Clinical-pathologic and Biochemical Aspects of the Sphingolipidoses and their Impact on the Nervous System
Drs. Reid Heffner and Alastair Brownie

Assigned Reading: Lippincott’s Biochemistry pp. 208-210
Robbins. pp. 158-164 and 1397-1398

Lecture Content:
Sphingolipid Biosynthesis and assay of catabolic enzymes
Biochemical and clinico-pathological aspects of disorders of sphingolipids leading to neurologic syndromes –
  Gangliosidosis GM1
  Tay-Sachs Disease and Sandhoff Disease
  Gaucher’s Disease
  Niemann-Pick Disease
The Leukodystrophies:
  Krabbe Disease
  Metachromatic Leukodystrophy
  Adrenoleukodystrophy
Learning objectives

• Be familiar with biochemical, clinical, pathologic and genetic features of diseases discussed
• Know definitions and significance of:
  – Cherry red spot
  – Hyperacusis
  – Meganeurite
  – Selected EM inclusions [MCBs, myelin-like, tubular, lamellar]
  – Leukodystrophy
  – Sea blue histiocyte
  – Gaucher cell
Sphingomyelins are the most abundant sphingolipid found in mammalian membranes especially the plasma membrane. They are found in nerve tissue and erythrocytes.

Cerebrosides (galactosylceramide and glucosylceramide) are found predominantly in the brain and PNS with high concentrations in the myelin sheath. They are major constituents of oligodendrocytes.

Gangliosides are found primarily in the ganglion cells of the CNS, particularly at nerve endings. They are concentrated in rafts in the plasma membrane (signal transduction!). The brain has 20-500 times more gangliosides than most non-neural tissues. The nomenclature is that of Lars Svennerholm! 5 minus the subscripted number = the number of CHOs

Sulfatides are cerebrosides with sulfated galactosyl residues. They are synthesized primarily in oligodendrocytes of the CNS.
SPHINGOLIPID BIOSYNTHESIS

SERINE + PALMITYL-CoA → SPHINGOSINE

Fatty acyl-CoA

PC

CERAMIDE

SPHINGOMYELIN

UDPG

UDPGal

Galactosyl-Ceramide

Glucosyl-Ceramide → GLOBOSIDES

Sialic acid

PAPS

SULFATIDE

GANGLIOSIDES
Ganglioside GM2
SPHINGOLIPIDOSES

• This group of inborn errors of metabolism occurs because of the lack of an enzyme in the breakdown (turnover) of sphingolipids

• Tay-Sachs disease (TSD), involving fatal neurological deterioration in early life, has been most studied

• Inheritance of TSD is autosomal recessive as it is for all other sphingolipidoses with the exception of Fabry disease

• Screening for carriers of TSD and other disorders has been done with significant success – how would you detect a carrier?
SPHINGOLIPIDOSES

How can we explain the accumulation of sphingolipids in these disorders?

SYNTHESIS \[\rightarrow X \rightarrow \text{CATABOLISM}\]

OR

SYNTHESIS \[\rightarrow X \rightarrow \text{CATABOLISM}\]

The sphingolipidoses are lipid storage diseases and in each of them the defect is in an enzyme of the degradative pathway occurring in lysosomes.
Inheritance of Autosomal Recessive Disorders
Diagnosis/Testing in TSD

- The diagnosis of HEX A deficiency relies upon the demonstration of deficient beta-hexosaminidase A activity in the serum of white blood cells of a symptomatic individual in the presence of elevated activity of the beta-HEX B isoenzyme.
- Mutation analysis is done for genetic counseling and to distinguish pseudodeficiency alleles from disease-causing alleles.
- Heterozygotes are identified through testing individuals with a positive family history or through population screening programs directed to people of Ashkenazi Jewish heritage.
ASSAY OF HEXOSAMINIDASE ACTIVITY

1. Enzyme assays of HEX-A and HEX-B
   only HEX-A hydrolyzes GM2 \textit{in vivo}

2. Use an artificial substrate to facilitate screening
   \textit{It is a substrate for both HEX-A and HEX-B}

3. Must distinguish between HEX-A and HEX-B \textbf{WHY?}
   HEX-B elevated in TSD heterozygotes

4. HEX-A is heat-labile!
   \textbf{What assays would you carry out?}

5. Electrophoretic separation

6. Assays on \textbf{live} cells!

7. Protein vs. Activity!!

8. GM2 activator
Heterozygote frequency of TSD in Ashkenazi Jewish populations worldwide as shown by mass screening program over the period 1971-92

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SPHINGOLIPID TURNOVER

GM₁ gangliosidosis

NAGA-GAL-GLC-CER

NANA

GM₂

NAGA-GAL-GLC-CER

NANA

GM₃

GAL-GLC-CER

NANA

Lactosyl-CER

Glucosyl-CER

Sphingomyelin

Lactosyl-CER

Glucosyl-CER

Sphingosine

CERAMIDE

Farber’s disease

So₃H-GAL-CER

Sulfatide

GM₁ gangliosidosis

TSD

SD

GM₂

GM₃

GM₃

GM₁ gangliosidosis

TSD

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GM₁ gangliosidosis

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GM₁ gangliosidosis

TSD

SD
Sphingolipidoses
General Principles

• Generally cause neuronal or white matter disease
• Onset typically in childhood
• Progressive fatal course
• No definitive treatment
General principles

- Neuronal disease
  - Brain and other structures to some extent
- White matter disease
  - Leukodystrophy
- Loss of cells/tissue
- Reactive gliosis
- Atrophy of brain in later stages
- Characteristic EM findings
Neuronal storage

Cytoplasm swollen
  Often foamy or vacuolated
Nucleus eccentric

Normal neuron
In later stages, neurons are lost and replaced by reactive glia (gliosis)
White matter disease

Normal white matter

Leukodystrophy
Gitter cells (macrophages)

White matter disease associated with myelin breakdown products
**GM₁ Gangliosidosis**

GM₁ \[\text{GAL-NAGA-GAL-GLC-CER}\]

\[\text{NANA}\]

\[\beta\text{-Galactosidase}\]

GM₂ \[\text{NAGA-GAL-GLC-CER}\]

\[\text{NANA}\]

**Inheritance:** Autosomal recessive

**Biochemical Findings:**

- Disease of lysosomes;
- GM₁ ganglioside and its derivative asialo-GM₁ ganglioside (GA₁) accumulate
- Also, glycoprotein-derived oligosaccharides, and keratan sulfate are found at elevated intracellular concentrations
GM$_1$ gangliosidosis

- Rare
- Several forms
- Most common is infantile onset form
- Storage in CNS and viscera
- Symptoms noted at or shortly after birth
- Psychomotor retardation
  - Reduced cognitive and motor function
- Skeletal abnormalities
- Organomegaly
- Death by age 2
GM₁ gangliosidosis

Cherry red spot
Retinal disease

Skeletal abnormalities
Frontal bossing, depressed nasal bridge
Long bones widened
GM\(_1\) pathology

- Very diffuse neuronal storage in brain
- Also astrocyte storage
- Also ganglion cells in intestine, etc
- Organomegaly, esp liver and spleen
- EM picture similar to Tay-Sachs disease

Vacuolated lymphocytes
GM$_1$-brain

Neurons swollen, filled with ganglioside
Tay-Sachs Disease and Sandhoff Disease

**GM\textsubscript{2}**

NAGA-GAL-GLC-CER

\textit{NANA}

\textit{Hexosaminidase A} \(\alpha\beta\) subunits

\textit{GAL-GLC-CER}

\textit{NANA}

\textit{Globoside}

NAGA-GAL-GAL-GLC-CER

\textit{Hexosaminidase B} \(\beta\beta\) subunits

\textit{GAL-GAL-GLC-CER}

**Inheritance:** Autosomal Recessive

**Biochemical Findings:**

In TSD Hex A is defective and Hex B levels are elevated

In Sandhoff, \textit{both} Hex A and B are defective
Tay-Sachs disease

- More common in eastern Europeans, Ashkenazis, French-Canadians, Irish
- Baby normal at birth
- Onset at 3 months
- Psychomotor retardation, impaired vision
- **Hyperacusis** = exaggerated startle response
- Death by age 3-4 years
Tay-Sachs pathology

• No visceral organ involvement
• Storage in neurons
  – Retina, brain (esp cerebellum)
• Brain becomes enlarged
  – There may be megencephaly
• With neuronal loss, brain undergoes atrophy
Retina

- Sclera
- Choroid
- Rods
- Cone nuclei
- Cones
- Pigment cells
- Outer limiting membrane
- Amacrine cells
- Horizontal cells
- Bipolar cells
- Ganglion cells
- Muller’s fibers
- Photoreceptor cells
- Rod nuclei
- Retina
Macula

Fig. 12 Light microscopic image depicting the choroid-RPE-retina interface. A section shows the normal anatomical relationships of the macular choroid (Ch) and its blood vessels (boxed area), retinal pigment epithelium (RPE) and retina (R). The section passes through the fovea (F).
Cherry red spot

Red spot is the vascular choroid at macula
Stands in contrast to pale surrounding retina with swollen ganglion cells

Storage in ganglion cell layer
Tay-Sachs disease

Enlarged neurons with foamy cytoplasm
Meganeurites

Swollen neuronal processes filled with ganglioside
Membranous cytoplasmic bodies

EM shows typical inclusions in Tay-Sachs disease
Sandhoff disease

- No ethnic tendency
- Neurologic picture similar to Tay-Sachs
- Also storage in visceral organs
- Swollen hepatocytes, pancreatic acini, renal tubules
- Foamy macrophages in spleen, LN, marrow and lungs
Gaucher Disease

Glucosyl-CER

\[ \text{\(\beta\)-Glucosidase} \]

CERAMIDE

Inheritance: Autosomal recessive

Biochemical findings:
\(\beta\)-glucosidase deficiency
Glucosyl-CER (glucocerebroside) accumulates
Gaucher disease

• Several forms of disease
• In 1882 Gaucher described a “tumor” of spleen
• Adult form most common
• 50% cases in Ashkenazis
• Usually no CNS symptoms
• Onset in late childhood or adulthood
• Hepatosplenomegaly
  – Hypersplenism with pancytopenia
• Live well into adulthood with variable prognosis
Gaucher pathology

Cerebroside stored mainly in macrophages (histiocytes)
Derived in large part from breakdown of blood cells
Gaucher cell has eccentric nucleus, wrinkled tissue paper cytoplasm

EM shows membrane-bound tubules
Gaucher bone lesion

Often involves the femur
Looks lytic or osteopenic
Has been described as flask deformity
Infantile Gaucher disease

- Rare
- Onset 3-6 months
- Psychomotor retardation
- Hepatosplenomegaly + CNS involvement
- Poor prognosis with death in 1-2 years
CNS Gaucher disease

Perivascular macrophages and Gaucher cells
NIEMANN-PICK DISEASE

CERAMIDE $\xrightarrow{Sphingomyelase}$ Sphingomyelin

Inheritance: Autosomal recessive
Biochemical findings:
In Types A and B sphingomyelin accumulates in lysosomes.
In Type C there is a defect in cholesterol trafficking. More common than Types A and B.
Niemann-Pick disease

- Heterogeneous group of diseases
  - Some are not sphingolipidoses
- Infantile form (type A) is classical type
  - About 75% cases
  - Severe neurologic disease + organomegaly
  - Cherry red spot in one third of patients
  - Early death by age 3 years
- Adult form (type B) usually without CNS symptoms
  - Patients survive into adulthood
Infantile Niemann-Pick disease

Marked splenomegaly
Infantile Niemann-Pick disease

Protuberant abdomen
Niemann-Pick disease

Widespread involvement of monocyte/macrophage system like Gaucher disease
Niemann-Pick disease

Signature cell is **sea blue histiocyte**

Seen with Wright’s stain

EM shows myelin-like inclusion
Metachromatic Leukodystrophy

\[ \text{So}_3\text{H-GAL-CER} \xrightarrow{\text{Arylsulfatase A}} \text{GAL-CER} \]

Inheritance: Autosomal recessive
Biochemical Findings:
Accumulation of sulfatide
Metachromatic Leukodystrophy (Greenfield’s disease)

• Late infantile form most common
  – Several types including juvenile and adult type
• Motor symptoms predominate early
  – Hypotonia, weakness, gait disturbances
• Peripheral neuropathy
• Death within 2-10 years
Leukodystrophy
Lesions are bilateral and diffuse
White matter is firm, gray, cavitated
LFB stain shows marked loss of myelin and sparing of U fibers
White matter disease

U fibers involved
Metachromatic staining in white matter
Gitter cells filled with sulfatide

Metachromatic material stains a different color from origin stain (purple)
Lamellar inclusions
MLD neuropathy

Metachromatic staining in nerve
Hunter’s Hope
Krabbe Disease

GAL-CER \[\rightarrow\] CERAMIDE

\[\beta\text{-Galactosidase}\]

Psychosine

Krabbe’s disease KD):
Krabbe disease has 4 clinical subtypes, distinguished by age of onset –
Type 1 - infantile
Type 2 - Late infantile
Type 3 - Juvenile
Type 4 - Adult
N.B. The late and adult onset forms are more common in Southern Europe.
Infantile globoid cell leukodystrophy (GLD). Rapidly progressive; invariably fatal disease of infants.

Inheritance: AR

Biochemical Findings:
Defect is in \(\beta\)-galactosidase
Psychosine is implicated in the disease
Gal-Cer levels are not elevated in the brain*

*Psychosine is galactosyl-sphingosine
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NBS: Criteria for Inclusion

• Disorder is serious & reasonably frequent
• Difficult to Dx clinically & requires a test
• Failure to treat causes irreversible damage
• Test is rapid, sensitive & specific
• Feasible intervention improves outcome
• Public health program is in place to inform families, physicians & public about disorder
• NBS program is cost effective
Measurement of psychosine in dried blood spots

A possible improvement to newborn screening programs for Krabbe disease

*Turgeon et alia, J. Inherit. Metab. Dis. March 2015*

Measurement of psychosine in dried blood spots could serve as a 2nd tier assay in newborn screening for Krabbe disease.
Krabbe disease

• Onset 3-6 months
• Marked psychomotor retardation
  – Irritable, stiffness, fever
• Rapid progression
• CNS and PNS involved
• Average survival 2 years
Krabbe disease
Krabbe disease

Myelin loss
Loss of oligodendroglia
Have been poisoned
Neurons are spared
Globoid cells
Globoid cells (macrophages)

Clusters of fat macrophages, often around blood vessels, are the diagnostic feature of Krabbe disease
**SPHINGOLIPIDOSES**

- **GM1 gangliosidosis**
- **Tay-Sachs disease**
- **Sandhoff disease**
- **Gaucher disease**
- **Niemann-Pick**
- **Sphingomyelin**
- **CERAMIDE**
- **Sphingosine**

**Key Components**

- **GAL-NAGA-GAL-GLC-CER**
- **NANA**
- **So$_3$H-GAL-CER**
- **MLD**
- **KD**

**Pathways**

1. **GAL-NAGA-GAL-GLC-CER**
   - NANA → **GM1 gangliosidosis**
   - NANA → **Tay-Sachs disease**
   - GAL-GLC-CER → **Lactosyl-CER**
   - NANA → **Gaucher disease**

2. **NAGA-GAL-GLC-CER**
   - NANA → **Sandhoff disease**
   - GAL-GLC-CER → **Glucosyl-CER**
   - NANA → **Sphingomyelin**

3. **So$_3$H-GAL-CER**
   - MLD → **GAL-CER** → **CERAMIDE**
   - KD → **Niemann-Pick**

4. **Sphingosine**