The Effects of Cyclodextrins on the Disposition of Intravenously Injected Drugs in the Rat

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Naproxen and flurbiprofen form complexes with hydroxypropyl-β-cyclodextrin; with stability constants of 2207 and 12515 M^{-1}, respectively. However, only small fractions of the drug remain complexed when the drug–cyclodextrin complex is added to plasma *in vitro*. This result can be explained by albumin effectively competing with cyclodextrin for drug binding and by the simultaneous displacement of the drug from cyclodextrins by plasma cholesterol. Naproxen and flurbiprofen were administered intravenously to rats as cyclodextrin complexes. The disposition in the body of naproxen was not significantly altered by the complexation. This indicates that immediately after administration all drug is removed from the cyclodextrin complex. However, the initial distribution of flurbiprofen was changed upon complexation. Drug concentrations in liver, brain, kidney, and spleen were increased, indicating that hydroxypropyl-β-cyclodextrin may improve the presentation of the flurbiprofen to biomembranes, as compared with plasma proteins. The effect was transient; 60 min after injection the differences in tissue concentration compared with controls were dissipated. Finally, the importance of protein binding in determining the mode of interaction of cyclodextrins on drug disposition is discussed.

KEY WORDS: hydroxypropyl-β-cyclodextrin; naproxen; flurbiprofen; intravenous administration; tissue concentration; protein binding.

INTRODUCTION

Cyclodextrins change the physicopharmaceutical properties of lipophilic drugs through inclusion complex formation. In parenteral dosage forms the inclusion of the drug may have several advantages, such as an increased aqueous solubility and stability (1–7) or a reduction of unwanted side effects (8–12).

However, cyclodextrins should not be regarded as simple excipients or solubility enhancers, since the formation of inclusion complexes might also change the pharmacokinetic behavior of the included drug and thereby its therapeutic effects. Recent reports provide conflicting results; complexation of hexobarbital with β-cyclodextrin decreased the drug concentrations in brain and liver but increased the blood and kidney concentrations after intravenous administration to mice. In rats these effects were less pronounced than in mice (13,14). On the other hand, the plasma kinetics in rabbits of intravenously administered prednisolon were not affected at all by complexation with β- or γ-cyclodextrin (15). Further, the concentration of the dihydropyridine salt of estradiol in rat brain was the same after intravenous administration of either the hydroxypropyl-β-cyclodextrin complex or a solution in dimethyl sulfoxide (1).

In previous papers we described the pharmacokinetics of intravenously administered cyclodextrins (16) and their effect on the disposition of cholesterol (17). This paper describes the effects of complexation with hydroxypropyl-β-cyclodextrin on the pharmacokinetics of two model drugs, naproxen and flurbiprofen, after intravenous administration to rats. The two model drugs were used because of their high plasma protein binding, in contrast to the barbiturates used by Nagai *et al.* (13,14). The general aim of the study is to obtain more detailed information on the pharmacokinetics of cyclodextrin–drug complexes in biological fluids, both *in vitro* and *in vivo*.

MATERIALS AND METHODS

Materials

The 2-hydroxypropyl-β-cyclodextrin was a gift from Prof. Szejtli, Chinoin, Budapest, Hungary. The average molar degree of substitution of the cyclodextrin molecule was 2.7. Flurbiprofen was a gift from The Boots Company, Nottingham, U.K. Naproxen was obtained from Sigma, St. Louis, MO. The physiological saline used to prepare the injections was sterile and pyrogen free. The water used 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 Waters Model 510 pump, a Promis autosampler, and a Waters Associates Model 484 tunable absorbance detector.

Complex Stability Constants

The complexation of naproxen and flurbiprofen with hydroxypropyl-β-cyclodextrin was studied by the phase-solubility method (18). To an aqueous suspension of drug, increasing amounts of cyclodextrin were added. The suspensions were then shaken until equilibrium was reached (7 days). After filtration (0.2 μm) the concentration of dissolved drug was determined spectrophotometrically, naproxen at 272 nm and flurbiprofen at 248 nm. The amount of dissolved drug was plotted against the cyclodextrin concentration. The complex stability constants (K_c) were calculated from these lines, using the equation

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

in which S_0 is the solubility of the drug without cyclodextrin.

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Plasma Protein Binding

Rat plasma was spiked with appropriate amounts of flurbiprofen, naproxen, and/or hydroxypropyl-β-cyclodextrin (Tables I and II). Drug protein binding was determined by ultrafiltration using the Amicon MPS-1 filtration system (Amicon Corp., Danvers MA) at 37°C. The drug concentrations in the ultrafiltrate were measured with HPLC, using the method described below for plasma.

Naproxen Bioanalysis

To an exactly weighted amount of 1 g tissue, 20.0 ml of a 2-μg/ml flurbiprofen solution in water (internal standard) and 100 μl of a 4 *M* sodium hydroxide solution were added. The tissue was homogenized with an ultra turrax high-efficiency disperser. After centrifugation (1800*g*) 2.0 ml of the supernatant was acidified with 100 μl of a hydrochloric acid solution (6 *M*). Subsequently 6.0 ml dichloromethane was added. The mixture was vortexed for 60 sec and centrifuged (1800*g*). The water layer was removed. The tube was placed in liquid nitrogen for 15 sec and the dichloromethane layer was transferred to a centrifuge tube. The tubes were placed in water of 40°C and the dichloromethane was evaporated under a gentle stream of nitrogen. The residue was dissolved in 500 μl acetonitrile with 2% acetic acid and this was used as a sample for HPLC analysis.

To 100 μl of plasma, 300 μl acetonitrile containing the internal standard was added. After mixing and centrifugation (1800*g*) the clear supernatant was used as sample for HPLC analysis. When necessary samples were diluted with acetonitrile.

The analytical column used was a Chrompak spherical C_{18} column (250 × 4.6 mm) preceded by a Chrompack RP guard column. The mobile phase was acetonitrile:water:acetic acid (49.9:49.9:0.2), used at a flow rate of 1.5 ml/min. The effluent was monitored at 229 nm. Sample volumes of 100 μl were injected onto the column. Peak height ratios of naproxen:flurbiprofen were measured. A calibration graph was prepared by the addition of known quantities of naproxen to blank samples.

Flurbiprofen Bioanalysis

To an exactly weighed amount of 1 g tissue, 20.0 ml of a 2 μg/ml ibuprofen solution in water (internal standard) and 100 μl of a 4 *M* sodium hydroxide solution were added. The tissue was homogenized with an ultra turrax high-efficiency disperser. After centrifugation (1800*g*) 2.0 ml of the supernatant was acidifed with 100 μl of a hydrochloric acid solution (6 *M*). Subsequently 6.0 ml dichloromethane was added. The mixture was vortexed for 60 sec and centrifuged (1800*g*). The water layer was removed. The tube was placed in liquid nitrogen for 15 sec and the dichloromethane layer was transferred to a centrifuge tube. The tubes were placed in water of 40°C and the dichloromethane was evaporated under a gentle stream of nitrogen. The residue was dissolved in 500 μl acetonitrile with 2% acetic acid and this was used as a sample for HPLC analysis.

To 100 μl of plasma, 300 μl of acetonitrile containing the internal standard was added. After mixing and centrifugation (1800*g*) the clear supernatant was used as sample for HPLC analysis. When necessary samples were diluted with acetonitrile.

The analytical column used was a Chrompak Spherical C_{18} column (250 × 4.6 mm) preceded by a Chrompack RP guard column. The mobile phase was acetonitrile:water:acetic acid (57.9:42:0.1), used at a flow rate of 1.5 ml/min. The effluent was monitored at 232 nm. Sample volumes of 100 μl were injected onto the column. Peak height ratios of flurbiprofen:ibuprofen were measured. A calibration graph was prepared by the addition of known quantities of flurbiprofen to blank samples.

Table I. The Effect of Hydroxypropyl-β-cyclodextrin on Plasma Protein Binding of Naproxen (Mean Values, n = 3)

Naproxen conc. (μg/ml)	hp-β-cd conc. (mg/ml)	Unbound fraction of naproxen (%)
30	—	0.1
30	1.0	0.1
30	5.0	0.1
30	10.0	0.3
30	50.0	0.6
300	—	0.1
300	10.0	3.6

Table II. The Effect of Hydroxypropyl-β-cyclodextrin on Plasma Protein Binding of Flurbiprofen (Mean Values, n = 3)

Flurbiprofen conc. (μg/ml)	hp-β-cd conc. (mg/ml)	Unbound fraction of flurbiprofen (%)
50	—	0.3
50	0.1	2.0
50	0.5	3.8
50	1.0	4.1
50	10.0	9.6
50	50.0	25.7
300	—	1.3
300	10.0	39.3

Hydroxypropyl-β-Cyclodextrin Bioanalysis

The hydroxypropyl-β-cyclodextrin concentrations were determined by the HPLC method described in earlier publications (16,19).

Drug Disposition Studies

The following injections were prepared: naproxen, 1.50 mg/ml, dissolved in rat plasma; naproxen, 1.50 mg/ml, and hydroxypropyl-β-cyclodextrin, 70 mg/ml, dissolved in saline; flurbiprofen, 1.50 mg/ml, dissolved in rat plasma; and flurbiprofen, 1.50 mg/ml, and hydroxypropyl-β-cyclodextrin, 70 mg/ml, dissolved in saline.

Male Wistar rats (254–276 g) were anesthetized with pentobarbital sodium (60 mg/kg administered intraperitoneally). For each injection a volume of 1.0 ml was administered intravenously (dorsal penile vein) to 12 rats. Ten minutes after injection a blood sample was taken from the vena cava from six rats of each group. The blood sample was heparinized and plasma was obtained after centrifugation (1800*g*). Immediately following blood sampling, the rats

were perfused transcardially with 50 ml physiological saline, in order to remove blood from the vascular system. Liver, kidneys, spleen, brain, and muscle (m. adductor longus) were excised from the body and stored frozen until analysis. The remaining six rats of each group received the same treatment 60 min after injection.

The tissue and plasma concentrations found after administration with and without hydroxypropyl-β-cyclodextrin were compared using Student's t test. Differences were considered to be significant if $P < 0.05$.

RESULTS AND DISCUSSION

Complex Stability Constants

The phase solubility diagrams of both drugs with hydroxypropyl-β-cyclodextrin give typical A_l-type diagrams, indicating that the solubility of the complex is high (Fig. 1). The low aqueous solubility of the pure drugs is strongly increased by complexation with hydroxypropyl-β-cyclodextrin. Flurbiprofen exhibited a sharper increase in solubility than naproxen. This is directly reflected in the sixfold-higher complex stability constant of flurbiprofen, 12500 M^{-1}, compared with naproxen, 2200 M^{-1}.

Plasma Protein Binding

Flurbiprofen and naproxen are both nonsteroidal anti-inflammatory drugs (NSAIDs), belonging to the group of propionic acid derivatives. This group of drugs is characterized by the high degree of plasma protein binding as an essential determinant of the pharmacokinetic properties (20). The high plasma protein binding of both drugs is decreased only slightly by the complexation with hydroxypropyl-β-cyclodextrin (Tables I and II). The limited effect of the cyclodextrin complexation can be attributed to two simultaneously occurring processes. The first is the competition between the cyclodextrin molecule and the plasma proteins for binding of the drug. Albumin is the major binding site for NSAIDs, and the association constants are high, 5.32×10^5 and 1.1×10^6 M^{-1} for flurbiprofen and naproxen, respectively (20). The second process is the competitive displacement of the drug from the complex by cholesterol. In a previous article (17) it was described that cholesterol is the major endogenous lipid that is complexed by hydroxypropyl-β-cyclodextrin in plasma and that the complex stability constant is high: 19,000 M^{-1}. Further cholesterol occurs at much higher concentrations in plasma than the drugs.

The differences between naproxen and flurbiprofen can be ascribed to the larger complex stability constant of flurbiprofen with hydroxypropyl-β-cyclodextrin and its lower association constant with plasma proteins. The protein binding of flurbiprofen already changes at hydroxypropyl-β-cyclodextrin concentrations, in the range found after intravenous administration (<2 mg/ml) (16). Although the decreases in protein binding are small, they may be of significant importance, since generally only the nonbound drug fraction is thought to produce pharmacological effect at the receptor sites in extravascular tissues. Although the hydroxypropyl-β-cyclodextrin is able to pass the vascular endothelium, it is incapable of passing cell membranes (16), and it is not clear whether the cyclodextrins carry the drug to the cells in an effective way. Consequently the implications of the observed changes in plasma protein binding, caused by cyclodextrins, for the drug disposition should be investigated *in vivo*. Furthermore, the results of the protein binding studies reflect an equilibrium situation, whereas *in vivo* pseudo-equilibrium situations will occur during the distribution phase. The equilibrium between free and complexed drug is known to establish itself within seconds (21), but the occurrence of small deviations from the equilibrium situation immediately after injection cannot be excluded.

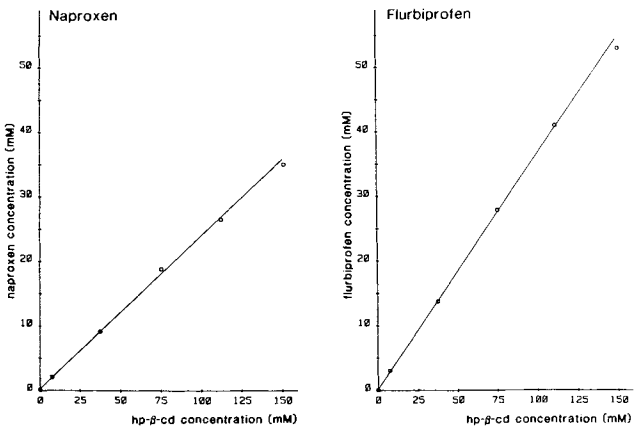

Fig. 1. Phase-solubility diagrams of naproxen (left) and of flurbiprofen (right) with hydroxypropyl-β-cyclodextrin.

In Vivo Drug Disposition

In the hydroxypropyl-β-cyclodextrin-containing injections, naproxen or flurbiprofen could easily be dissolved through complexation with the cyclodextrin. However, since the solubility of the pure drug is far too low to prepare reference injections of pure dissolved drug in physiological saline, rat plasma was used as solvent. The tissue and plasma concentrations were measured shortly after injection, because the effects of the cyclodextrin complexation were anticipated to be largest in the distribution phase. As described previously (16), hydroxypropyl-β-cyclodextrin is rapidly eliminated from the body and cyclodextrin concentrations, high enough to exert any effect, were expected to occur only in the first 30 min after injection.

Lack of effect of hydroxypropyl-β-cyclodextrin on naproxen disposition (Tables III and IV) suggests that naproxen is rapidly displaced from the cyclodextrin complex by the cholesterol and competitive binding to plasma proteins, resulting in a situation which is not different from that after injection of noncomplexed naproxen. In contrast, 10 min after flurbiprofen injection, drug concentrations in all tissues, except the muscle, are higher after administration of the complex than after flurbiprofen alone (Table V). The differences are thought to result either from complexation of flurbiprofen by hydroxypropyl-β-cyclodextrin or through an effect of hydroxypropyl-β-cyclodextrin on membrane permeability. However, this last possibility was not considered very likely, since changes in membrane permeability should also have affected naproxen disposition. The finding that hydroxypropyl-β-cyclodextrin has an effect on the disposition of flurbiprofen and not on the disposition of naproxen

Table III. Naproxen Concentrations in Tissue and Plasma Found 10 min After Intravenous Administration of Naproxen with and Without Hydroxypropyl-β-cyclodextrin and the Significance Level of the Difference Between the Two Administrations (Mean ± SD; $n = 6$)

	With hp-β-cd (μg/g)	Without hp-β-cd (μg/g)	Difference
Brain	0.626 ± 0.070	0.670 ± 0.114	n.s.[a]
Liver	6.81 ± 0.71	6.50 ± 0.87	n.s.
Kidney	7.41 ± 1.03	6.78 ± 0.95	n.s.
Spleen	4.93 ± 0.45	5.03 ± 0.68	n.s.
Muscle	3.81 ± 0.79	3.15 ± 0.48	n.s.
Plasma (μg/ml)	51.1 ± 6.4	47.2 ± 3.4	n.s.

[a] Not significant.

Table V. Flurbiprofen Concentrations in Tissue and Plasma Found 10 min After Intravenous Administration of Flurbiprofen with and Without Hydroxypropyl-β-cyclodextrin and the Significance Level of the Difference Between the Two Administrations (Mean ± SD; $n = 6$)

	With hp-β-cd (μg/g)	Without hp-β-cd (μg/g)	Difference
Brain	0.439 ± 0.053	0.375 ± 0.031	$P < 0.05$
Liver	7.39 ± 0.54	4.97 ± 0.67	$P < 0.001$
Kidney	5.20 ± 0.77	4.55 ± 0.51	$P < 0.05$
Spleen	3.50 ± 0.30	3.06 ± 0.30	$P < 0.05$
Muscle	3.24 ± 0.42	3.14 ± 0.63	n.s.[a]
Plasma (μg/ml)	79.3 ± 3.7	76.8 ± 1.9	n.s.

[a] Not significant.

agrees with the results of the protein binding studies. The higher tissue concentration of flurbiprofen in the brain, after injection of the complexed flurbiprofen, was unexpected because the cyclodextrins are considered to be hydrophilic drug carriers. Further, no hydroxypropyl-β-cyclodextrin could be detected in the brain tissue. From these results it was inferred that the hydroxypropyl-β-cyclodextrin can be regarded as a drug carrier which is able to deliver flurbiprofen to biological membranes in a more efficient way than the plasma proteins. However, since only a small fraction of the drug remains complexed in the plasma, the final effect will be small.

Ten minutes after intravenous administration the hydroxypropyl-β-cyclodextrin concentration in plasma is about 500 μg/ml (16). However, the 10-fold increase in free flurbiprofen fraction found in the *in vitro* experiment at this cyclodextrin concentration (Table II) is not reflected in the increase in tissue concentrations, which is at most 1.5-fold. This apparent discrepancy may have several causes. As described above the free drug fraction measured *in vitro* contains also the fraction complexed with cyclodextrin and the uptake of drug in tissue from the complex could be different from that of the free drug. Further, the *in vitro* measurements were performed in plasma and not in blood. Although observed trends are generally the same in both fluids, they might differ in quantity. For example, the cholesterol content in blood is higher than in plasma.

The differences in tissue concentration found 10 min after injection compared with controls were no longer detectable 60 min after injection (Table VI). At this time the cyclodextrin plasma concentrations are low [about 100 μg/ml (16)] and no significant effect, on the pharmacokinetic behavior of the flurbiprofen, was expected anymore. Very likely the normal distribution of the drug between tissues and plasma has been restored at this time. The slightly higher brain concentration of flurbiprofen 60 min after the hydroxypropyl-β-cyclodextrin complex may result from slow drug efflux from the brain.

These results indicate binding competition between cyclodextrin and plasma proteins for the drug. Simultaneously a competition between cholesterol and drug for the cyclodextrin also occurs. As a result, only small fractions of the drug remain complexed by the cyclodextrins once the complex enters the circulation. A significant effect of cyclodextrin complexation on drug disposition can therefore be expected only for those drug–cyclodextrin complexes that possess a relatively high complex stability constant, as compared with the association constant with plasma proteins and do not undergo displacement from the cyclodextrin complex by endogenous lipids.

Since the concentrations of drug and cyclodextrin also influence the complexation equilibrium, factors such as the administered dose and the volume of distribution will also influence the magnitude of the effect of cyclodextrin on drug disposition. In larger animals, for example, the larger dilu-

Table IV. Naproxen Concentrations in Tissue and Plasma Found 60 min After Intravenous Administration of Naproxen with and Without Hydroxypropyl-β-cyclodextrin and the Significance Level of the Difference Between the Two Administrations (Mean ± SD; $n = 6$)

	With hp-β-cd (μg/g)	Without hp-β-cd (μg/g)	Difference
Brain	0.622 ± 0.159	0.613 ± 0.122	n.s.[a]
Liver	5.64 ± 0.50	5.45 ± 0.77	n.s.
Kidney	5.98 ± 0.98	5.56 ± 0.51	n.s.
Spleen	3.68 ± 0.54	3.41 ± 0.41	n.s.
Muscle	2.68 ± 0.32	3.06 ± 0.62	n.s.
Plasma (μg/ml)	36.6 ± 3.3	35.2 ± 1.9	n.s.

[a] Not significant.

Table VI. Flurbiprofen Concentrations in Tissue and Plasma Found 60 min After Intravenous Administration of Flurbiprofen with and Without Hydroxypropyl-β-cyclodextrin and the Significance Level of the Difference Between the Two Administrations (Mean ± SD; $n = 6$)

	With hp-β-cd (μg/g)	Without hp-β-cd (μg/g)	Difference
Brain	0.275 ± 0.027	0.218 ± 0.043	$P < 0.05$
Liver	3.31 ± 0.87	3.90 ± 0.75	n.s.[a]
Kidney	3.05 ± 0.59	3.18 ± 0.33	n.s.
Spleen	2.59 ± 0.50	2.19 ± 0.17	n.s.
Muscle	2.51 ± 0.35	2.41 ± 0.25	n.s.
Plasma (μg/ml)	48.9 ± 3.1	50.4 ± 5.2	n.s.

[a] Not significant.

tion of the complex containing injection might decrease the complexed fraction of drug and thereby the effects of the cyclodextrins. This may explain the observed differences between rats and mice, concerning the effects of cyclodextrins on barbiturate disposition (13).

The results obtained in this study differ from the results reported by Nagai *et al.* (13) and Shirakura *et al.* (14), concerning the effects of β-cyclodextrin on the disposition of hexobarbital. The brain and liver concentrations of hexobarbital were decreased by β-cyclodextrin, whereas in the present study the flurbiprofen concentrations in these organs were increased by hydroxypropyl-β-cyclodextrin. This difference might be due to differences in protein binding of the drugs. Barbiturates have a relatively low affinity for plasma proteins and high partition into tissues (22,23). The free fraction of the barbiturates is relatively high (40–60%). Therefore, the drug fraction complexed by cyclodextrins will be relatively high and originate mostly from the non-protein-bound fraction. Since the affinity of the complex for membranes in compartments such as brain and liver is likely to be lower than that of the free drug, complexation results in decreased tissue concentrations in liver and brain. In contrast, flurbiprofen has a very high plasma protein binding (>99%) and drug complexed by cyclodextrin originates largely from the protein-bound fraction. Since the cyclodextrins are more efficient carriers of the drug to membranes than the proteins, the tissue concentrations will generally increase. The impact of cyclodextrin complexation on the disposition of a certain drug consequently may depend both on its relative affinity for plasma proteins and on its tissue affinity.

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