Summary of research performed for "The Fellowship of the Cats"

To more rigorously evaluate the mechanistic, pharmacologic, and toxicity issues associated with 2-hydroxy-propylbetacyclodextrin (HPßCD) therapy in Niemann-Pick type C disease (NPC), we have utilized the spontaneous feline NPC model which harbors a missense mutation in *NPC1* (pC955S), orthologous to the most common mutation in juvenile-onset patients. Disease progression in this model recapitulates both the neuropathological and biochemical abnormalities observed in this subset of human patients. Grant funding from the NNPDF provided partial salary support for the veterinarian (Dr. Bagel) who is responsible for the daily care of these animals and responsible for administering the HPßCD; funding also supported the breeding of cats for the experimental studies. We have performed the following experiments with the following results:

Determine the minimum dose of intrathecal HPßCD sufficient for ameliorating neurological signs of NPC disease and the minimum dose resulting in altered hearing threshold. Cats with the *NPC1* gene mutation were treated beginning at 3 weeks of age IT at different dose levels (120 mg, 60 mg, 30 mg, 15 mg, 7.5 mg, and 3 mg) of HPβCD administered every 14 days and compared to cats left untreated. All cats dosed with 120 mg once every 2 weeks IT remained clinically normal over 52 weeks in regards to coordination and physical appearance. Specialized staining of brain tissue demonstrated retention of Purkinje cells, and more normal levels of cholesterol and GM2 in the IT treated cats when compared to untreated cats at 24 weeks. Cohorts of cats treated with lower doses of HPβCD continue to be evaluated. Doses of 30 mg IT (7.5 mg/ml CSF) or more every 2 weeks resulted in cats which are clinically normal to at least 24 weeks of age; cats treated at lower doses showed mild neurological deficits at 24 weeks of age. Regarding the hearing deficit, cats dosed at 7.5 mg HPβCD or higher showed elevations in hearing threshold consistent with negative effects on the auditory system, although more significant changes to hearing threshold were found with higher doses. In summary, 30 mg IT HPβCD was determined to be efficacious with only mild to moderate effects on hearing threshold.

Treat ten NPC disease cats with a combination of 120 mg intrathecal HPßCD and 1000 mg/kg SQ HPßCD to evaluate the long-term effects of treating both CNS and hepatic disease on outcome measures. Four cats in this cohort are currently over 2 years of age. A complete serum biochemical profile is obtained monthly to assess hepatic disease (ALT, AST, albumin, and bilirubin). Toxicity is assessed through monthly radiographs, blood gas studies as well as through routine serum biochemical testing of other systemic functions (renal, electrolyte, glucose, etc). There is no evidence of toxicity. However, these cats show gaze palsy and have deficits in swallowing. It appears that in chronically treated cats, some aspect of the NPC phenotype become evident.

NNPDF funding has also allowed us to produce cats for biomarker studies with Dr. Ory. IT HPBCD (30 mg and 120 mg) or saline vehicle was administered to 3-week-old and 16-week-old NPC1 cats (n=3/group), and dosing continued biweekly. Plasma samples were obtained for 24(S)-HC measurements immediately prior to HPBCD dose and at 1, 2, 3 and 7 days post therapy. Plasma 24(S)-HC showed up to a 70% increase, peaking at 2-3 days post therapy and remaining elevated above pre-treatment levels at 7 days. The 24(S) response attenuated with successive doses, suggesting that the cholesterol did not re-accumulate to pre-treatment levels. Both naïve 3- and 16-week-old cats showed similar responses, and no differences were observed between dosing levels. CSF samples were obtained pre-treatment and 3 days post-treatment from the HPBCD-treated cats and triol concentrations measured. While triol concentrations did not change acutely (i.e., at 3 days) in response to therapy, triol concentrations steadily reduced over time in the pre-treatment samples in all treatment groups. These findings indicate that HPBCD is acting to reduce free cholesterol stores in brain tissue, and provide a potential biomarker for monitoring the chronic effects of HPBCD therapy. CSF samples were obtained pre-treatment and 3 days post-treatment from the HPBCD-treated cats and CE species quantified. Three days post-therapy, CE species were increased 2.1-5.7-fold (16:0, 2.2-fold; 18:0, 5.7-fold; 18:1, 2.1-fold; 18:2, 3.3-fold, 20:4, 2.8-fold) as compared to baseline, providing compelling evidence that increased cholesterol esterification in the brain tissue resulting from HP-β-CD exposure is reflected in lipid secretion (likely lipoproteins) into the CSF.

Peripheral nervous system disease, not expected to be influenced by IT therapy, was evaluated in NPC cats. We investigated peripheral nerves in the naturally occurring feline model of NPC disease. Electrodiagnostic studies revealed significantly slowed motor and sensory nerve conduction velocities in affected cats in the absence of altered M-wave amplitude. Histologic and ultrastructural analyses showed thin myelin sheaths, membranous debris, myelin figures, lipid vacuolization of Schwann cell cytoplasm, and expanded paranodal areas. Axonal degeneration was not identified. There was a shift to small myelinated fibers in affected cats, and there were significant decreases in fiber diameter, axon diameter, and myelin thickness. These changes were similar to those described in the murine NPC disease model and in rare patients in whom nerve biopsy has been performed. These results were published in J Neuropathology and Experimental Neurology in 2013. Characterization of the demyelinating neuropathy is necessary for evaluating clinical trials that target only the CNS aspects of NPC.

Lay summary.

The only colony of NPC cats in the world exists at the School of Veterinary Medicine of the University of Pennsylvania. The disease in cats is very similar to the disease in humans with similar clinical, chemical, and microscopic abnormalities; it is thought to be a more accurate model of the disease than is the mouse model. We have been treating affected cats with several doses of HPβCD into the spinal fluid and have determined that 30 mg (7.5 mg/ml CSF) intrathecally given every two weeks is sufficient to result in substantial amelioration of neurological disease with only mild-moderate negative effects on hearing. While treated cats are normal at 1 year of age (untreated cats die by 6 months of age), we have determined that long-term therapy of NPC cats (for over two years) results in no toxicity, however, some signs of NPC disease begin to manifest in older cats. Also, in collaboration with Dr. Dan Ory, we are evaluating the accuracy of plasma and CSF biomarkers to judge whether the dose we are giving is adequately treating disease or whether the dose should be increased. These studies are continuing. Finally, we have evaluated electrodiagnostic biomarkers of peripheral nerve disease. Disease of nerves occur with NPC disease but does not result in clinically significant signs of disease. However, as patients live longer, signs of nerve dysfunction may occur. We have studied peripheral nerve disease in untreated cats with NPC disease and are in the process of evaluating the efficacy of therapy on nerves.