ELSEVIER

Clinica Chimica Acta 354 (2005) 131–139

www.elsevier.com/locate/clinchim

G_{M1}-ganglioside degradation and biosynthesis in human and murine G_{M1}-gangliosidosis

Renata Sano[a,b,c], Vera M.T. Trindade[a], Alessandra Tessitore[c], Alessandra d'Azzo[c], Matheus B. Vieira[a,b], Roberto Giugliani[b,d], Janice C. Coelho[a,b,*]

[a]*Department of Biochemistry, ICBS, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil*
[b]*Medical Genetics Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, RS, Brazil*
[c]*Department of Genetics, St Jude Children's Research Hospital, Memphis, TN, USA*
[d]*Department of Genetics, IB, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil*

Received 6 October 2004; received in revised form 19 November 2004; accepted 19 November 2004

Abstract

Background: Gangliosides are building blocks of cell membranes and their biosynthesis and degradation have been extensively studied in the past. Regulation of the metabolism of these glycolipids controls fundamental cell functions. G_{M1}-gangliosidosis, a neurodegenerative glycosphingolipid storage disease, is caused by deficiency of lysosomal β-galactosidase with consequent disruption of the normal degradative pathway of G_{M1}-ganglioside. We studied the impact of G_{M1}-ganglioside accumulation on its biosynthetic enzyme in cells and tissues from human patients and from the G_{M1}-gangliosidosis mouse model.

Methods: We tested the qualitative and quantitative pattern of gangliosides by thin layer chromatography and *N*-acetylneuraminic acid dosage, respectively. Regulation of G_{M1}-ganglioside biosynthesis was evaluated by G_{M1} synthase assay in human and murine samples.

Results: G_{M1}-ganglioside accumulation has an inhibitory effect on the human but not on the mouse G_{M1} synthase. We present evidence that G_{M1} synthase activity in human and murine cells are regulated by different mechanisms.

Conclusions: Alternative pathways in the mouse may account for these results and possibly explain some of the phenotypical differences between the human and mouse forms of this disorder.

Keywords: G_{M1} synthase; G_{M1}-ganglioside; Acid β-galactosidase; G_{M1}-gangliosidosis

Abbreviations: β-gal, β-galactosidase; *β-gal*$^{-/-}$, mouse model of G_{M1}-gangliosidosis; C:M, chloroform:methanol; ER, endoplasmic reticulum; Gal T-II/G_{M2} β-1,3-galactosyltransferase, G_{M1} synthase; GlcCer, glycosylceramide; GSL, glycosphingolipid; HMF, hind-, mid- and forebrain; NANA, *N*-acetylneuraminic acid; TLC, thin layer chromatography; UPR, unfolded protein response.

* Corresponding author. Serviço de Genética Médica, Hospital de Clínicas de Porto Alegre, Rua Ramiro Barcelos, 2350, Porto Alegre-RS, 90035-003, Brazil. Tel.: +55 51 21018011; fax: +55 51 21018010.

E-mail address: jcoelho@hcpa.ufrgs.br (J.C. Coelho).

0009-8981/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.cccn.2004.11.035

1. Introduction

The pathways of glycosphingolipid (GSL) metabolism involve enzymes that reside in subcellular organelles of the endocytic and exocytotic systems. Therefore, the biosynthesis and degradation of glycosphingolipids strictly depend on the enzymes involved and the way they are regulated; the transport of GSLs through the endocytic and exocytic pathways; the rate of plasma membrane turnover and finally the occurrence of external stimuli that influence second messengers/bioregulators [1].

The biosynthesis of gangliosides takes place on intracellular membranes (endoplasmic reticulum; Golgi apparatus) and is catalyzed by membrane-bound enzymes. Transport of de novo produced compounds to the plasma membrane occurs via vesicles following the exocytotic flow [2]. Ganglioside (and GSLs in general) degradation occurs within the endocytic route in the acidic compartments of the cell (late endosomes, lysosomes) and is carried out by hydrolytic enzymes (mostly soluble) with the aid of activator proteins and negatively charged lipids [3,4]. Final products of GSL degradation can be reutilized for their biosynthesis and constitute a metabolic salvage process that saves energy. Knowledge of the mechanisms regulating GSL degradation has come from studies of the human diseases that are caused by genetic defects of GSL metabolism [5].

The non-redundant activity of lysosomal β-galactosidase (β-gal) ensures the correct and timely breakdown of β-galactosyl-containing glycoconjugates in lysosomes and contributes to the maintenance of cellular homeostasis. The enzyme cleaves with high affinity the sialoglycolipid G_{M1}-ganglioside. Genetic defects that affect β-gal function in humans, result in the neurodegenerative, glycosphingolipid storage disease G_{M1}-gangliosidosis [6,7]. Clinically, early infantile, late infantile/juvenile and adult/chronic forms are distinguished. The β-gal activity in affected individuals correlates with disease onset and severity of the symptoms, ranging from little or no activity in the infantile- and juvenile-onset forms to measurable activity in adult-onset forms [7]. Infantile-onset patients usually succumb to the disease in the first few months of life.

The mouse model of G_{M1}-gangliosidosis (β-$gal^{-/-}$) closely recapitulates the severe neurodegenerative features of the early onset form of the disease [8]. Massive, age-dependent accumulation of G_{M1}-ganglioside and asialo-G_{A1} is widespread, with almost all neurons being affected to some extent, and is accompanied by gradual deterioration of motor function [8]. It was recently found that neuronal cell death in these mice results from the activation of an unfolded protein response (UPR), as direct consequence of G_{M1}-ganglioside accumulation in the endoplasmic reticulum (ER) and depletion of ER calcium stores [9]. Whether the accumulated metabolites have a physiological impact also on the biosynthesis of GSLs in lysosomal disorders is currently unknown.

G_{M1}-ganglioside is one of the most investigated gangliosides. It plays important role in the development and functions of neural system [10]. G_{M1} synthase is responsible for the synthesis of G_{M1}/G_{D1b} from G_{M2}/G_{D2} in the ganglioside biosynthetic pathway. As in glycoprotein-glycosyltransferases, ganglioside glycosyltransferases are type II membrane proteins having a short N-terminal cytosolic domain, a transmembrane domain, a stem region and a large C-terminal domain oriented toward the luminal space of the Golgi cisternae, the location of catalytic activity [11]. The enzyme transfers a galactose residue from the sugar nucleotide donor UDP-gal to the terminal *N*-acetylgalactosamine residue of the neutral glycolipid G_{A2} or of the gangliosides G_{M2}-ganglioside and G_{D2}-ganglioside. Recent studies have shown that *N*-acetylgalactosaminyl-transferase and G_{M1} synthase associate physically in the distal Golgi membranes of CHO-K1 cells. In these complexes, the product of the first enzyme (G_{M2}-ganglioside) is channeled as substrate for the second enzyme in G_{M1}-ganglioside formation [12]. This association improve the efficiency of glycolipid synthesis by chanelling the intermediates from the position of product to the position of acceptor along the transfer steps. Furthermore, G_{M1} synthase depends on *N*-glycosylation for its activity and for proper trafficking to the Golgi complex. G_{M1} synthase cDNA cloned from mouse brain demonstrated amino acid sequence identity of 93% with the human form [13]. Differences between human and mouse expression of G_{M1} synthase may reflect the species- and tissue-specific glycosylation of glyco-