# Safety of Parenteral Hydroxypropyl β-Cyclodextrin

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**Abstract** □ Post-treatment data were collected on a patient who received intravenous hydroxypropyl β-cyclodextrin in a dose of 1.5 g/kg in 1985. Although no untoward effects were observed in this patient, rarely occurring agitation and pulmonary edema have been noted after injections into rabbits and dogs, respectively. These complications are analyzed here on the basis of symptoms and on the effects of hydroxypropyl β-cyclodextrin on the biochemistry of a representative lipid, cholesterol, which were studied in rats. It is hypothesized that these untoward effects of parenteral hydroxypropyl β-cyclodextrin are due to complex formation, with lipid mediators of pathological responses, of which prostaglandins are one example. These mediators normally have brief and localized functions; if hydroxypropyl β-cyclodextrin happens to be injected when these mediator systems are activated, their influence and the responses of the organism may be increased.

## Introduction

Conversion of cyclodextrins into their hydroxypropyl ethers by a reaction of low selectivity reduces some of the problems of cyclodextrins in pharmaceutical uses, namely low water solubility and parenteral toxicity.[1-4] Consequently, with hydroxypropyl cyclodextrin mixtures, one takes full advantage of the formation of inclusion complexes (host–guest complexes), and lipophilic drugs can be dissolved and made freely and rapidly accessible for absorption into the organism.[2-4] Hydroxypropyl cyclodextrins have very low toxicity by the parenteral route,[2,5] and no adverse effects have been seen in humans;[6] nevertheless their use is not without occasional distress/agitation in rabbits and pulmonary edema in dogs. In this work we analyze the short-term adverse effects and present a hypothesis to explain them, which also may be helpful for minimizing them. Because these effects have been observed only occasionally, we could not study them directly but addressed them by analyzing symptoms and by studying the effect of hydroxypropyl cyclodextrins on homeostasis and conversions of lipids. Furthermore, we present systematic documentation of the absence of any complications up to 8 years after the intravenous administration of hydroxypropyl β-cyclodextrin to humans.[6]

## Experimental Section

*Materials*—Hydroxypropyl β-cyclodextrin was purchased from Pharmatec/Pharmos Inc., Alachua, FL, Batch 069-32-24A. [$^{14}$C]Acetic acid was purchased from Moravek Biochemicals, Inc., Brea, CA, and had a specific activity of 56 mCi/mmol. [$^{14}$C]Cholesterol was purchased from New England Nuclear, Inc., Boston, MA, and had specific activity of 52 mCi/mmol.

*De Novo Synthesis of Cholesterol*—We followed an established procedure.[7] Male rats, HSD/SD strain, were used in the experiments. The average weight of a rat was 325 g in experiment A and 337 g in experiment B. Animals had unlimited access to food (open formula rat and mouse ration, NIH 07) and to water. Treatment was by intravenous injection with hydroxypropyl β-cyclodextrin dissolved in isotonic phosphate buffered saline; doses used are in Table I.

In Experiment A, the treatment was performed on the first day at 10 a.m. and 4 p.m., and on the second and third days only at 10 a.m. One hour after the last treatment, an injection of sodium [$^{14}$C]acetate was administered intraperitoneally, 78 μCi per kg of body weight. Rats were exsanguinated 1 h after the radiolabel was administered, and the plasma was processed as follows. At first the radioactivity in the plasma was measured after adding an aliquot of the plasma (25 or 50 μL) to the aqueous hydrochloric acid (0.5 mL, 0.5 M). To that sample, Ready-Gel (8 mL) was added, and radioactivity was measured by liquid scintillation counting. The newly synthesized cholesterol in the plasma was measured by adding an aliquot (1 mL) to the solution of aqueous potassium hydroxide (0.5 mL, 50% w/w) and ethanol (2 mL). Cholesterol esters then were saponified at 70 °C for 80 min under argon gas. After the mixture was cooled to room temperature, the total cholesterol was extracted with hexane (three times, 3 mL), the combined extracts were evaporated, the residue was dissolved in benzene (0.1 mL), and radioactivity determined by liquid scintillation counting. Background corrected counts of extracts from rats injected with phosphate buffered isotonic saline were averaged and the counts of all experiments, after correction for background, were expressed as a percentage of the above average, which was assigned a value 100%. The newly synthesized cholesterol in solid tissues was measured after prolonged hydrolysis of samples by alcoholic potassium hydroxide (50 mg/mL), together 80 h at room temperature, 40 h at 37 °C, and 2 h at 80 °C. After further dilution of the samples with aqueous ethanol, the cholesterol was extracted with petroleum ether and its radioactivity was determined as above. All the reported data are averages of duplicate samples. Experiment B was performed using the same protocol, but doses of hydroxypropyl β-cyclodextrin were changed, as described in Table 1.

*Lecithin:Cholesterol Acyltransferase (LCAT) Activity in Serum*—We used slightly modified established procedure.[8] Hydroxypropyl β-cyclodextrin was added to the freshly collected citrated (0.0025 M) rat plasma (0.5 mL) up to the stated concentration, and the run was started by the addition of [$^{14}$C]cholesterol (1 μCi) in ethanol (10 μL). The addition caused formation of a small amount of gel which was more apparent at higher concentrations of hydroxypropyl β-cyclodextrin. The gel was dispersed by shaking on a vortex mixer, and the mixture was incubated at 20 °C. At the times specified in Figure 1, small aliquots (2–4 μL) were withdrawn and spotted on a thin-layer chromatography plate (Merck Silica gel 60). The plate was developed with a mixture of hexane, diethyl ether, and acetic acid (83:16:1). We scraped off the silica gel from the location where the cholesteryl esters were expected, and determined the radioactivity by liquid scintillation counting. All data reported are averages of duplicate samples.

*Hydroxypropyl β-Cyclodextrin Used in Human*[6]—The condensation of β-cyclodextrin with propylene oxide was performed using aqueous sodium hydroxide (12.4% w/w) as a solvent and product was purified by extraction with acetone (to remove nonpolar impurities) and by a dialysis against distilled water. The average degree of substitution by 252 Cf mass spectrometry was 5.6 (no β-cyclodextrin was detectable in this spectrum) and 4.9 by nuclear magnetic resonance.[9,10] The distribution of substituents on glucose residues was, in molar percentages, as follows: $S_0$, 42.8; S2, 13.6; S3, 5.7; S6, 15.5; S2, 3, 5.2; S2, 6, 6.0; S3, 6, 2.6; S2, 3, 6, 3.0 (measured after hydrolysis by nuclear magnetic resonance).[11] The content of monopropylene glycol was 0.02% (w/w) and of dipropylene glycol was 0.02% w/w.[11]

## Results

*Regulation of Biosynthesis and Esterification of Cholesterol—De novo* biosynthesis of cholesterol from acetic

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Table 1—Effects of Intravenous Hydroxypropyl β-Cyclodextrin on *De Novo* Cholesterol Synthesis in Rats

| | Experiment A | | | | Experiment B | | | |
|---|---|---|---|---|---|---|---|---|
| intravenous treatment | 4 × PBS[a] | | 4 × 0.5 g HPBCD[b] per kg | | 3 × PBS | | 3 × 1.5 g HPBCD per kg | |
| Animal | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| *De novo* cholesterol (cpm/mL of plasma) | 391 | 772 | 849 | 590 | 1433 | 599 | 1080 | 6466 |
| % of averaged controls | 67 | 133 | 146 | 102 | 141 | 60 | 106 | 46 |
| Liver weight (g) | 12.5 | 9.3 | 8.9 | 10.9 | | | | |
| % of averaged controls | 115 | 85 | 82 | 100 | | | | |
| *De novo* cholesterol (cpm/g of liver) | 887 | 1415 | 2011 | 1699 | | | | |
| % of averaged controls | 77 | 123 | 175 | 148 | | | | |

[a] Phosphate-buffered isotonic saline. [b] Hydroxypropyl β-cyclodextrin.

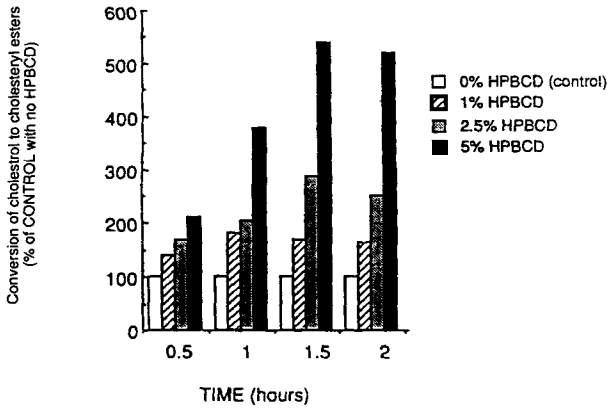


**Figure 1**—Effects of hydroxypropyl β-cyclodextrin (HPBCD) on the conversion of [$^{14}$C]cholesterol into [$^{14}$C]cholesteryl esters in rat plasma.

acid can be measured easily in the rat, and it is an accurate indicator of the overall availability of cholesterol in an organism.[7] The level of biosynthesis varies with the overall state of the animal, but any such variations are much smaller than the changes routinely observed upon cholesterol depletion in animals (e.g., by bile binding agents in food); these changes may occur over 1 order of magnitude.[7] The results of experiments A and B (Table 1) indicate that a series of intravenous administrations of hydroxypropyl β-cyclodextrin did not greatly change the levels of biosynthesis of cholesterol from those seen in controls.

Another parameter that gives pertinent information on the effects of parenteral hydroxypropyl β-cyclodextrin is the rate of conversion of cholesterol into cholesteryl esters. This process is affected by the rate of transfer of lipids between the lipoproteins through the aqueous phase; the respective measurements can be made *ex vivo* in plasma.[8,12,13] Using sera from the same strain of rats as above, we measured the time dependencies of the conversion (Figure 1). The results show a moderate increase proportional to the concentration of hydroxypropyl β-cyclodextrin added.

*Short-Term Adverse Effects in Animals*—No short-term adverse effects of parenteral hydroxypropyl β-cyclodextrin have been observed in mice and rats in this laboratory (ref 5 and unpublished materials) or recorded in the literature.[2,14] In a rabbit (New Zealand, white), one adverse reaction was observed during the period when about 100 boluses in all were administered to about a dozen animals.[15] The rabbit in question became distressed and agitated immediately after the start of the injection; when the injection was interrupted, the rabbit recovered. Tests for irritation and deviation from isotonicity of the injection solution were negative, and the same rabbit tolerated the solution well when it was administered as eye drops. In dogs, parenteral hydroxypropyl β-cyclodextrin was recorded to cause pulmonary edema in two animals out of three.[16,17] Cardiovascular function was followed closely but was found not to be disturbed in any of the three dogs;[16,17] thus edema could not be due to defective heart activity. Pathological leakage of liquid into the alveoli was thus a probable culprit. In monkeys, intravenous hydroxypropyl β-cyclodextrins did not cause any adverse effects.[14]

*Absence of Adverse Effect in Humans*—A young boy suffering from severe hypervitaminosis A, probably due to an inherited variance in the metabolism of that vitamin (patient 2, of ref 6), received an infusion of hydroxypropyl β-cyclodextrin in June 1985. The dose was 470 mg/kg/day, a total of 30 g over 4 days in the form of a 5% solution in water. (Characterization of the hydroxypropyl β-cyclodextrin used by the presently available methods is described in the Experimental Section). Clinical observations and those laboratory results which were related to the disease and which were collected at the time of infusion therapy had been published previously;[6,18] the distribution of retinoids in lipoproteins was described later.[15] Table 2 displays the clinical chemistry data documenting the lack of short-term adverse effects of the infusion. The data show that in spite of a badly balanced injection solution, no hemolysis was seen. A number of related derivatives of β-cyclodextrin were shown to have somewhat lower hemolytic activity *in vitro*; the present results indicate that the improvements which occur when these are used instead of hydroxypropyl β-cyclodextrin *in vivo* may not be significant. Studies on animals have shown that intravenous hydroxypropyl β-cyclodextrin causes morphological changes in kidneys which are repaired only slowly. Table 3 shows the clinical chemistry data related to kidney function after the infusion; no damage is seen.

## Discussion

Hydroxypropyl β-cyclodextrins, free or containing complexed drug, equilibrate rapidly with surrounding lipids after entering an organism.[15,19] Thus a new reservoir—a pool of circulating and rapidly accessible lipids—is formed. Formation of this pool increases the total concentration of dissolved lipids, but because these newly dissolved lipids are in complex forms, their physicochemical activities are not changed. Cholesterol is probably the major lipid present in this new pool; this suggestion follows from the observation that it was mainly this lipid which was excreted with hydroxypropyl β-cyclodextrin into urine.[15]

Presently we are interested in learning how formation of such a new pool of cholesterol will affect its biosynthesis; this biosynthesis is regulated on the basis of cholesterol availability or of a preception of availability. Biosynthesis of cholesterol can be measured easily. In a rat it occurs at a basal level; thus both increases and decreases in the conversion of acetic acid to cholesterol can be measured. The present results show that parenteral hydroxypropyl β-cyclodextrin has no strong effects on the biosynthesis of cholesterol; formation of a new circulatory pool of a lipid is not perceived as an excess or a lack of that lipid. Although these results are relevant

**Table 2—Clinical Chemistry Data Collected during the Infusion of Hydroxypropyl β-Cyclodextrin into the Patient**

| | Preinfusion | Infusion of HPBCD | | | | Day after the Infusion | | |
|---|---|---|---|---|---|---|---|---|
| Day | −1 | 1 | 2 | 3 | 4 | 1 | 2 | 3 |
| White blood count | 6900 | | | 8400 | | 7700 | | |
| Hemoglobin | 11.5 | | | 10.5 | | 10.2 | | |
| Sodium (mequiv/L) | 133 | | | | 138 | | | |
| Potassium (mequiv/L) | 3.6 | | | 4.6 | | | | |
| Chloride (mequiv/L) | 107 | | | 107 | | | | |
| Bicarbonate (mequiv/L) | | | | 21 | 14 | | 16 | |
| Urea (mmol/L) | 2.4 | 1.7 | | 3.2 | | | 3.2 | |
| Creatine (μmol/L) | 35 | | | 44 | | | 35 | |
| Proein (g/L) | 56 | 63 | 57 | 63 | 64 | 66 | 65 | |
| Albumin (g/L) | 40 | 41 | 38 | 40 | 40 | 42 | 41 | |
| Glucose (mg/dL) | 72 | 104 | 94 | | 103 | | 113 | |
| Total calcium (mmol/L) | 2.00 | 2.07 | 1.98 | 2.07 | 1.90 | 2.10 | 2.05 | |
| Ionized calcium (mmol/L) | 1.06 | 1.14 | 1.04 | 1.13 | 1.05 | 1.07 | 1.08 | 1.09 |
| pH | 7.48 | 7.38 | 7.46 | 7.42 | 7.41 | 7.45 | 7.46 | 7.40 |
| Phosphate (mmol/L) | 1.55 | 1.67 | 1.71 | 1.77 | 1.64 | 1.80 | 1.77 | |
| Cholesterol | 199 | 214 | 187 | 178 | 179 | 209 | 211 | |
| Uric acid (mmol/L) | 0.23 | 0.25 | 0.22 | 0.18 | 0.15 | 0.27 | 0.17 | |
| Alkaline phos (IU/L) (<300) | 950 | >700 | 647 | >600 | 611 | 559 | 650 | |
| T bilirubin (μmol/L) | 29 | 32 | 27 | 29 | 30 | 34 | 29 | |
| Conjugated bilirubin | 2 | 1 | 2 | 2 | 2 | 2 | 3 | |
| ALT U/L (<35) | 32 | 43 | 65 | 35 | 36 | 33 | 47 | |
| AST U/L (<40) | 67 | 74 | 62 | 70 | 35 | 55 | 82 | |
| γGT U/L (50) | 52 | 59 | 15 | 54 | 56 | 53 | 55 | |
| LDM U/L (<200) | | | | 536 | | | | |

**Table 3—Renal Function Tests of Patient**

| Date | BUN (mg/dL) | UREA (mM) | Creatine (μM) |
|---|---|---|---|
| May 1984 | 9 | 3.2 | 9 |
| June 11, 1985 | 6.7 | 2.4 | 26 |
| June 12, 1985 | 4.8 | 1.7 | 35 |
| First day of HPBCD infusion | | | |
| June 14, 1985 | 9 | 3.2 | 44 |
| Third day of HPBCD infusion | | | |
| June 17, 1985 | 9 | 3.2 | 35 |
| Second day after infusion | | | |
| October 29, 1990 | 13.7 | 4.9 | 67 |
| April 2, 1991 | 9.8 | 3.5 | 46 |

strictly to cholesterol, other lipids form analogous new pools, and their biosynthesis is probably also not affected.

Also investigated were the effects of this new pool on phenomena that depend principally on the rates of transfer of lipids through the aqueous phase of circulating blood. Esterification of cholesterol in plasma is such a process because it depends on rates of transfer of lipids between lipoprotein particles.[12,13] Formation of a new, rapidly accessible pool of cholesterol may be expected to increase the transfer, and indeed the rate of conversion was observed to rise.

On the basis of the above results, we can propose a hypothesis to explain the short-term adverse effects seen in animals. The results suggest that although the hydroxypropyl β-cyclodextrin in circulation may not affect the overall balances of the compounds with which it forms complexes, the processes that are controlled by time may be changed. The above-mentioned adverse effects may be caused by a wider distribution of signal lipid mediators of pathological responses; this distribution is controlled principally by the rate of blood flow and the rate of metabolism of the mediators. Some of these mediators are called "local hormones" and are synthesized in many organs in response to specific stimuli. They exert their effects mostly at the site of their synthesis, unlike endocrine hormones.[20] If a new pool of these local hormones is formed through complex formation, they may escape inactivation through the rapid local metabolism, may be carried to other organs, and may mediate pathological responses there.

Other results also support this hypothesis. Parenteral cyclodextrins and cyclodextrin derivatives were shown previously to assist distribution of foreign toxins. Toxic effects of retinoic acid, injected intraperitoneally, were accelerated by intravenous injections of 2,6-di-*O*-methyl-β-cyclodextrin.[21] Subcutanous injections of β-cyclodextrin aided the cancerogenic effects of *N*-ethyl-*N*-(2-hydroxyethyl)nitrosoamine, which was administered orally.[22] Parenteral cyclodextrins thus may assist in the distribution of externally introduced toxins; largely the same can be expected for potential toxins formed by the organism itself. Furthermore, the above-mentioned lipid mediators, of which the products of oxygenation of arachidonic acid are examples, are known to form complexes with cyclodextrins[23] and thus their distribution in the organism may be expected to be affected by parenteral cyclodextrin derivatives. Our hypothesis also is compatible with the sporadic or species-specific character of the adverse effects. According to the hypothesis, cyclodextrins exert these effects by aggravating the response of the organism to some specific stimuli that may be unrelated to the cyclodextrin administration; therefore these effects are bound to be irregular.

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