Gene Expression-Targeted Isoflavone Therapy: Facts, Questions and Further Possibilities

Grzegorz Wegrzyn
Department of Molecular Biology
University of Gdansk
Gdansk, Poland
Lysosomal storage diseases (LSD)

• A group of over 50 diseases
• Each disease is caused by a deficiency in:
  (i) a specific lysosomal hydrolase (leading to an inability to degrade particular macromolecules, for example: sphingolipids, glycoproteins, glycosaminoglycans)
  (ii) a protein involved in transport of particular compounds through lysosomal membranes
  (iii) an enzyme that modifies lysosomal proteins, ensuring their proper localization and function
  (iv) an activator of particular lysosomal enzyme
Normal fibroblast      LSD fibroblast

lysosomes

nucleus
The deposition of undegraded substrates in tissues throughout the body leads to a multisystemic disease.

- Growth deficiency (dwarfism)
- Central nervous system and sensory organs affected
- Bones and joints problems (Disostosis multiplex)
- Visceral organs’ dysfunctions
Pathogenic cascades in LSD

Primary cause of an LSD

Genetic defect that leads to an altered protein activity and the accumulation of metabolite(s) in lysosomes

Secondary cause

Changes in cellular processes

Secondary biochemical pathway(s)

Downstream pathways that are affected by either the primary or secondary causes

Tissue pathology

Altered gene expression

Tertiary biochemical pathway(s)

Cell/tissue damage and death

Nature Reviews | Molecular Cell Biology
Mucopolysaccharidoses (MPS): a group of lysosomal storage diseases

Glycosaminoglycans (GAGs) are accumulated in MPS

Different kinds of GAGs, whose degradation is inhibited at different steps, are accumulated in various MPS types.
Degradation of dermatan sulfate

I2S enzyme
α-L-iduronidase
N-acetylglactosamine 4-sulfatase
β-hexosaminidase A, B, S
β-glucuronidase

I2S = iduronate-2-sulfatase
NAc = N-acetylgalactosamine
MPS are chronic, progressive and life-threatening diseases

Clinical features include:
- organomegaly (e.g. liver, spleen, tongue)
- dysostosis multiplex
- obstructive airway disease
- impaired cardiovascular functions
- impaired hearing and vision
- joint stiffness
- hernias
- spinal cord compression
- hydrocephalus
- mental retardation (in some cases)

SIGNIFICANTLY SHORTENED LIFE SPAN (death usually within a childhood)
Potential therapies for LSD:

- Enzyme replacement therapy (ERT)
- Bone marrow (or stem cell) transplantation
- Gene therapy
- Stop codon read-through
- Small chaperones
- Substrate optimization therapy
- Substrate reduction therapy
Potential therapies for LSD:

- Enzyme replacement therapy (ERT)
- Bone marrow (or stem cell) transplantation
- Gene therapy
- Stop codon read-through
- Small chaperones
- Substrate optimization therapy
- Substrate reduction therapy
The idea of substrate reduction therapy

NORMAL (Synthesis = Degradation)
The idea of substrate reduction therapy

LSD (Synthesis > Degradation)
The idea of substrate reduction therapy

Precursors (for synthesis) → Substrate (for degradation)

SYNTHESIS

Inhibited by therapeutics

DEGRADATION

Inhibited due to mutations

SRT for LSD (Synthesis = Degradation)
Facts
Inhibition of glycosaminoglycan synthesis using rhodamine B in a mouse model of mucopolysaccharidosis type IIIA

Roberts A.L., Thomas B.J., Wilkinson A.S., Fletcher J.M., Byers S.
Rodamine B is an inhibitor of GAG synthesis but the mechanism of its action is unknown.
Reduction of GAG accumulation in MPS fibroblasts treated with rhodamine B for 14 days
MPS IIIA mouse treated with rhodamine B (weekly infusions of 1 mg/kg) for 6 months

Untreated MPS IIIA mouse

Control (a healthy mouse)
Genistein-mediated inhibition of glycosaminoglycan synthesis as a basis for gene expression-targeted isoflavone therapy for mucopolysaccharidoses

Ewa Piotrowska, Joanna Jakobkiewicz-Banecka, Sylwia Baranska, Anna Tylki-Szymanska, Barbara Czartoryska, Alicja Wegrzyn and Grzegorz Wegrzyn
5, 7-dihydroxy-3- (4-hydroxyphenyl)-4H-1-benzopyran-4-one
4', 5, 7-trihydroxyisoflavone

Genistein
Inhibition of glycosaminoglycan (GAG) synthesis by genistein

Inhibition of EGF receptor phosphorylation
(long term experiment - 24 h)
EGF receptor

Signal transduction (kinases’ cascade) including action of protein tyrosine kinases

Activation of transcription factors

Stimulation of gene expression (transcription)

MPS IIIB mouse model
Short-term experiment

**AIM:** To establish the most effective and non-toxic dose of genistein which can significantly reduce GAG storage in MPS IIIB mice and be used as substrate reduction therapy for Sanfilippo syndrome.

Reduction of GAG storage in liver of MPS IIIB mice

Wild-type

MPS IIIB untreated

MPS IIIB genistein (160 mg/kg for 8 weeks)
Electron Microscopy

Hair morphology males

Hair morphology females

Graphs show the score changes in hair morphology with varying genistein concentrations for males and females.
Long-term experiment

- Soy free diet
- Diet with genistein 160 mg/kg

Weight, Blood, Urine, Hair

Histology, Biochemistry, MassSpec

TopScan, Circadian rhythm, Bar crossing, Inverted Screen

Malinowska et al. (2010) PLoS ONE
Behavioral tests

Inverted screen test

Bar crossing test

Home cage behavior
Behavioral tests

- Entry to centre area frequency
  - MPS IIIB: Control > Genistein (p=0.001)
  - WT: No significant difference

- Centre speed (mm/s)
  - MPS IIIB: Control > Genistein (p<0.001)
  - WT: No significant difference

- Side speed (mm/s)
  - MPS IIIB: Control > Genistein (p<0.001)
  - WT: No significant difference

- Distance travelled (m)
  - MPS IIIB: Control > Genistein (p<0.001)
  - WT: No significant difference

- Speed over 90 mm/s frequency
  - MPS IIIB: Control > Genistein (p<0.001)
  - WT: No significant difference

- Speed > 90 mm/s duration (s)
  - MPS IIIB: Control > Genistein (p<0.001)
  - WT: No significant difference

- Immobility frequency
  - MPS IIIB: Control = Genistein
  - WT: Control > Genistein (p=0.001)

- Immobility duration (s)
  - MPS IIIB: Control = Genistein
  - WT: Control < Genistein (p>0.001)

- Bar crossing score
  - MPS IIIB: Control > Genistein (p=0.001)
  - WT: No significant difference
A Genetic Model of Substrate Reduction Therapy for Mucopolysaccharidosis

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William C. Lamanna, Roger Lawrence, Stéphane Sarrazin, Carlos Lamed-Diaz, Philip L. S. M. Gordts, Kelley W. Moremen, and Jeffrey D. Esko

From the Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California at San Diego, La Jolla, California 92030-0687 and the Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30602-4712

Background: Treatment of neuropathology in mucopolysaccharidoses may be possible by substrate reduction therapy.

Results: A genetic model of substrate reduction therapy for mucopolysaccharidosis type IIIa ameliorates disease pathology in the brain.

Conclusion: Partial inhibition of glycosaminoglycan biosynthesis may be useful for treating mucopolysaccharidoses type III.

Significance: Proof of principle is presented that inhibition of heparan sulfate synthesis might prove beneficial for treating mucopolysaccharidoses.
Mice:

$Sgsh^{-/-}$ (Illa)
$Sgsh^{-/-}Ext1^{+/+}$ (IllaE1)
$Sgsh^{-/-}Ext1^{+/+}Ext2^{+/+}$ (IllaE1E2)
gSRT ameliorates lysosomal storage in MPS IIIa mice


- Brain
  - WT: 7 ± 1.4
  - IIIa: 5.3 ± 0.3
  - IIIaE1: 3.9 ± 0.4
  - IIIaE1E2: 29 ± 2.0

- Liver
  - WT: 5 ± 0.2
  - IIIa: 16.5 ± 2.6
  - IIIaE1: 11.3 ± 1.8
  - IIIaE1E2: 12 ± 1.8

- Spleen
  - WT: 0.3 ± 0.1
  - IIIa: 18 ± 2.4
  - IIIaE1: 12 ± 1.4
  - IIIaE1E2: 40 ± 2.9

- Kidney
  - WT: 10 ± 0.7
  - IIIa: 10.4 ± 2.4
  - IIIaE1: 7.4 ± 0.7
  - IIIaE1E2: 14.2 ± 1.5

- Heart
  - WT: 0.14 ± 0.01
  - IIIa: 5.4 ± 0.6
  - IIIaE1: 10 ± 1.4
  - IIIaE1E2: 8.2 ± 0.9

- Lung
  - WT: 5 ± 0.2
  - IIIa: 10 ± 1.4
  - IIIaE1: 10 ± 1.4
  - IIIaE1E2: 8.2 ± 0.9
gSRT reduces hepatomegaly in MPS IIIa mice

gSRT ameliorates markers of neuropathology in MPS IIIa mice

Ext1/Ext2 heterozygosity improves the efficacy of ERT in MPS IIIa cells and mice

Questions
Storage correction in cells of patients suffering from mucopolysaccharidoses types IIIA and VII after treatment with genistein and other isoflavones

Audrey Arfi · Magali Richard · Christelle Gandolphe · Daniel Scherman

Received: 1 October 2009 / Revised: 7 December 2009 / Accepted: 8 December 2009
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Brief Communication

Genistein reduces heparan sulfate accumulation in human mucolipidosis II skin fibroblasts

Takanobu Otomo *, Mohammad Arif Hossain, Keiichi Ozono, Norio Sakai

Department of Pediatrics, Osaka University Graduate School of Medicine, 2-2 Yamada-oka, Suita, Osaka 565-0871, Japan

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KEYWORDS:

ABSTRACT

Genistein, a soy isoflavone, reduces glycosaminoglycan synthesis and its effect on mucopolysaccharidoses has been tested. In this report, we examined the effect of genistein in human mucolipidosis II skin fibroblasts in vitro. Heparan sulfate was accumulated within both cells and in extracellular spaces in mucolipidosis II. Genistein reduced the amount of heparan sulfate in cultured cells dose dependently and also inhibited cell growth dose dependently.
RESEARCH PAPER

Genistein reduces glycosaminoglycan levels in a mouse model of mucopolysaccharidosis type II

A Friso, R Tomanin, M Salvalaio and M Scarpa

Department of Pediatrics, University of Padova, Padova, Italy
Genistein increases glycosaminoglycan levels in mucopolysaccharidosis type I cell models

Sandra D. K. Kingma · Tom Wagemans · Lodewijk IJlst · Frits A. Wijburg · Naomi van Vlies

Received: 10 December 2013 / Revised: 24 February 2014 / Accepted: 10 March 2014
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Abstract Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder characterized by diminished degradation of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate, which results in the accumulation of these GAGs and subsequent cellular dysfunction. Patients present with a variety of symptoms, including severe skeletal disease. Genistein has been shown previously to inhibit GAG synthesis in MPS fibroblasts, presumably through inhibition of tyrosine kinase activity of the epidermal growth factor receptor (EGFR). To determine the potentials of genistein for the treatment of skeletal disease, MPS I fibroblasts were induced into chondrocytes and osteoblasts and treated with genistein. Surprisingly, whereas tyrosine phosphorylation levels (as a measure for tyrosine kinase inhibition) were decreased in all treated cell lines, there was a 1.3 and 1.6 fold increase in GAG levels in MPS I chondrocytes and fibroblast, respectively ($p<0.05$). Sulfate incorporation in treated MPS I fibroblasts was 2.6 fold increased ($p<0.05$), indicating increased GAG synthesis despite tyrosine kinase inhibition. This suggests that GAG synthesis is not exclusively regulated through the tyrosine kinase activity of the EGFR. We hypothesize that the differences in outcomes between studies on the effect of genistein in MPS are caused by the different effects of genistein on different growth factor signaling pathways, which regulate GAG synthesis. More studies are needed to elucidate the precise signaling pathways which are affected by genistein and alter GAG metabolism in order to evaluate the therapeutic potential of genistein for MPS patients.
The Phytoestrogen Genistein Modulates Lysosomal Metabolism and Transcription Factor EB (TFEB) Activation*  

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Marta Moskot‡, Sandro Montefusco§, Joanna Jakóbkiewicz-Banecka¶, Paweł Mozolewski‖, Alicja Węgrzyn¶, Diego Di Bernardo§, Grzegorz Węgrzyn¶, Diego L. Medina§, Andrea Ballabio§**,†††‡‡ and Magdalena Gabig-Cimińska‡‡  

From the ‡Laboratory of Molecular Biology (affiliated with the University of Gdańsk), Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Wita Stwosza 59, 80-308 Gdansk, Poland, the §High Content Screening Facility, Telethon Institute of Genetics and Medicine (TIGEM), Via P. Castellino 111, 80131 Naples, Italy, the ¶Department of Molecular Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland, the ‖Department of Microbiology, University of Szczecin, Felczaka 3c, 71-412 Szczecin, Szczecin, Poland, the **Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, the ‡‡Jan and Dan Duncan Neurological Research Institute, Texas Children’s Hospital, Houston, Texas 77030, and the §§Medical Genetics, Department of Pediatrics, Federico II University, Via Pansini 5, 80131 Naples, Italy  

**Background**: Genistein is a potential drug for certain inherited lysosomal disorders.  
**Results**: Genistein influences molecular cross-talk in the cell responsible for lysosomal enhancement.  
**Conclusion**: Genistein potentiates lysosomal metabolism by activating transcription factor EB (TFEB).  
**Significance**: The explanation of genistein action offers more adequate therapeutic procedures for the treatment of some lysosomal storage diseases.
Gene expression modulation

<table>
<thead>
<tr>
<th>Gene expression modulation</th>
<th>Modulation targets</th>
<th>No. of genes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Genistein concentration [μM]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>▲ up-regulated genes</td>
<td>whole genome</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>GAG metabolism</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>GSL metabolism</td>
<td>0</td>
</tr>
<tr>
<td>▼ down-regulated genes</td>
<td>whole genome</td>
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<tr>
<td></td>
<td>GAG metabolism</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>GSL metabolism</td>
<td>0</td>
</tr>
</tbody>
</table>

Microarray analyses using HumanHT-12 Expression BeadChip

Transcriptomes of human fibroblasts treated with genistein
Expression of genes coding for enzymes involved in GAG synthesis and degradation is response to genistein, as assessed by microarray analyses and qRT-PCR.

Relative to *GAPDH* as a control.

Relative to *TBP* as a control.
GAG synthesis pathways

GAG degradation pathways

Stimulation by genistein

Inhibition by genistein

No strong effect of genistein
Expression of genes coding for enzymes involved in glycosphingolipids’ (GSL) synthesis and degradation is response to genistein, as assessed by microarray analyses and qRT-PCR.

Relative to GAPDH as a control

Relative to TBP as a control
Expression TFEB, a gene coding for the master positive regulator of lysosomal biogenesis, is stimulated by genistein.
Genistein stimulates lysosomal biogenesis in HDFa cells

- Control – no genistein
- 30 µM genistein
- 60 µM genistein
- 100 µM genistein
Genistein stimulates lysosomal biogenesis in MPS I cells
Brief Report


Ewa Piotrowska, MSc¹; Joanna Jakóbkiewicz-Banecka, PhD¹,²; Anna Tylki-Szymanska, MD, PhD, DSc³; Anna Liberek, MD, PhD⁴; Agnieszka Maryniak, PhD, DSc³; Marcelina Malinowska, MSc¹; Barbara Czartoryska, PhD⁵; Ewa Puk, PhD⁶; Anna Kloska, MSc¹; Tomasz Liberek, MD, PhD, DSc⁷; Sylwia Baranska, PhD¹; Alicja Wegrzyn, PhD, DSc²; and Grzegorz Wegrzyn, PhD, DSc¹

¹Department of Molecular Biology, University of Gdansk, Gdansk, Poland; ²Laboratory of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Gdansk, Poland; ³The Children's Memorial Health Institute, Warsaw, Poland; ⁴Department of Pediatrics, Children's Gastroenterology and Oncology, Medical University of Gdansk, Gdansk, Poland; ⁵Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland; ⁶Biofarm, Poznan, Poland; and ⁷Department of Nephrology, Transplantation, and Internal Medicine, Medical University of Gdansk, Gdansk, Poland
Figure 1. Urinary heparan sulfate (HS) concentrations in patients with mucopolysaccharidosis (MPS) IIIA or IIIB treated with a genistin-rich isoflavone extract. (Median values are shown by the dashed horizontal bars at baseline and 12 months.) $P = 0.028$ versus baseline. Significant changes were found in patients IIIA-1, IIIA-2, IIIA-3, IIIA-4, IIIA-5, IIIB-3, and IIIB-5.
Table II. Hair morphology assessed using electron microscopy and cognitive function assessed using the Brief Assessment Examination (BAE) at baseline and after 12 months of treatment with genistin-rich isoflavone extract.\textsuperscript{21–23}

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Hair Morphology Score*</th>
<th>BAE Score†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>12 Months</td>
</tr>
<tr>
<td>IIIA-1</td>
<td>1</td>
<td>0\textsuperscript{†}</td>
</tr>
<tr>
<td>IIIA-2</td>
<td>1</td>
<td>0\textsuperscript{†}</td>
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<tr>
<td>IIIA-3</td>
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<td>1\textsuperscript{†}</td>
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<tr>
<td>IIIA-5</td>
<td>3</td>
<td>1\textsuperscript{†}</td>
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<tr>
<td>IIIIB-1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IIIIB-2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>IIIIB-3</td>
<td>2</td>
<td>1\textsuperscript{†}</td>
</tr>
<tr>
<td>IIIIB-4</td>
<td>3</td>
<td>2\textsuperscript{†}</td>
</tr>
<tr>
<td>IIIIB-5</td>
<td>3</td>
<td>1\textsuperscript{†}</td>
</tr>
</tbody>
</table>

\*Scale: 0 = normal to 5 = most abnormal.
\textsuperscript{†}Scale: 0 = no contact with the tested child to 52 = normal score for properly developed child at the age of 3 years.
\textsuperscript{‡}\textit{P} = 0.012 versus baseline.
Genistein supplementation in patients affected by Sanfilippo disease

Verónica Delgadillo • Maria del Mar O’Callaghan • Rafael Artuch • Raquel Montero • Mercedes Pineda

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Table 3  Clinical and biochemical results at baseline and after 12 months of treatment

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>12 months</th>
<th>Wilcoxon test</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Standard deviation</td>
<td>Reference values</td>
</tr>
<tr>
<td>GAGs (mg/mmol creat.)</td>
<td>27.54</td>
<td>13.94</td>
<td>(0.36–6.4) 6\textsuperscript{a}</td>
</tr>
<tr>
<td>CoQ10 (μmol/L)</td>
<td>0.42</td>
<td>0.16</td>
<td>(0.41–1.12) 0.70</td>
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<tr>
<td>Cholesterol (mmol/L)</td>
<td>3.8</td>
<td>0.75</td>
<td>(2.47–5.20) 2.73</td>
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<tr>
<td>Vitamin E (μmol/L)</td>
<td>20.16</td>
<td>5.89</td>
<td>(13.4–36.4) 23</td>
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<tr>
<td>Disability score</td>
<td>9.5</td>
<td>6.27</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a}GAG Glycosaminoglycan, n.s. not significant

\textsuperscript{b}GAG reference values are average values for 6 year old

\textsuperscript{b}Significant worsening in disability scores was observed after 12 months of therapy
Table 4 Hair morphology determined with electron microscopy at baseline and after 12 months of treatment with genistein-rich isoflavone extract

<table>
<thead>
<tr>
<th>Patient</th>
<th>Hair morphology score&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Baseline</td>
<td>After 1 year</td>
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<td>III A - 1</td>
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<td>0</td>
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<td>III A - 2</td>
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<td>III A - 3</td>
<td>3</td>
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<td>III A - 4</td>
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<td>III A - 6</td>
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<td>III A - 7</td>
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<td>III A - 8</td>
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<td>III A - 9</td>
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<tr>
<td>III A - 10</td>
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<td>III B - 2</td>
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<td></td>
</tr>
<tr>
<td>III C - 1</td>
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<td>0</td>
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<tr>
<td>III C - 3</td>
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<tr>
<td>III C - 5</td>
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<td>0</td>
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</tr>
<tr>
<td>III C - 6</td>
<td>2</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Scores range from 0 (normal) to 5 (most abnormal)
Improvement in the Range of Joint Motion in Seven Patients With Mucopolysaccharidosis Type II During Experimental Gene Expression-Targeted Isoflavone Therapy (GET IT)

Jolanta Marucha,¹ Anna Tylki-Szymańska,¹ Joanna Jakóbkiewicz-Banecka,² Ewa Piotrowska,² Anna Kloska,² Barbara Czartoryska,³ and Grzegorz Węgrzyn¹*

¹Department of Metabolic Diseases, The Children’s Memorial Health Institute, Warsaw, Poland
²Department of Molecular Biology, University of Gdańsk, Gdańsk, Poland
³Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland

Received 25 May 2010; Accepted 23 April 2011
Genistein in Sanfilippo Disease: A Randomized Controlled Crossover Trial

Jessica de Ruijter, MD, Marlies J. Valstar, MD, PhD, Magdalena Narajczyk, PhD, Grzegorz Wegrzyn, PhD, Wim Kulik, PhD, Lodewijk IJlst, BASc, Tom Wagemans, BASc, Willem M. van der Wal, PhD, and Frits A. Wijburg, MD, PhD

Objective: Sanfilippo disease (mucopolysaccharidosis type III [MPS III]) is a rare neurodegenerative metabolic disease caused by a deficiency of 1 of the 4 enzymes involved in the degradation of heparan sulfate (HS), a glycosaminoglycan (GAG). Genistein has been proposed as potential therapy but its efficacy remains uncertain. We aimed to determine the efficacy of genistein in MPS III.

Methods: Thirty patients were enrolled. Effects of genistein were determined in a randomized, crossover, placebo-controlled intervention with a genistein-rich soy isoflavone extract (10mg/kg/day of genistein) followed by an open-label extension study for patients who were on genistein during the last part of the crossover.

Results: Genistein resulted in a significant decrease in urinary excretion of total GAGs (p = 0.02, slope −0.68mg GAGs/mmol creatinine/mo) and in plasma concentrations of HS (p = 0.01, slope −15.85ng HS/ml/mo). No effects on total behavior scores or on hair morphology were observed. Parents or caregivers could not predict correctly during which period of the crossover a patient was on genistein.

Interpretation: Genistein at 10mg/kg/day effectively reduces urinary excretion of GAGs and plasma HS concentration in patients with MPS III. However, the absolute reduction in GAGs and in HS is small and values after 12 months of treatment remain within the range as observed in untreated patients. No clinical efficacy was detected. Substantially higher doses of genistein might be more effective as suggested by recent studies in animal models.

ANN NEUROL 2012;71:110–120
Genistein decreases levels of total GAGs and heparan sulfate in MPS III patients.
The Use of Elevated Doses of Genistein-Rich Soy Extract in the Gene Expression-Targeted Isoflavone Therapy for Sanfilippo Disease Patients

Věra Malinová • Grzegorz Węgrzyn • Magdalena Narażycki

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Glycosaminoglycans (GAGs)

Precursors

SYNTHESIS

DEGRADATION

Primary GAG storage

Secondary storage

Inflammation

Oxidative stress

P-tau accumulation

Apoptosis

Genistein

Synapse disappearance and dysfunction

Dysfunctions of cells, tissues and organs

Symptoms

Further Possibilities
High dose genistein aglycone therapy is safe in patients with mucopolysaccharidoses involving the central nervous system

Katherine H. Kim a,b, Charlotte Dodsworth a, Andrea Paras a,b, Barbara K. Burton a,b,*

a Ann & Robert H. Lurie Children’s Hospital of Chicago, 225 E. Chicago Ave, Chicago, IL 60611, USA
b Department of Pediatrics, Feinberg School of Medicine, Northwestern University, 303 E. Chicago Ave, Chicago, IL 60611, USA

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ABSTRACT
Genistein (4,5,7-trihydroxyisoflavone), a soy derived isoflavone, has been proposed as a substrate therapy in patients with mucopolysaccharidoses (MPS) disorders with central nervous system involvement based on studies in cultured fibroblasts demonstrating that this agent inhibits glycosaminoglycan synthesis. Several studies have reported treatment of MPS III patients with low dose genistein (5–15 mg/kg) yielding no serious adverse effects and variable neurocognitive outcomes. Mice with MPS IIIB treated with high dose (160 mg/kg/day) genistein exhibited a significant decrease in heparan sulfate accumulation and neuropathology in the brain and improvement of the behavioral phenotype. No study to date has been performed using high dose genistein treatment in MPS patients. We initiated an open label study to assess the safety of high dose genistein treatment in MPS patients with neurological impairment. Twenty-two eligible patients were treated with pure synthetic genistein at a dose of 150 mg/kg/day. Safety labs, urine GAG levels, and neurocognitive markers were monitored for 12 months.
Sanfilippo Clinical Trial

A Phase III, Double Blinded, Randomised, Placebo Controlled Clinical Trial for
High Dose Oral Genistein Aglycone in Patients with Sanfilippo Syndrome
(Mucopolysaccharidosis III A, B and C) - GENiSIS2013.

Released November 26 2013

Stem Cell & Neurotherapies Group, Centre for Genomic Medicine, University of Manchester
Willink Biochemical Genetics Unit, Centre for Genomic Medicine, St Mary’s Hospital, C
Manchester University Hospitals NHS Foundation Trust

We will soon be recruiting for a phase III, double blinded, randomised, placebo controlled clinical trial
high dose oral genistein aglycone in Sanfilippo diseases (MPSIIIA, B and C). This is funded by the
society for Mucopolysaccharide Diseases, The National MPS society and the GEM appeal in a grant
Brian Bigger and sponsored by the Central Manchester Universities Hospitals NHS Foundation Trust.
expect to begin recruiting in early 2014, primarily from within the UK.

The trial will be regulated by the UK MHRA, performed at the NIHR/Wellcome Trust Clinical Research
facility in Manchester using GMP grade genistein aglycone. The trial will be one year placebo control
with one year open label extension with robust efficacy and safety endpoints.
Effects of flavonoids on glycosaminoglycan synthesis: implications for substrate reduction therapy in Sanfilippo disease and other mucopolysaccharidoses

Anna Kloska • Joanna Jakóbkiewicz-Banecka • Magdalena Narajczyk • Zyta Banecka-Majkutewicz • Grzegorz Węgrzyn

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**Fig. 3** Effects of mixtures of natural flavonoids (K—kaempferol, N—naringenin, D—daidzein, G—genistein, at 10 μM concentration each) on kinetics of glycosaminoglycan synthesis in fibroblasts. Relative $^{35}$S incorporation into GAGs after 3-day exposure to mixtures of various flavonoids is presented. Labeling was conducted for 24 h with 20 μCi/ml H$_2$$^{[35]$S$]}$O$_4$. Radioactivity of incorporated $^{35}$S was measured in a scintillation counter, calculated per DNA amount [dpm/ng DNA], and expressed as the percentage of control (ctrl = cell culture treated with 0.05% dimethylformamide). The results presented are average values obtained for three different cell lines with bars indicating standard deviation. Statistical analysis was performed by using the $t$-Student two-tailed test. Values of $p<0.05$ (*) or $p<0.01$ (**) are indicated.
Synthetic genistein derivatives as modulators of glycosaminoglycan storage

Anna Kloska¹, Magdalena Narajczyk², Joanna Jakóbkiewicz-Banecka¹, Grzegorz Gryniewicz³, Wiesław Szeja⁴, Magdalena Gabig-Cimińska¹,⁵ and Grzegorz Węgrzyn¹*
Table 3 Effect of synthetic derivatives of genistein at 30 μM concentration on the number of different lysosomal structures in MPS IIIA and MPS IIIB fibroblasts

<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of structures per 100 μm² of cellular cross section ± SD</th>
<th>Total number</th>
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<tr>
<td></td>
<td>lamellar</td>
<td>complexed</td>
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<tr>
<td>MPS IIIA</td>
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<tr>
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<td>0.40 ± 0.17</td>
<td>0.35 ± 0.35</td>
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<tr>
<td>IFG-032</td>
<td>0.14 ± 0.08 *</td>
<td>0.22 ± 0.11</td>
</tr>
<tr>
<td>IFG-034</td>
<td>0.17 ± 0.09 *</td>
<td>0.27 ± 0.14</td>
</tr>
<tr>
<td>IFG-036</td>
<td>0.19 ± 0.14 *</td>
<td>0.28 ± 0.16</td>
</tr>
<tr>
<td>IFG-038</td>
<td>0.32 ± 0.16</td>
<td>0.27 ± 0.13</td>
</tr>
<tr>
<td>IFG-066</td>
<td>0.13 ± 0.10 *</td>
<td>0.16 ± 0.08</td>
</tr>
<tr>
<td>IFG-071</td>
<td>0.28 ± 0.13</td>
<td>0.26 ± 0.09</td>
</tr>
<tr>
<td>IFG-072</td>
<td>0.20 ± 0.10 *</td>
<td>0.28 ± 0.10</td>
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</table>

MPS IIIB

<table>
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<tr>
<th>Compound</th>
<th>Number of structures per 100 μm² of cellular cross section ± SD</th>
<th>Total number</th>
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<tr>
<td>None</td>
<td>0.44 ± 0.18</td>
<td>0.14 ± 0.10</td>
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<tr>
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<td>0.06 ± 0.05</td>
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<td>0.12 ± 0.11</td>
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<td>0.11 ± 0.14</td>
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<tr>
<td>IFG-071</td>
<td>0.07 ± 0.07 *</td>
<td>0.07 ± 0.06</td>
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<tr>
<td>IFG-072</td>
<td>0.11 ± 0.12 *</td>
<td>0.16 ± 0.08</td>
</tr>
</tbody>
</table>

Asterisks (*) indicate statistically significant differences (one-way ANOVA with Tukey’s multiple comparisons as a post-hoc test, p < 0.05) relative to control MPS IIIA and MPS IIIB cells (None) where no tested compound was added into culture medium.
Microarray analyses using HumanHT-12 Expression BeadChip

Transcriptomes of human fibroblasts treated with genistien, kaempferol, daidzein, and their combinations

<table>
<thead>
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<td>30 + 30 60 100</td>
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<td>6 10 0 5 0 4</td>
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<tr>
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<td>GSL metabolism</td>
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<td>6 12 5 13 11 19</td>
<td>8 20 1 5 3 10</td>
<td>4 15</td>
<td></td>
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<tr>
<td>▼ down-regulated genes</td>
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<td>236 370</td>
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