Progress Report
Project Title: Influence of a ketogenic diet and a novel eliglustat tartrate CCG222628 on brain GM2 storage, behavior, and survival in mice with Sandhoff Disease
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Research Objective

To determine if delivery of the novel eliglustat tartrate CCG222628 **(**3f) to the brain, liver, and heart of *Hexβ* +/- mice is greater when the mice are administered 3f together with a restricted ketogenic diet than when administered with a standard high carbohydrate diet.

Progress

The progress to date involves the increase of the Sandhoff mouse colony that will be used to collect the tissue samples for the proposed research and for completion of preliminary data. We present our most recent preliminary data showing the effect of the novel eliglustat tartrate CCG222628 **(**3f) on the total brain gangliosides, GM2 content, and GA2 content in the heterozygous normal *Hexβ* +/- mice and in the knockout *Hexβ* -/- mice (**Table 1**).

The mice were injected daily with either phosphate buffered saline (PBS controls) or with 3f from postnatal day 2 (p-2) to either p-21 or p-60. Although tissue samples have been collected from the control and 3f-treated *Hexβ* -/- mice from p-2 to p-60, we have not yet isolated or purified the lipids from these samples. Consequently, these data are not yet added to the table, but will be included over the next phase of the grant period.

Table 1. Influence of Ketogenic Diet and CCG-222628 (3f) on Ganglioside Concentration in Sandhoff Mice[a]

					Ganglioside Sialic Acid (µg/100 mg Dry Weight)				Neutral Lipid (ug/100 mg Dry Weight)	
Treatment	Genotype	Age (Days)	Dosage (mg/kg Body Weight)	n[b]	Total	Reduction (%)	GM2	Reduction (%)	GA2	Reduction (%)
PBS	+/-	21	-	2	528	-	1.9	-	-	-
PBS	-/-	21	-	4	563 ± 5	-	58.3 ± 0.8	-	271 ± 0.07	-
3f	-/-	21	30, 15, 5	3	489 ± 13**	13	30.1 ± 2.5**	48	134 ± 0.03**	51
PBS	+/-	60	-	4		-		-		
PBS	-/-	60	-	2		-		-		
3f	-/-	60	30, 15, 5	3						
KD-R + 3f	-/-	60								

* Asterisks indicate that the value is significantly different from the -/- control group at *P<0.05 **P<0.01

[a] Mice treated intraperitoneally(IP) 1x/day from p-2 to final age

[b] n, the number of independent samples per group

[c] Mice treated at 30 mg/kg/day from p-2 to p-7, 15 mg/kg/day on p-8, and 5 mg/kg/day from p-9 to p-21

The data show that **3f** caused significant reductions in total brain ganglioside content (13%) the content of GM2 (48%), and the content of GA2 (51%) in the treated *Hexβ* -/- mice relative to the PBS controls. No major

adverse effects on viability, body weight, brain weight, or brain water content were found in the mice at the dosages used. However, we needed to reduce the 3f dosage from 30 mg to 15 mg, and finally to 5mg/kg in order to maintain a normal body weight in the treated mice (Table 1). We are the first research group to evaluate the therapeutic potential of this new small molecule inhibitor as a SRT for Sandhoff disease.

Our plan now is to determine if the calorie restricted ketogenic diet (KD-R) can enhance the therapeutic action of 3f in the brain, heart, and liver of *Hexβ* -/- mice compared to the *Hexβ* -/- mice that receive the standard high carbohydrate diet. This information will be added to the last row in Table 1 after we collect the data. We will also determine if the amount of 3f is greater in the tissues of the 3f-treated *Hexβ* -/- mice than in the tissues of the control mice. Finally, we will evaluate the delivery of 3f to the *Hexβ* -/- mice using the Alzet pump technology. The Alzet pump, implanted subcutaneously, is designed to deliver drug continuously to the mice for a 30-day period. This delivery system avoids the stress of daily intraperitoneal injections and can put a constant stream of drug on target for longer periods than can daily ip injections. We will compare and contrast the therapeutic efficacy of 3f following daily ip injections or delivery through the Alzet pump.

Goals for next phase of the study

1. Complete evaluation of tissue ganglioside content for the 60-day old control and 3f-treated *Hexβ* -/- mice.
2. Evaluate the tissue content of 3f in the treated mice.
3. Compare the influence of 3f on tissue gangliosides following administration using ip injection and drug delivery through the Alzet pump.
4. Begin treatment of mice using calorie restricted ketogenic diet.