Pharmacokinetics associated with an efficacious dose of trehalose, an autophagy enhancing disaccharide in development as a treatment of Parkinson's disease.

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Objective

- Trehalose (JNX3001), a disaccharide and known autophagy enhancer, is efficacious in providing neuroprotective benefit in several rodent models of Parkinson's disease when administered in the drinking water *ad libitum* (Sarkar et al., 2014, Wu et al., 2015, Ferguson et al., 2015, Tanji et al., 2015, He et al., 2016).
- Whilst demonstrating the potential of trehalose, these studies neither define a dosing regime nor target trehalose exposures that would aid the design of clinical trials.
- We present evidence that trehalose retains its efficaciousness when administered as a single, daily oral dose in a rat AAV α-synuclein model of Parkinson's disease.
- We also present plasma and brain levels of trehalose associated with efficacy in the rat and thus define target plasma and brain trehalose levels for future preclinical and clinical studies.

Methods

- Twenty-one female Sprague-Dawley rats were split into 3 groups (N=5-8 rats/group) and received a unilateral injection of AAV1/2 that expresses A53T α-synuclein (AAV1/2 αSyn) or empty vector (control) into the substantia nigra.
- Commencing on the day of surgery and continuing for 6 weeks, rats received either vehicle (sterile water) or trehalose (2.67 g/kg/day) by oral gavage.
- Behaviour was assessed at week 6 in the cylinder test to assess forelimb asymmetry with asymmetry indicating an imbalance in striatal dopaminergic function between the side injected with AAV1/2 αSYN and the contralateral side.
- After 6 weeks, the rats were killed and striatal tissue from both hemispheres were analysed for dopamine.
- In a separate study, 100 Sprague-Dawley rats received trehalose (2.67 g/kg/day) by oral gavage for 1 or 7 days. On days 1 and 7, groups of 5 rats were sacrificed at pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 8 and 12 h post-dose and plasma and brain samples collected.
- Plasma and brain samples were analysed for trehalose levels using a validated LC-MS/MS method and pharmacokinetic parameters calculated.

Results

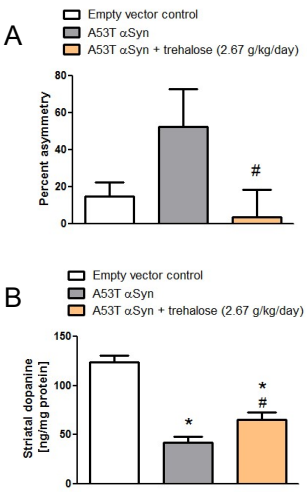
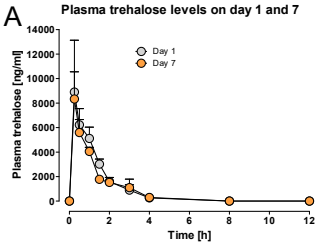
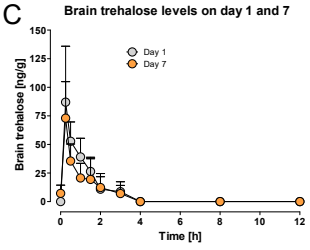


Figure 1. Effect of trehalose in the AAV1/2 αSyn rat model of Parkinson's disease. Daily administration of trehalose (2.67 g/kg/day, *p.o.*) for 6 weeks significantly reduced forelimb asymmetry measured in a cylinder test (**A**). Post-mortem analysis of striatal dopamine demonstrated that trehalose significantly reduced the AAV1/2 αSyn induced loss of dopamine (**B**). Data is mean ± s.e.mean, N=5-8 rats/group. *=P<0.05 vs. EV control, #=P<0.05 vs. A53T aSYN. One-way ANOVA followed by Fisher's LSD post-hoc test.



B

Parameter	Day 1	Day 7
T_{max} (h)	0.25	0.25
C_{max} (ng/ml)	8900	8336
$AUC_{0\text{-}12\ h}$ (h.ng/ml)	10851	9494
$AUC_{0\text{-}inf}$ (h.ng/ml)	11136	9876
$t_{1/2}$ (h)	0.76	0.89



D

Parameter	Day 1	Day 7
T_{max} (h)	0.25	0.25
C_{max} (ng/g)	87.0	73.0
$AUC_{0\text{-}12\ h}$ (h.ng/g)	86.8	65.3
$AUC_{0\text{-}inf}$ (h.ng/g)	97.0	76.2
$t_{1/2}$ (h)	0.81	1.06

Figure 2. Plasma (**A**) and brain (**C**) trehalose timecourse following 1 and 7 days oral administration of trehalose (2.67 g/kg/day). Data expressed as mean ± s.e.mean, N=5 rats/timepoint. Pharmacokinetic parameters of trehalose in the plasma (**B**) and brain (**D**) show that brain levels of trehalose were approximately 1% of plasma levels. The half-life of trehalose in both the plasma and brain was short (<1 h) and no trehalose was detected in either plasma or brain 8 h post-dose. No accumulation of trehalose occurred between days 1 and 7.

Summary

- Trehalose has previously been shown to be efficacious in protecting dopaminergic transmission in animal models of Parkinson's disease when administered *ad libitum* in the drinking water.
- We have demonstrated that the efficacy of trehalose is preserved when trehalose is administered as a single daily dose rather than in the drinking water.
  - Trehalose significantly reduced AAV1/2 αSyn induced forelimb asymmetry.
  - Trehalose significantly reduced AAV1/2 αSyn induced loss of striatal dopamine.
- We have determined the pharmacokinetics of trehalose that are associated with efficacy and have thus defined target exposure levels for future preclinical and clinical studies.
  - Plasma C_{max} and $AUC_{0\text{-}inf}$ were approximately 9,000 ng/ml and 11,000 h.ng/ml respectively.
  - Brain C_{max} and $AUC_{0\text{-}inf}$ were approximately 90 ng/g and 100 h.ng/g respectively.
- Together, these data demonstrate a route of administration that is amenable to take forward into clinical development (single, daily administration) and trehalose exposures, associated with preclinical efficacy, to target in future studies.

References

- Sarkar et al., (2014). Neurotoxicology **44**, 956-960.
- Wu et al., (2015). Neuroscience **284**, 900-911.
- Ferguson et al., (2015). Behav Brain Res **292**, 68-78.
- Tanji et al.,(2016) Biochem Biophys Res Comm **465**, 746-752.
- He et al., (2016). Mol Neurobiol. **53**, 2258-2268.

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