WIDESPREAD BRAIN DISTRIBUTION OF SGSH ENZYME IN CANINE BRAIN FOLLOWING WHITE MATTER INJECTION OF LYS-SAF302, AN AAVRH.10-SGSH VECTOR

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INTRODUCTION

No disease modifying treatment is currently available for Mucopolysaccharidosis type IIIA (MPS IIIA), a predominantly neurological lysosomal storage disease caused by an autosomal recessive defect in the N-sulfoglucosamine sulfohydrolase (SGSH) gene causing progressive neurodegeneration.

The safety and tolerability of intraparenchymal injections of AAV serotype rh.10 carrying the human SGSH cDNA has been demonstrated in a Phase 1/2 clinical study in 4 children with MPSIIIA. A Phase 2/3 multi-center clinical study is planned with LYS-SAF302, a more potent second-generation AAVrh.10-SGSH vector.

The purpose of the present study was to evaluate the SmartFlow® cannula (MRI Interventions, Inc.) for intraparenchymal injection of the clinical candidate, LYS-SAF302.

METHODS

LYS-SAF302, an AAVrh10 viral vector coding for the human SGSH gene, was co-administered with gadolinium (0,125 mmol/ml) in white fiber tracts of beagle dogs. One or two injections of 200µl to 500µl (at 5µl/min or 10µl/min) were performed per hemisphere leading to a total dose ranging from 6E11vg to 2E12vg per animal.

MRI was used for gadolinium distribution analysis immediately post-injection. One month after treatment, brains were sectioned in the coronal plane at 4 mm and numbered.

The even number slabs were placed in sterile petri dishes and 8 mm biopsy punches were immediately taken and frozen for qPCR and enzyme activity analysis.

RESULTS

MRI showed consistent patterns of distribution using the SmartFlow® cannula with no evidence of reflux up the needle track (Figure 1). The mean ratio of volume of distribution/ volume injected was 3,1 +/- 0,5 (n=19 hemispheres).

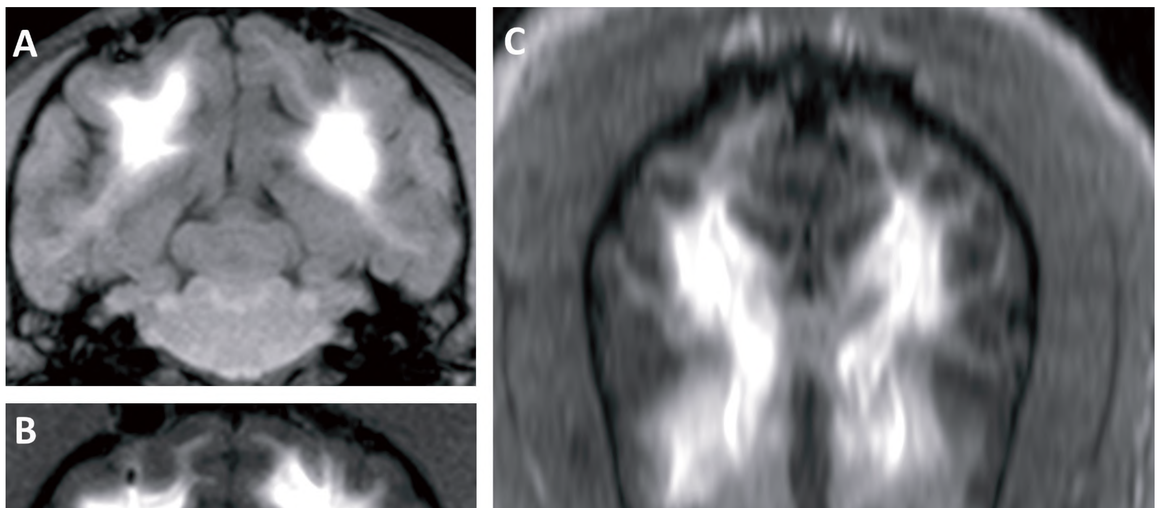

Figure 1: Gadolinium distribution: Coronal caudal (A), coronal rostral (B) and horizontal (C) MRI images following the injection of 500µl in two sites per hemisphere showing gadolinium spread along the rostro-caudal axis.

Each brain punch with a mean qPCR value > 0.1 vector copy/cell shows increased SGSH activity over endogenous levels.

Furthermore, SGSH enzyme activity was found in brain punches with low levels of vector (qPCR < 0.1 vector copy/cell) supporting secretion and spread of enzyme beyond transfected cells (Figure 2).

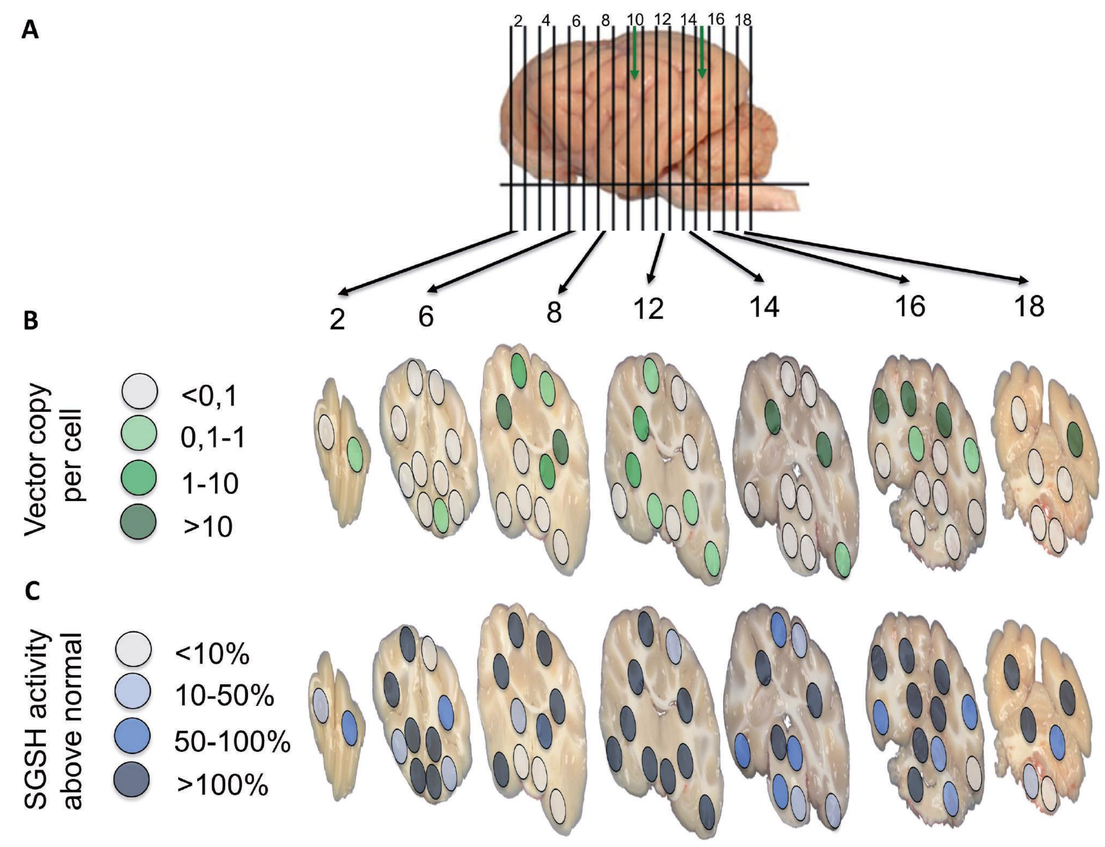

Figure 2: Vector and enzyme activity biodistribution. (A) Representation of brain slicing of a dog injected with 500µl in two sites per hemisphere (green arrows) for a total dose of 2E12 vector genomes. (B) qPCR analysis were performed using PCR TaqMan method using primers and probe specific of the transgene and results (green dots) are expressed as mean vector copy per cell. (C) Enzyme activity analysis were performed using the 2 steps method resulting in the release of measurable 4-MU fluorescent substance. Results (blue dots) were normalized as % of endogenous activity determined as mean value of 80 punches from 2 PBS injected hemispheres of 2 distinct dogs.

10% enzymatic activity increase in the brain of MPS IIIA has been shown to be sufficient to correct abnormal behavior. We have mapped the enzyme activity distribution observed in a dog brain following a single injection of 500µl of LYS-SAF302 at 1E12vg/ml per hemisphere onto a child's brain. This shows that 3 injections per hemisphere would be sufficient to cover the rostro caudal length of a child's brain (Figure 3).

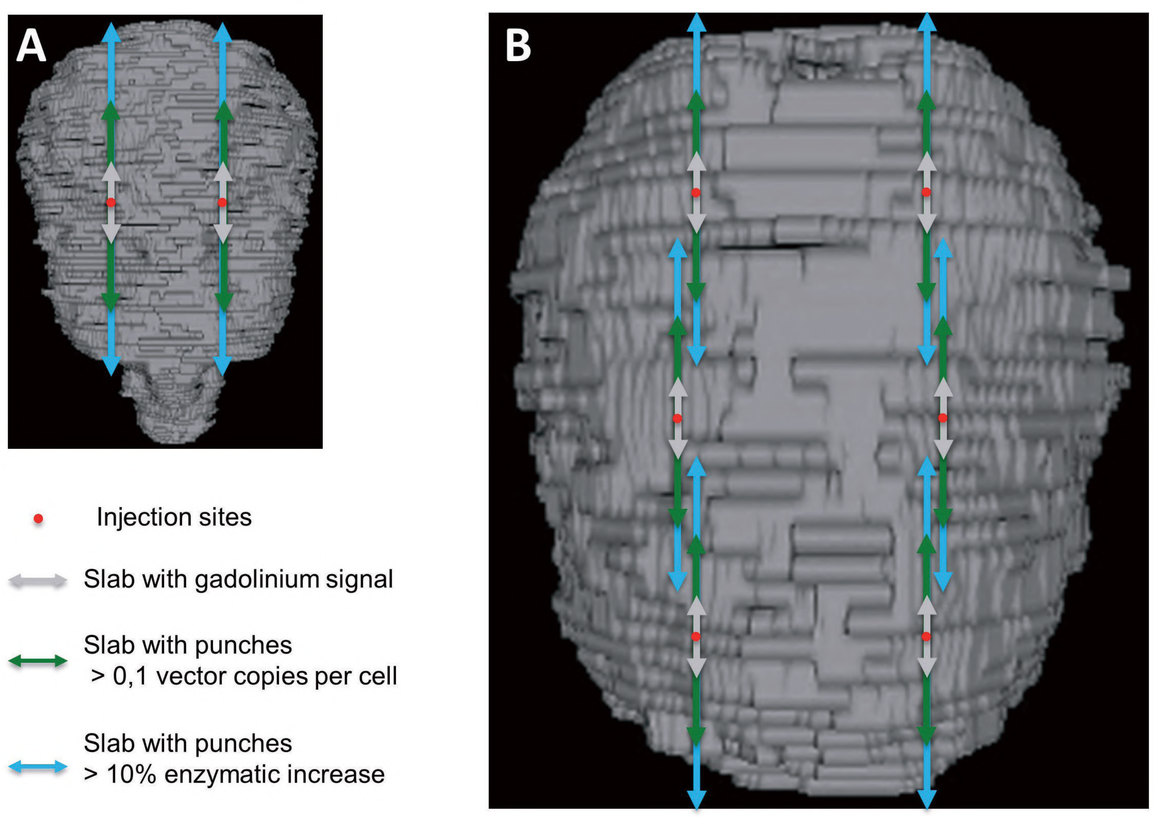

Figure 3: Rostro-Caudal spread of gadolinium, vector and SGSH activity in dog brain mapped onto child's brain. (A) Schematic representation of a 65cm3 brain from a dog injected with 500µl of LYS-SAF302 per hemisphere (total dose = 1E12 vector genomes). Slabs with positive gadolinium signal are represented with grey arrows, slabs containing punches with more than 0,1 vector copies per cell are represented with green arrows and slabs containing punches with an increase higher than 10% of SGSH activity are presented with blue arrows. (B) Schematic representation of a 1003 cm3 brain from a 6 year old MPSIIIA child with biodistribution of gadolinium, vector and enzyme observed in dog mapped onto.

CONCLUSIONS

Widespread brain distribution of both vector and SGSH enzyme activity was found following injections of LYS-SAF302 into dog white fiber tracts. This study supports the use of this route of administration in the upcoming clinical trial of LYS-SAF302 in children affected with MPSIIIA.

ACKNOWLEDGEMENTS

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