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Lipid composition of microdomains is altered in neuronopathic Gaucher disease sheep brain and spleen

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ABSTRACT

Gaucher disease is a lysosomal storage disorder caused by a deficiency in glucocerebrosidase activity that leads to accumulation of glucosylceramide and glucosylsphingosine. Membrane raft microdomains are discrete, highly organized microdomains with a unique lipid composition that provide the necessary environment for specific protein-lipid and protein-protein interactions to take place. In this study we purified detergent resistant membranes (DRM; membrane rafts) from the occipital cortex and spleen from sheep affected with acute neuronopathic Gaucher disease and wild-type controls. We observed significant increases in the concentrations of glucosylceramide, hexosylsphingosine, BMP and gangliosides and decreases in the percentage of cholesterol and phosphatidylcholine leading to an altered DRM composition. Altered sphingolipid/cholesterol homeostasis would dramatically disrupt DRM architecture making them less ordered and more fluid. In addition, significant changes in the length and degree of lipid saturation within the DRM microdomains in the Gaucher brain were also observed. As these DRM microdomains are involved in many cellular events, an imbalance or disruption of the cell membrane homeostasis may impair normal cell function. This disruption of membrane raft microdomains and imbalance within the environment of cellular membranes of neuronal cells may be a key factor in initiating a cascade process leading to neurodegeneration.

1. Introduction

Cellular membranes are complex structures of lipids and proteins, where interactions among different components are responsible for specific cell functions as well as serving a structural purpose. Membrane lipids and proteins are not randomly distributed, but instead are organized within specific domains. Cholesterol together with glycosphingolipids and proteins are organized into specialized membrane microdomains that are called 'lipid rafts' or 'membrane rafts' [1,2]. These membrane rafts are highly dynamic assemblies that are laterally mobile, floating freely within the liquid bilayer of cellular membranes but can also cluster to form larger, ordered platforms [2]. The molecular order of membranes, fluidity, and organization of membrane rafts are important for various cellular events and functions [3,4], including trafficking of membrane proteins, exo/endocytosis, cell-cell communication, signal transduction pathways [5], host–microbial pathogen interactions [6], immune recognition and intracellular vesicle trafficking [7]. Without the correct lipid environment, proteins do not function properly; hence maintenance of lipid homeostasis is increasingly recognized as a crucial factor for normal cell function.

The ability of glycosphingolipids to act as both hydrogen bond donors and acceptors, enables their interaction with other lipids and proteins to form membrane rafts with varying architecture and function in the same cell [8,9]. The ceramide moiety of the GSL also gives rise to heterogeneity within the membrane rafts, influencing interactions with proteins through their ceramide moiety such that interaction specificity is conferred by ceramide composition [10] and also influencing lipid environment and architecture [8,11,12]. Thus showing the emergence of glycosphingolipids as key regulators with membrane rafts to control cellular events, and that their deregulation may have a role in diseases such as cancers and neurodegeneration [8].

Membrane rafts are highly ordered and more tightly packed than non-raft domains due to intermolecular hydrogen bonding involving

Abbreviations: BMP, bis(monoacylglycero)phosphate; Cer, ceramide; DRM, detergent resistant membranes; DSM, detergent soluble membranes; DHC, dihexosylceramide; GalCer, galactosylceramide; GCase, β-glucocerebrosidase; GlcCer, glucosylceramide; GlcSph, glucosylsphingosine; GD1, disialoganglioside; GT1, trisialoganglioside 1; GT3, trisialoganglioside 3; GM1, monosialotetrahexosylganglioside; GM2, GalNAcβ4(Neu5Acα3)Galβ4GlcCer; GM3, Neu5Acα3Galβ4GlcCer; GT1, (GT1a, Neu5Acα8Neu5Acα3Galβ3GalNAcβ4(Neu5Acα3Galβ4GlcCer; GT1b, Neu5Acα3Galβ3GalNAcβ4(Neu5Acα8Neu5Acα3)Galβ4GlcCer; GT1c, Galβ3GalNAcβ4(Neu5Acα8Neu5Acα8Neu5Acα3)Galβ4GlcCer); GT3, Neu5Acα2,8Neu5Acα2,8Neu5Acα2, 3Galβ1,4Glcβ1Cer (II^3(NeuAc) LacCer) [trisialosyllactosylceramide]; HexSph, hexosylsphingosine; MBS, MES-buffered saline; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; SM, sphingomyelin; THC, trihexosylceramide.

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the saturated fatty acid side chains of the sphingolipids and cholesterol [13]. The tight interaction between cholesterol and sphingolipids results in these liquid-ordered domains being resistant to solubilisation with detergents [1], and are therefore also referred to as detergent-resistant membranes (DRM). Their characteristic trait of being insoluble in non-ionic detergents [14] as well as their low buoyant density, is used to isolate membrane microdomains, allowing analysis and characterization of the lipid composition of the DRM domains and the detergent-soluble membrane (DSM) domains in cells.

Alterations to the lipid composition of cellular membranes, in particular changes in DRM organization, have been implicated with neurodegenerative diseases [15,16]. Alterations in the ganglioside and/or cholesterol content of DRM microdomains have been associated with Alzheimer's, Parkinson's and, Huntington's diseases, amyotrophic lateral sclerosis and prion disease [15,17–20]. It is hypothesized that changes in, and disruption of, the DRM environment, contributes to the loss of neural function seen in these diseases [21]. To understand the organization and structural specificities of glycosphingolipids within membrane rafts, as well as the significance of interactions between glycosphingolipids and surrounding molecules, it is important to elucidate the physiological functions of glycosphingolipid-enriched membrane rafts and their related diseases.

Gaucher disease arises from mutations in the β-glucocerebrosidase gene which encodes the lysosomal enzyme β-glucocerebrosidase (GCase; acid β-glucosidase) [22]. GCase mediates the hydrolysis of glucosylceramide (GlcCer) to ceramide (Cer) and glucose within the lysosome [23–26]. A deficiency of GCase activity leads to lysosomal accumulation of GlcCer and its deacylated form glucosylsphingosine (GlcSph) primarily in tissue macrophages but also in other cells including neurons [27]. We have identified a naturally occurring mutation in the β-glucocerebrosidase gene in sheep that leads to acute neurological symptoms [28,29].

We reported initial characterization of this sheep model of Gaucher disease and demonstrated that reduced β-glucocerebrosidase activity (1–5% of wild-type) resulted in accumulation of GlcCer and hexoylsphingosine (HexSph), as well as secondary accumulation of bis(monoacylglycero) phosphate (BMP) and gangliosides (GM1, GM2, GM3), in the brain, liver and spleen [29]. Affected animals display an ichthyotic cutaneous appearance, akin to the collodian variant of lethal neonatal Gaucher disease in humans. Key clinical features of acute neuronopathic disease were evident. Characteristic saccadic impairment and extra ocular ophthalmoparesis were present, and motor examination confirmed a pattern of axial hypotonia and appendicular hypertonia of a spastic quality; hyper-reflexic myotatic reflexes and forelimb clonus were elicited. The animal could not support its body weight against gravity, and when positioned supine failed to right itself. Wild-type and heterozygous animals were normal on neurological examination and proved ambulant within 1 h of birth [29].

In this report, we examine the lipid composition of cell membrane domains in the occipital cortex of the brain and spleen of wild-type and Gaucher sheep. Preliminary analysis of total cell lipids [29] indicated that the occipital cortex was one of the most changed areas in the Gaucher brain, particularly the ratio of the different GlcCer species. In addition the spleen also showed a high level of GlcCer accumulation. Consequently, in this study we isolated DRM and DSM from occipital cortex and spleen tissue in the Gaucher sheep to assess whether lysosomal GlcCer accumulation alters membrane microdomain composition. This is the first study to examine the composition of DRM and DSM microdomains from Gaucher brain tissue.

2. Materials and methods

2.1. Reagents

Anti flotillin-1 (polyclonal) was purchased from Sigma (St. Louis, MO: Sigma-Aldrich Cat# F1180 Lot# RRID:AB_1078893). The WestFemto ECL blotting system and Micro BCA protein assay kit were purchased from Thermo Scientific (IL, USA). HRP-conjugated goat anti-rabbit immunoglobulin was purchased from Merck Millipore (Vic, Australia: Millipore Cat# AP307P Lot# RRID:AB_92641). The internal standards Cer 18:1/17:0 [*N*-heptadecanoyl-D-*erythro*-sphingosine], BMP 14:0/14:0 [bis(monomyristoylglycero)phosphate (S,R isomer) (ammonium salt)], phosphatidylcholine (PC) 13:0/13:0 [1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine], phosphatidylethanolamine (PE) 17:0/17:0 [1,2-diheptadecanoyl-*sn*-glycero-3-phosphoethanolamine] and phosphatidylglycerol (PG) 14:0/14:0 [1,2-dimyristoyl-*sn*-glycero-3-phospho-(1′-rac-glycerol) (sodium salt)] were purchased from Avanti Polar Lipids (Alabaster, AL); dihexosylceramide (DHC) 18:1/16:0 (d_3) [*N*-palmitoyl-d_3-lactosylceramide], GlcCer 18:1/16:0 (d_3) [*N*-palmitoyl-d_3-glucopsychosine], trihexosylceramide (THC) 18:1/17:0 [*N*-heptadecanoyl ceramide trihexoside] and GlcSph were purchased from Matreya LLC (Pleasant Gap, PA); phosphatidylcholine (PC) 14:0/14:0 [1,2-dimyristoyl-*sn*-glycero-3-phosphocholine] and cholesteryl heptadecanoate 17:0 were purchased from Sigma (St. Louis, MO). The d_{35}GM1 18:1/18:0 internal standard was purchased from S, Sonnino, Department of Medical Chemistry, University of Milan, Italy. C18 solid phase extraction columns (200 mg) were obtained from UCT (Bristol, PA). All solvents were of HPLC grade, except chloroform, which contained 1% ethanol.

2.2. Tissue preparation

All animal housing, breeding and experimental procedures were approved by the SA Pathology and South Australian Health and Medical Research Institute (SAHMRI) Animal Ethics Committees in accordance with the guidelines established for the use of animals in experimental research as outlined by the Australian National Health and Medical Research Council Code of Practice for the Care and Use of Animals for Scientific Purposes (8th edition, 2013). The neuronopathic Gaucher sheep model has been previously described with wild-type control and Gaucher lambs determined by genotyping [28,29]. The occipital cortex gray matter and spleen from three wild-type and three Gaucher newborn lambs (age-matched) were harvested, snap frozen in liquid nitrogen and stored at −80 °C.

2.3. Isolation of DRM and DSM microdomains

DRM and DSM microdomains were extracted from the occipital cortex gray matter and spleen using the method previously described by Hattersley et al., [30]. Briefly, tissues were cut into small pieces, placed into a glass Dounce homogeniser with 2 ml MES-buffered saline (MBS) (25 mM MES pH 6.5, 0.15 M NaCl), containing 1% (v/v) Triton X-100 and 1 mM PMSF and homogenised 20 times before being incubated on ice for 30 min. After incubation, homogenates were centrifuged at 425*g* for 5 min at 4 °C, after which a 50 μl aliquot of the supernatant was taken for protein determination using the Micro BCA protein assay kit. The remainder of the supernatant was put into the bottom of a Beckman (Palo Alto, CA) centrifuge tube and the sucrose concentration was adjusted to 40% (w/v) by the addition of 2 ml 80% (w/v) sucrose in MBS buffer containing 1% (v/v) Triton X-100 and 1 mM PMSF. The sample was overlayed with 5 ml 30% (w/v) sucrose in MBS buffer containing 1 mM PMSF and then 4 ml 5% (w/v) sucrose in MBS buffer containing 1 mM PMSF. Samples were centrifuged at 270,500*g* for 16–20 h at 4 °C in a SW40 rotor and 1 ml fractions were collected from the top of the gradient.

2.4. Western blot analysis of DRM and DSM microdomains

An aliquot of each membrane microdomain fraction (10 μl) was run on 12% SDS–PAGE gels according to the method of Laemmli [31]. The gels were transferred to a PVDF membrane at 35 V for 70 min. The membrane was incubated in block solution (TBS + 0.1% (v/v) Tween