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Alterations of membrane lipids and in gene expression of ganglioside metabolism in different brain structures in a mouse model of mucopolysaccharidosis type I (MPS I)

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ABSTRACT

Mucopolysaccharidosis I (MPS I) is a congenital disorder caused by the deficiency of α-L-iduronidase (IDUA), with the accumulation of glycosaminoglycans (GAGs) in the CNS. Although GAG toxicity is not fully understood, previous works suggest a GAG-induced alteration in neuronal membrane composition. This study is aimed to evaluate the levels and distribution of gangliosides and cholesterol in different brain regions (cortex, cerebellum, hippocampus and hypothalamus) in a model using IDUA knockout (KO) mice (C57BL/6). Lipids were extracted with chloroform–methanol and then total gangliosides and cholesterol were determined, followed by ganglioside profile analyses. While no changes in cholesterol content were observed, the results showed a tissue dependent ganglioside alteration in KO mice: a total ganglioside increase in cortex and cerebellum, and a selective presence of GM3, GM2 and GD3 gangliosides in the hippocampus and hypothalamus. To elucidate this, we evaluated gene expression of ganglioside synthesis (GM3, GD3 and GM2/GD2 synthases) and degradation of (Neuraminidase1) enzymes in the cerebellum and hippocampus by RT-sq-PCR. The results obtained with KO mice showed a reduced expression of GD3 and GM2/GD2 synthases and Neuraminidase1 in cerebellum; and a decrease in GM2/GD2 synthase and Neuraminidase1 in the hippocampus. These data suggest that the observed ganglioside changes result from a combined effect of GAGs on ganglioside biosynthesis and degradation.

1. Introduction

Mucopolysaccharidosis (MPS) is a monogenic disease classified as lysosomal storage disorder typically characterized by the total or partial deficiency of an enzyme involved in the degradation pathway of glycosaminoglycans (GAGs) (Fuller et al., 2004). Several clinical manifestations of MPS have been characterized and described, and are numerically classified from MPS I to MPS IX. Mucopolysaccharidosis type I (MPS I) is an autosomal recessive disease caused by a genetic defect that codifies a lysosomal hydrolase, α-L-iduronidase (IDUA) (EC. 3.2.1.76) (Clarke et al., 1997; Freeman and Hopwood, 1992). This enzyme degrades two particular GAGs, heparan and dermatan sulfate. The absence or the reduced amount of this enzyme leads to a storage of these GAGs in cells (Neufeld and Muenzer, 2001). The phenotypes may vary considerably from a more severe (Hurler syndrome), to a less severe (intermediate) (Hurler–Scheie syndrome) or else to a mild (Scheie syndrome) manifestation of the pathology. The patients have deleterious effects in childhood and preadolescence due to the underlying accumulation of GAGs in different organs, including the central nervous system, reticuloendothelial system and skeleton. In most cases the patients have progressive neurological dysfunction. These patients are severely affected as early as in their first 10 years of life, and death is a very common outcome (Neufeld and Muenzer, 2001; Zheng et al., 2003). This form of disease, as well as its other manifestations, is very complex. It is most typically defined by storage sites of heparan and dermatan sulfate, as previously mentioned (Liu et al., 2005; Neufeld and Muenzer, 2001; Zheng et al., 2003). Nevertheless, little is known about changes in cell dynamics caused by this accumulation (Holley et al., 2011).

Abbreviations: CNS, Central Nervous System; GAGs, glycosaminoglycans; HPTLC, high performance thin-layer chromatography; IDUA, α-L-iduronidase; KO, Knockout; MPS I, Mucopolysaccharidosis I; NANA, N-acetyl-neuraminic acid; Neu1, Neuraminidase 1; RT-sq-PCR, Semi quantitative Polymerase Chain Reaction; TLC, thin-layer chromatography.

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Nowadays, a significant lack of biomarkers is observed, whether biochemical or molecular biology levels. These biomarkers would be a starting point for the studies on physiopathological parameters of the disease (Campos and Monaga, 2012). The description of these factors would allow more thorough investigations of the possible treatment strategies, and would also provide additional tools for the follow-up stages of these studies and assist in the development of post-treatment applications. In this scenario, some previously studied factors might become the object of future research like the characterization and quantification of membrane lipid components (ganglioside and cholesterol) in different brain structures.

Gangliosides are the most complex glycosphingolipids. They contain oligosaccharides with one or more sialic acid residues, which account for their total negative charge. Gangliosides are mainly present in the plasma membrane of cells and are highly expressed in the vertebral central nervous system where they are involved in neural development. They are also related to cell differentiation and proliferation and to signal transduction. Of the several types characterized, the most significantly expressed gangliosides in the central nervous system are GM1, GD1a, GD1b, and GT1b (Yu et al., 2012; Zeller and Marchase, 1992). Gangliosides GM2 and GM3 are glycosphingolipids that build up when some lysosomal storage diseases occur, among them MPS I. Some studies have demonstrated the accumulation of these gangliosides in the total brain from the murine MPS I model (McGlynn et al., 2004; Russell et al., 1998; Walkley, 2004) or with GD3, in some brain regions (Heinecke et al., 2011; Wilkinson et al., 2012). On another hand, Walkey (1995) demonstrated a striking correlation between abnormally elevated levels of GM2 within pyramidal neurons and the presence of new dendritic sprouting.

This lipid accumulation can occur due to the changes on ganglioside biosynthesis and/or degradation processes (Fig. 1) (Tettamanti, 2004; Yu et al., 2009).

The present study analyzes the ganglioside content and profile in the cortex, cerebellum, hippocampus and hypothalamus of IDUA knockout mice (MPS I mice) in comparison to wild type animals. This report also describes, for the first time, in cerebellum and hippocampus of IDUA knockout mice, the gene expression of ganglioside synthesis (GM3, GD3 and GM2/GD2 synthases) and ganglioside degradation (Neuraminidase1-Neu1) enzymes (Fig. 1) by RT-sq-PCR. In addition, the cholesterol level, an important membrane fluidizer, was assessed (Simons and Ehehalt, 2002).

2. Material and methods

2.1. Animal model

C57Bl/6 knockout mice deficient for α-L-iduronidase (IDUA-KO) represent a murine model for human MPS I. MPS I mice were derived from animals kindly provided by Dr Elizabeth Neufeld (UCLA, Los Angeles, CA, USA). The mice were produced by targeted disruption of the murine IDUA gene (Ohmi et al., 2003). The colony was maintained by breeding heterozygous animals, and homozygous mutants were identified at birth by polymerase chain reaction. The animals were kept in standard conditions and were used in the experiments at 5 months of age (Reolon et al., 2006). All experimental procedures were in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH publication No. 85-2 3 revised 1996) and the procedures of the Brazilian College of Laboratory Animal and were approved by the institutional research ethics and animal care committee.

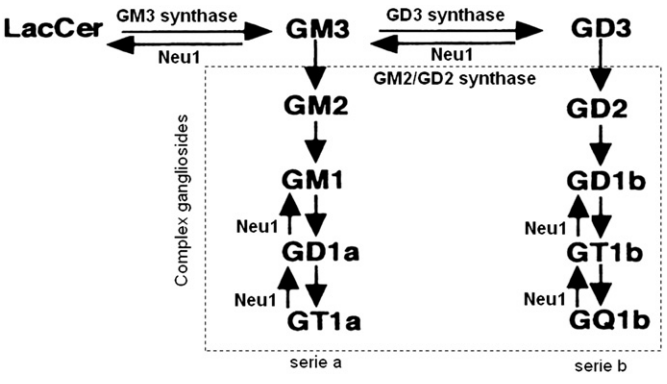

Fig. 1. Biosynthesis and degradation pathways of a-, b-series gangliosides. Neu 1: neuraminidase 1.

2.2. Materials

GM3, GM2, GM1, GD3, GD1a, GD2, GD1b and GT1b gangliosides and thiobarbituric acid were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Silica-gel 60 thin-layer chromatography (TLC) and silica-gel 60 high performance thin-layer chromatography (HPTLC) sheets were supplied by Merck (Darmstadt, Germany).

Total RNA was extracted using the Trizol method (Invitrogen, São Paulo, SP, Brazil). First strand cDNA was synthesized using SuperScript® III First-Strand Synthesis SuperMix (Invitrogen São Paulo/SP Brazil). Quantification of gene expression was carried out by RT-sq-PCR using Platinum® Taq DNA Polymerase (Invitrogen, São Paulo, Brazil), dNTPs (Ludwig Biotechnologies, Alvorada, Brazil), and primers. Specific primers for each gene were designed using the IDT Design Software (Integrated DNA Technologies Inc., USA).

All other chemicals and solvents used were of analytical grade.

2.3. Lipid extraction

Animals were decapitated and cortex, cerebellum, hippocampus and hypothalamus were removed, weighed and homogenized in a 2:1 mixture of chloroform:methanol (C:M, 2:1, v/v) to a 20-fold dilution of tissue mass and centrifuged at 800 ×*g* for 10 min. The pellet was re-homogenized in C:M (1:2, v/v) to a 10-fold dilution of original sample mass (Folch et al., 1957). The C:M extracts were combined and this pool was used for the following determinations. Considering the hippocampus and hypothalamus low mass, pools of five respective structures were used for this methodology.

2.4. Ganglioside evaluation

Aliquots from the total lipid extracts were used for ganglioside determination by the N-acetyl-neuraminic acid (NANA) quantification with the thiobarbituric acid method described by Skoza and Mohos (1976).

Ganglioside species were analyzed by thin layer chromatography (TLC) as a screening chromatography. Groups which presented ganglioside profile with a noticeable alteration were again analyzed using high performance thin layer chromatography (HPTLC) in order to confirm the preliminary results. TLC (cortex and cerebellum) or HPTLC (hippocampus and hypothalamus) was performed on 10 × 10 cm Merck plates of silica gel 60 using a developing tank described by Nores et al. (1994). Aliquots of total lipid extracts, containing 16 nmol of NANA suspended in 10 μL C:M (1:1), were spotted on 8 mm lanes. HPTLC was developed, sequentially, with two mixtures of solvents, first C:M (4:1, v/v) and second C:M: 0.25% $CaCl_2$ (60:36:8, v/v). The ganglioside profile was visualized with resorcinol reagent (Lake and Goodwin, 1976; Svennerholm, 1957). Chromatographic bands were quantified by scanning densitometry at 580 nm with a CS 9301 PC SHIMADZU densitometer. The terminology used herein for gangliosides was the one recommended by Svennerholm (1963).

2.5. Cholesterol quantification

Aliquots of total lipid extract were evaporated, suspended in isopropanol and quantified according to an enzymatic method (Bergmeyer, 1974) and using a commercial kit (Cholesterol Liquiform — Labtest, Lagoa Santa, MG, Brazil).

2.6. Protein quantification

Protein sediment obtained after lipid extraction was dissolved with NaOH 1 N and measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

2.7. Semi quantitative PCR (RT-sq-PCR) of GM3 synthase (EC 2.4.99.9), GD3 synthase (EC 2.4.99.8), GM2/GD2 synthase (EC 2.4.1.92) and Neuraminidase1 (EC 3.2.1.18) enzyme mRNA expression

Animals were decapitated and cerebellum and hippocampus were dissected out under sterile conditions, collected in RNAse-free polypropylene tubes, immediately frozen in liquid nitrogen and stored at −80 °C. Total RNA was extracted with Trizol according to manufacturer's instructions. RNA integrity, purity and concentration were checked by electrophoresis in 1% agarose gel and spectrophotometry. First strand cDNA was synthesized from 1.5 μg of total RNA. Each cDNA pool was then aliquoted and stored at −20 °C.

Gene sequence information of GM3 synthase (NCBI reference sequence NM_011375.2), GD3 synthase (NCBI reference sequence NM_011374), GM2/GD2 synthase (NCBI reference sequence NM_008080.5), and Neuraminidase1 (NCBI reference sequence NM_010893.3) was collected from databases (www.ncbi.nlm.nih.gov and www.ensembl.org). Specific primers for each gene were designed using the IDT Design Software (Integrated DNA Technologies Inc., USA) avoiding primers that could generate secondary structures (primers and template). The primers for GM3 synthase (forward: 5′ AAT GCA CTA TGT GGA CCC TG 3′; reverse: 5′ GTT GAT GCT GTA CCT GTC CTC 3′) were designed between exons 3 and 4; for GD3 synthase (forward: 5′ CGA TAA TTC CAC GTA CTC CCT C 3′; reverse: 5′ TTTGGAACCGACATCTCTGG 3′) between exons 2 and 4; for GM2/GD2 synthase (forward: 5′ TCAGGATCAAGGAGCAAGTG 3′; reverse: 5′ AAG GCTTTAGTGAGGTCAACC 3′) between exons 2 and 3 and for Neuraminidase1 (forward: 5′ TGC ATC CGA TGA GG GGGC CA 3′; reverse: 5′ CCA CAG CGC CCA GGT TCA GG 3′) between exons 2 and 3. The results were normalized using reference gene B2M (forward: 5′ ATT CAC CCC CAC TGA GAC TG 3′, exon 2 and reverse: 5′ TGC TAT TTC TTT CTG CGT GC 3′; exon 4; NCBI reference sequence NM_009735.3). All primer sequences were assessed for specificity using non-redundant basic local alignment search tools (www.ncbi.nlm.nih.gov/BLAST) and target-specific sequence alignment programs.

Analysis of ganglioside metabolism enzyme gene expression was carried out by RT-sq-PCR using 1× PCR Buffer, 1.5 mM MgCl2, 1 U Platinum® Taq DNA Polymerase, 0.2 mM dNTPs, and 0.2 mM of each transcript specific primer in a 25 ul final reaction volume. Reactions were conducted in a Verity thermocycler (Applied-Biosystems, USA) according to the thermocycling conditions: 1 min at 94 °C followed by 40 cycles of 20 s at 94 °C, 20 s at a 60 °C, and 20 s at 72 °C, followed by a final extension step of 7 min at 72 °C. The PCR products obtained were run in 1.0% electrophoresis agarose gel, and the PCR products were quantified using Alpha Easy FC Software (version 6.0.0; Alpha Innotech Corp.). All experiments were repeated at least twice.

2.8. Statistical analyses

The results are expressed as mean ± standard error for the mean (SE). All analyses were performed using the Statistical Package for the Social Sciences (SPSS) software in a PC-compatible computer. Student's *t*-test was used to compare means between IDUA knockout and wild type animals. Differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Ganglioside analysis

Total ganglioside contents were statistically higher in cortex and cerebellum of MPS I animals than in wild type mice (Fig. 2). On the other hand, in the hippocampus and hypothalamus of IDUA knockout mice, the amount of gangliosides was similar to that observed in the same structures of wild type animals.

The four main central nervous system gangliosides (GM1, GD1a, GD1b and GT1b) were detected in the cerebral structures studied. The profiles of the different ganglioside species from wild-type and MPS I animals, obtained by using TLC, were apparently unaffected in cortex and cerebellum. Relating this observation to total ganglioside contents, it could indicate an increase of the detected ganglioside species in cortex and cerebellum of MPS I mice (Table 1). However, especially GM3, GM2 and GD3 gangliosides have noticeable contents in hippocampus and hypothalamus of MPS I animals in comparison to wild-type (Table 1). Besides these, other minor gangliosides were also found in the hippocampus (GX) and hypothalamus (GX and GQ1b).

3.2. Cholesterol quantification

Cholesterol level from brain structures was quantified as described in the Materials and methods section. There was no statistical difference between MPS I and wild type animals for this lipid parameter (Fig. 3).

3.3. Relative expressions of ganglioside metabolism genes

As cortex and cerebellum like hippocampus and hypothalamus presented, respectively, similar results for ganglioside concentration and profile we decided to evaluate the relative expressions of ganglioside metabolism genes only in cerebellum and hippocampus. In both regions GM3 synthase expression was not altered when comparing KO and Wt animals. On the other hand, in cerebellum of KO mice, a reduction in gene expressions of GD3 synthase (43.2%), GM2/GD2 synthase (34.2%) and Neuraminidase1 (58.1%) was observed in comparison to their expressions in Wt animals (Fig. 4). However, in the hippocampus, there was a decrease in gene expression only for GM2/GD2 synthase (22.3%) and Neuraminidase1 (46.9%) enzymes in KO mice compared to the expressions in Wt animals, respectively (Fig. 4).

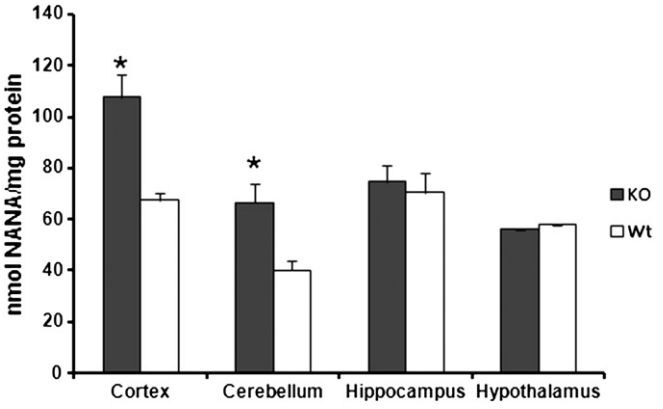

Fig. 2. Total ganglioside contents in the different brain structures analyzed. Data are expressed as mean ± SE for 5 animals in each group. Pools of five structures were used to obtain triplicate data from the hippocampus and hypothalamus. KO: Knockout; WT: Wild type. *Different from Wt, $p < 0.05$.

Table 1
Percentage distribution of gangliosides.

	Cortex		Cerebellum		Hippocampus		Hypothalamus	
	KO	Wt	KO	Wt	KO	Wt	KO	Wt
GM3	–	–	–	–	3.1 (±0.3)	–	10.2 (±2.0)	–
GM2	–	–	–	–	1.3 (±0.1)	–	3.5 (±1.0)	–
GM1	23.0 (±1.8)	24.8 (±1.9)	12.9 (±0.9)	13.6 (±0.7)	13.2 (±1.8)	15.8 (±2.1)	12.4 (±0.9)	13.7 (±0.8)
GD3	–	–	–	–	2.2 (±0.2)	–	*4.1 (±0.6)	2.1 (±0.5)
GD1a	41.8 (±1.9)	41.9 (±1.6)	28.6 (±2.3)	34.5 (±1.2)	36.9 (±2.7)	39.1 (±4.3)	23.3 (±2.2)	30.4(±2.8)
GX*	–	–	–	–	4.3 (±0.4)	4.1 (±0.4)	8.1 (±0.8)	7.4 (±0.9)
GD1b	15.9 (±0.7)	16.9 (±0.6)	19.7 (±1.7)	16.3 (±2.3)	8.6 (±1.3)	7.3 (±1.9)	8.3 (±0.9)	11.9 (±1.2)
GT1b	19.3 (±3.7)	16.6 (±0.9)	38.8 (±2.0)	35.6 (±3.0)	30.4 (±0.8)	32.7 (±4.7)	22.6 (±0.9)	25.8 (±0.8)
GQ1b	–	–	–	–	–	–	7.5 (±1.0)	8.7 (±0.7)

Values represent percentage mean ± SE.
GX* = could be GT1a or GD2 or LD1.
* Different from Wt, $p < 0.05$.

4. Discussion

Mucopolysaccharidosis type I is a lysosomal storage disease arising from a lack of activity of the α-L-iduronidase enzyme. This enzyme breaks down glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate. There is an involvement of multiple organs and some symptoms include growth delay, hepatosplenomegaly, coarse facial features, excessive urinary GAGs, skeletal abnormalities and neurological deficits (Neufeld and Muenzer, 2001).

Because MPS I is a degenerative disorder with a progressive accumulation of heparan sulfate and dermatan sulfate, it has become a key object for the analysis of neurochemical parameters. Considering the important roles played by membrane lipids in central nervous system development, and that some studies have sometimes reported alterations in total ganglioside content and others demonstrated the existence of GM3, GM2 and GD3 storage sites in patients and MPS I models, as well as of cholesterol secondary storage (Abraham et al., 1969; Heinecke et al., 2011; Ikeno et al., 1982; Ledeen et al., 1965; McGlynn et al., 2004; Russell et al., 1998; Walkley, 2004; Wilkinson et al., 2012), detailed investigations on these lipids, mainly on ganglioside composition and metabolism, are needed to elucidate issues related to the murine model.

The present study reveals an increase in total ganglioside content in the cortex and cerebellum of MPS I mice, while in the hippocampus and hypothalamus of IDUA knockout mice, the amount of gangliosides was similar to that observed in the same structures of wild type animals. It can be found in the literature that total brain ganglioside sialic acid content in patients is increased in some reports and decreased or unchanged in others (Abraham et al., 1969; Constantopoulos and Dekaban, 1978; Ikeno et al., 1982; Wherret, 1968). Our values for cerebellum are different from those determined by Heinecke et al. (2011), perhaps due to distinct evaluation procedures. Nevertheless, our data are the first report for hippocampus and hypothalamus.

The four main gangliosides present in the brain (GM1, GD1a, GD1b, and GT1b) were detected in the mouse cerebral regions studied. In cerebral cortex and cerebellum, the profiles of ganglioside species from IDUA knockout and wild-type groups were apparently unaffected. These observations, mainly for cerebellum, do not agree with that previously reported (Heinecke et al., 2011), probably due to different methodological analyses. However, GM3, GM2 and GD3 gangliosides have noticeable contents in hippocampus and hypothalamus of MPS I animals. Besides these, other minor gangliosides were found in the hippocampus (GX) and hypothalamus (GDx and GQ1b). Ganglioside GDx could be GT1a or LD1 as detected in mouse cerebellum (Heinecke et al., 2011; Ohsawa, 1989; Seyfried et al., 1978). The presence or the elevation of GD3, respectively in hippocampus or in hypothalamus from MPS I animals could suggest neurodegeneration since this ganglioside is enriched in reactive astrocytes and is considered a good parameter for neurodegeneration (Seyfried and Yu, 1985). Wilkinson et al. (2012) reported a detailed study comparing the brain of mouse models of mucopolysaccharidosis types I, IIIA and IIIB at 4 and 9 months of age. They showed interesting data about neuroinflammation, astrocytosis, microgliosis and synaptic disorganization. Besides these, they detected the GM2 ganglioside immunoreactivity in cortex slices of MPS mice and that it has an intense vesicular pattern in layers II/III and V/VI with less staining in layer IV and negligible in wild-type. The authors did not observe significant differences between the MPS genotypes and no overall time effect for GM2 immunoreactivity. Intense staining for

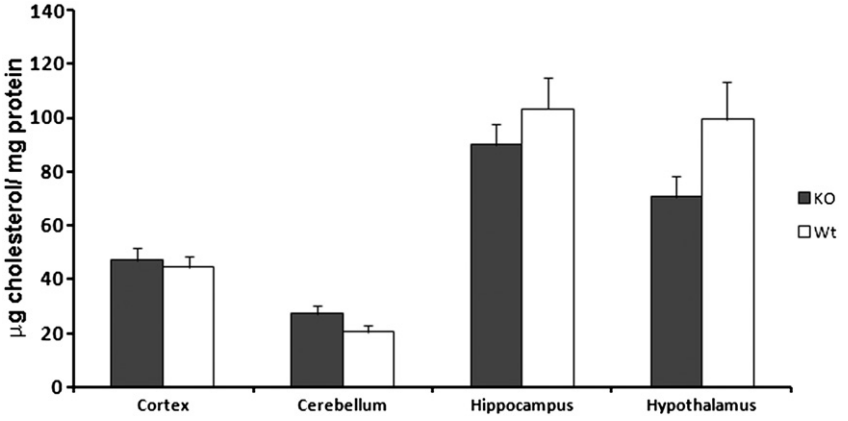

Fig. 3. Cholesterol levels in the different brain structures analyzed. Data are expressed as mean ± SE for 5 animals in each group. Pools of five structures were used to obtain triplicate data from the hippocampus and hypothalamus. KO: Knockout; Wt: Wild type. *Different from Wt, $p < 0.05$.

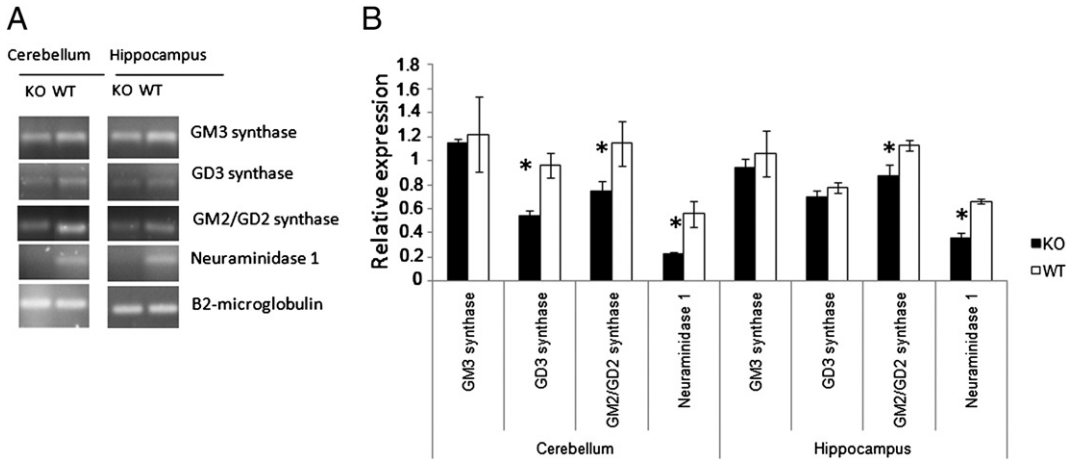

Fig. 4. Relative expressions of ganglioside metabolism genes. (A) Representative image of RT-sq-PCR gels; (B) quantification of the PCR bands. Data represent mean ± SE. n = 4. KO: Knockout, Wt: Wild type. *Different from Wt, $p < 0.05$.

this ganglioside was also found in other regions of the three MPS type brains such as amygdala, lateral septal nucleus, stria terminalis, preoptic area, hypothalamic areas and pyramidal cells of hippocampus. In addition, Walkley (1995) observed an elevated immunoreactivity for GM2 ganglioside in pyramidal neurons which showed an ectopic dendritogenesis. Sohn et al. (2006) demonstrated that an increase in GM3 might be directly associated with oxidative glutamate toxicity. In a previous study it has been shown that glutamate uptake and the number of glutamate receptors were reduced in MPS I animals (Camassola, 2008) Nevertheless, generally speaking, this increase in GM3 may be caused by the inhibition of the enzymes responsible for glycosphingolipid degradation, induced by GAGs stored in cells (McGlynn et al., 2004). As GM3 expression occurs predominantly during embryo development (Yu et al., 2009, 2012), accumulation of this ganglioside in MPS I brain patients could be involved in their slow neural development (Ikeno et al., 1982).

It is also known that glycosphingolipids are involved in electric effects and in the concentration of ions like Ca^{+2}, as well as in the formation of membrane rafts, a feature that has proved its importance in the proper function of CNS signaling routes (Furukawa et al., 2011; Harder and Simons, 1999). Thus, changes in glycosphingolipid composition and amount may influence these processes (Harder and Simons, 1999; Simons and Ehehalt, 2002; Yu et al., 2012). The findings described in the present study for the brain structures of MPS I animals may explain behavioral data already obtained for these animals, as described by Reolon et al. (2006) and improved by gene therapy as reported by Baldo et al. (2013).

In this report, the quantification of cholesterol levels in the different brain structures did not show any differences between healthy and MPS I animals. Considering that the characteristics of the MPS I tend to vary considerably with age (Russell et al., 1998; Zheng et al., 2003), other researchers (Heinecke et al., 2011) evaluated the total lipid distribution in the whole brain and cerebellum of MPS IH and control mice at 6 months and at 12 months of age. No other significant lipid differences (cholesterol, phospholipids) were found in either whole brain or in the cerebellum at either age. However, the content of total sialic acid and levels of gangliosides GM3, GM2, and GD3 were higher in the whole brains of mice than in control mice at 12 months of age. On the other hand, an immunohistochemical evaluation conducted by McGlynn et al. (2004), demonstrated that cholesterol storage sites were found only there where GM2 and GM3 were accumulated, but in separate vesicles within affected neurons from MPS I mice. This Important observation indicates that ganglioside storage was not simply due to the GAG-inhibition of specific lysosomal hydrolases, but suggests that other mechanisms, such as ganglioside synthesis and/or trafficking alterations could be responsible for their increase in MPS disorders.

Aiming to study the changes in the content and profile of the gangliosides observed in the present report, the gene expression of enzymes involved in ganglioside metabolism was investigated using the technique of RT-sq-PCR. For these analyses the cerebellum and the hippocampus were used. They have shown, respectively, as cortex and hypothalamus, an increase in the total ganglioside content and accumulation of GM3, GM2 and GD3 in KO animals for the IDUA gene. Ganglioside biosynthesis, in turn, was evaluated by the gene expression of 3 enzymes: GM3 and GD3 synthases involved in the synthesis of simple gangliosides (GM3 and GD3) which are precursors of complex gangliosides; and GM2/GD2 synthase, a common enzyme controlling the synthesis of complex gangliosides along different pathways (Maccioni et al., 1999; Yu et al., 2012). However, as ganglioside content is determined by a ratio between its synthesis and degradation, gene expression of Neuraminidase1, a key enzyme of lysosomal degradation of these glycosphingolipids, was also assessed (Miyagi and Yamaguchi, 2012; Tettamanti, 2004).

In the cerebellum, a decrease in the gene expression of three enzymes tested in KO animals was observed (GD3 synthase, GM2/GD2 synthase and Neuraminidase1) compared respectively to the expressions in wild type mice. The reduction of synthase expressions was smaller than Neuraminidase 1 suggesting that anabolic route could be favored and so explaining the increase in ganglioside concentration in KO mouse cerebellum. In the hippocampus, there was a decrease in gene expression for GM2/GD2 synthase and Neuraminidase1 enzymes in KO mice compared, respectively, to the expressions in Wt animals. These results are in agreement with the accumulation of GM3 and GD3 detected in this structure. However, at the moment there is not an explanation for GM2 accumulation.

Studies in the literature reported that an increase in intracellular GAGs, which occurs in MPS I, reduces neuraminidase activity in the lysosome (Walkley, 2004) and modifies glycosphingolipid synthesis in the Golgi complex (Campos and Monaga, 2012). This alteration could be associated to the impairment of activation of some intracellular transduction pathways linked to glycosyltransferase expression (Campos and Monaga, 2012; Maccioni et al., 1999; Morrison, 2012; Ngamukote et al., 2007). In addition, GAG accumulation alter the trafficking and/or recycling of gangliosides, and lipid raft composition and, therefore, damaging neuronal function (Campos and Monaga, 2012; Daniotti and Iglesias-Bartolomé, 2011; Walkley, 2004).

Taking together, the data from this report confirm, in part, previous suggestions that ganglioside changes are due to a combined effect on ganglioside biosynthesis and degradation. Considering the importance of gangliosides in cell biology and in the GM3 pro-apoptotic role (Sohn et al., 2006), these findings may be related to the neurological dysfunction of MPS I. The results obtained in the present study indicate

that ganglioside content and profile may be efficiently employed as follow-up parameters in MPS I models, during and after preclinical tests of gene therapy, cellular therapy, and enzyme replacement therapy (Baldo et al., 2013; Camassola et al., 2005; Zheng et al., 2003). Apart from this, ganglioside storage sites are also important in the investigations of new mechanisms of neuronal dysfunction in MPS I individuals (Campos and Monaga, 2012; Wilkinson et al., 2012).

5. Conclusions

The reported data add a new aspect to the ganglioside accumulation in patients and MPS I models. The alteration in the metabolism of gangliosides is not only given by the direct action of GAGs on the enzymes involved in their degradation, but also by the GAGs' influence on the gene expression of Neuraminidase 1 (degradation) and GD3 synthase, and GM2/GD2 synthase (biosynthesis).

Statement on conflicts of interest

There are no conflicts of interest between the authors.

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