Bile acid and sterol solubilization in 2-hydroxypropyl-β-cyclodextrin

Jeff De Caprio, Jaime Yun, and Norman B. Javitt[1]

Division of Hepatic Diseases, New York University Medical Center, 550 First Avenue, New York, NY 10016

Summary The use of 2-hydroxypropyl-β-cyclodextrin has made it possible to prepare stable aqueous solutions of cholesterol, 26-hydroxycholesterol, 7α-hydroxycholesterol, and monohydroxy bile acids such as lithocholic and 3β-hydroxy-5-cholenoic acids. These solutions are suitable for cell culture studies and for parenteral administration to animals.—**De Caprio, J., J. Yun, and N. B. Javitt.** Bile acid and sterol solubilization in 2-hydroxypropyl-β-cyclodextrin. *J. Lipid Res.* 1992. **33.** 441–443.

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Since the introduction of an experimental model for the rapid induction of cholestasis by intravenous infusion of monohydroxy bile acids (1, 2), a major technical problem has been to prepare an infusion mixture that maintains these compounds in aqueous solution (3), and we ourselves have tried numerous procedures without success. We now report that 2-hydroxypropyl-β-cyclodextrin provides a stable aqueous soluble vehicle for the solubilization and administration of monohydroxy bile acids and other steroids.

MATERIALS AND METHODS

The compound 2-hydroxypropyl-β-cyclodextrin was obtained from Pharmatec, Inc., Alachua, FL, as both a powder and a sterile 45% aqueous solution (w/v). The maximum solubilities of the compounds that were evaluated were determined by adding increasing amounts of each compound to a 45% solution and stirring overnight on a magnetic mixer until a residue was noted. The tubes were then centrifuged and an aliquot of the clear solution was taken for analysis by GLC after formation of the methyl ester acetate for bile acids and the mono- or diacetates for sterols using methods for their preparation that were described in detail previously (4, 5). Internal standards were 3α, 7α-dihydroxy-12-oxo-5β-cholanoic acid for bile acids and coprostanol for sterols.

The usefulness of some of these preparations was determined by evaluating the stability of 26-hydroxycholesterol in cell culture and the excretion of 3β-hydroxy-5-cholenoic acid in bile after parenteral administration in rats. Surgical preparation of the animals (male Wistar rats, 250–300 g) and techniques for monitoring bile flow and quantifying radioactivity were previously described in detail (1, 2).

RESULTS AND DISCUSSION

The solubilities of the bile acids and sterols in a 45% solution of 2-hydroxypropyl-β-cyclodextrin are listed in **Table 1**. These solutions were kept in the refrigerator for several weeks and no precipitates were noted.

Our interest in β-cyclodextrins began when we noted that precipitation of 26-hydroxycholesterol (initial concentration = 6 μM) dissolved in either ethanol or n,n,-dimethylformamide occurred slowly after addition to Dulbecco's Minimal Essential Medium. We therefore sought an alternative vehicle and found that addition of up to 20 μl of 2-hydroxypropyl-β-cyclodextrin to 5.0 ml of medium overlying a nonconfluent monolayer of HepG2 cells had no effect on cell morphology or growth. In contrast to the experience with the previous vehicles, no precipitates were noted when 10 μl of 2-hydroxypropyl-β-cyclodextrin containing 30 nmol of 26-hydroxycholesterol was added to 5 ml of the same medium. The concentration (6 μM) was verified by GLC analysis initially and again after the medium, which had been frozen for 2 weeks at –20°C, was thawed and centrifuged. As we reported previously (4), the major metabolite of 26-hydroxycholesterol in HepG2 cells is 3β-hydroxy-5-cholenoic acid. In parallel studies comparing the percentage conversion to 3β-hydroxy-5-cholenoic acid of identical amounts of 26-hydroxycholesterol added in n,n-dimethylformamide and in 2-hydroxypropyl β-cyclodextrin, the yield rose from 17.7 to 43.4%. In addition, the remaining nonmetabolized 26-hydroxycholesterol was recovered in the expended medium only when 2-hydroxypropyl-β-cyclodextrin was used.

To determine whether the solubilized monohydroxy bile acids sequester after parenteral administration, we injected 3β-hydroxy-5-cholenoic acid both intraperitoneally and intravenously into a rat with a bile fistula. Bolus injection of 0.3 ml of the vehicle had no observable deleterious effect on the status of the animal (convulsions, somnolence) and, as shown in **Table 2**, had no effect on bile flow.

After 30 min, 0.3 ml of the vehicle containing 3β-hydroxy-5-cholenoic acid was given by bolus injection and a plasma level of 144 μM was obtained 3 min later. The plasma level decreased progressively during the

Abbreviation: GLC, gas–liquid chromatography.

[1]To whom correspondence should be addressed.

TABLE 1. Solubility of sterols and bile acids in 2-hydroxypropyl-β-cyclodextrin

Compound	μmol/ml[a]
Cholesterol	15.5
7α-Hydroxycholesterol[b]	19.8
26-Hydroxycholesterol	27.4
Lithocholic acid	50.7
3β-Hydroxy-5-cholenoic acid	51.3
Sodium taurolithocholate[c]	39.5
Chenodeoxycholic acid[c]	50.8
Ursodeoxycholic acid[c]	50.8

[a]Maximum solubility at 20°C in 0.9% NaCl containing 45% 2-hydroxypropyl-β-cyclodextrin (w/v).
[b]Analyzed by GLC as the di- and trimethylsilyl ether.
[c]Maximum solubility was not determined; GLC analysis was not done since no residue was present.

ensuing hour and the bulk of the administered bile acid was recovered in bile during this period. By 90 min post-injection, 78% of the injected dose was recovered in bile and 90% by 3 h. It was concluded that no sequestration in lungs or other tissues occurred and that the bulk of the injected bile acid continued to circulate and thus was available for uptake and excretion by the liver.

Similar findings were obtained after intraperitoneal injection of 0.5 ml of the vehicle containing ^{14}C-labeled 3β-hydroxy-5-cholenoic acid (Table 2). Radioactivity appeared in the bile within 13 min and an apparently constant plasma level of the bile acid (250 μM) was found to occur between 30 and 185 min after administration. Excretion of radioactivity into bile was relatively constant during this period. It was concluded that, after intraperitoneal administration, the compound was slowly and continually absorbed from the peritoneal cavity.

In a recent study the maximum solubility for lithocholic acid that could be obtained using heat and 7.5% bovine serum albumin was 5.3 μM (3), a value slightly less than had been reported for both lithocholic acid and 3β-hydroxy-5-cholenoic acid (6 μM) using a mixture of propylene glycol and human serum albumin (2). Thus the above study would have required a bolus injection of 2.0 ml of the mixture with 150 mg of albumin. We are not aware of reports attempting to use the intraperitoneal or other routes of administration for studies of monohydroxy bile acids.

Administration of 2-hydroxypropyl-β-cyclodextrin by either the intrathecal or the intracerebral route was shown to be without toxicity(6) and we too have noted no adverse effects when bile acids solubilized in this vehicle are given either intraperitoneally or intravenously.

None of the vehicles commonly used to solubilize steroids and bile acids, such as propylene glycol, ethanol, dimethyl sulfoxide, or dimethyl formamide, is able to maintain solubility when diluted with aqueous media. Cyclodextrin-solubilized compounds do not precipitate upon aqueous dilution. The difference is attributable to the cyclic structure of this class of compounds, which provides a lipophilic interior (6) in which organic compounds that have limited aqueous solubility will form a soluble complex. Presumably, this mechanism accounts for the observation that solu-

TABLE 2. Recovery of [24-^{14}C] 3β-hydroxy-5-cholenoic acid in rat bile following intravenous and intraperitoneal administration

Elapsed Time	3β-Hydroxy-5-cholenoic Acid					
	Intravenous			Intraperitoneal		
	Bile Flow	Bile Acid	Serum	Bile Flow	Bile Acid	Serum
min	*μl/min*	*nmol/min*	μM	*μl/min*	*nmol/min*	μM
20	16.1			6.3		
40	17.3[a]			6.5		
60	17.4			6.4		
70	15.1[b]		144	7.5[c]	0.0	
80	14.7	63		7.0	26	
90	14.2	191	99	6.0	39	
100	13.9	149		14.3	64	250
110	13.0	112	56	11.0	53	
120	13.0	85		10.6	49	
130	13.0	86	4	9.2	37	
140	12.8	77		8.4	35	
150	12.2	68		7.1	36	
255	11.9	62		7.4	34	250
328	12.9	32		9.8	48	

[a]2-Hydroxypropyl-β-cyclodextrin (0.3 ml) injected intravenously.
[b]2-Hydroxypropyl-β-cyclodextrin (0.3 ml) containing 10.6 μmol 3β-hydroxy-5-cholenoic acid injected intravenously.
[c]2-Hydroxypropyl-β-cyclodextrin (0.5 ml) containing 17.8 μmol 3β-hydroxy-5-cholenoic acid injected intraperitoneally.

bilization in cyclodextrin solution requires as long as 24 h, during which time a steady state is established between the compound within the "cage" and the surrounding aqueous solution.

Determining the precise effect of this method of solubilization on the volume of distribution following intravenous infusion or injection and on the rate of uptake by the liver is beyond the scope of this study.

We believe that this new method of solubilization is technically easier, is more reliable, and permits the design of a wider range of studies directed at understanding the physiological effects of bile acids and other steroids.

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