The small molecule aldehyde trap NS2 represents a novel pharmacological approach for the treatment of SSADH deficiency.

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Introduction

- Succinic semialdehyde dehydrogenase (SSADH; ALDH5A1) deficiency (SSADHD; MIM 271980) is the most prevalent inherited disorder of 4-aminobutyrate (GABA) degradation.[1]
- Accumulation of the enzyme substrate, succinic semialdehyde (SSA), is associated with elevations in both GABA and the related neuromodulator, γ-hydroxybutyrate (GHB)(Figure 1).
- SSADHD is accompanied by elevations to a number of other potentially toxic metabolites, including the aldehyde 4-hydroxy-2E-nonenal that may contribute to the SSADHD phenotype (seizure, speech deficits and developmental delays).
- NS2 is an aldehyde-trapping molecule under clinical development, proven to form conjugates with aldehydes.[2]

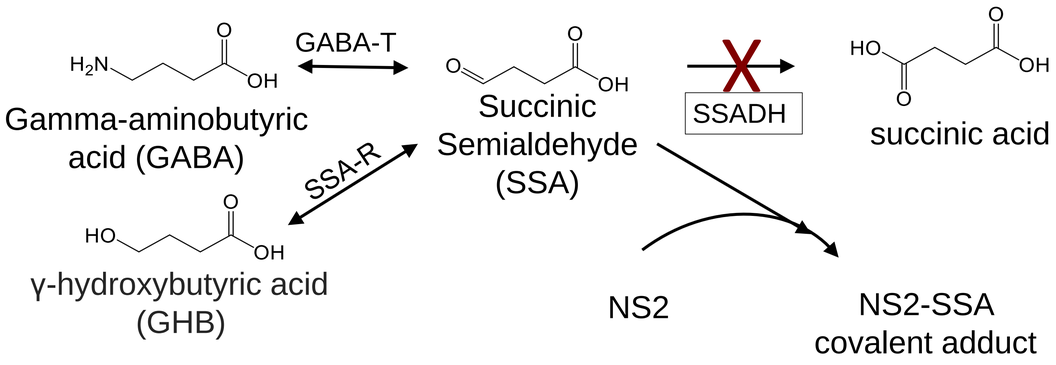

Figure 1. Metabolism of GABA proceeds via conversion to SSA by GABA transaminase (GABA-T). SSA may be metabolized by succinic semialdehyde dehydrogenase (SSADH) to form succinic acid, or by succinic semialdehyde reductase (SSA-R) to form γ-hydroxy-butyric acid (GHB). Detoxification of SSA results in formation of succinic acid, which enters the Krebs cycle. In SSADH-deficient patients, GABA, GHB and SSA accumulate to supernormal concentrations.

- NS2, is hypothesized to conjugate and diminish the effects of excess SSA.

Objective

The primary goal of this work is (1) to determine if the aldehyde-trapping agent, NS2, efficiently conjugates with succinic semialdehyde, and (2) obtain preliminary pharmacokinetic information about NS2 and its SSA conjugate.

Methods

Synthesis and LC/MS/MS optimization of the NS2-SSA adduct (in situ)

- NS2 (100 µl, 100 mM in methanol) and SSA (15% w/v in water) solutions were added to 9.4 ml of 95% water:5% acetonitrile, followed by the addition of 400 µl of 0.1 N HCl. Solutions were incubated at room temperature.
- Incubations were diluted 1000-fold then infused onto an ABI Sciex 6500 quadrupole ion trap mass spectrometer operated in positive ion mode, to obtain MRM parameters.

Treatment of wild type mouse hepatocytes with NS2

- Cells were harvested and freshly plated on Matrigel coated plates, allowed to attached, then treated with NS2 (100 µM) or vehicle (24 h). Cell media and pellets were collected separately and quantified for NS2 and NS2-SSA.

Pharmacokinetic study in wild type mice

- Mice (B57, aged 41-46 days) were administered vehicle (PBS and <1% DMSO) or NS2 (10 mg/kg, 100 µl, diluted in PBS), i.p. Mice (n=3) were sacrificed at predetermined timepoints following NS2 treatment; serum, liver, brain, and kidney were collected.

Acute NS2 exposure in wild type (wt) and mutant mice (mt, aldh5a1-/-)

- Mice (wild type or mutant, n=6-7) were randomized to receive NS2 (10 mg/kg) or vehicle.
- Animals were sacrificed 8 hours following treatment, and their tissues were harvested.

Acknowledgments/Conflict of interest

Garrett Ainslie is funded in part by the generous support from the SSADH Association (www.ssadh.net). The investigators received financial support from Aldeyra Therapeutics.

References

1. Pearl et al. (2014) Inherited disorders of gamma-aminobutyric acid metabolism and advances in ALDH5A1 mutation identification. *Developmental Medicine and Child Neurology*, December 29, 2014:
2. www.clinicaltrials.gov (NCT02402309)

Results

1. *In situ* and *in vitro* formation of the NS2-SSA conjugate

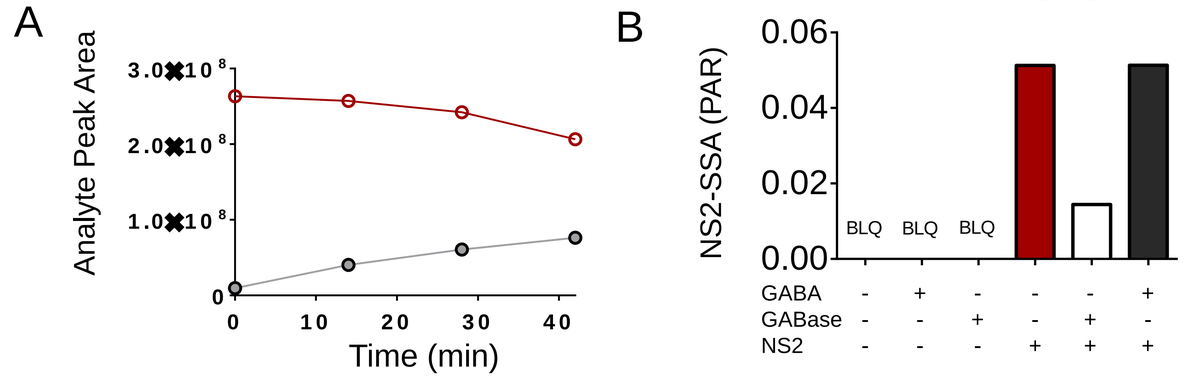

Figure 2. NS2 (crimson) depletion and NS2-SSA conjugate formation (grey) versus time, incubated at room temperature in acetonitrile/water (5:95) (A). NS2-SSA conjugate formation in plated hepatocytes from wild type mice (24 h, 100 µM NS2) with or without supplemental GABA or GABase (B). Bars denote the mean of duplicate incubations .PAR, peak area ratio.

2. NS2 and NS2-SSA formation pharmacokinetics *in vivo*.

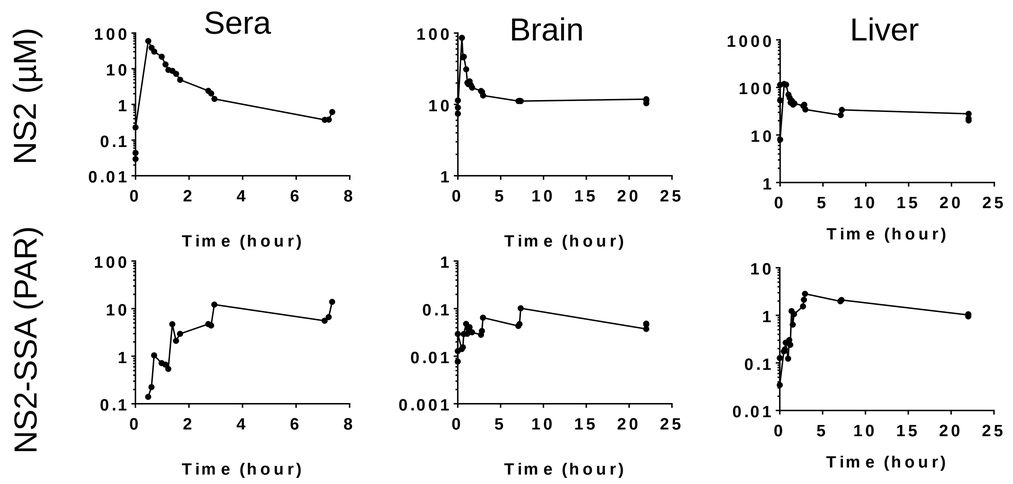

Figure 3. NS2 (top) and NS2-SSA (bottom) measured in wild type mouse sera (left), brain (middle), and liver (right) following i.p. administration of NS2 (10 mg/kg). Each data point represents the mean of triplicate analyses for an individual subject (CV%<5% for each point).
PAR = peak area ratio

3. NS2-SSA formation in aldh5a1-/- and wild type mice.

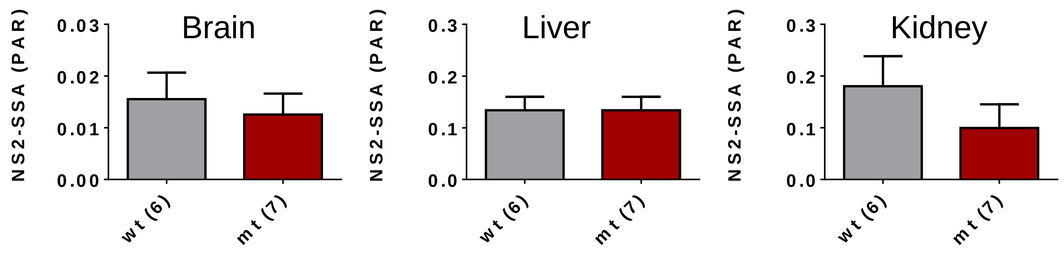

Figure 4. NS2-SSA in brain (left), liver (middle), and kidney (right) 8 h after an NS2 dose (10 mg/kg, i.p.) to wild type (grey) or mutant (crimson) mice. Parentheticals denote the number of biological replicates. Bars denote the mean and standard deviation.

Conclusions/Future Directions

- NS2 readily forms a stable conjugate with SSA *in situ*, *in vitro* and *in vivo* (both wt and aldh5a1-/- mice).
- Preliminary mouse pharmacokinetic data suggest that NS2 is rapidly absorbed and distributed among tissues, and the NS2-SSA conjugate is formed rapidly.
- The metabolic fate of the NS2-SSA adduct remains to be evaluated.
- A 5-day dosing study in aldh5a1-/- mice to examine the effects of NS2 on key GABA pathway metabolites is underway.