Origin of lysosomal enzymes in human cerebrospinal fluid

Third Assisi Workshop on Biomarkers in the early diagnosis of neurodegenerative disorders

Perugia May 21-23, 2015
Lysosomes

Macromolecules

Bacteria

Death cells
Lysosomes

- Acid hydrolases
  - Nucleases
  - Proteases
  - Glycosidases
  - Lipases
  - Phosphatases
  - Sulfatases

- Transporters
  - substrates
    - amino acids
    - di/tripeptides
    - monosaccharides
    - nucleosides
    - nucleotides
    - inorganic ions
    - vitamins

- ATP
  - \( H^+ \)
Lysosome Biogenesis
Lysosomal enzyme transport
glucocerebrosidase mechanisms of transport
Glucocerebrosidase Binds to its Target Cells via the Mannose Receptor
Lysosomal Storage Diseases, LSD

Caused by genetic mutations that reduce / disrupt
the activity of a specific lysosomal hydrolase

50 known LSDs (1/ 7,000-9,000 live births)
60% of them involve PNS and/or CNS
Niemann-Pick, Fabry, Tay-Sachs, Gaucher’s Disease (and others) share some neuropathological features (undegraded material, inflammatory mediators, morphological and functional abnormalities) with Parkinson’s and Alzheimer’s disease.

Gaucher’s disease (GD) is strongly associated to PD.
Enzymatic activity variations in CSF
Parkinson (PD) and Dementia with Lewy bodies (DLB)

- ↓ β-glucocerebrosidase in **CSF** of PD and DLB, ↓ α-mannosidase and β-mannosidase in **CSF** of PD (Balducci et al.; 2007) (Parnetti et al.; 2009)

- ↑ β-hexosaminidase in **CSF** of PD (Parnetti et al.; 2013)

- ↑ β-galactosidase and cathepsin E, ↓ α-fucosidase in **CSF** of PD (Van Dijk et al.; 2013)
# Enzymatic activity variations in brain

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Caudate</th>
<th>Frontal cortex</th>
<th>Substantia Nigra</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-exosaminidase</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>α-fucosidase</td>
<td></td>
<td>↓ (PD)</td>
<td></td>
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<tr>
<td>β-mannosidase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-mannosidase</td>
<td></td>
<td>↑ (DLB)</td>
<td></td>
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<tr>
<td>β-galactosidase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-glucorecebroside</td>
<td>↓ (PD)</td>
<td></td>
<td>↓ (PD) ↓ (DLB)</td>
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<tr>
<td>Cathepsin E</td>
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</table>
Lysosomal enzymes in the CSF could result directly from the secretory processes that occur in the brain tissue during the transfer of these enzymes from the Golgi apparatus to the lysosomes.

However, it is also possible that they are secreted from other tissues and reach the central nervous system through the plasma flow across the blood-brain barrier.
The aim of this study is to determine the type and origin of α-mannosidase activity in the CSF by comparison with alpha-mannosidase activity and isoenzyme expression in human plasma and frontal gyrus.
Among lysosomal enzymes, α-mannosidases are expressed in multiple forms in human tissues and body fluids with multiple functions in glycoprotein metabolism.
**Lysosomal \(\alpha\)-mannosidase**

- **Gene**: MAN2B1
- **Optimum pH**: 4.5
- **Isoforms**: A, B

**Plasmatic \(\alpha\)-mannosidase**

- **Gene**: ?
- **Optimum pH**: 5.5
- **Isoforms**: ?

\(\alpha\)-mannosidase Class II
α-mannosidase activity at pH 4.5 and pH 5.5
of human plasma, CSF, frontal gyrus

<table>
<thead>
<tr>
<th></th>
<th>α-mannosidase activity pH 4.5</th>
<th>α-mannosidase activity pH 5.5</th>
<th>ratio pH 4.5/pH 5.5</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Plasma (5)</strong></td>
<td>335 ± 98.8 mU/ml</td>
<td>1550.4 ± 255.3 mU/ml</td>
<td>0.21 ± 0.03</td>
</tr>
<tr>
<td><strong>CSF (5)</strong></td>
<td>141 ± 10.6 mU/ml</td>
<td>117.7 ± 17.8 mU/ml</td>
<td>1.21 ± 0.13</td>
</tr>
<tr>
<td><strong>Frontal gyrus (5)</strong></td>
<td>1442 ± 416 mU/mg</td>
<td>964 ± 74 mU/mg</td>
<td>1.49 ± 0.37</td>
</tr>
</tbody>
</table>

(number of sample analysed)
CSF

mU/ml

α-mannosidase activity pH 4.5

α-mannosidase activity pH 5.5

NaCl (M)

0.5

0

fraction number

0 10 20 30 40 50 60 70 80
Frontal gyrus

α-mannosidase activity pH 4.5

α-mannosidase activity pH 5.5

NaCl (M)

fraction number

mU/ml
CONCLUSIONS

• Lysosomal α-mannosidase is expressed in frontal gyrus in two forms A and B that can be separated by DEAE-cellulose chromatography.

• In human plasma the plasmatic α-mannosidase represents the majority of α-mannosidase activity and does not cross the Blood Brain Barrier.

• In CSF the A and B forms of lysosomal α-mannosidase derived from the brain.
Also for lysosome enzymes, CSF mirrors the pathological changes taking place in the brain.

Which enzymes for which pathology?

- region-specificity?
- disease-specificity?
Lysosomal β-hexosaminidase

Gene
- HEXA: Chr 15
- HEXB: Chr 5

Protein
- subunit α
- subunit β

Isoenzymes
- HexA: α β
- HexB: β β
β-hexosaminidase activity at pH 4.5
of human plasma, CSF, frontal gyrus

<table>
<thead>
<tr>
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<th>β-hexosaminidase activity pH 4.5</th>
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<tr>
<td><strong>Plasma (2)</strong></td>
<td>14.7 ± 2 U/ml</td>
</tr>
<tr>
<td><strong>CSF (2)</strong></td>
<td>3.9 ± 0.65 U/ml</td>
</tr>
<tr>
<td><strong>Frontal gyrus (2)</strong></td>
<td>121.5 ± 32.4 U/mg</td>
</tr>
</tbody>
</table>

(number of sample analysed)
Plasma

β-hexosaminidase activity pH 4.5

mU/ml

fraction number

β-hex A

β-hex B

NaCl (M)

0

0.5
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Optimum pH total β-hexosaminidase activity

Plasma

CSF
Lysosomal Storage Diseases, LSD

- Continuum of symptoms; onset at any age

- Typically classified in 3 subtypes: infantile, juvenile and adult/chronic

- **Infantile forms**: mental retardation, movement impairment, deafness and/or blindness, hepatomegaly, splenomegaly, respiratory and cardiac insufficiency

- **Adult forms**: often characterized by neurodegeneration and dementia
Optimum pH α-mannosidase activity

Frontal gyrus

Plasma

CSF
Lysosomal Storage Diseases

Normal Cell → Enzyme Deficiency → Lysosomal Storage Disease Cell

Liver and spleen → Bone → Bone marrow → Kidneys → Brain and nerves → Heart → Muscles & Joints

Facies/eyes → Skin
Gaucher’s Disease, GD

Rare autosomal recessive LSD (1/60,000 live births)

Three variants: type 1, 2, and 3

caused by mutations in the GBA gene, coding \textbf{beta-glucocerebrosidase} a lysosomal enzyme involved in the catabolic pathway of \textbf{glycosphingolipids}.

Macrophages accumulate the largest amount of glucocerebroside — “\textit{Gaucher’s cell}”

Schrier, S. Image bank 2002
American Society of Hematology

Lichtman’s Atlas of Hematology
Thus the cerebrospinal fluid might mirror the brain pathological changes linked to neurodegenerative disorders such as Parkinson’s disease.

Based on this result it is likely to deduce that other lysosomal enzymes present in the CSF might have the same origin from the brain and that their content could vary in pathological conditions.
The lysosomal system

Lysosomes contain up to 60 soluble **acidic hydrolases**, 55 membrane-associated and 215 integral membrane proteins.

Lysosomes are involved in membrane **repair**, **homeostasis**, **cell signaling**, and **apoptosis**.