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

Vish Koppaka\textsuperscript{a}, Jaclyn Cadaoa\textsuperscript{a}, Sean Cullen\textsuperscript{a}, Elida Gomez\textsuperscript{b}, Creobelle Guzman\textsuperscript{a}, Huimin Hu\textsuperscript{b}, Kartika Jayashankar\textsuperscript{a}, Mike Machado\textsuperscript{a}, Gabrielle Morris\textsuperscript{a}, Rosario Mosca\textsuperscript{b}, Arjun Natesan\textsuperscript{a}, Alessandra d’Azzo\textsuperscript{b}, Michael Vellard\textsuperscript{a}

\textsuperscript{a}Ultragenyx Pharmaceutical Inc., Novato, United States, \textsuperscript{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 in the lysosomal serine carboxypeptidase, Protective Protein/Cathepsin A (PPCA) and secondary deficiency of neuaminidase 1 (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\textsuperscript{-/-}) 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\textsuperscript{-/-}) mouse model

- Developed and characterized by Dr. Alessandra d’Azzo
- Progression is accompanied by equivalent loss of all three activities
- Progressive accumulation of sialylated oligosaccharides in urine
- Develops phenotype resembling the severe form of GS

<table>
<thead>
<tr>
<th>Group</th>
<th>Test Article(s)</th>
<th>Gender</th>
<th>Genotype</th>
<th>N/group</th>
<th>IV Dose (mg/kg)</th>
<th>Terminal collection time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Vehicle</td>
<td>M/F</td>
<td>WT</td>
<td>10</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>Vehicle</td>
<td>M/F</td>
<td>PPCA \textsuperscript{-/-}</td>
<td>10</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>3</td>
<td>Vehicle + CPH</td>
<td>M/F</td>
<td>PPCA \textsuperscript{-/-}</td>
<td>10</td>
<td>0</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>rhPPCA + CPH</td>
<td>M/F</td>
<td>PPCA \textsuperscript{-/-}</td>
<td>10</td>
<td>0.2</td>
<td>24</td>
</tr>
<tr>
<td>5</td>
<td>rhPPCA + CPH</td>
<td>M/F</td>
<td>PPCA \textsuperscript{-/-}</td>
<td>10</td>
<td>0.6</td>
<td>24</td>
</tr>
<tr>
<td>6</td>
<td>rhPPCA + CPH</td>
<td>M/F</td>
<td>PPCA \textsuperscript{-/-}</td>
<td>10</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>7</td>
<td>rhPPCA + CPH</td>
<td>M/F</td>
<td>PPCA \textsuperscript{-/-}</td>
<td>10</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>8</td>
<td>rhPPCA + CPH</td>
<td>M/F</td>
<td>PPCA \textsuperscript{-/-}</td>
<td>10</td>
<td>20</td>
<td>24</td>
</tr>
<tr>
<td>9</td>
<td>rhPPCA + CPH</td>
<td>M/F</td>
<td>PPCA \textsuperscript{-/-}</td>
<td>10</td>
<td>0</td>
<td>1 week recovery</td>
</tr>
</tbody>
</table>

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
  - CathA activity was measured with the synthetic substrate Z-Phe-Ala. The activities of β-galactosidase, neuaminidase, and hexosaminidase (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 hematoxylin 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.
- Sialic Acid Determination
  - Total sialic acid content was determined using an Enzyme 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 CathA activity and rescue of endogenous NEU1 and β-GAL activities in GS fibroblasts after uptake of rhPPCA. In untreated fibroblasts, CathA 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.

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.

CONCLUSIONS

- CathA deficiency is accompanied by equivalent loss of neuaminidase activity and elevated β-Galactosidase and hexosaminidase A activities in untreated GS mice relative to WT control
- Dose-dependent increase in CathA activity in affected tissues such as liver, spleen, kidney and heart; brain tissue showed increase in CathA activity to a smaller extent
- Improvement of neuaminidase 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

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

Conclusions

- CathA deficiency is accompanied by equivalent loss of neuaminidase activity and elevated β-Galactosidase and hexosaminidase A activities in untreated GS mice relative to WT control
- Dose-dependent increase in CathA activity in affected tissues such as liver, spleen, kidney and heart; brain tissue showed increase in CathA activity to a smaller extent
- Improvement of neuaminidase 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