Bis(monoacylglycero)phosphate: a secondary storage lipid in the gangliosidoses

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Abstract  Bis(monoacylglycero)phosphate (BMP) is a negatively charged glycerophospholipid with an unusual sn-1:sn-1′ structural configuration. BMP is primarily enriched in endosomal/lysosomal membranes. BMP is thought to play a role in glycosphingolipid degradation and cholesterol transport. Elevated BMP levels have been found in many lysosomal storage diseases (LSDs), suggesting an association with lysosomal storage material. The gangliosidoses are a group of neurodegenerative LSDs involving the accumulation of either GM1 or GM2 gangliosides resulting from inherited deficiencies in β-galactosidase or β-hexosaminidase, respectively. Little information is available on BMP levels in gangliosidosis brain tissue. Our results showed that the content of BMP in brain was significantly greater in humans and in animals (mice, cats, American black bears) with either GM1 or GM2 ganglioside storage diseases, than in brains of normal subjects. The storage of BMP and ganglioside GM2 in brain were reduced similarly following adeno-associated viral-mediated gene therapy in Sandhoff disease mice. We also found that C22:6, C18:0, and C18:1 were the predominant BMP fatty acid species in the gangliosidoses brains. The results show that BMP accumulates as a secondary storage material in the brain of a broad range of mammals with gangliosidoses.—Akgoc, Z., M. Sena-Esteves, D. R. Martin, X. Han, A. d’Azzo, and T. N. Seyfried. Bis(monoacylglycerol)phosphate: a secondary storage lipid in the gangliosidoses. J. Lipid Res. 2015. 56: 1006–1013.

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Bis(monoacylglycero)phosphate (BMP) is a negatively charged glycerophospholipid that is mainly localized in late endosomes/lysosomes (1–3). Many cell types have low amounts of BMP, which comprises less than 1% of total cellular phospholipid content. However, BMP content is higher in alveolar macrophages (18% of total phospholipids) than in other cell types (4). BMP is a structural isomer of phosphatidylglycerol (PG) and is synthesized through a series of acylation and deacylation steps involving a transacylase, which reorients the glycerol backbone (5). This reorientation gives BMP its unusual sn-1:sn-1′ structural configuration (based on the phosphate-linked glycerol carbon), which is not observed in other phospholipids (5, 6) (Fig. 1). This unusual configuration is thought to play a role in the resistance of BMP to many phospholipases, and its stability in late endosomes/lysosomes (7, 8). BMP stimulates the degradation of glycosphingolipids in coordination with lipid binding proteins (saposins) in lysosomes (9).

Lysosomal storage diseases (LSDs) are a group of inherited disorders that are caused by defective/deficient lysosomal enzymes (10). Sandhoff disease (SD) and GM1 gangliosidosis are caused by genetic deficiencies of lysosomal β-hexosaminidase (HexB) and acid β-galactosidase (β-gal), respectively (10–14). SD is characterized by the storage of ganglioside GM2 and its asialo derivative GA2, whereas GM1 gangliosidosis involves the storage of ganglioside GM1 and its asialo derivative GA1 (11–14). Elevated levels of BMP are observed in many LSDs, including mucopolysaccharidosis, Niemann-Pick disease type A/B/C, Gaucher disease, and Fabry disease (15–26). However, this elevation is not present in all LSDs, such as the Spielmeyer-Sjögren type of neuronal ceroid-lipofuscinosis (20). Previous studies showed that BMP was elevated in the brain tissue of a SD human patient, and in serum obtained from GM2 gangliosidoses patients (20, 27). However, a detailed study of BMP content in the GM2 and GM1 gangliosidoses brain is lacking (28). We found that the levels of BMP were significantly higher in humans and animals with either GM1 or GM2 gangliosidoses than in brain tissue from

Abbreviations:  AAV, adeno-associated viral; BMP, bis(monoacylglycerol)phosphate; β-gal, β-galactosidase; HexB, β-hexosaminidase; HPTLC, high-performance TLC; LSD, lysosomal storage disease; p15, postnatal day 15; PG, phosphatidylglycerol; SD, Sandhoff disease.

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species-matched control samples. The data indicated that BMP is a secondary storage material along with gangliosides in the gangliosidoses brain.

MATERIALS AND METHODS

Mice

The SV129 Hes6 (+/−) mice were obtained from Dr. Richard Proia (National Institutes of Health). β-gal (+/−) mice were derived by homologous recombination and embryonic stem cell technology, as previously described (29, 30). Homozygous (−/−) mouse pups were derived from crossing heterozygous females with heterozygous male mice. Genotypes were determined as described previously (31, 32). Cortex brain samples were collected at humane end point and stored at −80°C. Humane end point for Hes6 (−/−) mice (SD) was about 100 days and for β-gal (−/−) (GM1 gangliosidosis) mice was about 180 days. All mice were propagated in the Boston College Animal Care Facility and were housed in plastic cages with filter tops containing Sani-Chip bedding (P. J. Murphy Forest Products Corp., Montville, NJ). The room was maintained at 22°C on a 12 h light/12 h dark cycle. Food (PROLAB R/M/H/3000) and water were provided ad libitum. All animal experiments were carried out with ethical committee approval in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care Committee.

Cats

SD and GM1 cats were obtained from the Baker colony at Auburn University, AL, and result from naturally occurring mutations as previously described (35–38). SD cats had <3% normal hexosaminidase activity in the cerebral cortex (39). GM1 cats had <10% of β-gal activity. Both feline models showed stereotypical clinical disease progression including corresponding ganglioside storage (GM2 for SD cats and GM1 for GM1 cats). Feline models also showed all brain and peripheral organ pathologies of the ganglioside disease and represented an authentic model to study disease progression (34, 40). According to the recommendations of the American Veterinary Medical Association Panel on Euthanasia, animals were euthanized by pentobarbital overdose, followed by transcardial perfusion with heparinized cold saline (0.9% NaCl) until jugular perfusate was clear. Cerebral cortex samples were collected at humane endpoint from gangliosidosis cats (SD, 4–5 months; GM1, 7–8 months) and from age-matched normal cats. Samples were frozen in liquid nitrogen and stored at −80°C. The Auburn University Institutional Animal Care and Use Committee approved all performed animal procedures.

Bears

American black bears with GM1 gangliosidosis were found in the Northeastern United States. The bears were in poor clinical condition at 10–14 months in age and humanely euthanized, as previously described (41). The bear brain tissue was obtained as a gift from Joseph Alroy, Tufts University, Boston, MA.

Humans

SD human cortex samples and their age-matched control were obtained from the National Institute of Child Health and Development Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore.

Adeno-associated viral vector design, preparation, and delivery to brain

The following vectors were used in this study: adeno-associated viral (AAV)1-CBA-Hexα, and AAV1-CBA-Hexβ. AAV vectors encoding α- or β-subunits of human Hexβ were constructed by PCR amplification of the respective cDNAs in the Mammalian Gene Collection clones 14125 (IMAGE 335324; Genbank: BC018927) and 1725 (IMAGE: 296735; Genbank: BC017378) obtained from the American Type Culture Collection (Manassas, VA). The primers used for PCR amplification were: Hexα1-ATCCACTAG-TGGAGCACCACATGACAAGTTCAGCTGGTTGTG7; Hexα2-AAT-TCCTGAGGCAGCTTCTGTCTCAGAAACTCTCTGTCCAC7; Hexβ1-AT-CCACTAGTGAGACCATCGTGGAGGTCGCTGGCCCGTGG; Hexβ2-ATTCTGAGATCAGTTCTCATGTCATGTTACATAATAGC7. PCR products were digested with Spe I and XhoI (sequences underlined in the primers above) and cloned into pAAV-CBA-MBG-W (42) in place of the mouse β-gal cDNA. All vectors used in this study carry the woodchuck hepatitis virus posttranscriptional regulatory element. AAV1 vector stocks were produced as described (42).

Six-week-old SD mice were injected with 2 μl of a 1:1 formulation of AAV1-CBA-Hexα + AAV1-CBA-Hexβ vectors (7.2 × 1012 gc/ml for each vector in the formulation) stereotaxically into left and right thalamus (n = 4) (coordinates from bregma in millimeters: anteroposterior, −2.0; medial lateral, ±1.5; dorsal ventral, −2.5) at a rate of 0.2 μl/min as previously described (43).

Lipid isolation, purification, and quantification

Total lipid extraction. Total lipids were extracted from the cortex of lyophilized whole brains with chloroform and methanol (1:1, v/v). Samples were further purified and prepared for column chromatography using previously described procedures (44, 45).

Column chromatography. DEAE-Sephadex (A-25; Pharmacia Biotech, Uppsala, Sweden) columns were used to separate neutral and acidic lipids (46). Total lipid extract was applied to the DEAE-Sephadex column that was equilibrated in solvent A (chloroform:methanol:water, 30:60:8, v/v/v). Neutral lipids were eluted with solvent A. Acidic lipids, including gangliosides, were eluted with solvent B, comprised of chloroform:methanol:0.8 M Na acetate (30:60:8, v/v/v).

Ganglioside purification. Acidic lipids were dried by rotary evaporation and further separated to acidic lipid and ganglioside fractions by Folch partitioning, as previously described (45, 47). An aliquot was taken from the ganglioside fraction for sialic acid determination and sialic acid was quantified by resorcinol assay. Gangliosides were further purified with base treatment and desalting, as previously described (12, 13, 45). Treatment of gangliosides with mild base is needed to remove contaminating phospholipids and any ganglioside internal esters or salt forms that might arise as artifacts of the lipid isolation procedures (48, 49).

BMP storage in the gangliosidoses

Fig. 1. Structure of 2,2′ diacyl-sn-1:sn-1′ BMP as previously described (56).
HPTLC. HPTLC was used to separate and visualize acidic and neutral lipids as previously described (12, 45, 50) BMP was purchased from Avanti Polar Lipids (Alabaster, AL). Lipid standards were either purchased from Matreya Inc. (Pleasant Gap, PA), Sigma (St. Louis, MO), or were the courtesy of Dr. Robert Yu (Medical College of Georgia, Augusta, GA). For BMP visualization, plates were run by a single ascending run with chloroform:methanol:ammonium hydroxide (30% by volume) (65:35:5, v/v/v) for 5 min and visualized by spraying with 3% cupric acetate in 8% phosphoric acid solution, followed by heating in an oven at 165°C for 7 min. The Camag HPTLC scanner with WinCATS software (Wilmington, NC) was used to estimate individual band densities. Concentrations of individual lipids were calculated based on standard curve obtained by standard lanes on HPTLC.

The amount of gangliosides spotted per lane was equivalent to 1.5 μg of sialic acid. Gangliosides were separated by a solution of chloroform:methanol:CaCl₂ (0.02%) (55:45:10, v/v/v) and visualized by spraying the dried plates with the resorcinol reagent, followed by heating at 95°C. Following quantification of ganglioside bands, total brain gangliosides were normalized to 100%, and sialic acid values were quantified by percent distribution of each ganglioside band (51).

Sialic acid quantification. The Folch upper phase, including the ganglioside fraction, was desalted and the amount of sialic acid was measured before and after desalting by the resorcinol assay, as previously described (13, 39). Three aliquots of each ganglioside sample were dried under vacuum. A resorcinol:dH₂O (1:1, v/v) solution (resorcinol reagent-HCl:0.2 M resorcinol:dH₂O:0.1 M CuSO₄, 40:5:5:0.125, v/v/v/v) was added to each sample, followed by submersion in a boiling water bath for 17 min. After cooling on ice, the reaction was stopped with butyl acetate:Nbutanol, (85:15, v/v). Each sample was vortexed and centrifuged at 700 g for 2 min. The absorbance of the upper aqueous layer was recorded at 580 nm using a Shimadzu UV-1601 spectrophotometer (Shimadzu, Tokyo, Japan). Sialic acid values were fit to a standard curve using N-acetylneuraminic acid as a standard (32).

Gas chromatography

Preparative HPTLC was used to separate BMP with chloroform:methanol:ammonium hydroxide (30% by volume) (65:35:5, v/v/v). Lipids were detected after spraying HPTLC with acetone:water (80:20, v/v) containing 5% primulin. The BMP fraction was scraped from the plate and esterified with acetyl chloride:methanol (1:4, v/v), and neutralized with K₂CO₃ (4%) in a sealed borosilicate tube under nitrogen. Separation of fatty acid methyl esters was carried out using gas-liquid chromatography (HP 6890) using a 30 m × 0.25 mm × 0.25 μm Omegawax 250 fused silica capillary column (Supelco). Area under the curve values were used to calculate the percentages of individual fatty acids compared with sum area of all fatty acids.

Mass spectrometry

Preparative HPTLC, using the solvent system chloroform:methanol:ammonium hydroxide (30%) (65:35:5, v/v/v), was used to separate BMP from other phospholipids. Shotgun lipidomics analyses of BMP were performed on a triple-stage quadrupole mass spectrometer (Thermo Scientific, San Jose, CA) equipped with an ionspray ion source, as previously described (53).

Statistical analysis

Statistical significance of data was analyzed by the two-tailed Student's t test between the normal and the diseased samples. Interquartile range was approximated between the maximum and minimum elements in the data set divided by two.

RESULTS

BMP accumulates in the brains of mice, cats, bears, and humans with GM2 (Sandhoff disease) or GM1 gangliosidoses

Our objective was to evaluate the content and fatty acid composition of BMP in the brains of mice, cats, bears, and humans with GM2 (Sandhoff disease) and/or GM1 gangliosidosis. BMP accumulation (up to 32-fold increase) was found in all samples of gangliosidosis brain tissue (Fig. 2, Table 1), showing that BMP accumulates as a secondary storage material in the gangliosidosis brain. BMP levels...
were also higher in juvenile [postnatal day 15 (p15)] β-gal (−/−) (GM1 gangliosidosis) mouse brains than inagematched β-gal (+/−) (normal) brains. We also observed that BMP was stored in the livers of Hexβ (−/−) (SD mice) (Table 1), but not in the livers of β-gal (−/−) GM1 gangliosidosismice (data not shown).

**Fatty acyl species of BMP**

We analyzed the fatty acyl species of BMP by gas chromatography. Major fatty acid species in BMP from gangliosidoses brain samples included stearic acid (C18:0), oleic acid (C18:1), arachidonic acid (20:4), and DHA (22:6) (Table 2). C22:6 was the predominant (57–62%) fatty acid present in the brains of GM1 and GM2 gangliosidosismice and in GM1 gangliosidosis American black bears. These species contained 16–22% of C18:0 and C18:1. In contrast, C18:0 and C18:1 were the predominant (37–57%) fatty acids in the brains of GM1 and GM2 gangliosidosiscats and GM2 gangliosidosis humans. These species had lower amounts (17–37%) of C22:6.

**BMP separates from its structural isomer PG by HPTLC and mass spectrometry**

BMP has the same molecular weight as PG, therefore it is challenging to separate BMP from PG in mass spectrometry without an initial chromatography procedure. In our solvent system, BMP (RF = 0.71) separated from PG (RF = 0.57) clearly. In order to further validate BMP as a separate lipid from PG, we analyzed the isolated BMP from HPTLC plates by a triple-stage quadrupole mass spectrometer. Analysis of commercial standards from Avanti showed BMP to have a distinct peak around m/z 92, while PG standard had two distinct peaks at m/z 171 and m/z 227. The BMP isolated from GM2 gangliosidosis mouse brains also showed the distinct peak at m/z 92, while these samples had very low peaks at m/z 171 and m/z 227 (data not shown). Also, the predominant BMP species in GM2 gangliosidosis mice had a molecular weight of 865.5 corresponding to 22:6-22:6 BMP (data not shown), which correlates well with the gas chromatography data showing that 22:6 is the predominant BMP fatty acid species in mice with GM2 gangliosidosis.

**AAV-mediated gene therapy reduces storage of brain BMP and ganglioside content**

The relationship of BMP storage to ganglioside storage was evaluated in the brains of Hexβ (+/−) (normal), Hexβ (−/−) (SD) mice that were treated or not treated with AAV gene therapy. Both GM2 and BMP levels were lower in the AAV-treated SD mice than in the untreated SD mice (Table 3). GM2 content was significantly correlated with the BMP content in the 13 brain samples analyzed.

### Table 2. Fatty acid distribution of brain BMP from mice, cats, bears, and humans with ganglioside storage disease

| Fatty Acid | Mouse (n = 3) | Cat (n = 2) | Bear (n = 1) | Human (n = 1) |
|------------|--------------|------------|--------------|--------------|
| C16:0      | 9.0 ± 1.8    | 5.4 ± 0.5  | 5.4 ± 1.1    | 10.9 ± 0.6   |
| C18:0      | 10.4 ± 1.8   | 8.7 ± 0.8  | 15.4 ± 2.7   | 24 ± 0.9     |
| C18:1      | 11.5 ± 1.9   | 11.7 ± 0.6 | 21.7 ± 3.3   | 22.6 ± 0.9   |
| C18:2n-6   | 1.5 ± 0.4    | 1.2 ± 0.1  | 5.2 ± 0.4    | 2.0 ± 0.5    |
| C20:2      | 2.0 ± 0.6    | 2.6 ± 1.2  | 0.5 ± 0.4    | 1.6 ± 0      |
| C20:4n-6   | 7.2 ± 0.4    | 8.0 ± 0.4  | 6.6 ± 0.7    | 5.9 ± 0.2    |
| C20:5n-3   | ND           | 4.4 ± 1.7  | 8.5 ± 3.0    | 0.4 ± 0.2    |
| C22:6n-3   | 58.4 ± 4.8   | 57.9 ± 2.4 | 36.7 ± 2.9   | 32.7 ± 1.4   |
| C24:0      | ND           | ND         | ND           | ND           |

ND, not detected.
was used to represent trace amounts on the HPTLC plate.

...noses, mucopolysaccharidosis (MPS I and II), Fabry disease, and Gaucher disease (16, 17, 19–25). However, no detailed studies of BMP content and composition have been conducted in the brains of gangliosidosis diseases (28). We observed a dramatic increase of BMP levels in GM1 and GM2 gangliosidosis brain samples in humans, American black bears, cats, and mice compared with their nondiseased counterparts. LSDs frequently involve a secondary storage material in addition to the primary storage material (28, 54). Examples include a secondary ganglioside storage in Gaucher disease and mucopolysaccharidosis, and secondary cholesterol storage in Niemann-Pick disease (55). We found that BMP was stored as a secondary material in the brains of GM1 and GM2 gangliosidoses brain samples in humans, American black bears, cats, and mice compared with their nondiseased counterparts. LSDs frequently involve a secondary storage material in addition to the primary storage material (28, 54). Examples include a secondary ganglioside storage in Gaucher disease and mucopolysaccharidosis, and secondary cholesterol storage in Niemann-Pick disease (55).

...storage was observed in the livers of SD mice (Table 1), where N-glycolylneuraminic acid-GM2 is also stored (58). This observation indicates that BMP co-accumulates with ganglioside storage, rather than as a downstream by-product of a nonfunctional enzyme.

Kobayashi and colleagues showed that BMP plays a role in the formation, structure, and trafficking of endosomal/lysosomal compartments in cells under normal conditions (3, 57, 59). Late endosomes/lysosomes form multivesicular bodies under normal conditions, but form multilamellar vesicles in pathological cases such as the gangliosidoses (60, 61). Both gangliosides and BMP are thought necessary for the formation of these aberrant lamellar bodies. It is possible that the high BMP levels we observed in the brains from the GM1 and GM2 gangliosidoses contribute to the formation of these multilamellar storage vesicles.

Glycosphingolipids are degraded in lysosomes with the aid of hydrolases and lysosomal lipid binding proteins, e.g., saposins (7, 62). The lipid composition of lysosomes is thought to be important in this degradation, and anionic phospholipids such as BMP are thought to facilitate the degradation of glycosphingolipids in the limiting membrane of endosomal/lysosomal compartments (63). BMP facilitates cholesterol transport, and the application of anti-BMP antibodies leads to cholesterol accumulation in lysosomes, mimicking the Niemann-Pick phenotype (64). BMP becomes limiting in Niemann-Pick disease fibroblasts, and exogenous feeding of BMP decreases excessive cholesterol storage (65). Also Hein, Duplock, and Fuller (15) show that selective decrease of BMP reduces the storage material, glucosylceramide, in TPH-1 Gaucher macrophages. Further studies will be needed to determine whether modulation of BMP levels can decrease ganglioside storage in the GM1 and GM2 gangliosidoses.

Inflammation is a hallmark of the gangliosidoses (66). Inflammation is observed with the onset of behavioral symptoms (about 2.5 months) in SD [HexB(−/−)] mice and GM1 gangliosidosis [β-gal(−/−)] mice (66, 67). Because BMP is a major lipid in alveolar macrophages, it is possible that microglia/macrophage infiltration could

| Sample | Phenotype | Treatment | GM2^b | BMP^b |
|--------|-----------|-----------|-------|-------|
| 1      | Normal    | None      | Trace | 66.8  |
| 2      | Normal    | None      | Trace | 72.1  |
| 3      | Normal    | None      | Trace | 61.9  |
| 4      | SD        | None      | 357.7 | 180.0 |
| 5      | SD        | None      | 376.7 | 162.5 |
| 6      | SD        | None      | 347.8 | 179.3 |
| 7      | SD        | None      | 391.1 | 187.5 |
| 8      | SD        | AAV       | 4.4   | 74.3  |
| 9      | SD        | AAV       | 16.4  | 74.5  |
| 10     | SD        | AAV       | 34.9  | 81.8  |
| 11     | SD        | AAV       | 23.0  | 81.4  |
| 12     | SD        | AAV       | 39.1  | 85.2  |
| 13     | SD        | AAV       | 4.1   | 70.2  |

^bGM2 values are expressed as micrograms of sialic acid per 100 mg of tissue dry weight.

^bBMP values are expressed as micrograms per 100 mg of tissue dry weight.

DISCUSSION

Although BMP comprises a small portion of total phospholipids in normal tissues, BMP levels increase in many LSDs, such as Niemann-Pick, neuronal ceroid lipofuscinoses, mucopolysaccharidosis (MPS I and II), Fabry disease, and Gaucher disease (16, 17, 19–25). However, no detailed studies of BMP content and composition have been conducted in the brains of gangliosidosis diseases (28). We observed a dramatic increase of BMP levels in GM1 and GM2 gangliosidosis brain samples in humans, American black bears, cats, and mice compared with their nondiseased counterparts. LSDs frequently involve a secondary storage material in addition to the primary storage material (28, 54). Examples include a secondary ganglioside storage in Gaucher disease and mucopolysaccharidosis, and secondary cholesterol storage in Niemann-Pick disease (55). We found that BMP was stored as a secondary material in the brains of GM1 and GM2 gangliosidoses. An explanation for the secondary storage of BMP in gangliosidosis brain has not been established (56). Because BMP is localized to endosomal/lysosomal membranes (57), a lysosomal expansion from stored gangliosides could simply increase the amount of a lipid localized in these compartments. However, BMP does not increase proportionally with lysosomal size, and it is not stored as a secondary material in all LSDs (56). Hence, lysosomal expansion might not be the only mechanism for BMP elevation (20, 56).

It is also unlikely that BMP storage is linked directly to the primary enzyme deficiency in LSDs, as BMP storage was not observed in the livers of GM1 gangliosidosis [β-gal (−/−)] mice despite the presence of ganglioside storage in the brains of these mice (data not shown). GM1 is not stored in the livers of β-gal (−/−) mice, as GM1 synthesis does not occur in mouse liver (58). On the other hand, BMP storage was observed in the livers of SD mice (Table 1), where N-glycolylneuraminic acid-GM2 is also stored (58). This observation indicates that BMP co-accumulates with ganglioside storage, rather than as a downstream by-product of a nonfunctional enzyme.

Kobayashi and colleagues showed that BMP plays a role in the formation, structure, and trafficking of endosomal/lysosomal compartments in cells under normal conditions (3, 57, 59). Late endosomes/lysosomes form multivesicular bodies under normal conditions, but form multilamellar vesicles in pathological cases such as the gangliosidoses (60, 61). Both gangliosides and BMP are thought necessary for the formation of these aberrant lamellar bodies. It is possible that the high BMP levels we observed in the brains from the GM1 and GM2 gangliosidoses contribute to the formation of these multilamellar storage vesicles.

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\( r = 0.9822, P < 0.01 \). For the correlation analysis, an arbitrary value of 0.5 μg sialic acid/100 mg of tissue dry weight was used to represent trace amounts on the HPTLC plate.
contribute, in part, to the increased BMP levels observed in the brains of the gangliosidoses. To address this possibility, we analyzed BMP levels in young Hexβ (−/−) mice at p15, well before the onset of behavioral symptoms and inflammation. However, GM2 ganglioside storage is observable in the p15 Hexβ (−/−) mice (58). We found that brain levels of BMP were higher in the p15 Hexβ (−/−) mice than in the normal age-matched controls. These findings suggest that BMP storage is not likely associated with inflammation, but is associated with ganglioside storage.

AAV gene therapy has been successfully used to treat GM1 and GM2 gangliosidosis in both mice and cats (42, 43, 68). Vectors expressing the deficient enzymes needed for corresponding ganglioside degradation are delivered intracranially. AAV treatments can successfully restore the deficient enzymatic activity and eliminate most of the corresponding ganglioside storage (42, 43, 68). We observed that AAV treatment also eliminated secondary storage of BMP in SD mouse brains. A significant positive correlation was observed between BMP storage and GM2 storage in the AAV-treated and untreated mice. These results show that AAV therapy that targets the primary storage successfully clears the secondary BMP storage as well.

Another property of BMP is its unique fatty acid composition. C18:1 is a major BMP fatty acid in many cell types (69–74). In alveolar macrophages, BMP predominantly contains n-6 fatty acids, such as linoleic acid (18:2) and arachidonic acid (20:4) (75, 76). Interestingly, DHA (C22:6, n-3) comprises a significant portion of BMP fatty acids in many cell types in drug-induced phospholipidosis, and also in many LSDs (22, 70–73, 77–82). We also found that DHA was a major fatty acid species in BMP from the brains of mice, cats, and bears with gangliosidosis. This predominance was most dramatic in mouse and bear samples where DHA comprised 57–62% of the BMP fatty acids. The reason for this high DHA percentage is unclear. Polyunsaturated fatty acids can influence membrane fluidity, which might be important for control of endosomal sorting and membrane fusion. Bouvier et al. (77) showed that 22:6/22:6 BMP can be oxidized in the presence of oxygen radicals, thus protecting cholesterol from oxidation. However, the specific function of 22:6 in LSDs remains unclear (56).

Here we analyzed the content and fatty acid composition of BMP in humans and in animals (mice, cats, and American black bears) with either GM1 or GM2 ganglioside storage disease. Our results showed that BMP was a significant secondary storage material in the gangliosidoses. BMP storage might be linked to lysosomal size, or might have a functional role in clearing excess storage material. Further studies will be needed to address these issues.

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