0031-3998/10/6801-0052
PEDIATRIC RESEARCH
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Vol. 68, No. 1, 2010
Printed in U.S.A.

2-Hydroxypropyl-β-Cyclodextrin Raises Hearing Threshold in Normal Cats and in Cats With Niemann-Pick Type C Disease

SARAH WARD, PATRICIA O'DONNELL, STEVEN FERNANDEZ, AND CHARLES H. VITE

Departments of Clinical Studies [S.W., C.H.V.] and Pathobiology [P.O., S.F.], School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT: 2-hydroxypropyl-β-cyclodextrin (HPβCD) is a promising experimental therapy for Niemann-Pick type C disease that improved intracellular cholesterol transport, substantially reduced neurodegeneration and hepatic disease, and increased lifespan in *npc1*$^{-/-}$ mice. On the basis of favorable treatment outcome in mice, HPβCD is being evaluated as a therapy in children with Niemann-Pick type C (NPC) disease. We evaluated the efficacy of HPβCD in the feline model of NPC disease and recognized a dose-dependent increase in hearing threshold associated with therapy as determined by brain stem auditory evoked response (BAER) testing. To further assess the effect of HPβCD on hearing threshold, normal cats were administered the drug s.c. at either 4000 mg/kg or 8000 mg/kg body weight, or intrathecally at a dose of 4000 mg/kg brain weight. HPβCD caused a significant increase in hearing threshold following one dose of 8000 mg/kg s.c. or 120 mg intrathecally, and the effect was maintained for at least 12 weeks. Repeated weekly s.c. administration of 4000 mg/kg HPβCD resulted in a similar increase in hearing threshold. These studies are the first to describe a specific negative effect of HPβCD on the auditory system and suggest the need for auditory testing in patients receiving similar doses of HPβCD. **(*Pediatr Res* 68: 52–56, 2010)**

Cyclodextrins are cyclic oligosaccharides with hydrophobic interiors used as formulation vehicles to increase the amount of drug, including hormones and vitamins, which can be solubilized in aqueous vehicles (1). 2-hydroxypropyl-β-cyclodextrin (HPβCD) was extensively studied in rodents, dogs, and monkeys where it was generally well tolerated at low doses (1,2). Daily i.v. administration of greater than 200 mg/kg caused reduced body weight, foamy macrophage infiltration of the lungs, elevations in hepatic enzymes, increased Kuppfer cells in the liver, and renal cortical tubular vacuolization in rodents (1,3,4). All of these changes were reversible following cessation of HPβCD administration (1).

Niemann-Pick type C (NPC) disease is an incurable lysosomal storage disorder characterized by the intralysosomal accumulation of unesterified cholesterol, hepatosplenomegaly, progressive neurologic dysfunction, and early death (5,6). Weekly intraperitoneal administration of 1500 mg/kg of HPβCD to *npc*$^{-/-}$ mice resulted in improvement in hepatic disease with no effect on neurologic disease or lifespan (7). In contrast, the administration of a single s.c. dose of 4000 mg/kg of a 20% solution of HPβCD to 7-d-old *npc*$^{-/-}$ mice reversed the defect in the lysosomal transport of cholesterol and significantly improved hepatic dysfunction, decreased neurodegeneration, and prolonged lifespan (8). Every other day s.c. administration of 4000 mg/kg of a 20% solution of HPβCD to *npc*$^{-/-}$ mice was the most effective treatment regimen at ameliorating clinical disease and increasing lifespan, and also significantly decreased neuronal cholesterol, ganglioside, and sphingosine accumulation, and decreased neuroinflammation (9). It was hypothesized that high doses of HPβCD were needed to ameliorate neurologic dysfunction because higher blood levels allowed more drug to cross the blood-brain barrier. An alternative hypothesis was that increased serum levels of HPβCD could bind enough circulating sterols to result in enhanced cholesterol egress from the CNS by an undefined mechanism (9). In each of these animal studies, no significant toxicity was observed after the administration of HPβCD except for increased macrophage infiltration of the lungs found at postmortem examination (9). On the basis of these data from the murine model, HPβCD has been approved for use in a group of children with NPC disease by the Food and Drug Administration (FDA).

Naturally occurring NPC disease occurs in cats which have a mis-sense mutation in *NPC1* (2864G-C) with clinical, neuropathological, and biochemical abnormalities similar to those present in juvenile-onset patients making this model homologous to the most common form of the disease seen in human patients (10,11). Brain stem auditory evoked response testing (BAER) of cats with NPC disease showed a prolongation in central conduction time with no significant alteration in hearing threshold compared with wild type cats (11). While evaluating the efficacy of HPβCD to treat NPC disease in cats, we noted a significant elevation of hearing threshold in animals receiving repeated s.c. doses of 4000 mg/kg. To our knowledge, a negative effect of HPβCD on auditory function has not been evaluated in any species. This study investigated the effects of the s.c. and intrathecal administration of HPβCD treatment on the BAER of both normal cats and cats with NPC disease.

METHODS

Animals. Cats were raised in the animal colony of the School of Veterinary Medicine, University of Pennsylvania, under National Institutes of Health and USDA guidelines for the care and use of animals in research. The experi-

Received January 11, 2010; accepted March 7, 2010.

Correspondence: Charles H. Vite, D.V.M., Ph.D., Department of Clinical Studies-Philadelphia, School of Veterinary Medicine, University of Pennsylvania, 3900 Delancey Street, Philadelphia, PA 19104; e-mail: vite@vet.upenn.edu

Supported by grants from the Ara Parseghian Medical Research Foundation, Dana's Angels Research Trust and NIH grant RR02512.

Abbreviations: BAER, brain stem auditory evoked response; **HPβCD,** hydroxypropyl-beta-cyclodextrin; **NPC,** Niemann-Pick type C

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mental protocol was approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

All animals examined were produced from the same line bred to produce cats with autosomal recessively inherited Niemann-Pick type C (NPC) disease. All cats were housed at 21°C with *ad libitum* food and water, 12-h light cycles, with 12–15 air changes per hour. Peripheral blood leukocytes from all cats were tested at 1 day of age for the *NPC1* mis-sense mutation using a PCR-based DNA test (10). Cats with two copies of the mis-sense mutation were classified as affected with NPC disease while cats with one or no copies of the mutation were classified as normal. Heterozygote to heterozygote breedings produced ~25% affected and ~75% normal offspring.

Study groups. Normal cats were placed in one of five study groups (Table 1). Group 1 ($n = 7$) were 6 months old and received no drug. Group 2 ($n = 3$) received one dose of 4000 mg/kg body weight HPβCD s.c. at 6 months of age. Group 3 ($n = 3$) received one dose of 8000 mg/kg body weight HPβCD s.c. at 6 months of age. Group 4 ($n = 3$) received one dose of 4000 mg/kg brain weight (120 mg for a 30-g brain weight) HPβCD intrathecally at 6 months of age. Intrathecal adminstration was achieved by anesthetizing cats with i.v. propofol (up to 6 mg/kg; Abbott Laboratories, Chicago, IL). A 20-gauge spinal needle was placed into the cerebellomedullary cistern and 1.0 mL of spinal fluid was removed. 0.6 mL of 20% HPβCD in saline was injected intrathecally over a 2-minute time period into the cerebellomedullary cistern. Group 5 ($n = 3$) received weekly 4000 mg/kg body weight HPβCD s.c. beginning at 8 weeks of age for a total of seven doses.

Cats affected with NPC disease were already involved in a clinical study evaluating the efficacy of HPβCD to treat disease. All cats were first administered the drug at 3 weeks of age, before the onset of clinical signs of disease, and continued to receive the drug weekly thereafter. Cats were placed into one of five groups (Table 2). Group 6 ($n = 8$) received no HPβCD and served as the control group for cats with NPC disease. Groups 7 ($n = 5$), 8 ($n = 2$), and 9 ($n = 5$) received a weekly dose of 1000 mg/kg HPβCD body weight s.c., 4000 mg/kg body weight HPβCD s.c., and 8000 mg/kg body weight HPβCD s.c., respectively. Group 10 ($n = 2$) received 4000 mg/kg brain weight (120 mg for a 30-g brain weight) HPβCD intrathecally every 2 wk (intrathecal administration methods described earlier).

HPβCD formulations. All HPβCD was administered in a 20% (wt/vol) solution dissolved in 0.9% sodium chloride. HPβCD was received from Sigma Chemical Co. and the powdered form (HPβCD-H107; Sigma Chemical Co. Aldrich, St. Louis, MO) was used in all s.c. administrations and the cell culture tested form (HPβCD-C0926; Sigma Chemical Co. Aldrich) was used for all intrathecal administrations. As a control for the saline injection, additional normal cats were injected one time s.c. ($n = 2$) and intrathecally ($n = 2$) with similar volumes of saline.

To control for possible differences between HPβCD available from Sigma Chemical Co., the product used in published mouse studies (8,9) and the FDA-approved formulation for use in patients (Trappsol–Pharm grade, Cyclodextrin Technologies Development, Inc, High Springs, FL), Trappsol was administered to four cats: one dose of 8000 mg/kg body weight s.c. ($n = 2$) and one dose of 4000 mg/kg brain weight intrathecally (120 mg for a 30-g brain weight; $n = 2$).

Brain stem auditory evoked response testing. All measurements of the BAER were obtained from cats given atropine sulfate (0.02 mg/kg; Butler Animal Health Supply, Dublin, OH) and then anesthetized with i.v. propofol (up to 6 mg/kg). The BAER data were recorded using 12 mm, 29-gauge subdermal needle electrodes and a Nicolet Viking Quest signal analyzer (Nicolet Biomedical, Madison, WI). The active electrode was placed over the osseous bulla of the stimulated ear, the reference electrode was situated over the vertex of the skull, and the ground electrode over the contralateral osseous bulla. All recording electrodes were placed s.c. Alternating rarefaction and condensation clicks were produced by connecting a square wave pulse (0.1 ms in duration) to a speaker (Model TIP-300; Nicolet Biomedical, Madison, WI). The stimuli were presented monaurally at a rate of 11.1 Hz using a 25-cm plastic tube of 1.5 mm diameter which was connected to the speaker at one end, with the other end laid in the unsealed ear canal (an open field stimulus). The stimulator delivered a 125 dB pSPL click to the recorded ear and delivered an 85 dB SPL white noise to the contralateral ear. The high pass filter on the amplifier was 20 Hz and the low pass cutoff was 3 kHz. A sensitivity of 1 uV/cm was used to record the responses and the averaging epoch was 10 ms with a sampling resolution of 0.01 ms. One thousand evoked responses were averaged for each BAER response obtained. Central conduction time was defined as the time between the first and the fifth peak. Wave V/I amplitude was determined by dividing the amplitude of the fifth wave by the amplitude of the first wave and multiplying by 100; amplitude was measured from peak to trough and expressed as microvolts. A modified method of limit procedure was used to estimate threshold. When a clearly defined BAER was identified at the reference stimulus of 125 dB, the attenuator was then increased in 3 dB steps and a signal averaged response was sought at each step. If an evoked response was observed, the attenuator was then increased by another 3 dB and the BAER response again observed. This continued until a sound level was reached at which an averaged evoked response could not be identified.

Table 1. *Summary of groups of normal cats treated with HPβCD*

Group	No. of cats	Dose and method of administration of HPβCD
1	7	NA
2	3	4000 mg/kg body weight subcutaneously once
3	3	8000 mg/kg body weight subcutaneously once
4	3	4000 mg/kg brain weight intrathecally once
5	3	4000 mg/kg body weight subcutaneously weekly for 7 doses

NA, not applicable.

Table 2. *Summary of groups of cats with NPC disease treated with HPβCD*

Group	No. of cats	Dose and method of administration of HPβCD
6	8	NA
7	5	1000 mg/kg body weight subcutaneously weekly
8	2	4000 mg/kg body weight subcutaneously weekly
9	5	8000 mg/kg body weight subcutaneously weekly
10	2	4000 mg/kg brain weight intrathecally every two weeks

NA, not applicable.

In normal cats, BAER studies were performed every other week following the administration of HPβCD in groups 2, 3, and 4 for a total of 12 wk, and were performed every week in group 5 for a total of 12 wk. In cats with NPC disease, BAER studies were performed at 16 weeks of age.

Statistical methods. The mean and SD of the thereshold, central conduction time, and wave V/I ratio estimates in each group were calculated to describe the data and an unpaired 2-tailed t test was used to compare data between various groups. Significance values of $p < 0.05$ (*) are given. Threshold differences between groups were considered statistically reliable if the probability of chance occurrence was 0.05 or less.

RESULTS

Normal cats. None of the normal animals that received either s.c. or intrathecal HPβCD injections showed evidence of loss of balance or ataxia at any point during the study. No clinical signs were attributable to HPβCD administration aside from pain at the s.c. injection site which was common in cats receiving weekly doses. Subjective evaluation of hearing was difficult to perform because normal, untreated colony-bred animals frequently do not respond repeatably to sound. Detailed behavioral testing was not performed.

A single s.c. dose of 4000 mg/kg HPβCD evoked waveforms the same as in cats which received no HPβCD (Fig. 1, groups 1 and 2). In contrast, a single s.c. dose of 8000 mg/kg HPβCD resulted in diminished wave form amplitude with changes severe enough to make specific waveforms difficult to identify (Fig. 1, group 3). Similarly, a single intrathecal dose of 120 mg HPβCD resulted in altered evoked responses characterized by reduced amplitude (Fig. 1, group 4). A single injection of intrathecal saline left the BAER unchanged in two cats (data not shown).

Hearing threshold, wave V/I amplitude, and central conduction time were measured for groups 1–5 (Table 3). Cats in groups 3 and 4 showed a significant increase in hearing threshold 2 weeks after injection compared with uninjected cats (group 1). The average click BAER threshold in control cats was 66 dB, whereas in groups 3 and 4 that were treated with 4000 mg/kg and 8000 mg/kg HPβCD, repectively, the

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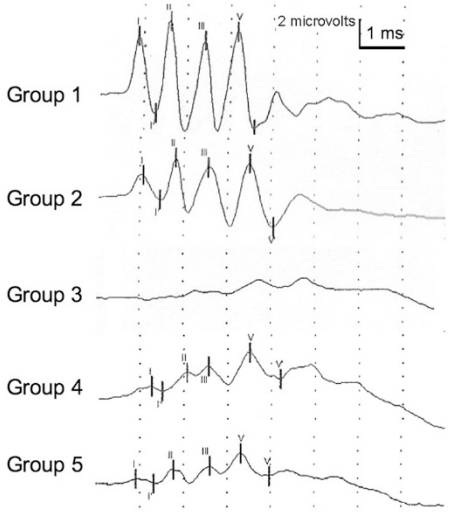

Figure 1. Representative brainstem auditory evoked responses for normal cats following the administration of HPβCD (group 2–5). Recordings for groups 2–4 were made 2 weeks after a administering a single drug dose. The recording for Group 5 was made one week after the sixth weekly drug dose. A decrease in wave form amplitude was noted in groups 3, 4, and 5 compared with untreated cats (group 1). Changes in groups 3–5 were severe enough that waveforms could not always be reliably identified (an example is given for group 3).

Table 3. *Hearing threshold, wave V/I amplitude, and central conduction time 2 weeks after administering a single dose of HPβCD to normal cats (groups 2–4) and 1 wk after the sixth dose (group 5)*

	Hearing threshold (dB SPL)	Wave V/I amplitude	Central conduction time (ms)
Group 1 ($n = 7$)	66.4 ± 3.2	181 ± 16.7	2.41 ± 0.09
Group 2 ($n = 3$)	63.0 ± 5.2	212 ± 24.0	2.43 ± 0.02
Group 3 ($n = 3$)	79.0 ± 4.6*	545 ± 402	2.36 ± 0.02
Group 4 ($n = 3$)	81.0 ± 3.0*	525 ± 127	2.37 ± 0.05
Group 5 (six doses of HPßCD) ($n = 3$)	80.0 ± 1.7*	469 ± 423	2.38 ± 0.09

* $p < 0.05$.

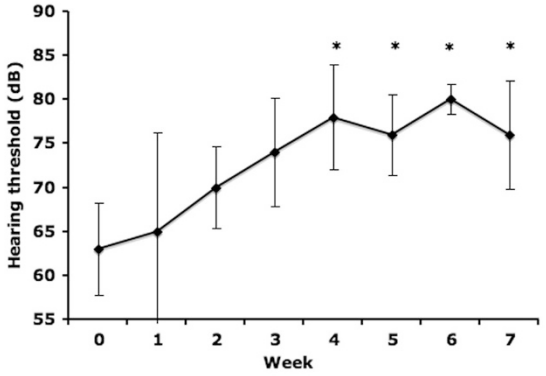

Figure 2. Weekly hearing thresholds following 4000 mg/kg weekly HPβCD administration (group 5; $n = 3$). The first dose was administered immediately after Week 0 threshold testing. Repeated HPβCD administration resulted in progressive elevation of the hearing threshold with a statistically significant ($p < 0.05$) increase from weeks 4 to 7 when compared with week 0.

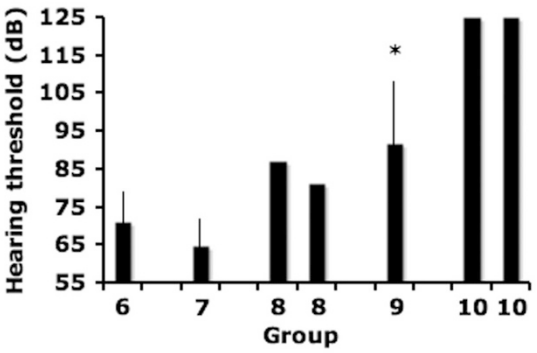

Figure 3. Cats with NPC disease cats showed no increase in hearing threshold after weekly administration of 1000 mg/kg HPβCD (group 7; $n = 5$) compared with untreated affected cats (group 6; $n = 8$). Both cats treated with weekly 4000 mg/kg (group 8; $n = 2$) showed an increase in hearing threshold and cats treated with weekly 8000 mg/kg (group 9; $n = 5$) had a statistically significant ($p < 0.05$) increase in threshold. No waveforms were evoked at the maximum stimulus intensity of 125 dB from two cats treated with every other week intrathecal HPBCD (group 10).

threshold increased to 79 and 81 dB, respectively. This approximately 13 dB difference between groups was statistically reliable ($p < 0.05$). Differences in wave V/I amplitude or in central conduction time were due to random sampling and were statistically insignificant among the groups. The BAER of cats in groups 2, 3, and 4 remained unchanged, neither improving nor worsening, during the 12-wk study following a single administration of HPβCD (data not shown).

Cats in group 5 received 7 weekly injections of 4000 mg/kg HPβCD s.c. Interestingly, although these cats showed no significant elevation in hearing threshold 2 weeks after the first injection, repeated weekly injections of the same dose resulted in a progressive elevation of hearing threshold with the threshold on the fourth through seventh week significantly greater than the hearing threshold observed before injection (week 0) (Table 3; Fig. 2).

Cats with NPC disease. Affected cats began weekly s.c. therapy with HPβCD at 3 weeks of age in an attempt to ameliorate disease progression. At 16 wk of age, hearing thresholds were statistically the same in normal cats (group 1, 66.4 dB ± 3/2 dB) and cats with NPC disease (group 6, 71.9 dB ± 7.9 dB). Interestingly, significant differences were absent between cats treated s.c. with 1000 mg/kg HPβCD (65 dB ± 7.5 dB; group 7) and untreated cats (group 6) (Fig. 3). However, both animals treated with weekly s.c. administered 4000 mg/kg (group 8) had higher hearing thresholds than any untreated cats with NPC disease. Similarly, cats treated with weekly s.c. administered 8000 mg/kg (91.8 dB ± 16.4 dB; group 9) had significantly greater hearing threshold compared with untreated cats with NPC disease. Finally, both cats given HPβCD intrathecally (every other week) had no click evoked waveforms even at the highest sound intensity of 125 dB. Where waveforms could be reliable discerned, the wave V/I amplitude and the central conduction time did not differ between untreated cats with NPC disease and cats treated with HPβCD.

Normal cats receiving trappsol. Trappsol was administered s.c. to two normal cats as a single dose of 8000 mg/kg body weight and intrathecally to two normal cats as a single dose of 4000 mg/kg brain weight (120 mg for a 30-g brain weight) in the same manner as the Sigma Chemical Co. product was

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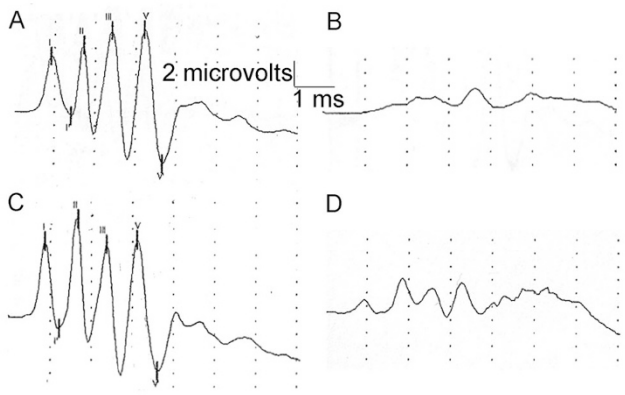

Figure 4. Normal cats treated with either intrathecal or s.c. Trappsol showed abnormal waveforms 1 week after administration. *A*, shows BAER tracing immediately before intrathecal injection of 120 mg Trappsol; *B*, shows BAER in same cat 1 week later. *C*, shows BAER tracing immediately before s.c. injection of 8000 mg/kg Trappsol; *D*, shows BAER in same cat 1 week later.

administered. BAER recordings were first made within 15 min of s.c. and intrathecal administration and no changes in the waveforms were noted (data not shown). Figure 4*A* and *C* show the BAER recordings before Trappsol administration in two cats. Figure 4*B* shows the BAER recording 1 week after the administration of HPβCD intrathecally, and Figure 4*D* shows the tracing obtained 1 week after the administration of s.c. HPβCD. The administration of Trappsol to normal cats resulted in changes to the BAER similar to that seen with the Sigma Chemical Co. product. Hearing threshold in two cats treated intrathecally increased from 69 dB to 72 dB before drug administration to 87 dB and 90 dB following administration. Hearing threshold in two cats treated s.c. increased from 69 dB and 72 dB before drug administration, to 87 dB in both cats following drug administration.

DISCUSSION

Niemann-Pick type C disease has an incidence of 1:150,000, with >250 disease-causing mutations identified (5). Natural history studies and therapy trials are difficult to perform on this disorder because of the relatively low incidence and the heterogeneity of disease in human patients. One particular mis-sense mutation represents >20% of mutant alleles and >50% of patients have a juvenile onset of neurologic disease. The feline model of NPC disease has a spontaneously occurring mis-sense mutation in *NPC1* (2864G-C) and exhibits clinical, neuropathological, and biochemical abnormalities similar to those present in juvenile-onset patients. Thus the cat model is homologous to the most common form of disease seen in human patients (10,12–17). The feline model has been useful for identifying the late endosomal/lysosomal accumulation of unesterified cholesterol and gangliosides (GM2 and GM3) (18), for evaluating the association of GM2 storage with meganeurite formation and abnormal dendritogenesis (19), for correlating neuroaxonal dystrophy with neurologic dysfunction (16), and for evaluating efficacy of experimental therapies (13,19). The onset and progression of neurologic dysfunction in the feline model has been well characterized (11,15). A regular onset of progressive cerebellar and vestibular dysfunction occurred in affected cats beginning with intention tremors and ataxia at 6 weeks of age. This dysfunction progressed until cats could no longer maintain sternal recumbency at ~24 wk of age. Changes in hearing threshold were not found although a delay in central conduction time and a decrease in wave V/I amplitude ratio was observed in 16- and 24-wk old affected cats compared with wild type cats (11).

HPβCDs are cyclic oligosaccharides consisting of seven β-(1–4) glucopyranose units (7). HPβCDs have a hydrophilic exterior and a hydrophobic interior making them useful for increasing the aqueous solubility of hydrophobic molecules such as cholesterol, steroids, and vitamins (20). *In vitro* studies using β-cyclodextrins have shown a marked removal of cholesterol from cultured neuronal (21,22) and nonneuronal cell lines (23–25). HPβCDs were shown to cross the blood brain barrier in *in vitro* (25) and in *in vivo* with difficulty (7,26). However, β-cyclodextrins were safely administered intrathecally in rodent studies and used to improve the delivery to the brain of drugs including anesthetic agents, galanin-like peptide, and estradiol (27–29).

Recently, HPβCD was shown to release cholesterol from NPC-deficient lysosomes and allowed unesterified cholesterol to be available to the NPC cell. This resulted in the amelioration of disease and the prolongation of life in the murine model (8,9,30). However, high doses of HPβCD (at least 4000 mg/kg weekly) appeared necessary to retard the progression of neurologic disease. Studies in *npc1*$^{-/-}$ mice showed that 1500 mg/kg HPβCD administered weekly caused a decrease in hepatic unesterified cholesterol concentrations without substantial effect on neurologic signs (7). Increasing the dose to either 4000 mg/kg weekly or every other day delayed clinical disease onset, increased survival time, corrected cholesterol metabolism, and improved biochemical and histologic disease (8,9). Because β-cyclodextrins do not easily penetrate the blood brain barrier (7,25), these studies suggested that parenteral administration of high doses of HPβCD are necessary to get sufficient amounts of HPβCD to cross the blood brain barrier and to have an effect on neurologic disease. Unfortunately, the pharmacokinetics of HPβCD are not well understood particularly in the nervous system. A plasma elimination half-life in rats was 0.4 h and in dogs was 0.8 h, although the concentration in cerebrospinal fluid after systemic administration was not described (1). Serum and cerebrospinal measurements of unlabeled HPβCD are technically difficult to perform, and these concentrations were not determined in the recent murine articles (8,9,30). Clearly, the kinetics of HPβCD in serum and spinal fluid will be necessary to clarify how HPβCDs effect neurologic dysfunction in NPC disease and to determine what dose is most efficacious while also limiting toxicity.

As a result of the dramatic improvement in clinical signs seen in the mouse model of NPC disease, HPβCD was given recent FDA approval for use in a small number of patients with NPC disease. This has increased the urgency to more fully characterize any dose-related potential toxic effects of the drug. In humans, i.v. administration of up to 3 g in healthy volunteers was well tolerated and doses of 16 g per day given with itraconazole did not result in hearing abnormalities (4). The authors could not find examples of doses of 1000 mg/kg and higher being used in human patients, although these are being proposed to treat patients with NPC disease. The authors are aware of no previous

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study examining the effect of HPβCD on auditory function and yet we were able to determine an effect on hearing using a small number of normal cats and cats with NPC disease. Our data show that 1000 mg/kg had no effect on the BAER response when given weekly for 14 doses between the ages of 3 and 16 wk of age. Doses of 4000 mg/kg body weight resulted in an increase in hearing threshold only after repeated dosing and doses of 8000 mg/kg body weight resulted in significant increases in hearing threshold in both normal cats and cats with NPC disease following the administration of a single dose. Interestingly, the doses needed to negatively impact the BAER response were the same dose necessary to retard nervous system dysfunction in mice. Our preliminary data in cats affected with NPC disease suggest a similar requirement for doses equal to or >4000 mg/kg to positively affect neurologic disease (data not shown). One conclusion that suggested itself is that doses of >4000 mg/kg are necessary for HPβCD to cross the blood brain barrier and that the effect on hearing is related to the ability of the drug to enter the CNS. Our data on the effect of intrathecal administration of HPβCD on hearing threshold supported the conclusion that the drug had its negative effect on hearing only after it entered the spinal fluid. Importantly, whether the drug was given s.c. or intrathecally, the negative effect of HPβCD on the auditory system was not ameliorated up to 12 wk after the cessation of drug administration suggesting that the effects may be irreversible.

The increased hearing threshold with no change in central conduction time suggested that the damage from HPβCD occurred in the peripheral auditory pathway (cochlea or eighth nerve) and that potential mechanisms of action for the hearing loss observed include a direct effect on the stria vascularis and its role in maintaining the ionic environment of the inner ear fluid space, the transduction and motility mechanisms of inner and outer hair cells, and/or the excitation patterns in the auditory nerve discharges. Identifying the site of action of HPβCD within the peripheral auditory system will likely be the first step in overcoming toxicity of HPβCD applications. Otoacoustic emission testing would be a useful method for evaluating outer hair cell function in cats but was unavailable for these studies. Histopathology of the cochlea should be performed in the future to identify any pathologic changes.

In summary, hearing impairment following HPβCD administration appeared to be both dose dependent and long lasting and may be a limiting factor in the use of this drug at high doses to treat Niemann-Pick type C disease. Auditory testing is recommended for patients receiving doses of 4000 mg/kg HPβCD or greater to evaluate the effect on hearing threshold in these patients.

Acknowledgments. We acknowledge the critical review of the manuscript by Drs. Shel Steinberg and James Saunders. Trappsol was provided by Dr. Rick Stratton.

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