Usefulness of Hexose Tetrasaccharide as a Biomarker for Monitoring Glycogen Accumulation in Peripheral Tissues and Brain in Pompe Disease.

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Introduction

- Pompe disease, also known as glycogen storage disease type II, is caused by mutations in acid α-glucosidase (GAA) gene, resulting in an accumulation of lysosomal glycogen throughout the body. The infantile form is characterized by hypertrophic cardiomyopathy, motor developmental delay, and respiratory insufficiency. Although enzyme replacement therapy is currently available for the treatment of patients with Pompe disease, its efficacy in the skeletal muscles and brain is limited.
- We have developed JR-162, a fusion protein consisting of a Fab fragment of anti-human transferrin receptor (hTfR) antibody and GAA, which has potential to cross the blood-brain barrier, utilizing receptor-mediated transcytosis of transferrin, to reach the brain parenchyma (Figure 1.). The TfR binding ability may also facilitate uptake of the drug to skeletal muscle cells.
- Hexose tetrasaccharide (Hex4) has been reported to be a biomarker for monitoring of disease progression and the therapeutic response to enzyme replacement therapy in Pompe disease. We evaluated the usefulness of Hex4 in the urine and cerebrospinal fluid (CSF) as a biomarker for monitoring glycogen accumulation in peripheral tissues and the brain, respectively, using Gaa-knockout mice, an animal model of Pompe disease.

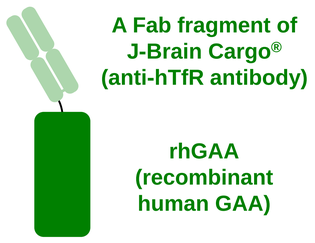
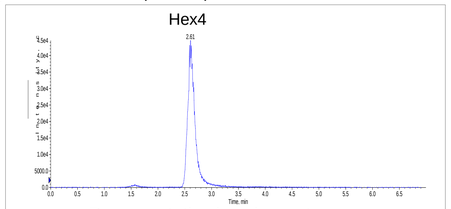


Figure 1. Structure of JR-162

LC/MS/MS Analytical Method of Hex4 (Hexose tetrasaccharide)

Table. LC/MS/MS analytical method of Hex4

HPLC	:	Nexera X2 (Shimadzu)
Column	:	ACQUITY UPLC BEH Amide Column, 2.1 mm X 50 mm (Waters) ACQUITY UPLC BEH Amide VanGuard Pre-column, 2.1 mm X 5 mm (Waters)
Mobile phase A	:	Water, 0.1% ammonium hydroxide
Mobile phase B	:	Acetonitrile, 0.1% ammonium hydroxide
%B	:	70%(0-3 min), 60%(3-5 min), 70%(5-7 min)
Flow rate	:	0.4 mL/min
Injection volume	:	10 µL
Column temperature	:	40 °C
MS/MS	:	QTRAP 5500 (AB Sciex)
Ion Source	:	ESI
Polarity	:	Negative
m/z	:	665.1/161.0 (analyte) 671.1/167.0 (internal standard, ^{13}C labeled)



Representative ion chromatogram

Animal Experiment

- We evaluated the efficacy of JR-162 in the reduction of accumulated glycogen, Hex4 concentrations in the urine and CSF using hTfR-KI/Gaa-KO mice. JR-162 or rhGAA was administered intravenously to mice biweekly for 12 weeks.

Effect of JR-162 on Tissue Glycogen Concentration

- JR-162 decreased glycogen concentrations in the heart, liver, skeletal and respiratory muscles as or more effectively than rhGAA, and was also effective for type II fiber dominant muscles that were poorly responsive to rhGAA. Furthermore, JR-162 markedly decreased the glycogen concentrations in the CNS tissues (Figure 2.).

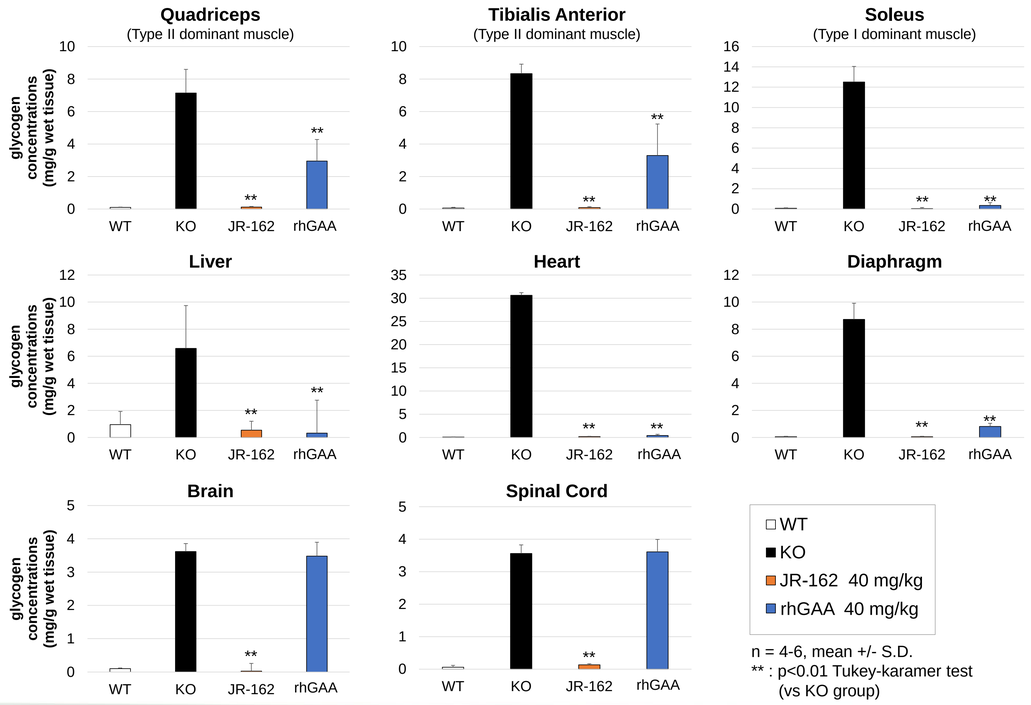


Figure 2. Glycogen concentrations in mouse tissues

Hex4 Concentrations in the Urine

- JR-162 decreased Hex4 concentrations in the urine much more than rhGAA (Figure 3.).
- The correlations between Hex4 concentrations in the urine and glycogen concentrations in peripheral tissues were evaluated. Hex4 concentrations in the urine were highly correlated with glycogen concentrations in peripheral tissues. These results suggest that Hex4 in the urine reflects glycogen level in peripheral tissues (Figure 4.).

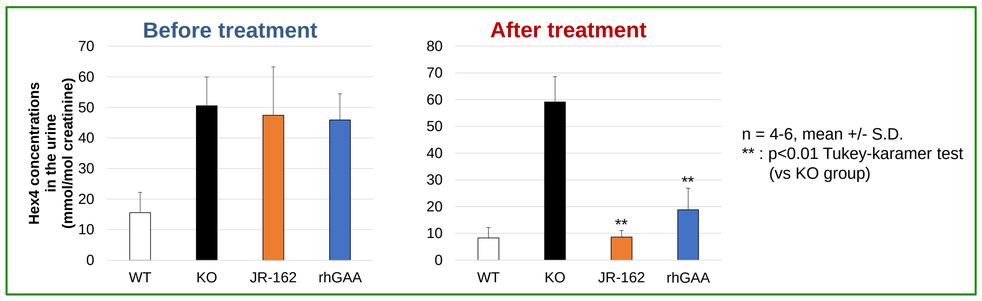


Figure 3. Hex4 concentrations in the urine before and after treatment

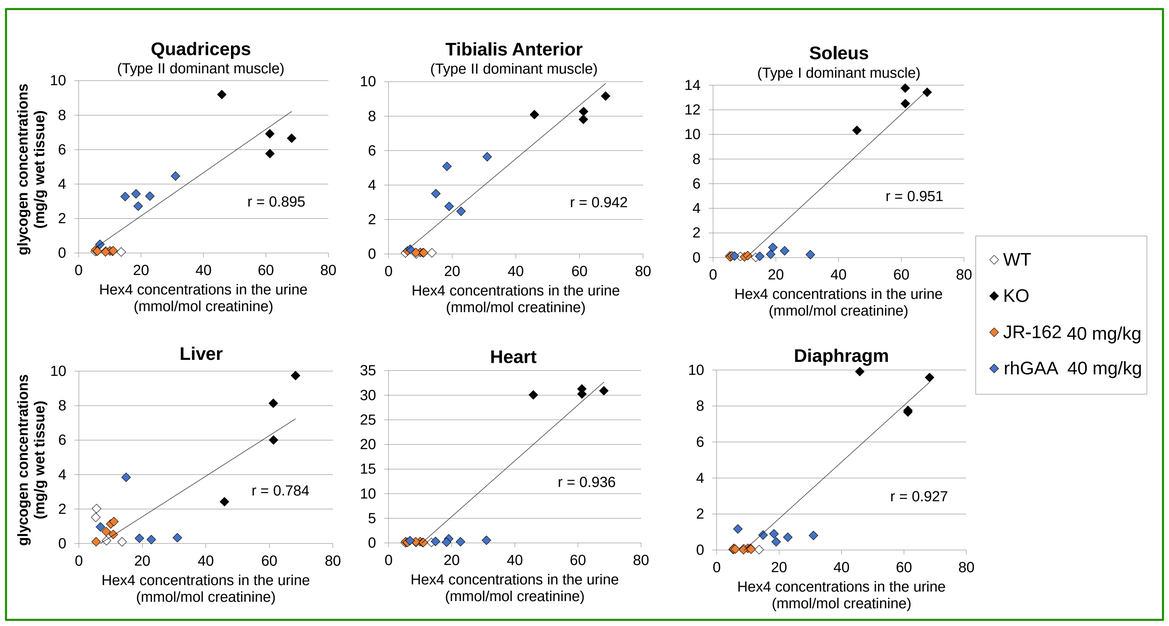


Figure 4. Correlation between urinary Hex4 and tissue glycogen

Hex4 Concentrations in the CSF

- JR-162 decreased Hex4 concentrations in the CSF much more than rhGAA (Figure 5.).
- We found that Hex4 concentrations in the CSF were highly correlated with glycogen concentrations in the brain. These data indicate that Hex4 in the CSF reflects glycogen level in the brain (Figure 6.).

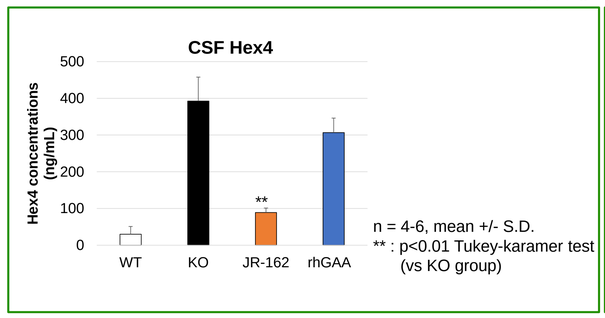


Figure 5. Hex4 concentrations in the CSF

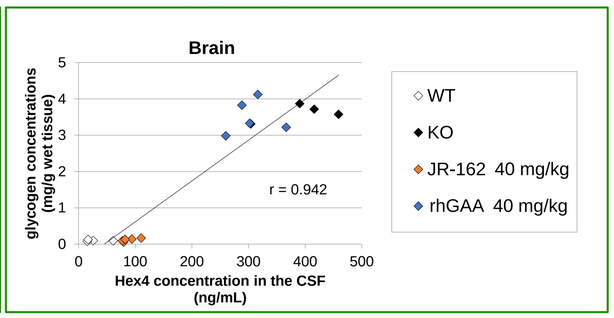


Figure 6. Correlation between CSF Hex4 and brain glycogen

Conclusions

- JR-162 markedly decreased glycogen accumulation in peripheral tissues and brain.
- Hex4 in the urine represents a predictor for systemic disease severity and drug efficacy in Pompe disease mice.
- Hex4 in the CSF is a biomarker for monitoring glycogen deposition in the brain and therapeutic response in Pompe disease mice.
- Hex4 may serve as a surrogate biomarker for the evaluation of drug effects in Pompe disease also in clinical studies.

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