SUBSTRATE REDUCTION THERAPY for LYSOSONAL STORAGE DISORDERS

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Research & Development Unit,
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INSA
Lysosomal Storage Disorders

- Genetic
- Rare
- Autosomal recessive (majority)

- Portugal - 1/4000
- Almost 60!
Lysosomal Storage Disorders (LSDs)

- Chronic
- Progressive
- Large spectrum of severity & symptoms

- Pathophysiology still unknown!
Lysosomal Storage Disorders (LSDs)
Lysosomal Storage Disorders (LSDs)

- Progressive accumulation
  
DISEASE
THE ENZYME AS A DRUG?

- 1969
- Elizabeth Neufeld

THE DEFECT IN HURLER AND HUNTER SYNDROMES, II. DEFICIENCY OF SPECIFIC FACTORS INVOLVED IN MUCOPOLYSACCHARIDE DEGRADATION

By Joseph C. Fratantoni, Clara W. Hall, and Elizabeth F. Neufeld

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland

Communicated by Christian B. Anfinsen, July 9, 1969

Abstract.—Cultured fibroblasts, derived from patients with the Hurler and Hunter syndromes, show defective degradation of sulfated mucopolysaccharide. The aberrant metabolism of Hurler cells can be corrected by secretions of fibroblasts of genotype other than Hurler, and similarly, the defect of Hunter cells can be corrected by secretions of fibroblasts of genotype other than Hunter. The active factors in these secretions, which are heat labile and associated with macromolecules, accelerate the degradation of mucopolysaccharide.
THE ENZYME AS A DRUG?
THE ENZYME AS A DRUG?

CI-MPR
clathrin-mediated endocytosis

plasma membrane

Late Endosome/
Lysosome

transport vesicle

clathrin coat

trans face
**Proof of Principle...**

- **Gaucher Disease** (GD)
  - Deficient enzyme: β-glucocerebrosidase
  - Gene: *GBA* (1q21)

- Most frequent LSD
Proof of Principle...

- **Gaucher Disease** (GD)
  - Deficient enzyme: β-glucocerebrosidase
  - Gene: *GBA* (1q21)

- Intravenous injections of the recombinant enzyme
- Excellent results in systemic disease

Original illustration by Marcos Bernardino for Cristiana Petriz’s “Gigi e a Doença de Gaucher”, 2010
# The **Enzyme** as a Drug?

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Available ERT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaucher</td>
<td>Cerezyme&lt;sup&gt;®&lt;/sup&gt; (Imiglucerase; Genzyme)</td>
</tr>
<tr>
<td></td>
<td>VPRIV&lt;sup&gt;®&lt;/sup&gt; (Velaglucerase alfa; Shire)</td>
</tr>
<tr>
<td></td>
<td>Elelyso&lt;sup&gt;®&lt;/sup&gt; (Taliglucerase alfa; Pfizer)</td>
</tr>
<tr>
<td>Fabry</td>
<td>Replagal&lt;sup&gt;®&lt;/sup&gt; (Agalsidase alfa; Shire)</td>
</tr>
<tr>
<td></td>
<td>Fabrazyme&lt;sup&gt;®&lt;/sup&gt; (Algalsidase beta; Genzyme)</td>
</tr>
<tr>
<td>MPS I</td>
<td>Aldurazyme&lt;sup&gt;®&lt;/sup&gt; (Laronidase; Genzyme)</td>
</tr>
<tr>
<td>MPS II</td>
<td>Elaprase&lt;sup&gt;®&lt;/sup&gt; (Idursulfase; Shire)</td>
</tr>
<tr>
<td>MPS IV A</td>
<td>Vimizim&lt;sup&gt;®&lt;/sup&gt; (Elosulfase alfa; Biomarin)</td>
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<tr>
<td>MPS VI</td>
<td>Naglazyme&lt;sup&gt;®&lt;/sup&gt; (Galsulfase; Biomarin)</td>
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<tr>
<td>Pompe</td>
<td>Myozyme&lt;sup&gt;®&lt;/sup&gt; (Lumizyme, Alglucosidase alfa; Genzyme)</td>
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<tr>
<td>LAL deficiency</td>
<td>Kanuma&lt;sup&gt;®&lt;/sup&gt; (Sebelipase alfa; Alexion)</td>
</tr>
</tbody>
</table>
THE **ENZYME** AS A **DRUG**?
THE ENZYME AS A DRUG?

- 1996
- Norman Radin


Treatment of Gaucher disease with an enzyme inhibitor

NORMAN S. RADIN

Mental Health Research Institute and Division of Nephrology MSBII. University of Michigan, 1550 W. Medical Centre Drive Ann Arbor, MI 48109-0876, USA

Received 23 May 1995, revised 20 June 1995

The hypothesis is offered predicting that Gaucher patients could be treated with a drug that slows the synthesis of glucosylceramide, the lipid that accumulates in this disorder. The present therapeutic approach involves augmenting the defective enzyme, glucosylceramide β-glucosidase, with exogenous β-glucosidase isolated from human thymus. This spectacularly expensive mode of treatment should be replaceable with a suitable enzyme inhibitor that simply slows formation of the lipid and matches the rate of synthesis with the rate of the defective, slowly working β-glucosidase. Several drugs that possess this ability are available, the best known of which is 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), a designer inhibitor that resembles the synthase's substrate and product. PDMP has been found to be effective in mice, rats, fish, and a wide variety of cultured cells. Its use, at suitable dosages, seems to be harmless, although long-term tests have not been made. The lack of suitable animal models of Gaucher disease has made it difficult to test the hypothesis adequately, but PDMP does rapidly lower the levels of glucosylceramide in normal animal tissues and the animals evidently do well with the lowered levels of glucosylceramide and its more complex glycolipid metabolites.
substrate reduction  enzyme replacement
N-Butyldeoxyojirimycin Is a Novel Inhibitor of Glucolipid Biosynthesis

Frances M. Platts, Gabrielle R. Neisse, Raymond A. Dwek, and Terry D. Butters

From the Glycobiology Institute, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, United Kingdom and the Monsanto Company, St. Louis, Missouri 63198

(Received for publication, October 1, 1993, and in revised form, November 15, 1993)

Safety, Tolerability, and Pharmacokinetics of Eliglustat Tartrate (Genz-112638) After Single Doses, Multiple Doses, and Food in Healthy Volunteers

M. Judith Peterschmitt, MD, MMsc, Amy Burke, MPH, Larry Blankstein, PhD, Sharon E. Smith, MD, Ana Cristina Puga, MD, PhD, William G. Kramer, PhD, James A. Harris, BSc, MRSC, David Mathews, MD, and Peter L. Bonate, PhD

Three phase 1 studies of eliglustat tartrate (Genz-112638), an oral inhibitor of glucosylceramide synthase under development for treating Gaucher disease type 1 (GD1), evaluated the safety, tolerability, and pharmacokinetics in healthy volunteers after escalating single doses (n = 99), escalating multiple doses (n = 36), and food (n = 24). Eliglustat tartrate was well tolerated at single doses ≤20 mg/kg and multiple doses ≤200 mg bid, with 50 mg bid producing plasma concentrations in the predicted therapeutically effective range of ~2 hours, followed by a monophasic decline with a ~6-hour terminal half-life. Unchanged drug in 8-hour urine collections was <1.5% of administered doses. Food did not significantly affect the rate or extent of absorption. Multiple-dose pharmacokinetics was nonlinear, showing higher than expected plasma drug concentrations. Steady state was reached ~60 hours after bid dosing. Higher drug exposure occurred in slower CYP2D6 metabolizers. Based on favorable results in healthy participants, a phase 2 trial in Gaucher disease patients commenced.
## SUBSTRATE REDUCTION THERAPY (SRT)

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Approved SRT</th>
</tr>
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<tbody>
<tr>
<td>Gaucher</td>
<td>Zavesca&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(Miglustat; Actelion)</td>
</tr>
<tr>
<td></td>
<td>Cerdelga&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(Eliglustat tartrate; Genzyme)</td>
</tr>
<tr>
<td>Niemann-Pick C</td>
<td>Zavesca&lt;sup&gt;®&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(Miglustat; Actelion)</td>
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</tbody>
</table>
1. NAME OF THE MEDICINAL PRODUCT

Zavesca 100 mg hard capsules

4.4 Special warnings and precautions for use

Tremor
Approximately 37% of patients in clinical trials and 40% of patients in a 20-month clinical trial in Niemann-Pick type C disease reported tremors. In many cases, these tremors were described as an exaggerated form of the patient’s own tremor, which began within the first month, and in many cases continued beyond 3 months. Dose reduction may ameliorate the tremor, but it may sometimes be required.

Gastrointestinal disturbances
Gastrointestinal events, mainly diarrhoea, have been observed in 59% of patients at the outset of treatment or intermittently during treatment. A high intake of carbohydrate is likely to lead to diarrhoea, which is caused by a reduction in the absorption of dietary disaccharides and other oligosaccharides that can lead to diarrhoea. Anti-diarrhoeal medicinal products such as loperamide may be necessary. Patients with chronic diarrhoea have not been evaluated in patients with a history of inflammatory bowel disease.

Effects on spermatogenesis
Male patients should maintain reliable contraception during treatment, as miglustat adversely affects fertility (see sections 4.6 and 5.3). Until further clinical data are available, male patients should cease Zavesca and maintain contraception for at least 3 months.

What are the risks associated with Cerdelga?

The most common side effect seen with Cerdelga (which may affect more than 1 in 10 people) is diarrhoea, in approximately 6 out of 100 patients. The majority of side effects are mild and transient.

For the full list of all side effects reported for Cerdelga, see the package leaflet.

Cerdelga must not be taken together with certain medicines that can interfere with the ability of the body to break down the medicine, which may affect the levels of Cerdelga in blood. For the full list of restrictions, see the package leaflet.
MUCOPOLYSACCHARIDOSES (MPS)

- Chronic
- Progressive
- Large spectrum of severity & symptoms
Mucopolysaccharidoses (MPS)

- Chronic
- Progressive
- Large spectrum of severity & symptoms

MPS III
(= Sanfilippo Syndrome)
Mucopolysaccharidosis (MPS) Type III

- Autosomal recessive
- Lysosomal Storage Disorders
  - Sub-type of MPSs; glycosaminoglycans (GAGs)
  - Accumulated substrate: heparan sulphate

- 4 different diseases:
  - III A
  - III B
  - III C
  - III D

  depending on the defective enzyme
AVAILABLE THERAPIES

iments None!

...only symptomatic!

ameliorate symptoms
support disabled patients

ERT for neurodegenerative MPS would require the introduction of active enzyme into the CNS

downarrow

extra difficulties!
AVAILABLE THERAPIES

⚠️ None!

...only symptomatic!

ameliorate symptoms
support disabled patients

ERT for neurodegenerative MPS would require the introduction of active enzyme into the CNS

Still, it’s being attempted with some promising results
Available Therapies

None!

...only symptomatic!

ERT for neurodegenerative MPS would require the introduction of active enzyme into the CNS.

Still, it’s being attempted with some promising results.

Perfect Target for Substrate Reduction Approaches!
gSRT FOR MucoPolySaccharidosis type III

*genetic substrate reduction*
gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

early stage of the HS biosynthetic cascade

Dermatan/Chondroitin Sulfate (DS/CS)

CHSY 1-3; CHPF

EXTL1,2; EXT1,3

Heparan Sulfate (HS)
gSRT for MucoPolySaccharidosis Type III

naturally occurring post-transcriptional gene silencing process

Designed to induce RNAi

siRNA

MPS III fibroblasts
**gSRT for Mucopolysaccharidosis Type III**

1. Control fibroblasts
2. siRNA
3. 24/48h incubation
4. Harvest cells
5. RNA extraction
6. cDNA synthesis
7. qRT-PCR (target gene expression assessment)
8. Livak method
gSRT FOR MUCO POLY SACCHARIDOSIS TYPE III

MPS III A

MPS III C

MPS III D

siRNA

mRNA

MPS III fibroblasts
gSRT FOR MucoPolySaccharidosis Type III

siRNA

MPS III fibroblasts

GAGs

% de GAGs detetada

<table>
<thead>
<tr>
<th></th>
<th>24h</th>
<th>48h</th>
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<tbody>
<tr>
<td>NT</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>MPS IIIc</td>
<td>40</td>
<td>30</td>
</tr>
<tr>
<td>MPS IIIc</td>
<td>30</td>
<td>20</td>
</tr>
</tbody>
</table>
gSRT for MucoPolySaccharidosis Type III

Further validation:

- ↑ nr of experiments;
- immunocytochemistry (anti-HS antibody)
- + tests in MPS IIIB
gSRT FOR MucoPolySaccharidosis TYPE III

Promising results!

Reasons to keep studying...
A LOOK FORWARD…

- Vector design & siRNA encapsulation into liposomes
  - ↑ bioavailability of siRNAs;
  - protection from degradation
  - control of
    - circulation time
    - release rate

- Coupling of specific ligands to siRNA-carrying liposomes
  - Transferrin (Tf)
  - Rabies virus peptide derivative (RGV-2r)

- Efficiency assessment + Targeting of brain cells
A LOOK FORWARD...

in vivo

studies
gSRT FOR MucoPolySaccharidosis TYPE III

SUMMARY

“early stages” GAGs biosynthesis gene

↓ GAG Storage
SUMMARY

“early stages” GAGs biosynthesis gene

Therapeutic use ✓
gSRT FOR MUCOPOLYSACCHARIDOSIS TYPE III

SUMMARY

“early stages” GAGs biosynthesis gene

Holds potential to benefit virtually all MPS!
ACKNOWLEDGMENTS

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Thank You!