One Year Follow-up on Chaperone Therapy for Two Siblings with Adult Tay-Sachs Disease

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ABSTRACT

Background: Adult Tay-Sachs Disease (ATSD) is caused by inadequate β-hexosaminidase-A (HexA) activity resulting in GM2 ganglioside accumulation. Individuals with ATSD develop ataxia and dysarthria by early teenage years and later lose ability to walk. No therapy has yet been shown clinically effective. The most prevalent ATSD mutation, αG269S, does not impact HexA enzyme function but rather causes post-translation misfolding and reduced enzyme stability. In-vitro studies have demonstrated that pyrimethamine (PYR) improves αG269S mutated HexA stability and transport to lysosomes. (Ref 1)

Case Report: Two brothers confirmed with ATSD and the αG269S mutation had been taking a substrate reduction therapy, miglustat (200mg, tid), for >4 years, but at age 24 and 25 years, showed accelerated decline in coordination and leg muscle strength resulting in inability to rise unassisted from a sitting position or climb stairs. PYR (75mg qd) at dinner was begun in combination with folic acid (5mg, qd). Repeat leukocyte HexA assays and hematology tests (CBC) were conducted. A prior short-term case study of the older brother reported leukocyte HexA activity increased (~3X) for up to 8 weeks following start of daily PYR. (Ref 2)

Results: Additional follow-up on the older brother showed decline of leukocyte HexA to baseline values after 13 weeks. The younger brother also showed an increase (~2X) in leukocyte HexA activity, but repeatedly encountered anorexia and vomiting after being on PYR for >4 weeks. Hematologic results remained normal for both. PYR (75mg, qd) was subsequently cycled 3 weeks ON followed by 3 weeks OFF for both siblings. Additional follow-up results are presented.

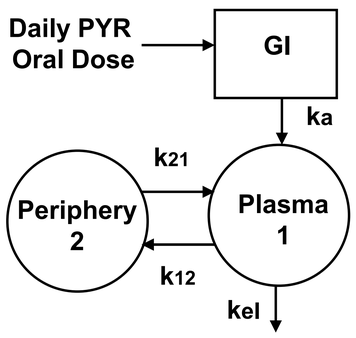
PYRIMETHAMINE

Pyrimethamine (PYR) was selected as a potential pharmacological chaperone drug for ADTS patients based on prior cellular studies showing improved stability of αG269S mutated HexA and increased transport to the lysosomes. (Ref 1) A dosage of 75 mg qd was based on the pharmacokinetic model (below) and selected to achieve a steady state "periphery" PYR concentration of 10 μM. PYR was given in combination with folic or folinic acid (5mg qd) to offset partial dihydrofolate reductase inhibition reported to be caused by PYR.

Pharmacokinetic Model: The PK model is based on the Weidekamm et al study (Ref 3) with repeat measurements taken on 14 normal adults (60 to 85 kg) each taking a single dose of 25 mg PYR (mean ± SD):

- Elimination half life (95.5 ± 30.6hr)
- Time to max concentration (4.2 ± 2.7 hr)
- Area under curve (19.1 ± 5.6 mg*hr / L)
- Volume of central compartment (75.9 ± 28.6 L)

Classical Two Compartment PK Model:



Derived First Order Rate Constants

k_a	0.693 /hr
k_{12}	0.071 /hr
k_{21}	0.065 /hr
k_{el}	0.016 /hr

PK Model Results:

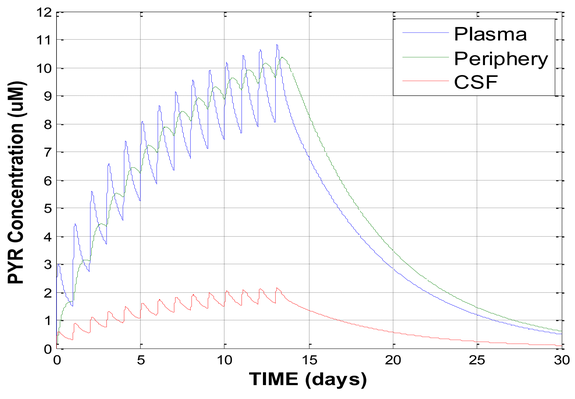


Figure 1. [PYR] simulated dose of 75 mg QD for two weeks. Time to steady state (~ 2 wks), steady state plasma concentration, and half-life (96 hrs) match published studies (Ref 3 & 4). The CSF/Plasma ratio is modeled at 20% (for reference only).

OLDER BROTHER WITH ATSD

Patient History: At age 25, a male confirmed with ATSD (alpha mutations: +1IVS12 (G>C) and G269S) showed accelerated decline in coordination and leg muscle strength, verified by EMG evaluations. The patient lost the ability to climb stairs and required assistance to maintain balance while walking. The patient had a history of periodic psychiatric events requiring hospitalization on the average of once per year since the age of 18.

Drug Regimen: PYR 25mg qd was begun for 2 weeks followed by 75mg qd for 8 weeks and then stopped to verify baseline HexA levels. PYR was again stopped in week 38 after observing a downturn in HexA level. PYR (75mg, qd) was subsequently cycled 3 weeks ON followed by 3 weeks OFF. Miglustat (200mg, tid) was stopped at week 45. Psychiatric medications were managed in the follow-up period as deemed appropriate by the patient's psychiatrist.

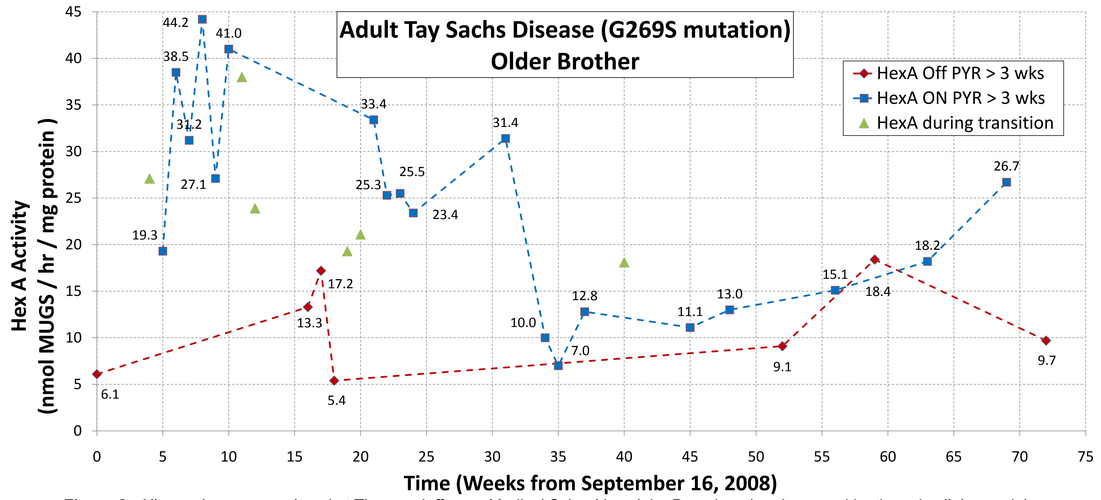


Figure 2. All samples were analyzed at Thomas Jefferson Medical School Lysolab. Based on the pharmacokinetic and cellular models (Ref 2), steady state response to PYR changes were assumed to require approximately three weeks. HexA activity is plotted showing samples taken after being on PYR (75 mg qd) for at lease three weeks ("ON") or samples taken after no PYR was taken for at least the previous 3 weeks ("OFF").

YOUNGER BROTHER WITH ATSD

Patient History: Similar to his older brother, at age 24, the younger brother showed accelerated decline in coordination and leg muscle strength. The younger brother also had a history of periodic psychiatric events requiring hospitalization. Psychiatric medications were managed though the follow-up period as deemed appropriate by the patient's psychiatrist.

Drug Regimen: PYR 75mg qd started at week 19 and stopped at week 31 after exhibiting anorexia and severe vomiting. The illness resolved within 4 days. PYR 75 mg qd was started again at week 36 but terminated at week 42 after again showing the same condition. PYR (75mg, qd) was subsequently cycled 3 weeks ON followed by 3 weeks OFF. Miglustat was stopped at week 45.

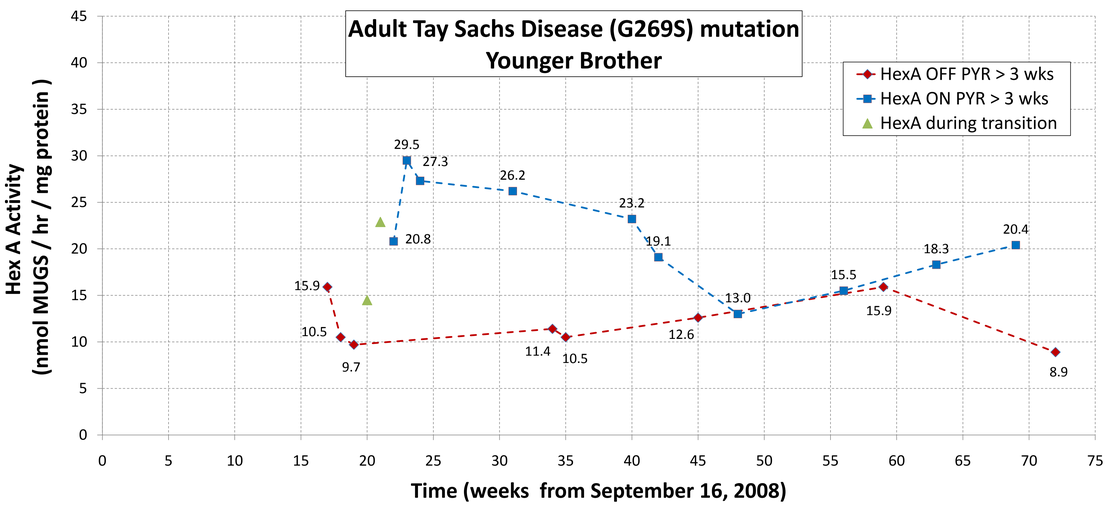
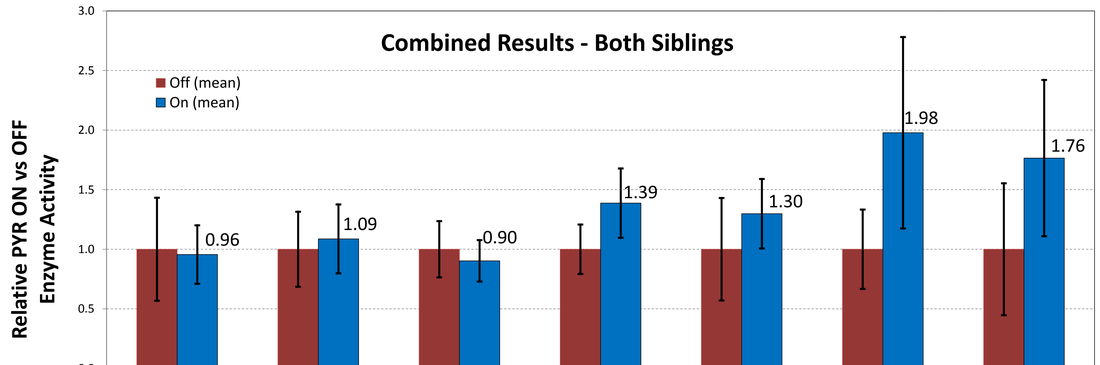


Figure 3. The definition of "ON" and "OFF" are the same as in Figure 2.

COMBINED RESULTS OF BOTH SIBLINGS



	Beta-galactosidase 4MU-Beta-D-galactoside Leukocytes	Beta-galactosidase 4MU-Beta-D-galactoside Plasma	Hex Total MUG Plasma	Hex Total MUG Leukocytes	Hex Total / Beta-gal Leukocytes	Hex A MUGS Leukocytes	Hex A / Beta-gal Leukocytes
Off (mean)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
On (mean)	0.96	1.09	0.90	1.39	1.30	1.98	1.76

Figure 4. Enzyme activity is shown comparing the average for blood samples taken after being on PYR at 75 mg/day for at lease three weeks ("ON") to the average of the samples taken when no PYR was taken for at least the previous three weeks ("OFF"). The samples used in the analysis for HexA are noted in Figures 2 and 3. Beta-galactosidase is shown as a reference. (Error bars are +/- 1 standard deviation of samples.)

RESULTS SUMMARY

Combined Count	*ON PYR* 29	*OFF PYR* 15
Hex A (nmol MUGS / hr / mg protein)		
Mean	23.02	11.64
Standard Error	1.74	1.00
Standard Deviation	9.35	3.88
Anova: Single Factor Analysis	*P-value < 0.0001*	

- HexA activity towards 4-methylumbelliferyl N-acetylglucosamine 6-sulfate (MUGS) increased when PYR (75 mg qd) was taken for more than 3 weeks ("ON") compared to when no PYR had been taken for at least 3 weeks ("OFF").
- HexA levels ON PYR declined for both siblings between week 34 (May 12, 2009) and week 56 (Oct 13, 2009).
- Other observations made following the initiation of PYR medication and throughout the remaining course of follow-up are below:
  - Neither sibling had significant changes in physical ability or coordination.
  - Neither sibling had a major psychiatric event requiring hospitalization.
  - The younger brother exhibited anorexia and vomiting after taking PYR (75 mg qd) for 6 to 8 weeks. Illness resolved within 4 days of discontinuing PYR. No other side effects were observed.
  - Marginally low % lymphocyte levels were measured in both siblings at baseline and throughout the follow-up period. Other blood cell count / differentials and serum folic acid remained within the normal range.

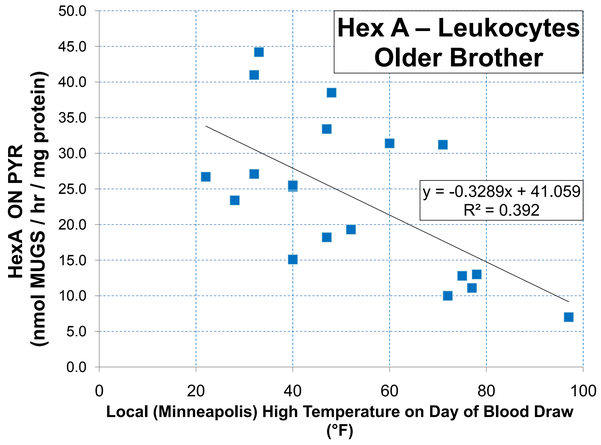


Figure 5: The relationship between HexA activity (MUGS) and the local high temperature (Ref 7) on the day of blood samples shipment.

DISCUSSION TOPICS

- The HexA αG269S protein is known to be unstable with a short half-life (Ref 5). Measurement of this enzyme level may require special processes and handling to obtain reliable results. Warm temperatures or delays during transport may affect measurement of this HexA. See Figure 5.
- While it has been hypothesized that increasing enzyme levels to 10% of normal may be sufficient to prevent ganglioside storage (Ref 6), an in-silico model has projected a possible long-term decline in HexA activity accompanying an effective chaperone therapy (Ref 2).
- While lack of additional significant decline in physical ability or coordination is encouraging for these two siblings, a biomarker specific to ganglioside storage levels within the CNS would be helpful in assessing efficacy and optimizing dose.
- Long-term clinical studies are required to establish PYR efficacy.

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