The Novel Aldehyde Trap ADX-102 Reduces Accumulations of GHB and GABA in Brain Tissue from Succinic Semialdehyde Dehydrogenase-Deficient Mice

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INTRODUCTION

Succinic semialdehyde dehydrogenase (SSADH; ALDH5A1) deficiency (SSADHD; MIM 271980) is an inherited disorder, associated with intellectual disability, seizures, ataxia, sleep disturbances, autism-like behavior, and anxiety. Loss of function of SSADH, which metabolizes succinic semialdehyde (SSA) to succinate, results in accumulation of gamma-hydroxybutyrate (GHB) and 4-amino butyrate (GABA) in fluids and tissues.

ADX-102 is a novel small molecule that forms covalent adducts with aldehydes, and has been shown to form stable conjugates with SSA *in vitro* and *in vivo*[1]. Thus, it is hypothesized that depletion of SSA by covalent sequestration with ADX-102 may result in reduced levels of GHB and GABA in SSADHD.

OBJECTIVE

Determine if ADX-102 treatment of SSADH mice reduces levels of GHB and GABA in plasma and brain tissue, and demonstrate the potential for aldehyde trapping in the treatment of SSADHD.

METHODS

Mice: SSADH-deficient (null) mice (B6.129-Aldh5a1$^{tm1Kmg/J}$) were generated by crossing mice heterozygous for SSADH deficiency.

In vivo treatment: Null mice and wild type (wt) littermates were given 50 mg/kg ADX-102 or vehicle, intraperitoneally (IP), once or twice daily from birth for 3.5, 7 or 15 days. After treatment, brains and plasma were harvested for analysis.

Ex vivo treatment: Following *in vivo* treatment with ADX-102 or vehicle for 3.5 days, brains were removed and divided sagittally. The left hemisphere was processed for assay of GHB and GABA. The right hemisphere was split coronally; the anterior portion was sliced into 0.5 mm sections, which were incubated in artificial cerebrospinal fluid (aCSF) containing ADX-102 (422 µM); the posterior portion was also sliced into 0.5 mm sections but was incubated in aCSF containing vehicle. All sections were incubated for 24 hours prior to assaying for GHB and GABA.

PK study: Wt mice were treated IP, once (qd) or twice daily (bid), with 30 mg/kg or 100 mg/kg ADX-102 for up to 30 days.

Analyses: GHB and GABA in plasma and brain tissue were measured by LC/MS/MS. ADX-102 in plasma and tissue of wt mice was measured by LC/MS/MS.

RESULTS

	Day 0			Day 1	Day 8	Day 15	Day 15/30
	null mice			ADX-102 (µM); wt mice			
	GHB (µM)	GABA (µM)	GHB + GABA (µM)	C_{max}	C_{max}	C_{max}	Trough
brain	361 ± 60	3678 ± 264	4039	ND	ND	ND	5 (qd) 11 (bid)
plasma	407 ± 124	48 ± 27	455	27 (qd) 34 (bid)	18 (qd) 35 (bid)	15 (qd) 15 (bid)	ND

Table 1: Concentrations of GHB and GABA in brain and plasma of null mice at birth, compared to interpolated ADX-102 concentrations in plasma and brain of wt mice after IP dosing 50 mg/kg, qd or bid (ND = no data).

		GHB (µM)	GABA (µM)
CSF	normal	< 3	<12
	SSADHD	116 - 1110	13.6 – 22.4
plasma	normal	< 3	0.126
	SSADHD	26 – 600	-

Table 2: Plasma and CSF levels of GHB and GABA[2,3] in humans with and without SSADHD.

REFERENCES

[1]Ainslie *et al.*, The small molecule aldehyde trap NS2 represents a pharmacological approach to enzyme replacement therapy for SSADHD (PgmNr 477/T), 65th annual ASHG meeting, 2015.
[2]Malaspina *et al.*, Neurochem Int. 99:72-84, 2016.
[3]Pearl *et al.*, Gene Reviews 2016.

RESULTS (continued)

At birth, GHB and GABA levels in brain and plasma of null mice were significantly higher than in wt mice and increased over time (Figure 1, Table 1). Levels of GHB and GABA in humans are shown in Table 2. The mouse phenotype was severe, with some null mice dying by post-natal Day 11. At the doses used, ADX-102 concentrations of up to 35 µM and 11 µM were achieved in plasma and brain, respectively (Table 1), in wt mice. At Day 7, plasma GHB and GABA levels were reduced numerically, but not statistically, in ADX-102 treated mice; plasma GABA levels were also reduced numerically but not statistically in ADX-102 treated mice at Day 3.5 (Figure 2). ADX-102 exposure to brain was maximized by incubating brain slices directly with ADX-102. *Ex vivo* ADX-102 treatment resulted in statistically significant reductions in GHB and GABA (Figure 3).

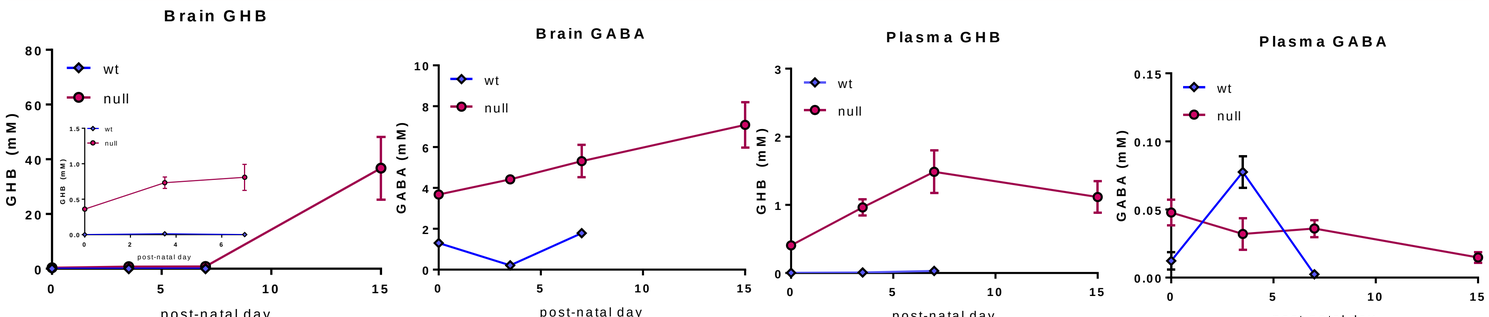

Figure 1: At birth, brain and plasma levels of GHB and GABA in null mice were significantly higher than in wt mice, and increased over time, with the possible exception of plasma GABA. Data represent mean ± SEM.

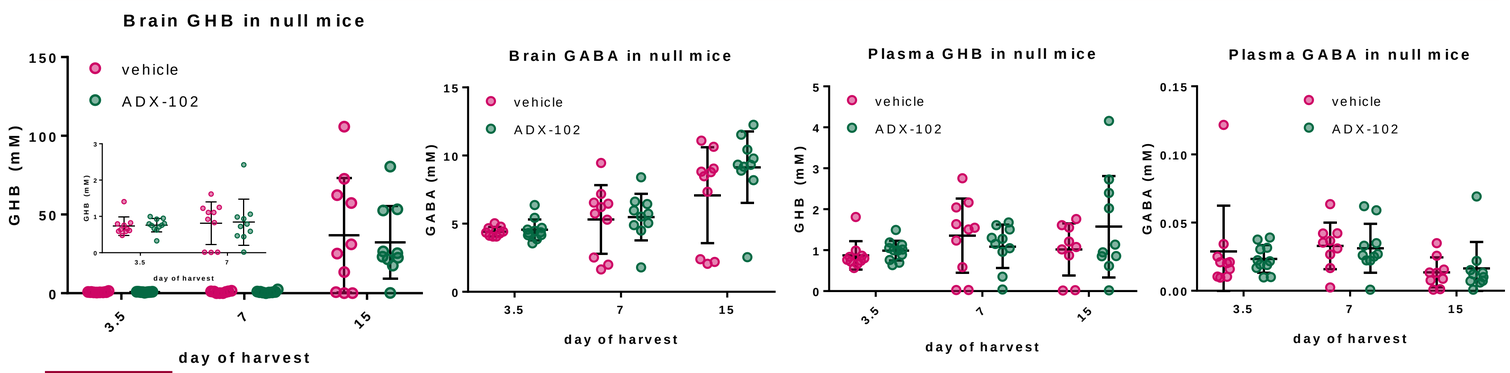

Figure 2: ADX-102 treatment of null mice resulted in small numerical, but not statistical, decreases in plasma GHB (Days 3.5 and 7) and GABA (Day 7) compared to vehicle. Mice treated for 3.5 days received 50 mg/kg ADX-102 IP bid, and those treated for 7 and 15 days received 50 mg/kg ADX-102 IP qd.

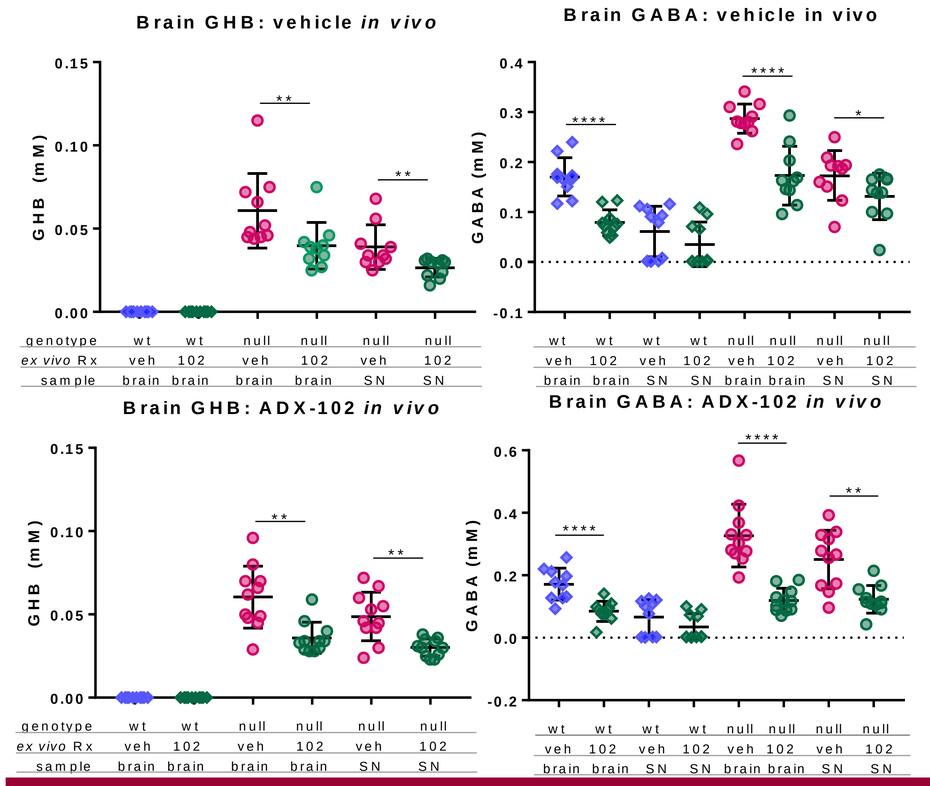

Figure 3: *Ex vivo* treatment of brain slices from null mice with ADX-102, resulted in statistically significant decreases of GHB and GABA in brain tissue, and in the incubation medium (SN). Mice were treated for 3.5 days with vehicle or ADX-102 (50 mg/kg, bid). Brains were then removed and sliced into 0.5 mm sections. Slices were treated *ex vivo* with vehicle or 422 µM ADX-102 for 24 hours. *p < 0.05; **p < 0.01; ****p < 0.0001

SUMMARY/CONCLUSIONS

ADX-102 can reduce GHB and GABA levels in SSADH-deficient mouse brain by covalent sequestration of SSA. Although the SSADHD mouse model is a severe representation of the human disease, the *ex vivo* demonstration that ADX-102 treatment can significantly reduce GHB and GABA levels in mouse brain, suggests that ADX-102 holds potential for treating SSDADH in humans. *In vivo* treatment of null mice with ADX-102 was not able to significantly reduce GHB and GABA levels, which is likely due to sub-stoichiometric levels of ADX-102 (at least 25-fold lower on Day 7), compared to GHB and GABA. There did appear to be a trend towards decreased GHB and GABA in plasma by Day 7 of treatment, however by Day 15, as GHB and GABA levels increased, ADX-102 activity was not detected. Sub-stoichiometric concentrations of ADX-102 in brain may, in part, have been a result of rapid peripheral adduction of plasma GHB and GABA by ADX-102, preventing active ADX-102 from reaching the brain. Early and persistent intervention with ADX-102 may be critical for effective treatment of SSADHD in humans.