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Metachromatic Leukodystrophy Clinical, Biological and Therapeutic Aspects

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1. Introduction

Scholz's disease or metachromatic leukodystrophy (MLD) is a lysosomal storage disease caused by a deficiency in arylsulfatase A (ARSA: EC 3.1.6.8). This enzyme is responsible for the degradation of sulfatides commonly called cerebroside-3-sulfate or 3-O sulfogalactosylceramide in galactocerebroside and sulfate. The success of hydrolysis of these sphingolipids by ARSA necessarily depends on the presence of saposine B forms a complex with the substrate. The pathological accumulation of sulfatides in the nervous system (myelin, neurons and glial cells) results most often neurological, mental retardation, nervous disorders, blindness. The metachromatic granules accumulated in the central nervous system and peripheral compounds are highly toxic. These are at high levels in the urine of patients affected by the MLD. Arylsulfatase A activity is collapsed in these patients. Unfortunately, the value of enzyme activity is not a predictor of clinical severity of the neuropathology. In contrast, the study of the gene that codes for the ARSA is seen as a way to diagnose the simplest and most reliable of the disease to avoid misdiagnosis due to the presence of pseudodeficit. The conventional therapeutic approaches are essentially symptomatic. They were made in order to restore the enzyme activity of arylsulfatase A and prevent the progression of the pathological accumulation of sulfatides and consequently reduce morbidity associated with MLD.

Key words: arylsulfatase A, metachromatic leukodystrophy, sulfatide, neurological affect.

The sphingolipidoses represent all lysosomal storage diseases that is characterized by the accumulation of sphingolipids essentially in lysosomes and that their etiopathogenic mechanisms are released (Murray et al., 1996).

These disorders are genetically caused by a deficiency of lysosomal protein or its activator (Beaudet et al., 2001). These proteins are involved in the catabolism of sphingolipids, which are complex lipid molecules. They derive from a common structural element, the ceramide. Ceramides are formed by the association of an amino alcohol to 18 carbons in a sphingosine called fatty long-chain acid which are saturated or not, and hydroxylated or not. Sphingosine has a center formed from a hydrophilic primary alcohol, the amine, a secondary alcohol function, and a pole with a hydrophobic acyl chain with a double bond. The structure of various sphingolipids nervous system corresponds to various

substitutions fixed on the hydroxyl group of carbon 1 of the sphingosine by ester or glycosidic bond (Métais et al, 1980a).

These sulfatide which are the major components of the myelin have the structure of cerebroside sulfate, the sulfo-galactosylceramide whose 3-hydroxyl of galactose is esterified by sulfuric acid which are the more important in this group (Fig. 1. Structure of sulfatide, Cui et al., 2008).

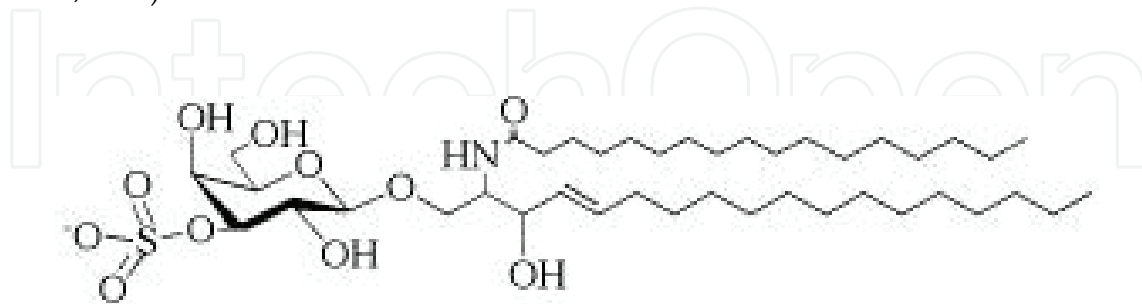


Fig. 1. Structure of sulfatide

The sphingolipidoses are classified according to the nature of the enzyme deficiency and substrate accumulated (Table 1. Classification of the main Sphingolipidosis, Borel et al., 1999)

Disease	Deficient enzyme	Accumulated products	Symptoms
Gaucher disease	β -Glucosidase	Glucosylceramide	Hepatosplenomegaly, Mental retardation, Bone pain
Krabbe disease	Galactocerebrosyl- β -galactosidase	Galacosylceramide	Mental retardation, Neurodegeneration, Decerebration-like
Fabry disease	α -galactosidase	Globotriaosylceramide	Ischemic infarction in affected organs, Angiokeratomas hypohidrosis, Mental retardation
Tay-Sachs disease	Hexosaminidase A	GM2-ganglioside	Neurodegeneration, Developmental disability, kidney and skin disorders
Metachromatic leukodystrophy	Arylsulfatase A	Sulfatide	Demyelination in CNS and PNS, Mental retardation
Niemann-Pick disease	Sphingomyelinase	Sphingomyelin	Hepatosplenomegaly, Mental retardation

Table 1. Classification of the main Sphingolipidosis

A great clinical and biological heterogeneity characterizes these metabolic diseases. Various Clinical forms, severe or moderate, may correspond to same enzyme deficiency, which explains the diagnosis difficulty of these disorders (Von Figura et al., 2001).

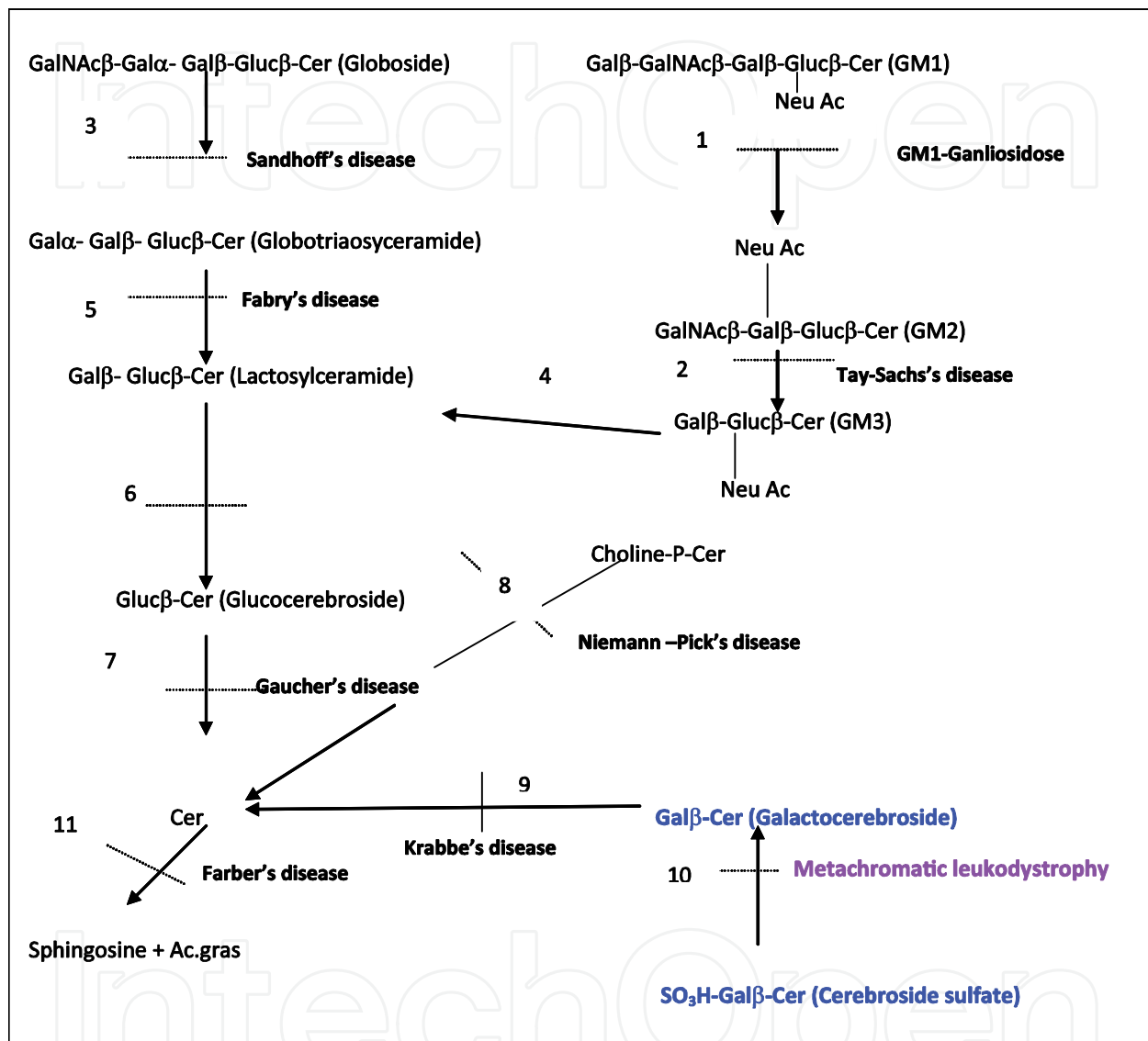


Fig. 2. Catabolism of sphingolipids and enzyme deficiencies responsible for sphingolipidoses. 1 : gangliosido- β -galactosidase ; 2 : β -hexosaminidase A ; 3 : β -hexosaminidase A et B ; 4 : neuraminidase ; 5 : α -galactosidase A ; 6 : lactosyl-ceramide- β -galactosidase ; 7 : cerebroside- β -glucosidase ; 8 : sphingomyelinase ; 9 : cerebroside- β -galactosidase ; 10 : arylsulfatase A ; 11 : ceramidase.

Metachromatic leukodystrophy (MLD) is a sphingolipidosis which corresponds to the loss of myelin in areas normally myelinated ago (Maria et al., 2003). The MLD or Scholz's disease was discovered by Scholz Greenfield in 1925. This is a recessive autosomal disease. This encephalopathy, which results from a deficiency of arylsulfatase A (ARSA, EC 3.1.6.8) is

involved in sulfatides hydrolysis to galactosyl-cerebroside and sulfate (Fig.2. Catabolism of sphingolipids and enzyme deficiencies responsible for sphingolipidoses, Métais et al, 1980b).

The accumulation of these toxic metabolites is the origin of lesions in the white matter in central and peripheral nervous system (Logowska et al., 2005). Some Rare cases of MLD are due to saposin B deficiency: sphingolipid activator protein B (Sap-B) (Deconinck et al., 2008).

The existence of arylsulfatase A pseudodeficiency in 7 to 15% of the population is causing by a collapsed ARSA activity without clinical signs (Bognar et al., 2002). The incidence of the MLD varies from 1 / 40 000 to 1 / 100 000. For Habbanites Jews, it's estimated at 1.3 percent (Kolodny & Fluharty, 1995a).

2. Clinical presentation

Clinically, three phenotypes were distinguished: Late infantile, juvenile and adult form. These phenotypes vary according to the absence or presence of neurological manifestations and their progression degree. The late infantile form is the most severe and frequent form, accounting for 60%. The symptoms appear before 4th age. The motor regression starts at the age of walking. Intellectual abilities are preserved until installing a state of terminal decerebration with pyramido-extrapyramidal syndrome.

Reflexes tendon's abolition is found early and reflects the association of peripheral neurological signs, the death often occurs 2-4 years after initial diagnosis (Kolodny & Fluharty, 1995b). The juvenile form symptoms (20 to 30% of cases) occurs between 4 and 16 years manifested most often by behavioral and walking disorders. It is characterized by a decreases in intellectual performance, emotional difficulties and language disorders. The motor regression is progressive. The majority of patients die at the age of 10 years, but survival is sometimes possible to more than 20 years (Maire et al., 2007). On the adult form (10 to 20% of cases) the symptoms occurs between 16 and 60 years, with a variable speed of evolution. The patient has generally a change of personality, behavior disorders, and then a decrease in intellectual and professional performance, memory loss and dementia (Bauman, 2002; Turpin, 1994a).

3. Pathophysiology of MLD

The sulfatides accumulate in the central and peripheral nervous systems are highly toxic compounds which found in high concentrations in the urine of patients affected by MLD (Whitfield et al., 2001). It was demonstrated that metachromatic granules accumulate in many organs: gall bladder, liver, pancreas, ovaries and lymph nodes.

The renal tubular epithelium is usually affected, they can be also found in the eye, in the dental pulp. Microscopically the demyelination is diffuse. It mainly affects the cerebral hemispheres, fibrillary gliosis is important. The metachromatic granules are alcianophiles, they can be extracellular and can also be situated on glial cells or macrophages. The deposit can reach 20 to 30 microns in diameter. However, these granules are more numerous in most affected white matter regions, but the gray matter is normal. The Peripheral nerve is always

affected by observing segmental demyelination and metachromatic substance deposits in Schwann cells, are showed in electron microscopy. (Turpin et al., 1994b)

4. The cerebroside-sulfatase (CSS) or cerebroside 3-sulfatase or arylsulfatase A (ARSA)

The arylsulfatase A belongs to the sulfatases family. These sulfatases ensure the hydrolysis of esters sulfate such that O-sulfates and N-sulfates with different specific substrates. In humans, there are 17 small sulfatases, 500 to 800 amino acids; ARSA protein has 507 amino acids. These enzymes are characterized by structural homology to the entire sequence, particularly in the N-terminal region. They have also a similar active site. The catalytic site of ARSA is located in a positively charge pocket and it acts as a ligand involving magnesium. The active site of this enzyme contains α helices that surround a large β sheet. The disulfure bridge (SS) is located between the aspartate (Asp335) and arginine (Arg370) residue. The sulfatases require posttranslational oxidation affecting cysteine leading to the formation of aldehyde which will facilitate the production of formyl glycine (FGL). Diez Roux and Ballabio showed that FGL is essential for the catalytic activity of the enzyme. (Cesani, 2009a; Diez-Roux & Ballabio, 2005a; Schestag, 2002).

The quaternary structure is pH-dependent; at neutral pH, the structure is dimeric, where as at lysosomal pH (acidic) homo-octameric form is predominant which formed by four dimmers.

The ARSA structure analysis is important to understanding the mutations and the genotype phenotype correlation studies. The ARSA is encoded by a gene located on 22q13.

Stein et al. have been cloned and sequenced the human ARSA gene. It spans 3,2 Kb genomic DNA and is separated into eight exons ranging in size from 116 to 362 bp. The ARSA gene is transcribed into three mRNA species of different sizes (2.1, 3.7 and 4.8 kb) (Lukatela et al., 1998).

5. Phenotype/genotype relationship

Since the identification of ARSA gene, more than 118 mutations have been described so far. Many point mutations (missense and non sense), many substitutions, splicing mutations, and some deletions and insertions have been reported (Cesani, 2009b). However, most common mutations in the general population: the IVS2 + 1G> A mutation which associated with the late infantile form, the P426L mutation is frequently associated with the juvenile form, I179S mutation is associated with adult form and pseudodeficiency: N350 and 1524 +95 A \rightarrow G (poly A-) (Clouter-Mackie & Gagnier, 2003). The distinction between the three phenotypes of MLD in the literature (late infantile, juvenile and adult form) posed a major problem for clinicians because they had just the age of clinical signs onset to typing different MLD forms. Recently, according to the study of Biffi et al., it is possible to establish phenotype-genotype relationship.

These authors proposed a classification of clinical forms of MLD based on the genotype, residual enzyme activity and the study of the expression of mutated proteins (Biffi et al., 2008).

6. Biological diagnosis

Various explorations are necessary in any acquired encephalopathy case resulting psychomotor regression. The biochemical diagnosis includes the following studies:

6.1 Urinary sulfatide analysis

The study of urinary excretion of sulfatides is possible by thin layer chromatography or spectrometry mass (Colsch et al., 2008).

6.2 Enzymatic study

The Confirmatory diagnosis is based on the demonstration of the ARSA deficiency. The determination of enzyme activity can be made on leukocytes circulating blood of sick patients, or skin fibroblasts in culture. The measurement of enzyme activity is possible by colorimetric method using a Synthetic substrate: paranitrocatechol sulfate (Dubois et al., 1975). However, the collapsed enzyme activity is not a predictor factor of MLD clinical severity. The study of heterozygotes subjects by measurement of enzyme activity is limited because there is an overlap between the values of ARSA activity in normal and heterozygotes subjects. The multiple sulfatase deficiency (MSD) leads to many problems in the result's interpretation (Diez-Roux & Ballabio, 2005b). In some clinically healthy subjects or suffering from neurological diseases other than MLD, the value of enzyme activity observed may be low, for the existence of the pseudodeficiency alleles. These alleles represent 7-15% of alleles in the general population. Two mutations described are responsible for that: N350 (PD1) 1524 95 A → G or poly A-(PD2). ARSA pseudodeficiency must be recognized to avoid misdiagnosis like for prenatal diagnosis in families where coexists one allele of MLD (Polten et al., 1991a).

6.3 Molecular diagnosis

Molecular diagnosis of MLD is the more accurate and uses samples more stable than those used for determining enzyme activity. This diagnosis can contribute to the identification of common mutations in the ARSA gene especially in the heterozygous subjects (Polten et al., 1991b). Genomic DNA was extracted from leukocytes, and was amplified by PCR (polymerase chain reaction) using oligonucleotide primers already described. Patients or their parents were screened for the presence of the most frequent MLD causal mutation by restriction endonuclease digestion and sequencing.

6.4 Prenatal diagnosis

Prenatal diagnosis is the best way to put heterozygous couple, especially in a who had couple already an affected child (index case). Two methods are proposed, the amniocentesis and chorionic amniocentesis. The chorionic villous biopsy is performed upon 8th or 9th week of pregnancy. This technique provides rapid results (maximum 3 days). Amniocentesis is achievable in the 14th week of gestation. Diagnostic confirmation is always performed after measurement of enzyme activity in amniotic cells crop, which will delay the results 3-4 weeks. Currently, the identification of fetal genotype and predicting the type of the disease

even in the absence of family history has become easier with the use of molecular technologies (Maire, 2004).

7. Treatment

7.1 Bone Marrow Transplant (BMT)

The BMT reconstitute the hematopoietic system of patient with stem cells from a healthy donor. Several patients with infantile and juvenile form of MLD received BMT. No signs of regression have been reported, with the exception of a child who had a neurological improvement post-BMT, but the BMT in patients with adult form was very beneficial. Improvement of neurological symptoms associated with a normalization of their ARSA activities and their sulfatide rate was noted. The post-BMT survival in patients suffering from adult form is estimated at 77% (Krivit et al., 2001).

7.2 Enzyme replacement

Enzymatic Therapy is to administer to the patient the active deficient enzyme which is responsible for his illness. Recently in Denmark, a test of enzyme replacement therapy was offered to children with the late infantile form. The clinical trial includes the study of safety and tolerance in the first phase. Patients are then followed in the second phase to study the dose-response. The enzyme which used in the test is similar to the human enzyme. To assess the Metazym[®] effect, the nervous system of patients has been evaluated at the beginning of therapy and after 6 to 12 months. These evaluations include MRI, cognitive tests, measurements of nerve conduction and motor function (Dali & Lund, 2009).

7.3 Substrate-reduction therapy with warfarin

The warfarin is an anticoagulant and an antagonist of vitamin K, The first Warfarin studies in mice showed that this compound inhibits the synthesis of sphingolipids leading to sulfatide reducing (Crowther et al., 2009).

7.4 Gene therapy

Gene therapy involves introducing into the body patient's a normal version of the defective gene which responsible of his illness. To date, several approaches are tested in animal models (Consiglio et al., 2000).

8. Conclusion

Metachromatic leukodystrophy is a sphingolipidosis caused by arylsulfatase A deficiency. This enzyme catalyses the first degradation step of the sulfatide.

Several mutations were identified in the ARSA gene which abolish the catalytic activity and may reduce the ARSA stability. Thus, the study of the ARSA gene three-dimensional structure associated with the search for the mutations responsible of MLD could establish phenotype-genotype relationship.

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