Non-Clinical Evaluation of a Blood-Brain Barrier-Penetrable N-sulfoglucosamine Sulfohydrolase in a Mouse Model of Mucopolysaccharidosis IIIA.

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Introduction

- Mucopolysaccharidosis type IIIA (MPS IIIA, also known as Sanfilippo syndrome type A) is a severe and progressive disorder that affects the central nervous system (CNS). The disease is caused by genetic defect of N-sulfoglucosamine sulfohydrolase (SGSH) gene, leading to accumulation of glycosaminoglycan heparan sulfate (HS) throughout the body, followed by severe neurological symptoms and mild skeletal deformities.
- Here we report the development of JR-441, a fusion protein consisting of anti-human transferrin receptor antibody (named J-Brain Cargo®) and rhSGSH (Figure 1). JR-441 has potential to cross the BBB, utilizing receptor-mediated transcytosis of transferrin, to reach the brain parenchyma.(Figure 2).

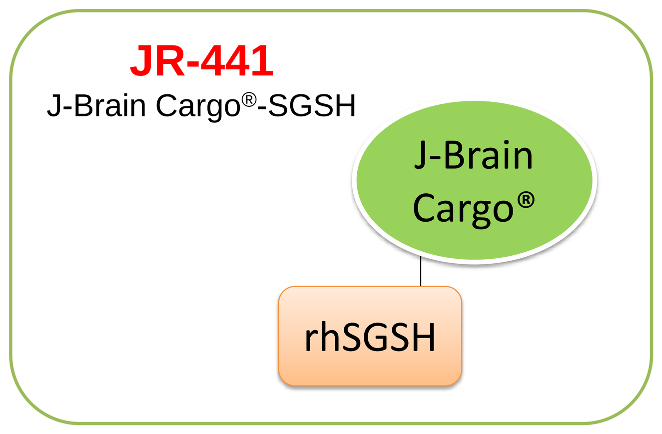


Figure 1. Structure of JR-441

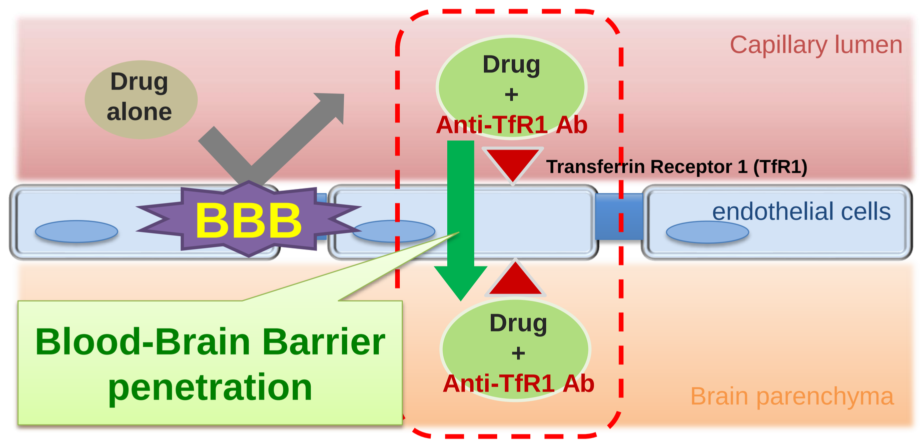


Figure 2. J-Brain Cargo® technology

Biodistribution to CNS of JR-441 in cynomolgus monkeys

- To compare biodistribution between JR-441 and rhSGSH, each drug was administered intravenously to cynomolgus monkeys. Tissues were collected and homogenized. Concentrations of the test substances were determined by electrochemiluminescent assay. Biotinylated anti-SGSH rabbit IgG antibody was coated to a blocked streptavidin plate. After each sample was added to the plate and reacted to a solid antibody , SULFO labeled anti SGSH rabbit IgG antibody was reacted. The test substances were quantified based on the luminescence intensity.
- When administered intravenously, concentrations of JR-441 in the plasma and the CSF were higher than those of rhSGSH (Figure 3).
- JR-441 were detected in all CNS tissues tested, and the concentrations decreased over time (Figure 4). On the other hand, rhSGSH was hardly detectable in the CNS tissues.
- Immunohistochemical analysis also showed delivery of JR-441 to neuronal cells (Figure 4).

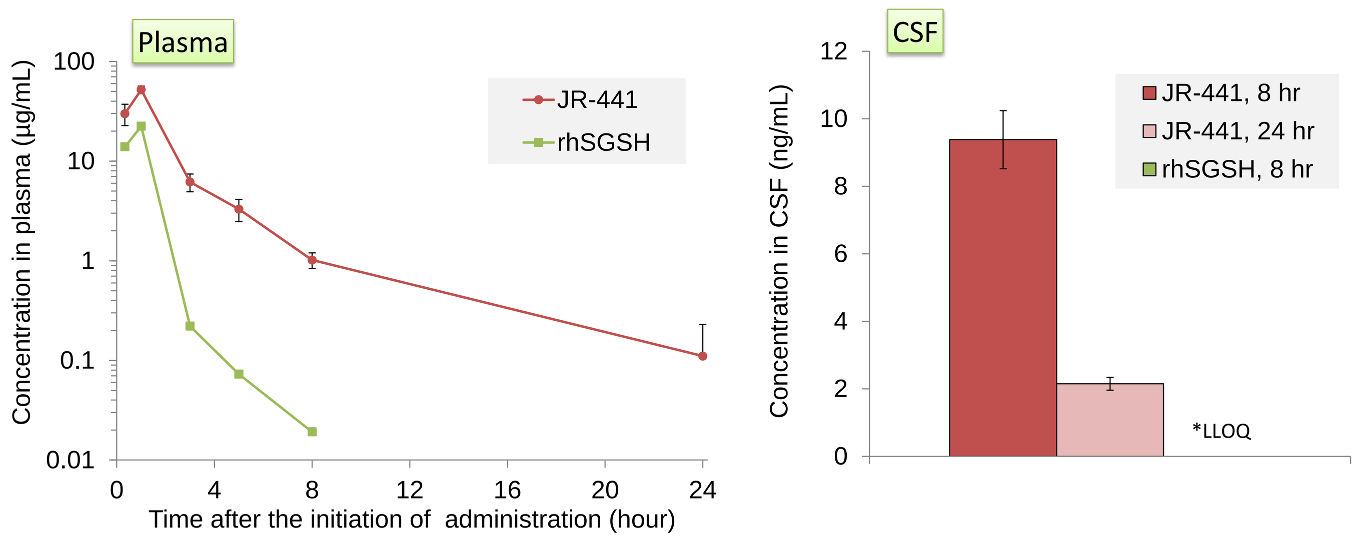


Figure 3. Concentration of JR-441 in plasma and CSF

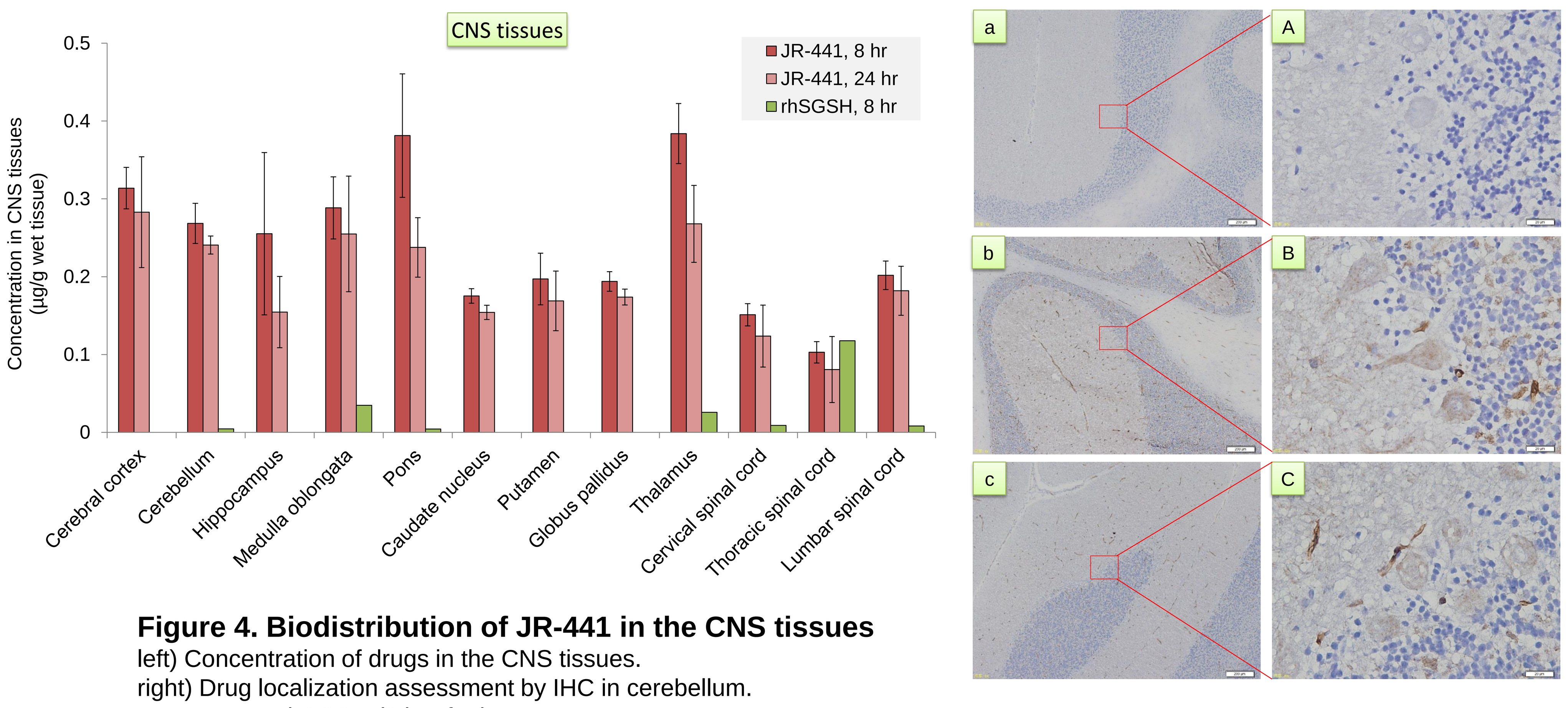


Figure 4. Biodistribution of JR-441 in the CNS tissues
left) Concentration of drugs in the CNS tissues.
right) Drug localization assessment by IHC in cerebellum.
a, A: rhSGSH (8 hr after)
b, B: JR-441 (8 hr after)
c, C: JR-441 (24 hr after)

A comparative biodistribution study of JR-441 and SGSH in mice

- To compare biodistribution and pharmacokinetics of JR-441 and rhSGSH, each drug was administered intravenously to human transferrin receptor knock-in (hTfR-KI) mice (n=3/group). Tissues were collected and homogenized to measure drug concentrations by electrochemiluminescent assay.
- Concentrations of JR-441 in plasma were remained higher than those of rhSGSH (Figure 5). JR-441, but not rhSGSH, was clearly detected in the brain of the mice (Figure 6). Concentrations of JR-441 in the heart and kidney were higher than those of rhSGSH at all sampling points while concentrations of these substances were similar in the liver (Figure 6).

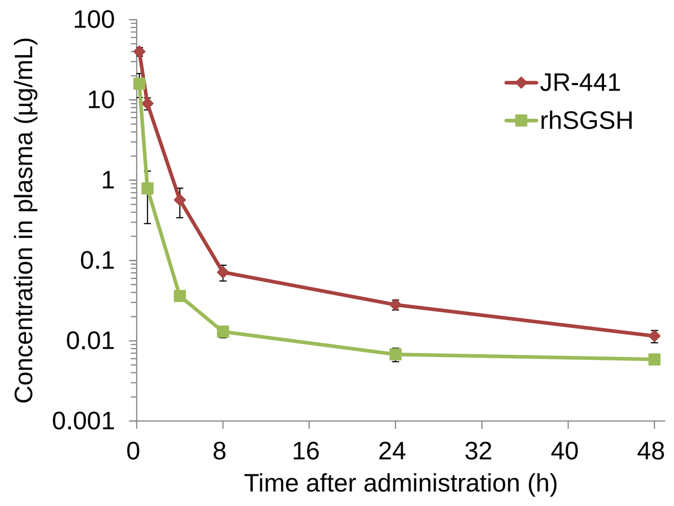
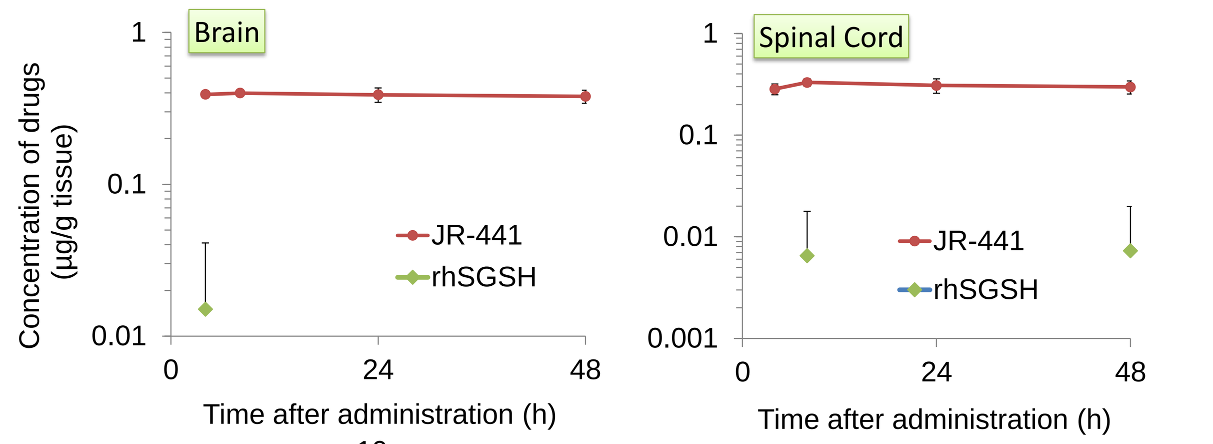


Figure 5. Concentration of JR-441 in plasma



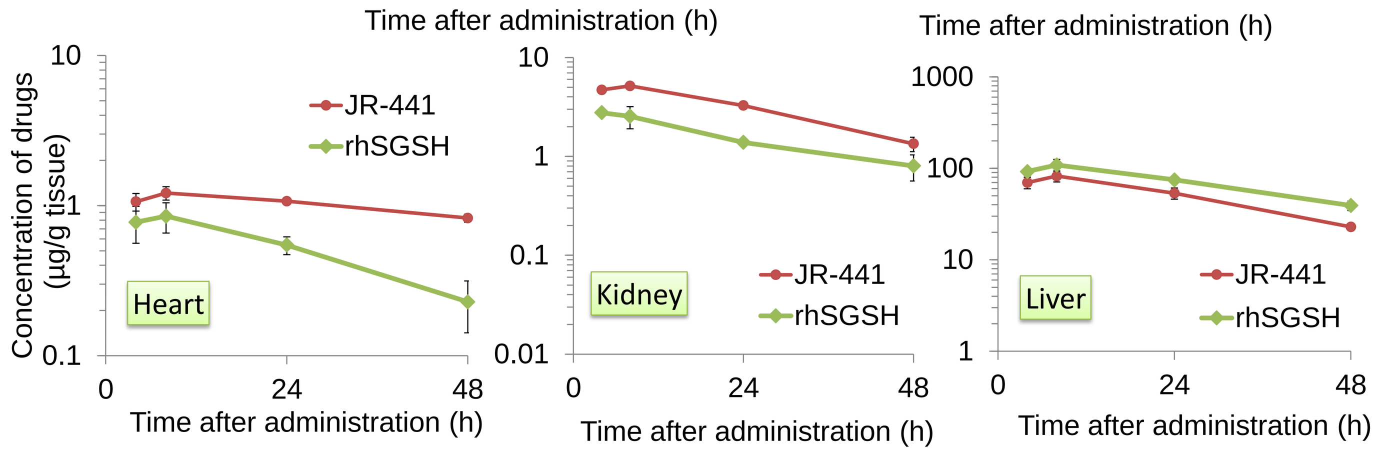


Figure 6. Concentration of JR-441 in tissues

Effect of JR-441 on HS reduction

- We then evaluated the efficacy of repetitive intravenous administration of JR-441 in the reduction of accumulated HS in the CNS and peripheral tissues of hTfR-KI/Sgsh-KO mice, an animal model of MPS IIIA.
- JR-441 dose-dependently decreased HS concentrations in the brain and CSF, in which rhSGSH failed to affect the concentration (Figure 7). The concentrations of HS in CSF correlated with that in the brain (Figure 8). Both JR-441 and rhSGSH decreased HS concentrations in the serum, heart, liver, and kidney (Figure 7).

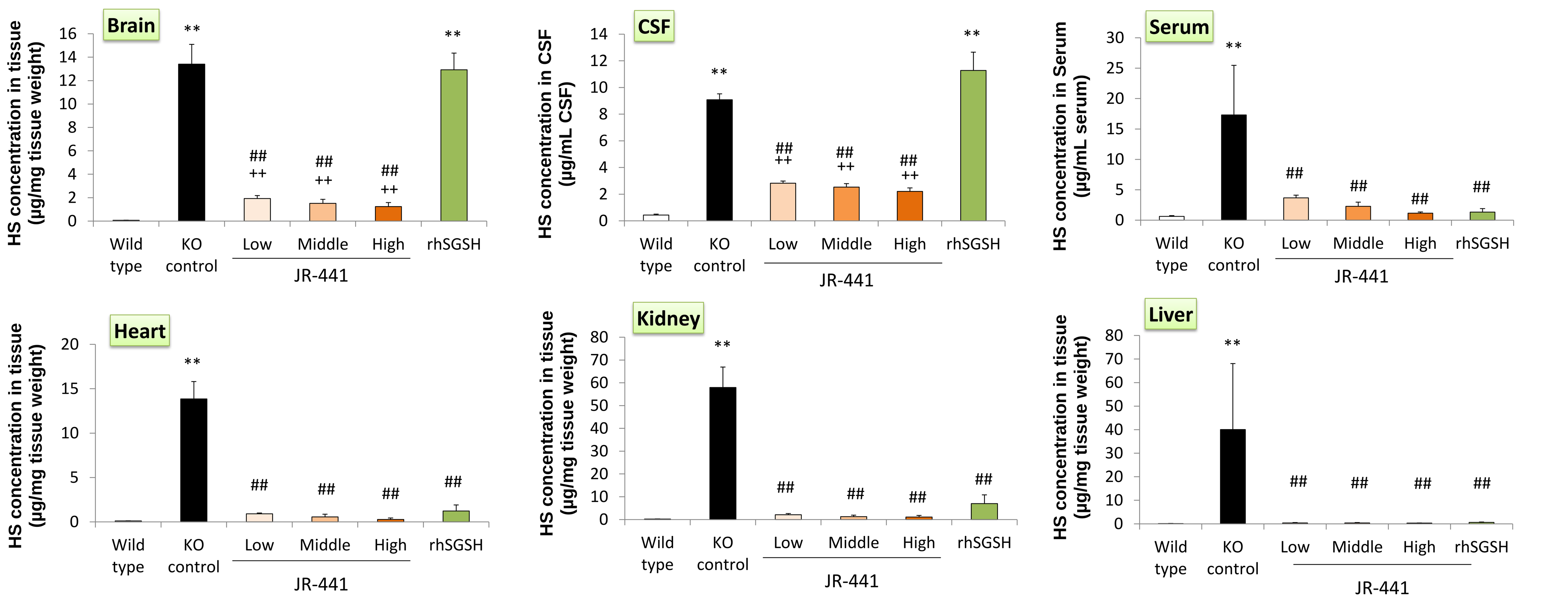
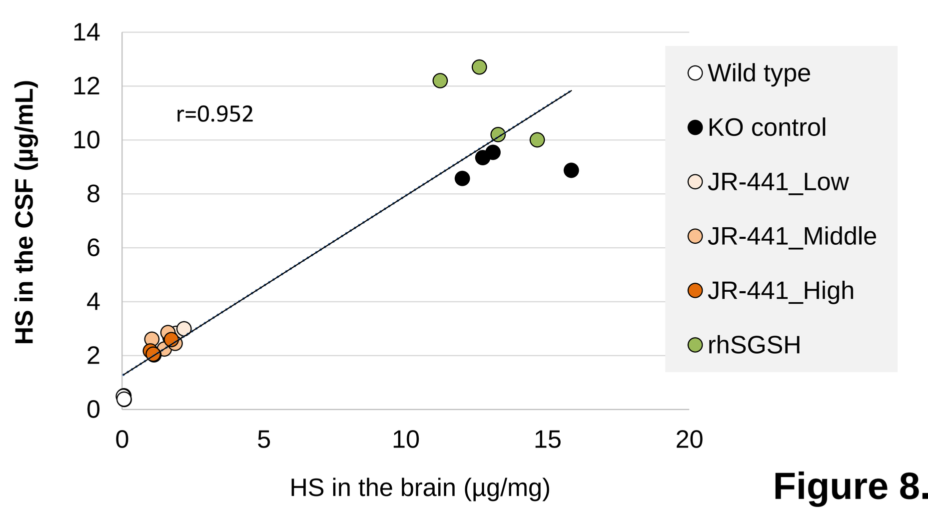


Figure 7. Results of evaluation of drug efficacy of JR-441 (HS concentrations in tissues)



N=3-4/group, mean +/- S.D.
**: p<0.01 (vs Wild type)
##: p<0.01 (vs KO control)
++: p<0.01 (vs rhSGSH) ,Tukey HSD

Figure 8. Correlation between HS in brain and CSF

Conclusions

- The anti-hTfR antibody-fused SGSH (JR-441) was delivered to the brain by crossing the BBB.
- JR-441 reduced the accumulated substrate in both CNS and peripheral tissues when administered intravenously to MPS IIIA mice.
- JR-441 has a potential to exert therapeutic benefit on the CNS sequelae in patients with MPS IIIA.