s and the dichloromethane layer was transferred to a centrifuge tube. The tubes were placed in water at 40 °C and the dichloromethane was evaporated under a gentle stream of nitrogen. To the residue 120 μl mobile phase was added and this was used as a sample for HPLC analysis. A calibration line was prepared by spiking plasma with known quantities of diazepam and analyzing these samples as described above. The peak-height ratio of diazepam : nitrazepam was plotted against the concentration of diazepam added. The concentration of diazepam in the test samples was calculated using the regression parameters obtained from the calibration line.

The analytical column was packed with Partisil 5 μm (150 mm × 3.0 mm i.d.). A guard column packed with Vydac (100 mm × 2.1 mm i.d.) was used before the analytical column. The mobile phase used was dichloromethane : tetrahydrofuran (94 : 6). The column was maintained at room temperature and the flow rate of the mobile phase was 1.0 ml/min. Column effluents were monitored at 254 nm.

Naproxen. To 1.0 ml of plasma, 2.0 ml of acetonitrile with 2% acetic acid was added. The mixture was shaken for 60 s and then centrifuged at 3000 rpm. The clear supernatant was used for HPLC analysis. A calibration line was prepared by spiking plasma with known quantities of naproxen and analyzing these samples as described above. The peak height of naproxen was plotted vs the concentration of naproxen added. The naproxen concentrations in the plasma samples were calculated using the regression parameters from this line.

The analytical column was a ChromSpher C18 column (Chrompack, mean particle size 5 μm, 250 mm × 4.5 mm i.d.) which was used with a Chrompack RP guard column (75 mm × 2.1 mm i.d.) at room temperature. The mobile phase used was water : acetonitrile (60 : 40) with 0.1% acetic acid, and the flow rate was 1.5 ml/min. The column effluents were monitored at 229 nm.

β-Cyclodextrin. The β-cyclodextrin concentrations in plasma were determined according to a previously described HPLC method (Frijlink et al., 1987).

In vivo study design

Eight healthy male volunteers (age 22–26, weight 67–90 kg) participated in the study with diazepam. Informed consent was obtained from each subject after they were informed about the nature of the study by an independent pharmacist. No drugs were taken 2 weeks prior to and during the experiment. The experiments were started at approx. 08:00 a.m., after an overnight fast. During the experiment the volunteers remained in a sit-

TABLE 1

The formulation of the micro-enemas and the fractions of drug dissolved and complexed

Drug	Enema no.	β-Cyclodextrin (mg)	Water (ml)	Fraction dissolved [a]	Fraction complexed
Diazepam					
0.5 mg	1	–	10	1.00	–
	2	23	10	1.00	0.24
	3	230	10	1.00	0.76
2.0 mg	4	–	10	0.28	–
	5	230	10	1.00	0.75
Naproxen					
8.0 mg	6	–	10	0.04	–
	7	200	10	1.00	0.96

[a] Total amount of dissolved drug, both free and complexed.

ting position. Each subject participated five times in the study. They received a diazepam-containing micro-enema (Table 1) at intervals of 7 days in a cross-over design. The enemas were administered after the first blood sample of 7 ml was taken. The other blood samples were taken at 5, 10, 20, 30, 60, 90, 120, 180 and 240 min after administration. Blood clotting was prevented by adding 15 mg disodium edetate to the samples. Plasma was obtained by centrifugation of the blood at 3000 rpm and was stored frozen until analysis.

Four healthy male volunteers (age 25–28, weight 72–79 kg) participated in the pilot study with naproxen. No drugs were taken 2 weeks prior to and during the experiment. The experiments were started at approx. 08:00 a.m., after an overnight fast. Each subject participated two times in the study. They received a naproxen-containing micro-enema (Table 1) at an interval of 7 days in a cross-over design. The further protocol was identical to that of the diazepam experiments.

Pharmacokinetic and statistical analysis

Both the plasma concentration vs time curves as well as the absorption profiles, calculated from the plasma data using numerical deconvolution (both drugs exhibit linear pharmacokinetics in the plasma concentration ranges found in these studies) (Proost, 1987), were used to study the absorption behaviour. For the diazepam study pharmacokinetic data following intravenous administration of 10 mg diazepam, as obtained by Moolenaar et al. (1980), were used as a reference.

For the naproxen study, plasma data following intravenous administration of 275 mg diazepam, as obtained by Accardo et al. (1982), were used as a reference.

To compare the different micro-enemas the fractions absorbed at the different times were tested with paired Student's *t*-test. Differences were considered to be significant if $P < 0.05$.

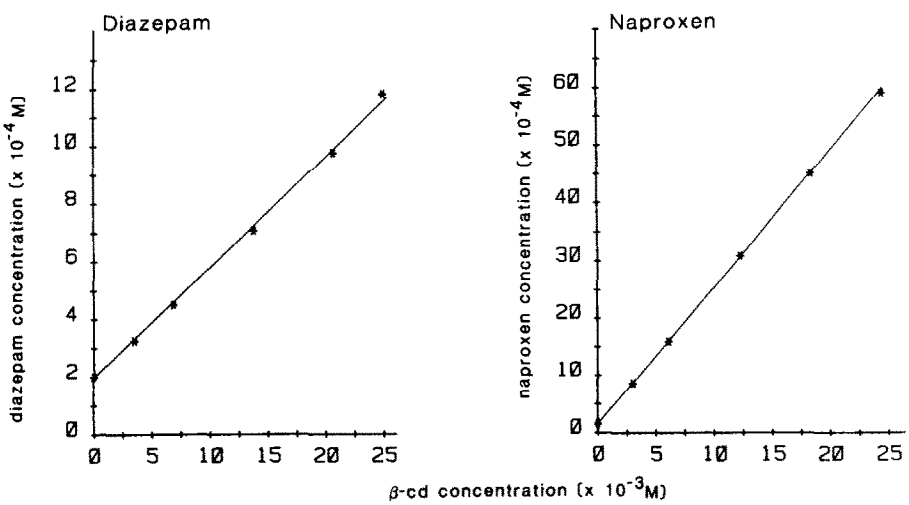

Fig. 1. Phase solubility diagrams of diazepam (left) and naproxen (right) with β-cyclodextrin.

Results and Discussion

Complex stability constants

The phase solubility diagrams of diazepam and of naproxen with β-cyclodextrin are presented in Fig. 1. In both cases, a so-called A_1-type diagram was obtained. The complex stability constants found for β-cyclodextrin with diazepam and naproxen were 179 and 2146 M^{-1}, respectively. The solubility of both drugs is increased by the complexation. The much greater complex stability constant of the naproxen-β-cyclodextrin complex, as compared with the diazepam-β-cyclodextrin complex, is reflected in the much larger increase of this drug's solubility.

Bioavailability studies

It is generally assumed that the dissolved drug-cyclodextrin complex must dissociate before gastrointestinal drug absorption can occur (Uekama and Otagiri, 1986). The reason for this is that both the complex and the pure cyclodextrin cannot be absorbed from the gastrointestinal tract. Only free drug can pass the membrane. Considering the above it might be expected that complexation of dissolved drug with cyclodextrins decreases the absorption rate. To study the effect of cyclodextrins on the absorption of dissolved drugs, the rectal route was the route of choice. This route was considered to be more appropriate than the oral route, since complicating factors, like a large dilution in the acid environment of the stomach and the absorption promoting effect of bile, are circumvented. The rectal route has the advantage that the solutions are directly exposed to the absorption membrane and the observed effects were considered to be a direct result of differences in drug absorption.

Moolenaar et al. (1980) have described the rectal absorption of diazepam. These investigators found that after rectal administration of dissolved diazepam the absorption was very rapid. Therefore, this drug was considered an appropriate model substance in order to investigate whether or not cyclodextrins decrease the absorption of dissolved drugs by forming inclusion complexes.

The formulation of the five different diazepam containing micro-enemas is given in Table 1. In Table 1, the amount of drug that is dissolved and the amount of dissolved drug that is complexed by β-cyclodextrin in the micro-enemas is also given. In the first three formulations 0.5 mg of diazepam was used. This amount was chosen, since it is the maximum that can be dissolved in 10 ml water. It was considered that pure drug completely dissolved in water was the best reference in this study. The use of cosolvents in order to obtain higher solubilities was thought inappropriate because they might influence the complexation of the drug and also membrane permeability in the rectum.

During all experiments none of the volunteers reported any irritation caused by the micro-enemas, not even at the highest β-cyclodextrin

TABLE 2

Diazepam plasma concentrations after administration of the different micro-enemas to healthy volunteers (mean ± S.D.; n = 8)

Time (min)	Diazepam plasma concentration (μg/l)				
	Enema 1	Enema 2	Enema 3	Enema 4	Enema 5
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5	5.3 ± 3.8	4.9 ± 3.2	4.1 ± 2.5	4.2 ± 1.8	14.2 ± 7.5
10	14.3 ± 6.5	15.9 ± 3.7	10.8 ± 4.0	17.9 ± 3.9	46.1 ± 13.5
20	17.9 ± 4.4	17.9 ± 3.8	16.8 ± 2.7	22.2 ± 3.1	61.6 ± 8.9
30	16.6 ± 4.7	17.0 ± 3.9	18.5 ± 2.9	23.1 ± 2.9	63.3 ± 9.4
60	15.2 ± 3.7	14.3 ± 3.9	17.5 ± 2.7	21.6 ± 3.7	54.7 ± 11.5
90	13.4 ± 3.5	11.7 ± 2.8	14.2 ± 3.2	17.1 ± 3.2	46.4 ± 12.0
120	12.3 ± 3.3	10.2 ± 1.5	12.5 ± 2.7	15.2 ± 3.9	42.3 ± 11.7
180	10.9 ± 2.5	10.0 ± 1.4	10.8 ± 2.2	13.5 ± 3.8	37.0 ± 8.4
240	10.5 ± 3.2	8.3 ± 0.8	10.6 ± 1.4	12.8 ± 2.5	33.4 ± 7.4

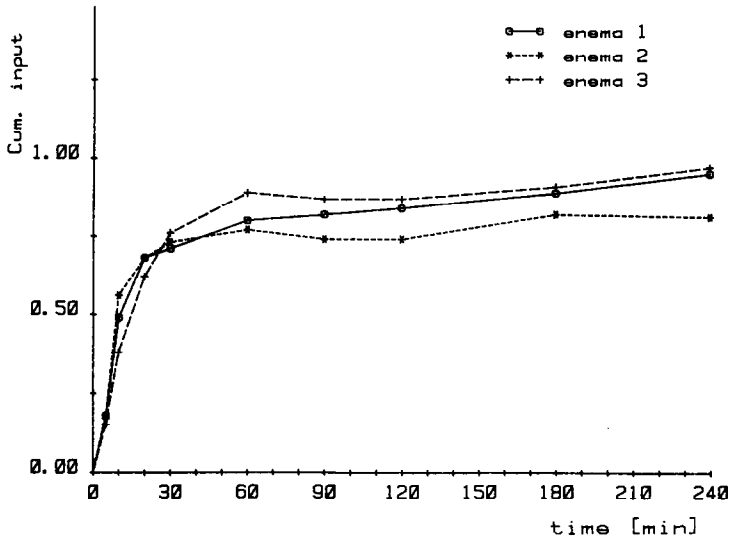

Fig. 2. Mean absorption profiles of diazepam calculated using numerical deconvolution. The cumulative input (fraction of the dose absorbed) is plotted vs time.

amounts used. β-Cyclodextrin might thus be considered as a non-irritating solvent enhancer. This in sharp contrast to many other cosolvents, like macrogols or glycofurol, for which irritancy of the rectal mucus membrane is reported (Moolenaar and Huizinga, 1981; Heers, 1986). The plasma levels of diazepam found in the volunteers are listed in Table 2. In order to gain a deeper understanding of the absorption behaviour, absorption profiles were calculated from the plasma data with numerical deconvolution. The results of these calculations are presented in Figs 2 and 3. The

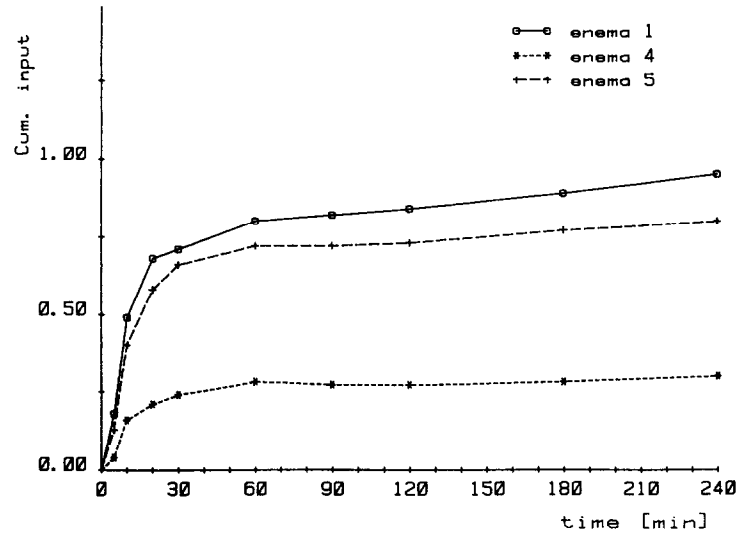

Fig. 3. Mean absorption profiles of diazepam calculated using numerical deconvolution. The cumulative input (fraction of the dose absorbed) is plotted vs time.

micro-enema containing 0.5 mg diazepam shows a rapid and complete absorption profile, which is not surprising as it might be expected that a lipophilic drug dissolved in pure water shows a strong tendency to pass over the rectum membrane to the circulation. The plasma levels determined are in good agreement with those found by Moolenaar et al. (1980) for a 10 mg-containing micro-enema. When the absorption profiles of the β-cyclodextrin-containing formulations with 0.5 mg diazepam are compared it is clear that, in spite of the fact that up to 75% of the diazepam is complexed by β-cyclodextrin, absorption is not decelerated. Even when an amount of 2 mg of diazepam is dissolved with the help of the cyclodextrins (formulation 5) no significant deceleration of drug absorption is observed ($P < 0.05$). In the 2 mg formulation without cyclodextrin only 28% of the total diazepam is dissolved and this amount is consequently rapidly absorbed. The remaining part is suspended in the water and is very slowly absorbed, as a result of the low dissolution rate of diazepam (intrinsic dissolution rate: 7.1 ± 0.4 $\mu g\ cm^{-2}\ min^{-1}$). The dissolution becomes rate limiting and the absorbed fraction is only 30% in 4 h.

In the plasma of the volunteers receiving micro-enema 3 no cyclodextrin plasma concentrations could be detected (detection limit 1.0 μg/ml). Based on this finding it was concluded that the diazepam is not absorbed in its complexed form and that complex dissociation had to occur before absorption. This implies that the diazepam which is complexed in the rectum lumen cannot be considered as a driving force for the membrane passage. The fact that cyclodextrin complexation did not decrease absorption rate needs further explanation.

The process of drug release and absorption of complexed drug in the lumen is influenced by the following factors:

– The rate of complex dissociation. The complex equilibrium is established within seconds (Cramer and Hettler, 1967; Habon et al., 1984) and dissociation is likely to be much faster than absorption. On the other hand, dissociation only replaces the absorbed free drug and thereby maintains the mass flux over the membrane at a relatively high level. Consequently, this process of complex dissociation never brings the mass flux to the level that would occur if all the drug were free.

– Dilution of the enema cannot be of importance, since the volume of the rectal fluid is only about 3 ml. This in sharp contrast to the oral route in which the dilution in the gastro-intestinal fluids significantly decreases the complexed drug fraction.

These two processes alone cannot explain the complete absence of absorption deceleration because a considerable drug fraction still remains complexed. Consequently, there must be a third process. This process was believed to be the displacement of drug from the complex, by lipids in the rectal mucus. Lipids are known to occur in the mucus layer (Clamp and Creeth, 1984); they might originate from endogenous as well as exogenous sources, like cell membranes, bile or food. Furthermore, lipids like cholesterol, phospholipids and bile salts are known to form β-cyclodextrin complexes with high stability constants (Miyajima et al., 1986). They might consequently be considered as strong competitors with the drugs for complex formation with cyclodextrins. The displacement of drug from the complex increases the free drug level (and consequently the driving force for absorption) in the mucus near the membrane. In fact, this is a process quite similar to the displacement of complexed drug by organic solvent near the interface occurring upon transfer of this drug from an aqueous to an organic solvent layer (Frijlink et al., 1989).

To test this hypothesis displacement studies were performed. As reported previously by Vikmon (1982), β-cyclodextrin decreases the visible absorption at 550 nm of phenolphthalein by forming inclusion complexes, with a high complex stability constant of 19200 M^{-1} (Frijlink et al., 1989). If other substances that form inclusion complexes are added to the solution they will compete with the phenolphthalein for complex formation. As a result of this competition a part of the phenolphthalein will be displaced from the complex leading to a smaller decrease in absorption. The decrease in absorption caused by bile and mucus samples thus provides a tentative measure of the ability of these samples to displace

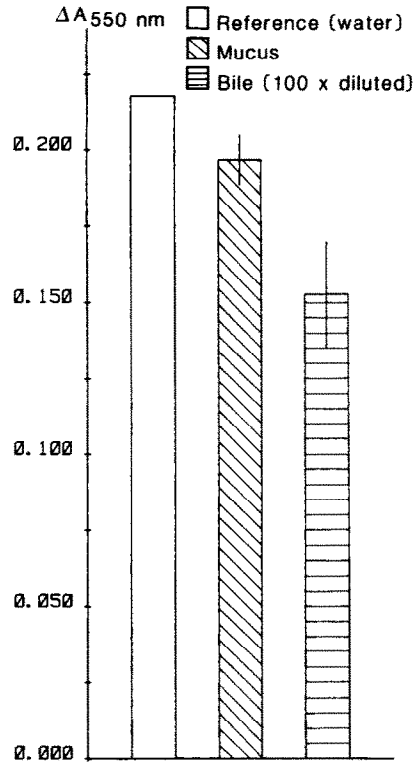

Fig. 4. Decrease in phenolphthalein absorption (550 nm) caused by β-cyclodextrin complexation, after addition of mucus and bile samples (mean ± S.D.).

drugs from the complex. The results of the displacement studies are presented in Fig. 4. Both mucus and bile were shown to displace phenolphthalein from the β-cyclodextrin complex. The mucus samples displaced about 10% of the complexed phenolphthalein. This does not appear to be very much but the following factors should be considered when evaluating this result. The volume of the enema (50 ml) already dilutes the mucus. Furthermore, the gel-like structure of mucus adheres strongly to the rectal wall, resulting in only small amounts of mucus being excreted with the enema. Finally, it should be noted that the stability constant of phenolphthalein is very high. The 10% displacement of phenolphthalein thus still indicates a considerable displacement ability, certainly towards most drugs, since their stability constants are usually 10 to 100-times lower than that of phenolphthalein.

A 100-times diluted bile sample displaced 30% of the complexed phenolphthalein, indicating an enormous displacement ability of bile. This may be explained by the fact that cyclodextrins are known to form stable complexes with steroid-like molecules, Miyajima et al. (1986), for example, found high complex stability constants for bile salts. Next to direct competition the surface tension decreasing effect of the bile may also decrease the complexed fraction. This result is in good agreement with the increase in absorption of complexed sulfamethizole from the rat intestinal closed-loop after addition of sodium cholate to the perfusate, recently reported by Nakanishi et al. (1989).

The displacement of complexed drug by lipids in the mucus explains why there is no deceleration of the diazepam absorption. In the mucus practically all diazepam is displaced from the complex leading to a free fraction equal to the total amount of diazepam, and consequently to a high driving force for the absorption. When large amounts of complexed drug are administered the displacement may result in free drug concentrations above saturation. This temporary supersaturation then causes a large mass flux of drug over the membrane. For example, this must be the case for enema 5 of which the absorption rate is as fast as for enema 1. Indicating a much larger absorption flux.

Rectal solutions of diazepam are effective in the acute treatment of convulsive attacks. However, doses larger than the 2 mg that can be dissolved by β-cyclodextrin are often needed. This problem might be resolved by the use of modified cyclodextrins. With these substances solutions containing high diazepam concentrations can be readily prepared (Müller and Brauns, 1985).

To determine whether the above-described mechanism was also valid for complexes with higher complex stability constants, a pilot study with naproxen micro-enemas in four volunteers was carried out. Naproxen is also known to have a rapid rectal absorption (Brogden et al., 1979; Gamst et al., 1984), but its complex stability constant is much larger than that of diazepam (2150 vs 179 M^{-1}).

The formulation of the different naproxen con-

TABLE 3

Naproxen plasma concentrations after administration of the different micro-enemas to healthy volunteers (mean ± S.D.; n = 4)

Time (min)	Naproxen plasma concentration (μg/l)	
	Enema 6	Enema 7
0	0.0± 0.0	0.0± 0.0
5	190 ±119	438 ±101
10	344 ±205	674 ±133
20	381 ±204	968 ±200
30	385 ±187	1123 ±222
60	364 ±150	1362 ±248
90	378 ±173	1480 ±297
120	326 ±114	1320 ±269
180	290 ± 91	1266 ±251
240	265 ± 83	1156 ±221

taining micro-enemas is given in Table 1. In Table 3 the plasma levels are presented and Fig. 5 displays absorption profiles calculated using numerical deconvolution. When no cyclodextrin is used less than 5% is dissolved. The absorbed fraction is much larger than this value of 5%, being about 25%. This difference is explained by the fast dissolution rate of naproxen in the rectal fluids (intrinsic dissolution rate 551 ± 10 $\mu g\ cm^{-2}\ min^{-1}$).

In the β-cyclodextrin-containing enema all naproxen is dissolved of which over 96% is complexed. It is clear that absorption from this micro-enema is rapid and complete. The absorption rate is hardly decreased by the complexation. This result indicates that a considerable displacement ability must exist in the rectum, because the complex stability constant is high and the amount of drug to be displaced is large. The absence of a decrease in absorption rate caused by cyclodextrin complexation is in good agreement with several papers which have also described an improved bioavailability of complexed drugs from different dosage forms (e.g., Iwaoku et al., 1980; Uekama et al., 1983; Vila-Jato et al., 1986). On the other hand, it is not in complete correspondence with the finding of Tokumura et al., (1985), that the addition of DL-phenylalanine is necessary to achieve complete absorption of cinnarizine from its β-cyclodextrin complex. Further research considering the preparation and dissolution of this complex is probably required to explain this discrepancy.

Conclusions

The complexation of slightly soluble drugs by cyclodextrins often increases the solubility of the drug. This increased solubility is directly reflected

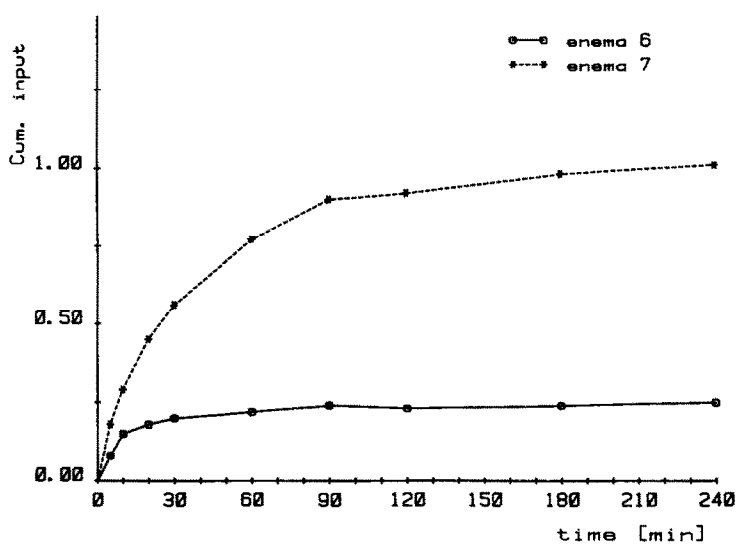

Fig. 5. Mean absorption profiles of naproxen calculated using numerical deconvolution. The cumulative input (fraction of the dose absorbed) is plotted vs time.

in the increased absorption rate of drug from micro-enemas, because the dissolution is no longer rate limiting.

The cyclodextrins or the cyclodextrin-drug complexes were not absorbed from the rectum. In spite of this, complexation did not significantly decrease the absorption rate. This phenomenon can be attributed to three factors. Probably most important of all, the displacement of the complexed drug from the complex by lipids in the rectum, the rapid replenishment of the absorbed free drug from the complex and the negligible dilution of the enema by the rectum fluid. The ability of mucus and bile samples to displace drug was demonstrated in vitro. The in vivo results demonstrate that even for drug-cyclodextrin complexes with high stability constants significant displacement occurs.

Cyclodextrins may consequently be considered as dissolution enhancers which can improve the absorption rate of slightly soluble drugs.

References

Accardo, S., Samanta, E., Cutolo, M. and Suffritti, G., Intravenous sodium naproxen: bioavailability on man and relief from pain in rheumatic diseases. *Curr. Ther. Res.*, 32 (1982) 952–962.

Bergeron, R.J., Cycloamylose-substrate binding. In Atwood, J.L., Davies, J.E.D. and MacNicol, D.D. (Eds), *Inclusion Compounds*, Academic Press, London, 1984, vol. 3, pp. 391–443.

Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S., Naproxen up to date. *Drugs*, 18 (1979) 241–277.

Clamp, R.J. and Creeth, J.M., Some non-mucin components of mucus and their possible biological roles. In *Mucus and Mucosa, Ciba Foundation Symposium 109*, Pitman, London, 1984, pp. 121–131.

Cramer, F. and Hettler, H., Inclusion compounds of cyclodextrins. *Naturwissenschaften*, 54 (1967) 624–632.

Duchêne, D., Glomot, F. and Vaution, C., Pharmaceutical applications of cyclodextrins. In Duchêne, D. (Ed.), *Cyclodextrins and Their Industrial Uses*, Editions de Santé, Paris, 1987, pp. 211–258.

Frijlink, H.W., Visser, J. and Drenth, B.F.H., Determination of cyclodextrins in biological fluids by high-performance liquid chromatography with negative colorimetric detection using post-column complexations with phenolphthalein. *J. Chromatogr.*, 415 (1987) 325–333.

Frijlink, J.W., Schoonen, A.J.M. and Lerk, C.F., The effects of cyclodextrins on drug absorption. I: In vitro observations. *Int. J. Pharm.*, 49 (1989) 91–102.

Gamst, O.N., Vesje, A.K. and Aarbakke, J., Bioavailability of naproxen sodium suppositories. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 22 (1984) 99–103.

Gerloczy, A., Fonagy, A., Keresztes, P., Perlaky, L. and Szejtli, J., Absorption, distribution, excretion and metabolism of orally administered ^{14}C-β-cyclodextrin in rat. *Drug Res.*, 35 (1985) 1042–1047.

Habon, I., Fritsch, S. and Szejtli, J., Simulation of pharmacokinetic behaviour of drug-cyclodextrin complexes. *Pharmazie*, 39 (1984) 830–834.

Heers, W., Beschreibung der einzelnen Suppositorienmassen. In Müller, B.W. (Ed.) *Suppositorien*, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1986, pp. 91–104.

Higuchi, T. and Connors, K.A., Phase-solubility techniques. *Adv. Anal. Chem. Instrum.*, 4 (1965) 117–212.

Iwaoku, R., Arimori, K., Nakano, M. and Uekama, K., Enhanced absorption of phenobarbital from suppositories containing phenobarbital-β-cyclodextrin inclusion complex. *Chem. Pharm. Bull.*, 30 (1980) 1416–1421.

Jansen, A.C.A., Hilbers, W., Poelma, F.G.J. and Tukker, J.J., The influence of inclusion by cyclodextrins on absorption kinetics of dantrolene in the rat. In Huber, O. and Szejtli, J. (Eds), *Proceedings of the Fourth International Symposium on Cyclodextrins*, Kluwer, Dordrecht, 1988, pp. 355–358.

Jones, S.P., Grant, D.J.W., Hadgraft, J. and Parr, G., Cyclodextrins in the pharmaceutical sciences, II. *Acta Pharm. Technol.*, 30 (1984) 263–277.

Lach, J.L. and Pauli, W.A., Interactions of pharmaceuticals with Schardinger dextrins. VI. *J. Pharm. Sci.*, 55 (1966) 32–38.

Lagas, M., Wettability and availability of drugs, Ph.D. Thesis, University of Groningen, Groningen, 1980.

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.

Moolenaar, F., Bakker, S., Visser, J. and Huizinga, T., Biopharmaceutics of rectal administration of drugs in man. IX. Comparative biopharmaceutics of diazepam after single rectal, oral, intramuscular and intravenous administration in man. *Int. J. Pharm.*, 5 (1980) 127–137.

Moolenaar, F. and Huizinga, T., Rectale irritatie van vehiculae geschikt voor diazepam micro-klysma's. *Pharm. Weekbl.*, 116 (1981) 33–34.

Müller, B.W. and Brauns, U., Solubilization of drugs by modified β-cyclodextrins. *Int. J. Pharm.*, 26 (1985) 77–88.

Nakanishi, K., Masada, M., Nadai, T. and Miyajima, K., Effect of the interaction of drug-β-cyclodextrin complex with bile salts on the drug absorption from rat small intestinal lumen. *Chem. Pharm. Bull.*, 37 (1989) 211–214.

Proost, J.H., Critical evaluation of the determination of bioavailability by numerical deconvolution, Ph.D. Thesis, University of Groningen, Groningen, 1987.

Saenger, W., Structural aspects of cyclodextrins and their inclusion complexes. In Atwood, J.L., Davies, J.E.D. and MacNicol, D.D. (Eds), *Inclusion Compounds*, Academic Press, London, 1984, vol. 2, pp. 231–259.

Tokumura, T., Tsushima, Y., Kayano, M., Machida, Y. and Nagai, T., Enhancement of bioavailability of cinnarizine

from its β-cyclodextrin complex on oral administration with DL-phenylalanine as a competing agent. *J. Pharm. Sci.*, 74 (1985) 496–497.

Uekama, K. and Otagiri, M., Cyclodextrins in drug carrier systems. *CRC Crit. Rev. Ther. Drug. Carrier Syst.*, 3 (1986) 1–40.

Uekama, K., Fujinaga, T., Hirayama, F., Otagiri, M., Yamasaki, M., Seo, H., Hashimoto, T. and Tsuruoka, M., Improvement of the oral bioavailability of digitalis glycosides by cyclodextrin complexation. *J. Pharm. Sci.*, 72 (1983) 1338–1341.

Vikmon, M., Rapid and simple spectrophotometric method for determination of micro-amounts of cyclodextrins. In Szejtli J. (Ed.), *Proc. First Int. Symp. on Cyclodextrins*, Budapest, Sept. 30-Oct. 2, 1981, D. Reidel, Dordrecht, 1982, pp. 69–74.

Vila-Jato, J.L., Blanco, J. and Vilar, A., Spironolactone/β-cyclodextrin complex: oral bioavailability in humans. *Acta Pharm. Technol.*, 32 (1986) 82–85.