LB-13

Recombinant Human Protective Protein/Cathepsin A: An Update on the Development of an Enzyme Replacement Therapy for Galactosialidosis

Vish Koppaka[a], Jaclyn Cadaoas[a], Sean Cullen[a], Elida Gomero[b], Creobelle Guzman[a], Huimin Hu[b], Kartika Jayashankar[a], Mike Machado[a], Gabrielle Morris[a], Rosario Mosca[b], Arjun Natesan[a], Alessandra d'Azzo[b], Michael Vellard[a]

[a]Ultragenyx Pharmaceutical Inc., Novato, United States, [b]St. Jude Children's Research Hospital, Memphis, United States

ABSTRACT

Background

Galactosialidosis (GS) is a rare, autosomal recessive, glycoprotein storage disease caused by a primary defect of the multifunctional lysosomal serine carboxypeptidase, Protective Protein/Cathepsin A (PPCA) and secondary deficiency of neuraminidase1 (NEU1) and β-galactosidase (β-GAL). The three enzymes form a high molecular weight lysosomal complex, and association with PPCA assures proper compartmentalization, catalytic activation and stability of the two glycosidases. Severe deficiency of NEU1 in GS patients causes progressive accumulation of sialylated glycoconjugates in tissues and body fluids.

Introduction

We have successfully developed a CHO cell line that overexpresses recombinant human PPCA protein, and developed a reliable process for purification of the 54 kDa-zymogen from the culture medium. We have demonstrated that rhPPCA is taken up by deficient human fibroblasts via the mannose-6-phosphate receptor pathway and subsequently rescues NEU1 and β-GAL activities. To develop an efficient and non-invasive therapy for the treatment of GS, we conducted an *in vivo* proof of concept study in GS (PPCA$^{-/-}$) mice to evaluate the efficacy of rhPPCA via biweekly intravenous administration for 8 weeks. The results of this study are described in this poster.

ANIMAL MODEL AND STUDY DESIGN

Galactosialidosis (PPCA$^{-/-}$) mouse model

- Developed and characterized by Dr. Alessandra d'Azzo
- Tissues show characteristic cytoplasmic vacuolization attributable to lysosomal storage
- Progressive accumulation of sialylated oligosaccharides in urine
- Develops phenotype resembling the severe form of GS

Group	Test Article(s)	Gender	Genotype	N/group	IV Dose (mg/kg)	Terminal collection time (hrs)
1	Vehicle	M/F	WT	10	0	24
2	Vehicle	M/F	PPCA -/-	10	0	24
3	Vehicle + CPH	M/F	PPCA -/-	10	0	24
4	rhPPCA + CPH	M/F	PPCA -/-	10	0.2	24
5	rhPPCA + CPH	M/F	PPCA -/-	10	0.6	24
6	rhPPCA + CPH	M/F	PPCA -/-	10	2	24
7	rhPPCA + CPH	M/F	PPCA -/-	10	6	24
8	rhPPCA + CPH	M/F	PPCA -/-	10	20	24
9	rhPPCA + CPH	M/F	PPCA -/-	10	20	1 week recovery

STUDY OBJECTIVES AND METHODS

Study Objectives

- Evaluate the tissue distribution of rhPPCA and normalize NEU1 and β-GAL activities
- Demonstrate reduction of lysosomal storage in affected tissues
- Demonstrate reduction in accumulation of sialylated glycoconjugates in urine

Methods

- **Enzyme Activity Assays**

Cathepsin A activity was measured with the synthetic substrate Z-Phe-Ala. The activities of β-galactosidase, neuraminidase, and hexosaminidase A (lysosomal control) were assayed with artificial 4-methylumbelliferyl substrates. All enzyme activities were normalized to total protein concentration by the bicinchoninic acid method (BCA).

- **Immunohistochemical Analyses**

Paraffin-embedded tissue sections were incubated overnight with an anti-PPCA antibody and subjected to standard immunohistochemical methods. Antibody detection was performed using diaminobenzidine (DAB) substrate and counterstained with hemotoxylin according to standard method.

- **Histopathological Analyses**

Tissues were isolated and fixed in 10% Neutral Buffered Formalin (NBF) then transferred to 70% Ethanol. Tissues were sectioned and stained with hematoxylin and eosin and analyzed by light microscopy to determine lysosomal storage as indicated by cytoplasmic vacuolization.

- **Urine Sialic Acid Determination**

Total sialic acid content was determined using an Enzychrome Sialic Acid Assay Kit from BioAssay Systems. All urine sialic acid measurements were normalized to creatinine concentration.

ENZYME ACTIVITY IN HUMAN GS FIBROBLASTS

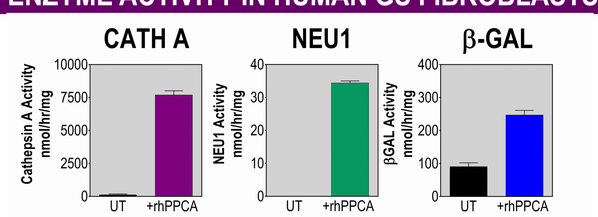

Figure 1. Uptake of rhPPCA in GS Patient-Derived Fibroblasts

Restoration of Cathepsin A activity and rescue of endogenous NEU1 and β-GAL activities in GS fibroblasts after uptake of rhPPCA. In untreated fibroblasts, Cathepsin A deficiency is accompanied by an equivalent loss of NEU1 activity, whereas β-GAL activity is only slightly reduced. In human GS fibroblasts, β-GAL is stable whether or not associated with PPCA, whereas the interaction of NEU1 with PPCA is clearly essential for its activity.

ENZYME ACTIVITY AND HISTOPATHOLOGY IN PPCA$^{-/-}$ MICE

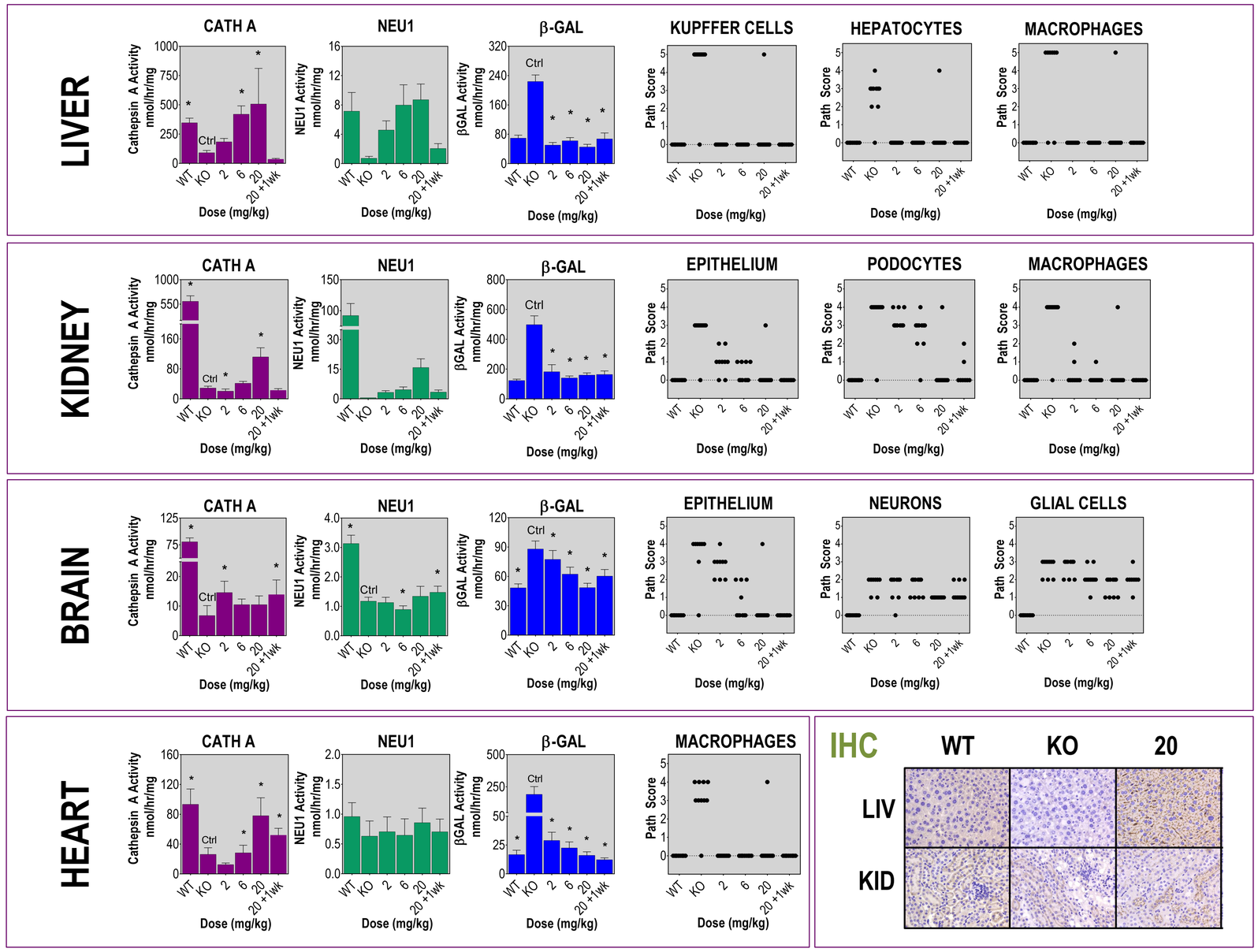

Figure 2. Enzyme activities in tissues and histopathology scores of GS (PPCA$^{-/-}$) mice

Cathepsin A, NEU1, and β-GAL activities assayed in tissue homogenates of GS (KO) mice after treatment with rhPPCA , with untreated GS and WT controls. Error bars indicate standard deviation. (*) denotes statistical significance from PPCA$^{-/-}$ KO Control. One-way ANOVA model and Dunnet's method were used to analyze data. Histopathology results of GS mouse tissue scored to determine lysosomal storage by cytoplasmic vacuolization. (Path scores: 0=Normal; 1=Minimal; 2=Mild; 3=Moderate; 4=Marked; 5=Severe). Immunostaining of hPPCA in liver and kidney tissue sections using a human PPCA-specific antibody. Brown punctate staining indicative of hPPCA was detected.

URINE SIALIC ACID

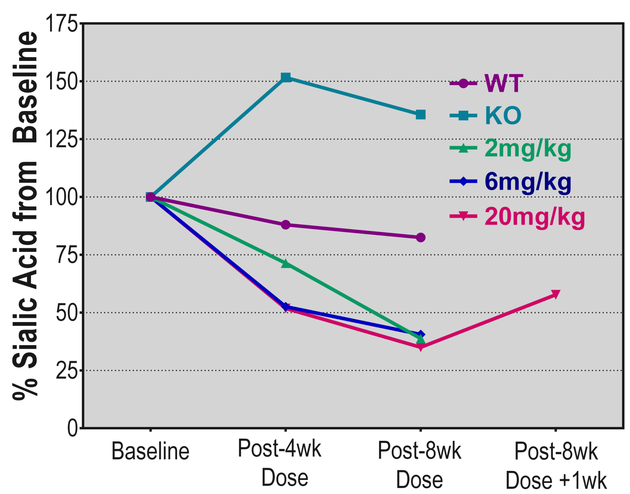

Figure 3. Analysis of total urine sialic acid in GS mice

Elevated urine sialic acid in untreated GS mice compared to WT control. Reduction of urine sialic acid in GS mice treated with rhPPCA.

SUMMARY

Conclusions

- Cathepsin A deficiency is accompanied by equivalent loss of neuraminidase activity and elevated β-Galactosidase and hexosaminidase A activities in untreated GS mice relative to WT control
- Dose-dependent increase in Cathepsin A activity in affected tissues such as liver, spleen, kidney and heart; brain tissue showed increase in Cathepsin A activity to a smaller extent
- Improvement of neuraminidase I activities and normalization of β-Galactosidase and hexosaminidase A activities with rhPPCA treatment
- At 20 mg/kg rhPPCA, the cytoplasmic vacuolization was no longer evident in nearly all tissue elements in all but one animal
- Decreased total sialic acid in urine
- No histopathologic findings associated with the test article up to 20 mg/kg were observed
- Overall improvement in efficacy with increasing dose of rhPPCA