CASE REPORT OF CHAPERONE THERAPY FOR ADULT TAY-SACHS DISEASE AND COMPARISON TO AN IN-SILICO PHARMACOKINETIC AND CELLULAR SIMULATION

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ABSTRACT

Background: Individuals with Adult Tay-Sachs Disease (ATSD) develop ataxia and dysarthria by early teenage years and later lose ability to walk. No therapy has yet been shown clinically effective. ATSD is caused by inadequate β-hexosaminidase-A (HexA) activity and GM2 ganglioside accumulation in neuronal lysosomes. The most prevalent ATSD mutation, αG269S, is not believed to impact HexA enzyme function but instead cause post-translation misfolding and reduced enzyme stability. Others have shown in vitro that pyrimethamine (PYR) is a potential pharmacological chaperone (PC) therapy for improving stability of G269S mutated HexA and increasing its transport to the lysosomes.

Model Description: Computer simulations were done to estimate PYR pharmacokinetics and GM2 response using three interlinked models: a pharmacokinetics model estimated neuronal PYR concentrations; an endoplasmic reticulum model predicted the relative amount of HexA transported to lysosomes; and a lysosomal model simulated GM2 degradation and HexA inhibition caused by PYR. The simulations also modulated HexA half-life as a function of accumulated GM2 substrate. Previously published parameter values were used when available. (Ref 5)

Case Report: A male confirmed with ATSD and the G269S mutation, had been taking a substrate reduction therapy, miglustat (200mg, tid), for greater than four years, but at age 25 showed accelerated decline in coordination and leg muscle strength, verified by EMG evaluations. At the request of the patient and parents, PYR 25mg QD was begun for two weeks followed by 75mg QD in combination with folic acid (5mg QD) to offset partial PRY dihydrofolate reductase inhibition. Weekly leukocyte HexA assays and monthly hematology tests (CBC) were conducted.

Results: No noticeable side-effects were observed during the first four weeks of treatment. Additional follow-up and comparisons to the modeled response will be presented.

MODEL OVERVIEW

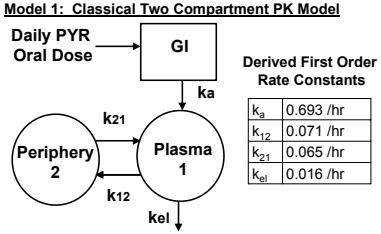
No animal models of ATSD exist. The use of pyrimethamine (PYR) as a potential pharmacological chaperone therapy for ADTS patients was therefore simulated using MATLAB® software. Simulations were based on three computer models. The models used 19 differential equations solved with the MATLAB "ode45" function.

PHARMACOKINETIC MODEL

The PK model is based on the Weidekamm et al study (Ref 8) 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)

Model 1: 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

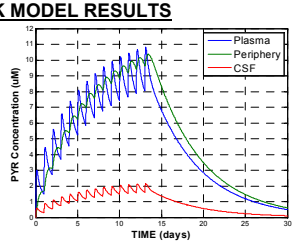
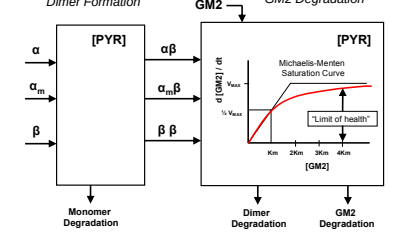


Figure 1. [PYR] simulated dose of 75 mg QD for two weeks. Time to reach steady state (~ two wks), steady state plasma concentration, and half-life (96 hrs) match published studies (Ref 4). The CSF/Plasma ratio is modeled at 20% (for reference only).

CELLULAR MODELS



Constant	Value	Units	Description
Km	46	μM	Michaelis Constant for Hex A ~ GM2
Ki	13	μM	Hex A ~ PYR Inhibition Constant
k_{CAT}	0.9	hr^{-1}	Hex A ~ GM2 Turnover Number
k_{GM2}	1.8	μM/hr	Influx of GM2 into LYS (see below)
$k_{\alpha\beta\text{-LYSd}}$	f * ln(2)/125	hr^{-1}	αβ degradation rate constant in LYS
$k_{\alpha m\beta\text{-LYSd}}$	f * ln(2)/8	hr^{-1}	$\alpha_m\beta$ degradation rate constant in LYS
$k_{\beta\beta\text{-LYSd}}$	ln(2)/200	hr^{-1}	ββ degradation rate constant in LYS
[PYR]	--	μM	Calculated peripheral [PYR]

ER Model Assumptions:

- The α, α_m (ATSD mutation), and β HexA monomers are translated at the same rate for each viable allele. For ATSD example, k_α= 0 cal, $k_{\alpha m}$= 1 x cal, and k_β= 2 x cal, with "cal" being a calibration constant.
- The α and β monomers are 10x more stable than the α_m monomers and 2x more stable than the α_m~PYR complex.
- The second order rate constants for αβ, $\alpha_m\beta$, and ββ dimer formation are set to the same value. Note that no αα (HexS) dimers are formed.

Lysosome Model Assumptions:

- HexA enzyme activity level at 10% of normal is sufficient to maintain health and avoid substrate accumulation ≥ 4 x Km. (Ref 3 & 9)
- At this "limit of health", lysosome volume is guessed to be 10% of total tissue volume and is held constant in this model.
- The short half-life of mutated HexA ($\alpha_m\beta$) is a major factor in ATSD severity. The Hex A (αβ, $\alpha_m\beta$) dimers have a half-life 125 and 8 hrs, respectively (similar to Ref 2 & 1). HexA is modeled as only degrading when in the "free" state (i.e., no PYR or GM2 in active site).

  "f" is the free to total enzyme concentration ratio:

  $$f = [E] / [ET] = ([ET] - [ES] - [EI]) / [ET]$$

  where: [E] ~ free enzyme concentration,
  [ET] ~ total concentration of enzyme,
  [ES] ~ enzyme-substrate complex concentration, and
  [EI] ~ enzyme-inhibitor complex concentration.

Model Calculations:

- Calculated GM2 entering lysosomes (assumed to be in complex with GM2 activator protein) is based on GM2 concentration within normal brain tissue at 20 nmol/g wet weight of tissue (Ref 6), half-life of GM2 for normal fibroblasts at 78 hrs (Ref 7), and all degradation occurring in lysosomes:

$$\therefore k_{GM2} \approx 20\,\mu M * \frac{\ln 2}{78hr} * \frac{1}{10\%} = 1.8\ \frac{\mu M\ GM2}{hr}$$

- At the "limit of health", the GM2 entering lysosomes is equal to the amount of GM2 being degraded.

$$\therefore k_{GM2} = \frac{V_{MAX} * [S]}{Km + [S]} \quad (\text{where } [S] = 4 * Km)$$

$$V_{MAX} \approx 2.2\ \frac{\mu M\ GM2}{hr}$$

and

$$[\alpha\beta]_{LYS} \geq \frac{V_{MAX}}{k_{CAT}} = 2.5\,\mu M$$

If this represents 10% of normal enzyme concentration, then the normal enzyme concentration is 25 μM.

- The models use repeated parallel calculations of 19 ordinary differential equations during each simulated day to estimate the molecular concentrations in each compartment. As an example, the calculation for the changes in lysosomal GM2 concentration in solution (without prior GM2 storage) is shown below. The equation accounts for the inhibition by PYR:

$$\frac{d[GM2]_{LYS}}{dt} = k_{GM2} - \frac{k_{CAT} * ([\alpha\beta]_{LYS} + [\alpha_m\beta]_{LYS}) * [GM2]_{LYS}}{[GM2]_{LYS} + Km * (1 + \frac{[PYR]}{Ki})}$$

CELLULAR SIMULATION RESULTS

Model Calibration:
A single constant calibration factor, "cal", sets the translation rate of α, α_m, and β HexA monomers. The factor was chosen such that, for normal individuals with no PYR present, the steady state lysosomal concentration of HexA is equal to the above calculated value of 25μM.

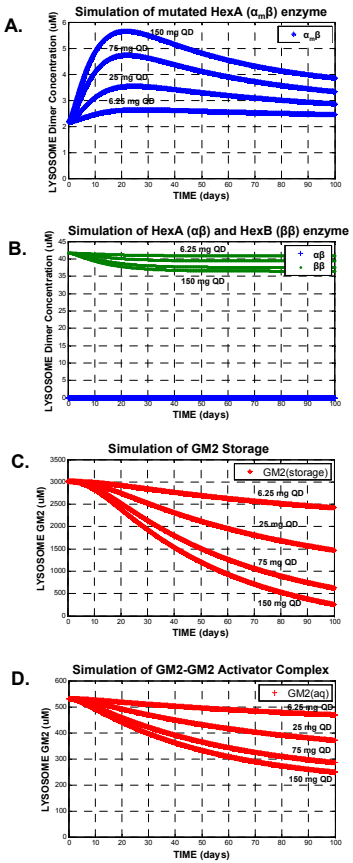


Figure 2. Simulated ATSD Patient – Shown are the results from the model simulations of an ATSD patient (i.e., translation rates of kα= 0, $k\alpha_m$= 1cal, and kβ= 2cal, with "cal" being a calibration constant). The simulations were run with four different PYR dose levels (6.25, 25, 75, and 150 mg QD) given continuously for 100 days. The initial values are the steady state values obtained from a two-year simulation with no PYR dose. **(A)** Simulation of mutated HexA ($\alpha_m\beta$) concentration in lysosomes. **(B)** Simulation of lysosomal HexA (αβ) and HexB (ββ). **(C)** Simulation of GM2 storage (aggregation). **(D)** Simulation of the GM2-GM2 activator complex concentration in lysosomes.

MODEL LIMITATIONS

- Although the PK plasma [PYR] closely matches the results found in published studies, the actual [PYR] availability for chaperone therapy within CNS lysosomes is not known.
- The simulations are based on classical PK and Michaelis-Menton theory and may be overly simplified.
- The GM2-GM2 activator complex "limit of health" concentration (4*Km) and the GM2-GM2 activator complex lysosomal solubility (5*Km) were based on the hypothetical example of Conzelmann and Sandhoff (Ref 3 & 9).
- A number of unknown parameter values were based on "best guess". No sensitivity analysis was done on these guesses.
- Parameter values were extracted from a number of different published studies, and thereby add to the inaccuracies.

CASE STUDY OF PYRIMETHAMINE IN AN ATSD PATIENT

Patient History:

A male confirmed with ATSD (alpha mutations: +1IVS12 (G>C) and G269S) had been taking a substrate reduction therapy, miglustat, for greater than four years, but at age 25 showed accelerated decline in coordination and leg muscle strength, verified by EMG evaluations. The patient lost the ability to climb stairs and required the use aids 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:

At the request of the patient and parents, pyrimethamine (PYR) 25mg QD was begun for two weeks followed by 75mg QD for eight weeks. Folic acid (5mg QD) was initiated with the first dose of PYR to offset partial dihydrofolate reductase inhibition caused by PYR. Psychiatric medications, miglustat (200mg, tid), and acetylcysteine (10 ml, bid) were continued without change. Weekly Hex enzyme assays and monthly hematologic tests (CBC) were conducted. The 75 mg QD was again initiated after being off PYR for eight weeks.

CASE STUDY RESULTS

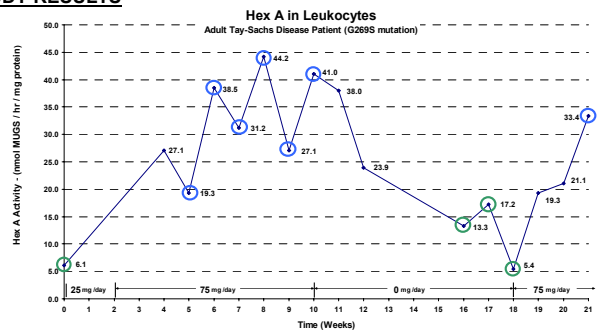
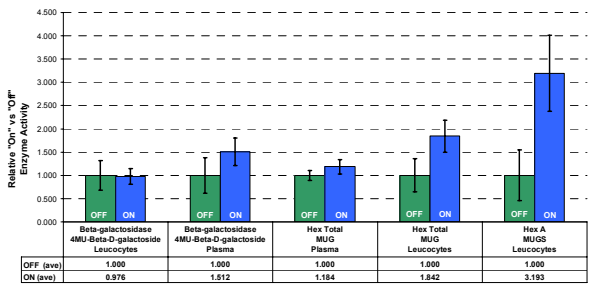


Figure 3. Blood samples were analyzed weekly (Thomas Jefferson University) except following the initiation of the study and for a period after the end of the first dosing period. Based on the pharmacokinetic and cellular models, it was hypothesized that the response to initiation or termination of the PYR would require approximately three weeks. The HexA values used in the analysis in Figure 4 are shown with a green ("OFF") and blue ("ON") circles.



	Beta-galactosidase 4MU-Beta-D-galactoside Leucocytes	Beta-galactosidase 4MU-Beta-D-galactoside Plasma	Hex Total MUG Plasma	Hex Total MUG Leucocytes	Hex A MUGS Leucocytes
OFF (ave)	1.000	1.000	1.000	1.000	1.000
ON (ave)	0.976	1.512	1.184	1.842	3.193

Figure 4. Enzyme activity are 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 Figure 3. The beta-galactosidase is shown as reference. (Error bars are +/- 1 standard deviation of samples.)

MODEL DISCUSSION

"Essentially, all models are wrong, but some are useful."
George E.P. Box (1987)

- The model incorporated an algorithm by which HexA is stabilized by both PYR and GM2. With this, reductions in [GM2] would also decrease the effective half-life of ADTS HexA and may thereby also limit chaperone therapy effectiveness.
- This simulated HexA half-life reduction suggests that measuring GM2 level may be required to assess long-term clinical efficacy.
- The in-silico models provide a means of creating hypotheses on the mechanisms and responses to PC therapy but should not be otherwise used until validated by clinical or experimental studies.

CASE STUDY DISCUSSION

- No noticeable side-effects were observed at any time, hematologic (CBC and differential) results remained in the normal range, and no neurological changes were observed.
- While the model anticipated a slight decrease in HexB with PYR, the patient's HexB tended to increase. This may imply that PYR has a stabilizing effect on the Hex A beta subunit.
- Hex A enzyme data from this case are encouraging, but large variations were observed week-to-week.
- Observation of clinical improvements in long-term studies or in proven CNS neuronal biomarkers of HexA or GM2 are required to establish PYR efficacy.

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