Pharmacological chaperone-mediated reduction of glucosylsphingosine in a Gaucher mouse model

Clark SW[1], Pellegrino L[1], Dungan L[1], Hamler R[1], Sanders A[2], Kaspieva O[2], Clarke L[2]

[1] *Amicus Therapeutics, Cranbury, NJ, USA*
[2] *The Child and Family Research Institute, University of British Columbia, BC, Canada*

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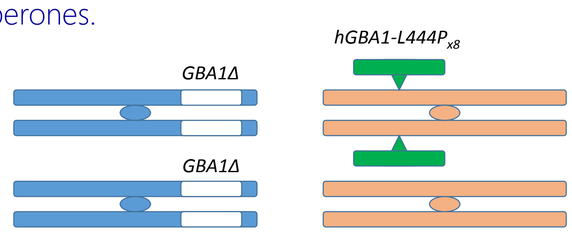
Introduction

Pharmacological chaperones (PCs) are highly specific small molecules that bind and stabilize their target protein, leading to increased enzyme activity in the proper cellular compartment. For diseases such as Gaucher, reduction of enzyme activity and concomitant elevation of the enzyme substrate is often due to a change in the protein arising from a single missense mutation. The enzyme affected in Gaucher disease, glucocerebrosidase (GCase), may be misfolded and/or unstable, resulting in degradation by the ER quality control system and reduced trafficking to its lysosomal site of action. Decreased GCase activity leads to accumulation of its substrates, glucosylceramide and glucosylsphingosine (GlcSph). Mouse models expressing the human L444P or N370S variants of GCase have been shown to accumulate GlcSph. We orally administered PCs specific for GCase to this model for up to two months, substantially elevating the tissue activity of both L444P and N370S GCase. A range of administration regimens and doses were explored that decreased the accumulation of GlcSph in several disease-relevant tissues, with reductions of more than 50%, 40%, and 30% seen in spleen, liver, and bone marrow, respectively. Taken together, these data suggest that orally-administered PCs may provide an alternative to ERT to reduce accumulated substrate in Gaucher disease.

Gaucher Mouse Models and PCs

These studies were conducted in a Gaucher mouse model (Saunders *et al.*, 2013) in which 4 copies of human N370S or 8 copies of human L444P are present as hemizygous transgenes on a homozygous *GBA1* null background. While these mice do not show overt accumulation of GlcCer until 1 year of age, GlcSph accumulation is present by 10 weeks of age in liver, spleen, and bone marrow. GlcSph is a poor substrate for the GCase enzyme and is thus a very sensitive marker of net activation or inhibition of GCase by pharmacological chaperones.



Two pharmacological chaperones were utilized in this study, AT2101 (isofagomine), and AT3375. Both are potent stabilizers of GCase, with AT3375 having higher specificity for GCase, greater bioavailability, improved PK, a 10-fold lower EC_{50}, and a lower pK_a.

Study 01 Design and Objectives

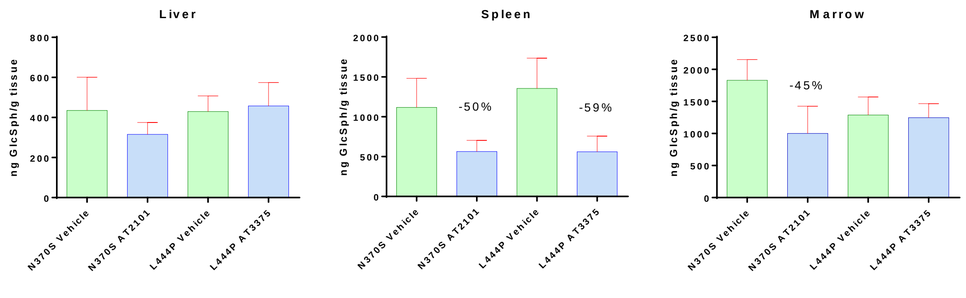
DESIGN

- 1-month drinking water study in 10-week-old mice
- 7 mice of mixed gender per group
- Groups
  - N370S with vehicle (water)
  - N370S AT2101 daily with 10-fold dose escalation per week
  - L444P with vehicle (water)
  - L444P AT3375 daily with 10-fold dose escalation per week
- Tissues harvested after a 24-hour washout

OBJECTIVES

- Demonstrate engagement of the GCase target in relevant tissues through modulation of sphingolipid levels

Study 01 Outcomes



Using an ascending dose of either AT2101 or AT3375 over a 28-day period of administration, there were no significant changes in liver GlcSph levels. Conversely, in spleen there were significant reductions in GlcSph of > 50% with both AT2101 and AT3375. Moreover, AT2101 produced a significant 45% reduction in GlcSph in the marrow.

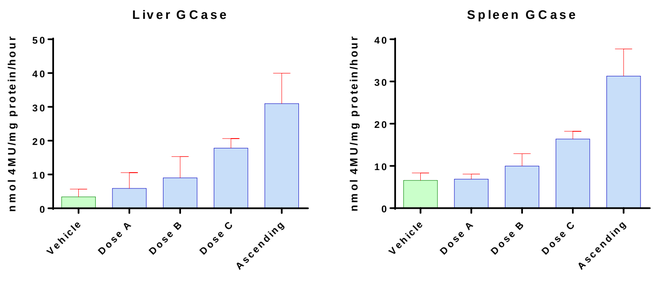
Study 02 Design and Objectives

DESIGN

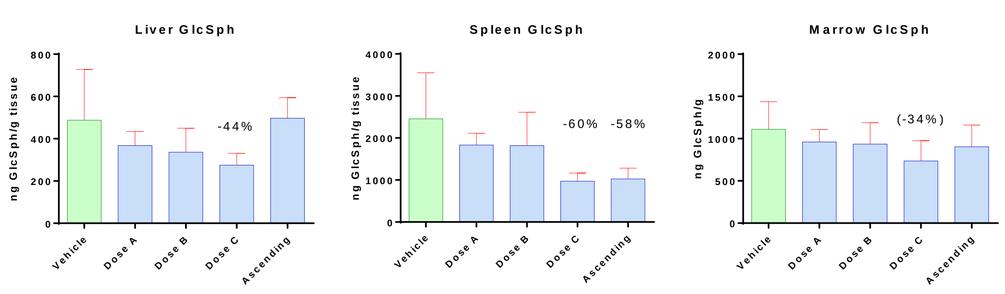
- 1-month drinking water study in 10-week-old mice
- 7 mice of mixed gender per group
- Groups
  - L444P with vehicle (water)
  - L444P AT3375 daily doses A - C
  - L444P AT3375 daily with 10-fold dose escalation per week
- Tissues taken after a 24-hour washout

OBJECTIVES

- Replicate the key findings of Study 01, and evaluate daily, fixed doses



Study 02 Outcomes



The ascending dose regimen of Study 01 was re-evaluated for AT3375 and was found to replicate the previous results; i.e., a significant 58% reduction of spleen GlcSph with no significant reduction of GlcSph in the liver or marrow. These findings were then extended to evaluate fixed-dose, daily administration of AT3375. GCase activity in liver and spleen were significantly elevated for both Dose C and the ascending dose regimen, with a trend that followed increasing AT3375 dose (marrow GCase activity was not determined due to limited sample). Study 02 extended the prior findings by identifying a fixed dose of AT3375 (Dose C) that significantly reduced GlcSph in spleen 60%, liver 44%, with a trend towards GlcSph reduction (34%) in marrow.

Study 03 Design and Objectives

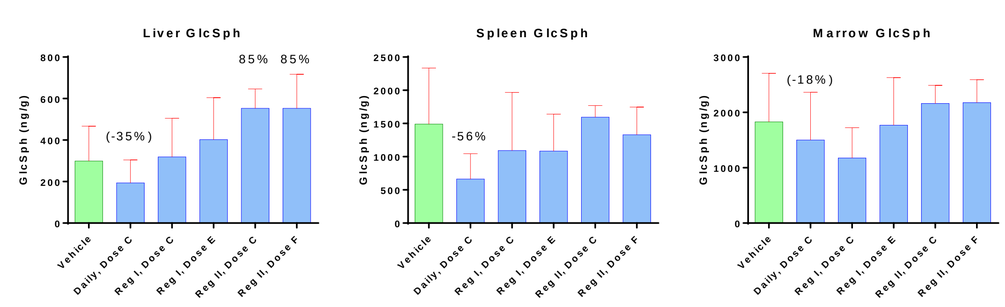
DESIGN

- 2-month drinking water study in 10-week-old mice
- 7 mice of mixed gender per group
- Groups
  - L444P with vehicle (water)
  - L444P AT3375 daily dose C
  - L444P AT3375 intermittent regimen I with dose C or E
  - L444P AT3375 intermittent regimen II with dose C or F
- Tissues taken after a 24-hour washout

OBJECTIVES

- Replicate the key findings of Study 02 and evaluate intermittent dosing

Study 03 Outcomes



The AT3375 daily administration of fixed dose, C, in Study 02 was re-evaluated in Study 03 for a two-month administration and was found to replicate the previous results; i.e., a significant reduction of GlcSph in spleen (56%) and a trend towards reduction of GlcSph in the liver (35%) and marrow (18%). These findings were then extended to evaluate intermittent administration of AT3375. GCase activity in liver and spleen were elevated with the fixed dose, C, as in Study 02; however, greater variation between individual mice precluded significance for a similar magnitude change. No intermittent regimen of administration of AT3375 significantly reduced GlcSph levels in liver, spleen, or marrow; however, intermittent regimen II significantly increased liver GlcSph levels 85% for both doses C and F. The extension of the administration period from one to two months increased the variation within groups, reducing the power of the study to detect significant changes. Future studies will employ gavage administration to avoid the intake variation inherent in drinking water administration.

Conclusions

- The pharmacological chaperone, AT3375, was evaluated in a Gaucher mouse model that accumulates GlcSph in multiple tissues by 10 weeks of age
- Across three independent studies, oral administration of AT3375 effected a consistent reduction of GlcSph levels in spleen by more than 50%
- AT3375 achieved similar reductions of GlcSph in the liver and marrow

Conflict of Interest

SW Clark, L Pellegrino, L Dungan, and R Hamler are full-time employees of Amicus Therapeutics