



A critical role for GM1 ganglioside in the pathophysiology and potential treatment of Parkinson's disease

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Abstract

Parkinson's disease (PD) is slowly progressing neurodegenerative disorder that affects millions of patients worldwide. While effective symptomatic therapies for PD exist, there is no currently available disease modifying agent to slow or stop the progression of the disease. Many years of research from various laboratories around the world have provided evidence in favor of the potential ability of GM1 ganglioside to be a disease modifying agent for PD. In this paper, information supporting the use of GM1 as a disease modifying therapeutic for PD is reviewed along with information concerning the role that deficiencies in GM1 ganglioside (and potentially other important brain gangliosides) may play in the pathogenesis of PD.

Keywords GM1 · Ganglioside · Parkinson's disease · Neuroprotection

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease of mid-to-late life, with approximately 1 in every 200 persons in the U.S. aged 60–69, 1 in 100 persons over 70, and 1 in every 35 persons over 80 estimated to have PD [1]. From 1990 to 2015, the number of people with PD worldwide doubled to over 6 million and this number is projected to double to over 12 million by 2040 [2]. Thus, with a significant and increasing global burden of PD, it is important to develop therapies that will slow disease progression and provide patients with a longer amount of time with a better quality of life. Although transient improvement of the most common motor symptoms of PD can be achieved with currently available dopaminergic pharmacotherapies, functional ability in PD patients deteriorates over time as the disease progresses [1–3]. There are no treatments that have been approved as disease modifying therapies for PD that significantly slow the progression of disease and symptoms.

GM1 ganglioside was first shown in two papers published in 1983 to enhance the survival of the damaged nigrostriatal

dopamine system in rats [4, 5]. It was suggested at that time that GM1 ganglioside might be a new type of drug for the treatment of PD [4]. Since that time, a wealth of preclinical data from diverse laboratories from around the world (ex., [6–18]) as well as Phase II clinical data in PD patients [3] support the suggestion that GM1 ganglioside is a promising potential disease modifying therapy for PD. This paper will review findings from preclinical studies that support a role of GM1 deficiency in the pathogenesis of PD as well as a role for GM1 administration as a disease modifying therapy for PD. The clinical experience with GM1 will then be reviewed, followed by a discussion of potential future directions.

The importance of GM1 for neuronal functional integrity

Gangliosides are sialic acid containing glycosphingolipids of which the major species in brain are a- and b-series gangliosides GM1, GD1a, GD1b, and GT1b. GM1 ganglioside, a major component of membrane signaling domains called lipid rafts, plays important roles in neuronal development and survival, exerts neurotrophic or neuroprotective effects under a variety of circumstances, and modulates a variety of critical cell functions including mitochondrial function, lysosomal integrity, and maintenance of Ca²⁺ homeostasis, among other processes critical for cell function and survival [19–22]. These functions of GM1 are important as disrupted Ca²⁺

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homeostasis and mitochondrial dysfunction are believed to play key roles in the pathophysiology of PD. There is a higher density of L-type voltage-gated Ca^{2+} channels (Cav1.3) in substantia nigra pars compacta (SNc) DA neurons than in ventral tegmental area (VTA) DA neurons, and this has been suggested to contribute to the selective vulnerability of SNc neurons in PD [23]. Interestingly, L-type Ca^{2+} channels associate with GM1-enriched membrane lipid rafts, where GM1 modulates their activity [24] and may act as a constitutive inhibitor. Thus, alterations in cellular GM1 content of SNc DAergic neurons could play an important role in modulating metabolic risk and contribute to the selective vulnerability of SNc neurons in PD. Additionally, several PD-relevant proteins (i.e., α -synuclein, LRRK2, PARKIN, and PINK1) colocalize with GM1 in lipid rafts [25–27], and α -synuclein aggregation, which is associated with PD pathology, is inhibited by GM1, dependent upon the amount of GM1 present [8, 25, 28]. The potential role of GM1, and in particular the decreased expression of GM1, in the pathogenesis of PD will be explored in the section below.

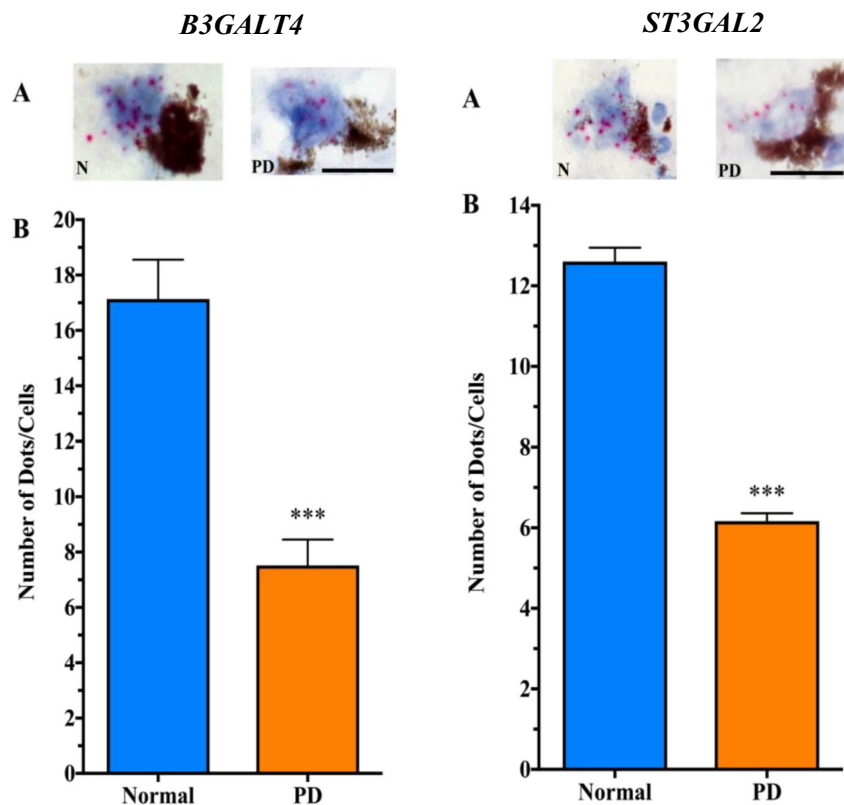
Reduced expression of GM1 as a risk factor for the development of Parkinson's disease

Although idiopathic PD is considered to be a disease of aging, the death of DA neurons in PD likely results from a process initiated many years earlier. For example, Calne and Langston [29] and others [30] have suggested that early life exposure to a neurotoxicant could induce a discrete but limited insult that reduces DA neuron numbers and that compensatory mechanisms engage to preserve normal DAergic function until later in life when age-related loss of DA neurons accumulates and cell loss exceeds a threshold over which compensatory mechanisms fail. However, it is also possible that early exposure to any number of possible neurotoxicants, particularly at levels that do not result in acute cell death, may alter the physiology of SN DA neurons in a manner that makes them less capable of defending against cumulative stressors that occur over the lifespan. Thus, a relatively unexplored potential pathogenic mechanism that may contribute to the degeneration of DA neurons in PD may involve exposure to an environmental neurotoxicant that alters the composition of the lipid (ganglioside) content of the cell, potentially by altering ganglioside biosynthesis, leading to increased vulnerability of DA neurons to various accumulating stressors over the lifespan and ultimately leading to sufficient neurodegeneration resulting in the development of PD. Whether PD develops or not could relate to the response of an individual's DA system to the initial insult (perhaps related to type of exposure, sex, and/or individual genetic susceptibility) and the degree to which ganglioside expression is affected. There are several lines of evidence that support this possibility.

Using heterozygous B4galnt1 knockout mice, Wu *et al.* reported decreases in SN GM1 and GD1a levels, with intact b-series gangliosides GD1b and GT1b, a somewhat surprising finding given that B4galnt1 is also important for b-series ganglioside synthesis. These mice showed a decrease in the number of SN DA neurons particularly as animals aged [6, 11]. Studies with this model suggested that partial removal of GM1 and other downstream gangliosides was sufficient for these animals to develop motor dysfunction, decreased striatal DA content, and elevated α -synuclein and α -synuclein aggregation, although α -synuclein aggregation was inferred and not directly assessed [6]. Decreased GM1 expression in the PD brain was also described [6, 31]. We further showed decreased levels of the four main brain gangliosides, GM1, GD1a, GD1b, and GT1b, in the PD SN [32], while levels of simple gangliosides GM2, GM3, and GD3 remained normal in the PD SN [32]. Decreased levels of the four main brain gangliosides in PD SN have also now been reported by Huebecker *et al.*, who additionally reported ante-mortem decreases in GM1, GD1a, GD1b, and GT1b in CSF from PD patients, compared to age-matched controls [33]. These authors also reported a significant decrease in neuraminidase activity in PD SN, that may contribute to the altered pattern of ganglioside expression observed [33]. The pattern of ganglioside loss that we observed in the PD SN suggested to us that the decreased expression of GM1, GD1a, GD1b, and GT1b could be related to altered expression of key biosynthetic enzymes involved in synthesis of GM1 and GD1b (B3galt4) and GD1a and GT1b (St3gal2). Using *in situ* hybridization histochemistry, we found that gene expression of *B3GALT4* and *ST3GAL2* in neuromelanin-containing neurons in the SN in PD cases was significantly decreased compared to that in age-matched control SN [34] (Fig. 1). These data suggest that dysfunctional ganglioside biosynthesis may be related to altered ganglioside levels which, even if resulting in relatively small reductions in GM1 expression, may contribute to heightened susceptibility of DA neurons to potentially damaging stressors over the course of the lifespan. Further support for this idea came from an additional study that showed that experimentally decreasing GM1 expression could enhance vulnerability to a neurotoxic exposure.

Using the human SK-N-SH DAergic cell line, we assessed the extent to which decreased *B3GALT4* and GM1 expression may lead to enhanced vulnerability for cell death [10]. SK-N-SH cells were differentiated and then transfected with human *B3GALT4* siRNA for 72 h., resulting in significant knock-down of *B3GALT4* gene expression, confirmed by qPCR, and approximately a 50% decrease in GM1 expression. Exposure of control SK-N-SH cells to 10 μM MPP⁺ for 24 h. did not result in any significant cell loss (assessed 48 h. later), while exposure of these cells to a higher concentration of the neurotoxin (100 μM MPP⁺) did result in significant (approximately 50%) cell loss. Exposure to siRNA alone also

Fig. 1 LEFT: *In situ* hybridization for *B3GALT4* gene expression (a) shows lower expression in residual neuromelanin-containing cells in the PD SN than in control SN neurons (b). RIGHT: *In situ* hybridization for *ST3GAL2* gene expression (a) shows lower expression in residual neuromelanin-containing cells in the PD SN than in control SN neurons (b). In both instances, each red dot represents a single RNA molecule. *** $P = 0.0001$ compared to Normal (Adapted from [34], reproduced with permission)



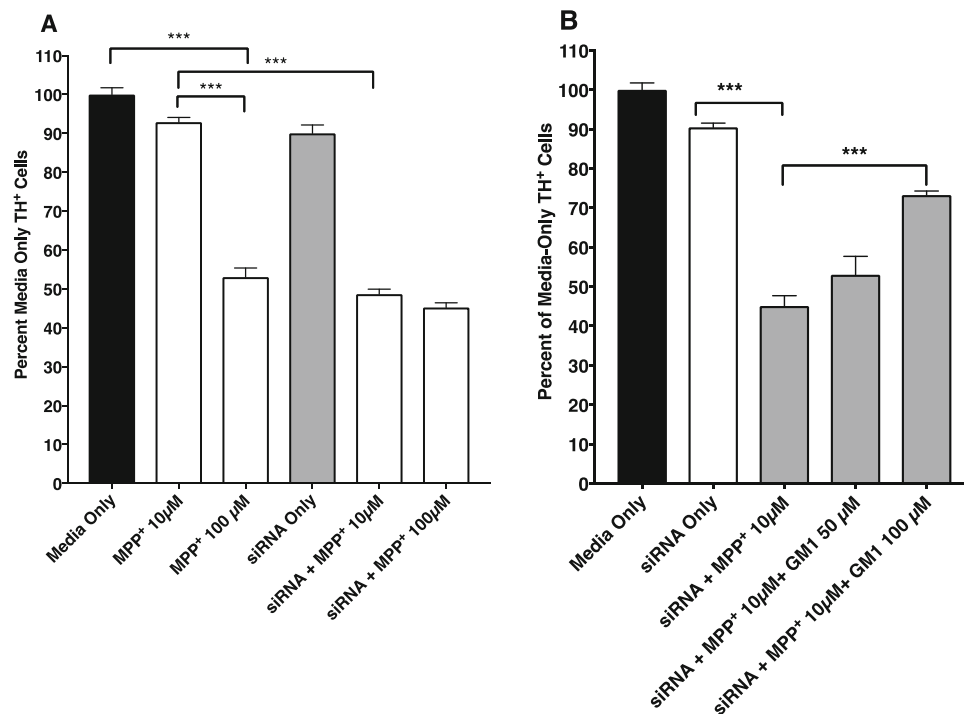
did not result in any significant cell loss. However, incubation with *B3GALT4* siRNA followed by exposure to 10 μM MPP⁺ for 24 h. resulted in decreased GM1 expression and cell loss of an extent similar to that observed with exposure to 100 μM MPP⁺ on intact cells [10]. Additionally, after treatment of cells with *B3GALT4* siRNA and enhanced toxicity from exposure to 10 μM MPP⁺, incubation of siRNA-treated cells with GM1 (100 μM) protected cells from the toxic effect of MPP⁺ [10] (Fig. 2).

These results showed that a decrease in GM1 expression can enhance vulnerability of cells to a previously nontoxic event. We suggested that a potential pathogenic mechanism that may contribute to the degeneration of DA neurons in PD is decreased levels of GM1 ganglioside (and perhaps other sphingolipids) due to an alteration in ganglioside/sphingolipid biosynthesis/degradation in response to endogenous or exogenous factors, leading to increased vulnerability of DA neurons to various stressors that accumulate over the lifespan, potentially leading to development of PD in certain susceptible individuals [10]. Together with the factors discussed above, findings that GM1 and GD1a levels in the brain decrease with aging [33, 35] further contribute to the risk of developing PD as one ages. In subjects ranging in age between 20 and 100 years, ganglioside (and other lipid) levels were assessed in frontal and temporal cortices and in subcortical white matter. Ganglioside levels were relatively stable between 20 and 70 years of age but decreased dramatically

between 70 and 100 years of age. The ganglioside pattern in frontal and temporal cortices changed significantly with aging, with decreasing amounts of GD1a and increasing amounts of GD1b, GM3, GM2, and GD3 between ages 20–39 and 90–99 years, with much larger decreases in GD1a than in GM1 [35]. More relevant to PD, a pattern of age-related decline in GM1 and GD1a levels (but not GD1b or GT1b levels) was also found in the SN of neurologically normal controls between ages in the mid-60s to 90s [33].

Although there are many possible contributors to the development of PD, exposures to environmental toxicants has received considerable attention. The risk of developing PD is increased with exposure to any pesticide (1.6–1.8-fold), to herbicides (1.3-fold), or to insecticides (1.5-fold) [36, 37]. Exposure to pesticides at levels lower than those eliciting acute toxicity and cell death may elicit neurotoxic effects through processes such as impaired neuritic outgrowth and impaired phenotypic differentiation and function [38], resulting in synaptic functional deficits and behavioral anomalies in later life [38–44]. Given the epidemiological association between pesticide exposures and PD risk, degeneration of nigrostriatal DA neurons has been examined in animals exposed to pesticides and related chemicals. However, most of these studies used exposure levels designed to demonstrate the ability of these chemicals to kill DA neurons. What has not been explored is the extent to which low level exposure to such chemicals that do not result in the death of DA neurons

Fig. 2 Quantification of effects of treatment of cells with B3GALT4 siRNA and enhanced toxicity from a low level of MPP⁺. (a) Exposure to 10 μ M MPP⁺ had no effect on normal cells while exposure to 100 μ M MPP⁺ caused about 50% cell loss. After treatment with B3GALT4 siRNA, exposure to 10 μ M MPP⁺ became just as toxic as an exposure to 100 μ M MPP⁺ under normal control conditions. (b) After treatment with B3GALT4 siRNA and enhanced toxicity from exposure to 10 μ M MPP⁺, incubation of siRNA-treated cells with GM1 protected cells from the toxic effect of MPP⁺. *** $P < 0.0001$ (From [10], reproduced with permission)



may instead prime them for increased vulnerability to a variety of possible insults that might occur throughout life. Even though idiopathic PD is a disease of aging, the pathological processes affecting DA neurons that ultimately result in PD are likely initiated many years earlier. Calne and Langston [29] and others [30] have suggested that early life exposure to a neurotoxicant could induce a discrete but limited insult that reduces DA neuron numbers and that compensatory mechanisms engage to preserve normal DAergic function until later in life when age-related loss of DA neurons accumulates and cell loss exceeds a threshold over which compensatory mechanisms fail. But, it is also possible that early exposure to a neurotoxicant that does not kill DA neurons per se, alters a physiological component of the cells in a manner that makes them less capable of defending against cumulative stressors that occur over the lifespan. Along these lines, it is suggested that a potential pathogenic mechanism contributing to the degeneration of DA neurons in PD, as a result of exposure to various environmental neurotoxicants, is altered composition of the ganglioside content of these cells, perhaps related to an alteration in ganglioside biosynthesis, leading to increased vulnerability of DA neurons to degeneration. The decrease in levels of gangliosides critical for cell function and survival, accentuated by an age-related decrease in GM1/GD1a levels, may ultimately lead to a loss of DA neurons sufficient for development of PD. Whether PD develops or not could relate to the individualized response of the DA system to the initial insult, perhaps related to type and extent of exposure, sex, and/or individual genetic susceptibility, and the degree to which ganglioside expression is affected. In support

of this possibility, we have shown in preliminary studies [45] that both *in vitro* and *in vivo*, low level pesticide exposure can decrease GM1 expression in otherwise healthy appearing DA neurons. The extent to which early life exposures to neurotoxic chemicals that do not kill DA neurons but may cause enduring alterations in ganglioside biosynthesis, decrease ganglioside expression, and increase the vulnerability to later degeneration, particularly in response to stimuli that alone would not be expected to stimulate neurodegeneration, needs to be furthered explored.

GM1, α -synuclein and Parkinson's disease

The functionality of lipid rafts, modulatory platforms for a variety of signaling pathways is influenced by GM1 content. Several PD-relevant proteins specifically associate with lipid rafts and co-localize with GM1. Alpha-synuclein (α -Syn) aggregation is believed to contribute to PD pathophysiology [46] and binding of α -Syn to GM1, *in vitro*, inhibits fibril formation, dependent upon the amount of GM1 present [25]. A similar effect of GM1 is also observed on the A53T mutant α -Syn [25]. Bartels, *et al.* [28] recently reported that the interaction of GM1 and α -Syn led to a complete resistance to fibrillar aggregation of acetylated α -Syn and suggested that binding to GM1-rich membranes could protect monomeric α -Syn from pathogenic aggregation [28]. Phosphorylation of α -Syn at Ser129 promotes α -Syn fibril formation [47] and the majority of α -Syn deposited in Lewy bodies in the PD brain is extensively phosphorylated at Ser129 [47, 48]. A53T α -Syn

as well as wild type α -Syn can form insoluble toxic fibrillar aggregates and mutant α -Syn may be more prone to aggregation and toxicity than wild type α -Syn [49, 50]. We recently reported that following AAV-A53T α -Syn injection into the SN in the rat, both early start (beginning 24 h. after surgery) and delayed start (beginning 3 weeks after surgery) administration of GM1 (30 mg/kg, IP daily for 5–6 weeks) reduced the size of α -Syn aggregates in the striatum and decreased pSer129 α -Syn expression in SN neurons (Fig. 3) [8]. *In vitro* studies have reported a direct association between GM1 and α -Syn, attributed to interaction between helical α -Syn and both the sialic acid and carbohydrate moieties of GM1, and that this association with GM1 inhibited fibrillation [25]. As we and others have shown decreased expression of GM1 and other complex gangliosides in the SN in PD [6, 33, 51] and decreased expression of genes involved in ganglioside biosynthesis in DAergic neurons in the PD SN [34], it is conceivable that reduced levels of gangliosides, and particularly GM1, in the PD SN may promote the accumulation of toxic α -Syn and that administration of GM1 may provide sufficient amounts of this important sphingolipid so as to at least partially inhibit the toxic aggregation of α -Syn and provide some level of protection to SN DA neurons. This possibility is supported by studies with B4galnt1 knockout mice, devoid of GM1 and other a-series gangliosides, in which increased amounts of α -Syn aggregation in knockout mice devoid of GM1 were reported and that this effect could at least be partially reduced by administration of GM1 and the semi-synthetic GM1 derivative LIGA-20 [6]. In our α -Syn over-expression study, GM1 was both neuroprotective and neurorestorative [8]. Both early start and delayed start use of GM1 protected against loss of SN DA neurons and striatal dopamine levels and reduced α -Syn aggregation. Early start GM1 administration protected against development of a motor deficit while delayed start administration of GM1 reversed the early appearing motor deficit [8].

Autophagy, Parkinson's disease and GM1

In PD, the presence of aggregated α -Syn is associated with accumulation of autophagosomes and reduction of lysosomal markers in SN DA neurons, suggesting a defect in lysosome-mediated clearance of α -Syn aggregates [52–54]. Thus, dysfunction in the autophagy-mediated clearance of α -Syn may contribute to the progressive degeneration of SN DA neurons in PD. In an AAV- α -Syn model in rats, stimulation of autophagy afforded significant protection against α -Syn toxicity [55].

Related to the potential neuroprotective effect of GM1 on PD and on α -Syn-related pathology is the role that GM1 may play in modulating autophagy and lysosomal pathology. *In vitro*, treatment of cells with D-Threo-1-phenyl-2-

decanoylamino-3-morpholino-1-propanol (D-PDMP), an inhibitor of glycosyl ceramide synthase that depletes endogenous gangliosides, resulted in reduced lysosomal activity, enhanced lysosomal permeability, accumulation of autophagic vacuoles in the cytoplasm and suppression of autophagic flux.

[22]. Additionally, D-PDMP-mediated inhibition of the autophagy-lysosomal pathway resulted in accumulation of α -Syn [22]. Importantly, the detrimental effects of D-PDMP on lysosomal pathology were significantly reversed by addition of GM1 to the cells, suggesting that levels of endogenous gangliosides and importantly GM1, may play a role in protecting against lysosomal pathology associated with synucleinopathy [22]. Decreased levels of GM1 and other gangliosides in the PD brain may promote lysosomal pathology, suppression of the autophagy-lysosome pathway, and accumulation of α -Syn, all of which could contribute to the pathophysiology of PD and all of which may be ameliorated by administration of GM1.

Microglia, neuroinflammation, Parkinson's disease and GM1

Microglial activation is believed to be an important contributor to the pathophysiology of PD [56, 57], with a variety of preclinical and clinical findings supporting the idea that microglial activation and microglia-mediated neuroinflammation play important roles in PD progression and DA neuron cell death [57–63]. Pathological conformations of α -Syn and the type of synuclein protein present have been suggested to trigger different cascades of signaling pathways within microglia to activate inflammatory responses that may differ as PD pathology progresses [57, 60]. Using administration of an AAV-wild type α -Syn vector into the SN, Sanchez-Guajardo *et al.* [63] demonstrated that the microglial response to α -Syn varied depending on the degree of α -Syn expression and neurodegeneration induced, and suggested that the microglial response was modulated by early events related to α -Syn expression in the SN and persisted long term [63].

As we have earlier described the neuroprotective/neurorestorative effects of GM1 in an α -Syn over-expression model in the rat [8], we have begun to examine if in addition to previously described effects of GM1 on α -Syn phosphorylation and aggregation, the neuroprotective effect of GM1 might also involve an influence on the microglial response to α -Syn. Striatal tissue from rats that received AAV-A53T α -Syn injection into the SN [8] was sectioned (30 μ m section thickness) and processed for Iba1 (Ionized calcium Binding Adapter molecule 1) immunohistochemistry (rabbit anti-Iba1, 1:5000, EnCor Biotechnology, Inc.) to visualize microglial cell bodies and processes, using Vectastain ABC reagent and DAB (Impact DAB Kit, Vector Laboratories, Inc.). Animals, vector delivery, and general

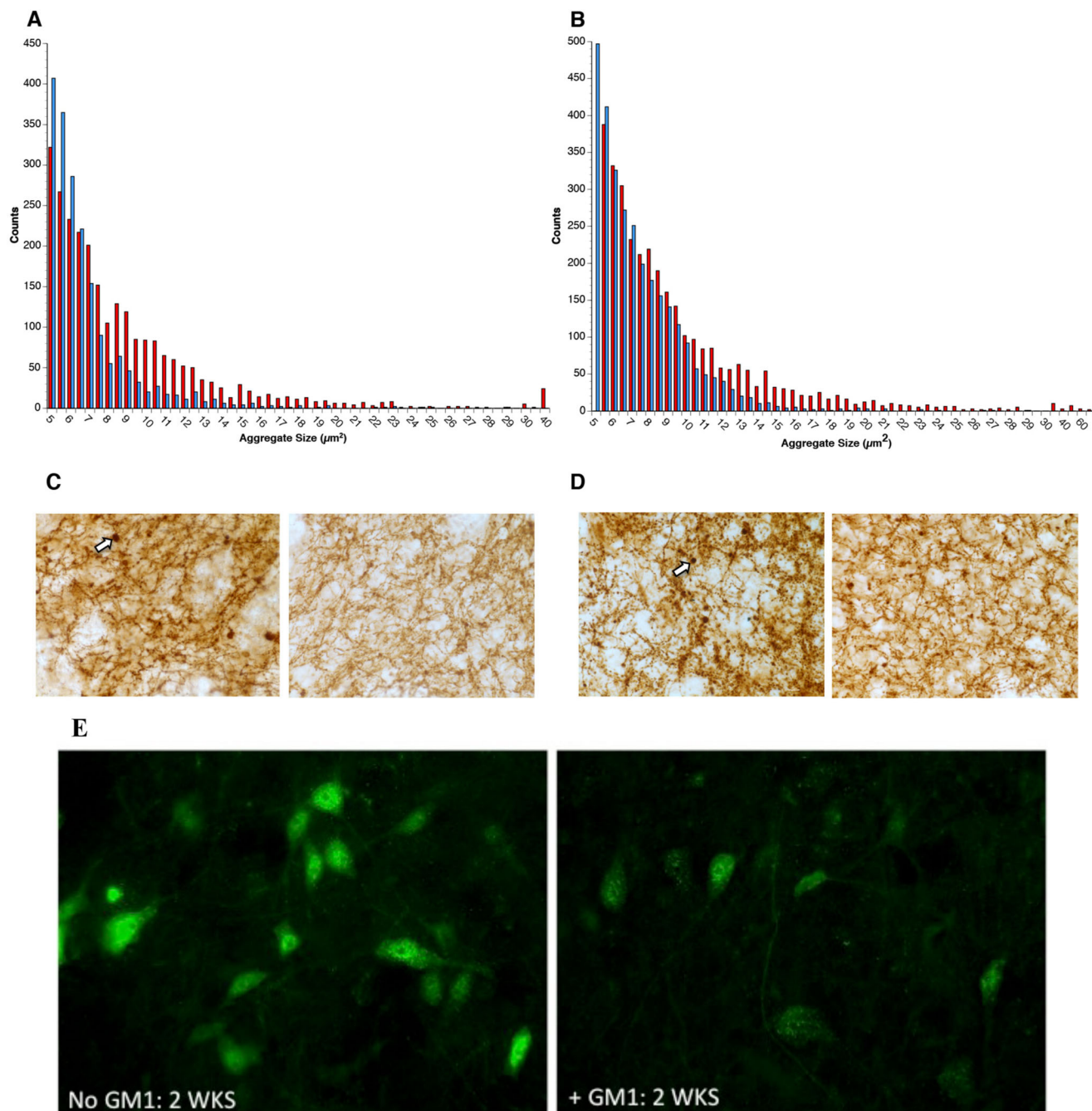


Fig. 3 GM1 treatment reduces the size of α -Syn-positive striatal aggregates. (a) The size distributions of α -Syn aggregates in saline-treated ($N=6$) (red bars) compared to early start GM1-treated (blue bars) animals ($N=6$), showed more smaller sized and fewer larger sized aggregates in the GM1 group ($P < 0.0001$); (b) Similar results were seen in the delayed GM1-treated group ($N=8$) (blue bars) vs. the saline-treated group ($N=7$) (red bars) ($P < 0.0001$); (c) Larger α -Syn aggregates are seen in saline-treated (left, arrow) vs. early start GM1 (right) animals; (d) similar findings in saline-treated (left) and delayed start GM1 (right) animals (From:

[8], reproduced with permission); (e) At the early symptomatic stage (2 wks after vector injection), insoluble pSer29 synuclein (following proteinase K digestion) is highly expressed in the SN in a saline-treated animal. In an animal that received GM1 (30 mg/kg/day) for 2 wks, starting 24 h after virus injection, there were fewer SN neurons expressing insoluble pSer129 and decreased expression per neuron, compared with saline-treated animals, suggesting decreased aggregation in the presence of GM1

immunohistochemical procedures have been described in detail previously [8]. Although studies are ongoing, preliminary

results show that at 4 weeks after AAV-A53T α -Syn injection, when striatal DA levels and SN tyrosine hydroxylase-

positive cell numbers are significantly decreased in saline control animals and when there is a significant neuroprotective effect from GM1 (30 mg/kg i.p. daily starting 24 h after AAV-A53T α -Syn injection), GM1 administration has a significant effect on microglial morphology [64]. Microglial morphology is closely related to functional state [65]. In the normal, healthy condition, microglia are mostly characterized by a ramified morphology whereas after injury or in the case of neuroinflammation, microglia are characterized as having shorter, thicker processes [66]. The number of process endpoints (an indication of the extent of process ramification) [67] were quantified from photomicrographs of Iba1-stained microglia in the striatum (control side and α -Syn injected side) of α -Syn/saline and α -Syn/GM1-treated animals using ImageJ and a method for quantifying microglia morphology described previously [67]. Results to date show that there is a significant decrease in the number of endpoints per microglial cell in the striatum on the α -Syn-injected striatum compared to the control side striatum in α -Syn/saline-treated animals. In comparison, there was no difference the number of endpoints per microglial cell on the striatum on the α -Syn-injected compared to the control side striatum in α -Syn/GM1-treated

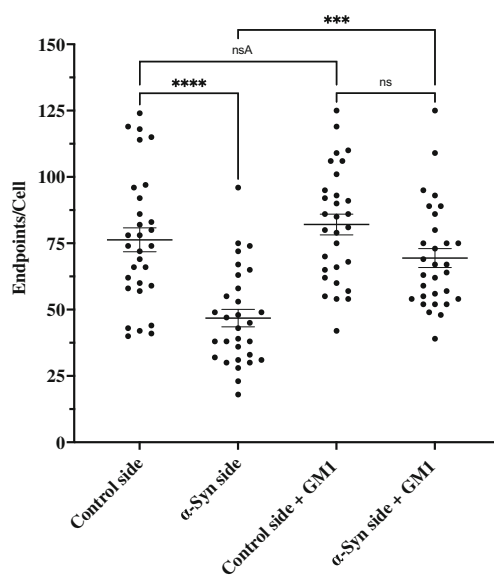


Fig. 4 Quantitative analysis of microglia morphology shows the microglial response to α -synuclein (α -Syn) toxicity in the AAV-A53T α -Syn over-expression model in the rat and its modification by GM1 administration. At 4 weeks after AAV-A53T injection in the substantia nigra (SN), microglial activation in the striatum coincided with decreased cell ramifications, resulting in a significant decreased number of endpoints per cell in comparison to the same measure taken from microglia from the control (non- α -Syn injected) side of the brain. GM1 treatment, initiated 24 h after AAV-A53T injection in the SN, resulted in no significant difference in the number of endpoints per cell in the striatum in the α -Syn injected side of the brain compared to the same measure from the control side of the brain. ($N=5$ animals per treatment group, 6 cells randomly selected from α -Syn and control sides and analyzed in each animal; **** $p < 0.0001$ α -Syn/saline vs. control/saline; *** $p < 0.001$ α -Syn saline vs. α -Syn GM1)

animals (Fig. 4). These preliminary findings suggest that inhibition of microglial activation and potentially damaging neuroinflammation by GM1 administration may be among the many factors that contribute to the neuroprotective/neurorestorative effects of GM1 in the AAV-A53T α -Syn PD model and potentially in human PD.

The rationale for the use of GM1 in the treatment of Parkinson's disease

Parkinson's disease is a progressive and debilitating neurodegenerative disease and thus, the need for a treatment that slows or stops disease progression is obvious. The rationale for developing a neuroprotective/neurorestorative therapy for PD rests in there being sufficient DAergic reserve early in the course of PD and that within the SN, intact DA neurons that have not degenerated may be potential targets for neuroprotection and some DA neurons that may be partially damaged and dysfunctional but still viable are potentially responsive to repair signals and are targets for neurorestoration. The initial rationale for considering GM1 as a neuroprotective/neurorestorative therapy for PD arose from the appreciation that PD was a complex disease involving multiple triggers/mechanisms/pathways that can lead to cell death and that an effective disease modifying therapy would likely need to influence a wide range of mechanisms involved in multiple critical cellular functions to successfully interfere with the disease process. Some of the many pathogenic triggers and mechanisms believed to be involved in PD are shown in Table 1 along with some of the many cellular pathways and processes known to be influenced by GM1. This, together with the information presented earlier in this paper, provide the rationale for the conduct of clinical trials to assess the use of GM1 as a potential disease modifying therapy for PD.

GM1 in the treatment of Parkinson's disease: the clinical experience

In consideration of the very positive preclinical findings in rodent and nonhuman primate models of PD, clinical trials of GM1 in PD were initiated in an effort to explore the translational relevance of the accumulated preclinical findings that had emanated from a variety of laboratories around the world [ex., [7, 9, 12–18]].

The first use of GM1 in PD patients was in a small, short-duration (18 week) open, single case design study, designed primarily to assess safety and to provide an initial readout on potential efficacy and to inform design of possible follow-up studies [68]. Effects of GM1 (bovine brain derived GM1, Fidia Pharmaceuticals, Inc) were tested in 10 PD patients (mean age 66.2 ± 9.8 years; range, 46 to 76 years; Hoehn

Table 1 Rationale for use of GM1 in Parkinson's disease

| Mechanisms potentially involved in PD pathogenesis | Functions attributed to GM1 ganglioside |
|--|--|
| Oxidative stress | Reduces oxidative stress reactions |
| Mitochondrial dysfunction | Regulates mitochondrial functions |
| Cytoskeletal disruption | Influences cytoskeletal dynamics |
| Fas-mediated cell death | May influence Fas recruitment into lipid rafts |
| Inflammation | Anti-inflammatory effects |
| Microglial activation | Reduces microglial activation |
| ATP/cellular energy depletion | Regulation of cellular bioenergetics |
| Excitotoxicity | Reduces excitotoxic damage |
| Decrease/loss of trophic support | Modulates neurotrophic factor responsiveness |
| Abnormal accumulation/aggregation of alpha-synuclein | Reduces aggregation of alpha-synuclein |
| Impaired autophagic mechanisms | Enhances autophagy |

and Yahr stage II-IV) who received 1000 mg GM1 i.v. once after the last of three baseline functional assessments. Patients then self-administered GM1 at 200 mg/day by subcutaneous injection for 18 weeks [68]. This study showed GM1, administered at this dose and this frequency, to be safe. Any adverse events were characterized as mild and transient, and no elevated anti-GM1 antibody titers were observed. Significant improvements in Unified Parkinson's Disease Rating Scale (UPDRS) motor scores and in performance of various timed motor tasks were observed, beginning after 4–8 weeks of treatment [68].

The next study of GM1 in PD was a double-blind, randomized, placebo-controlled study in which 45 patients (34 males, 11 females) with mild to moderate PD (Hoehn and Yahr stage I-III) received either placebo ($N = 23$, mean age 63.7 ± 8.8 years) or GM1 ($N = 22$, mean age 59.5 ± 10.1 years), again with an i.v. loading dose of 1000 mg of GM1 (or placebo) followed by 200 mg/day GM1 (or placebo) by subcutaneous injection for 16 weeks [69]. There were no statistically significant differences between the groups at baseline. The primary endpoint measure was change from baseline in UPDRS motor scores, assessed at study weeks 4, 8, 12 and 16. At 16 weeks, there was a significant difference between treatment and placebo groups in UPDRS motor scores as well as in several secondary outcome measures of timed motor activities [69]. Again, GM1 was well tolerated and no consistent alterations in clinical chemistry measures were observed, although mild to moderate local injection site reactions were reported. In preclinical studies, GM1 was shown to enhance dopamine synthesis and release and increase tyrosine hydroxylase levels in residual DAergic neurons in animal PD models [14, 70] and these functions of GM1 may at least in part underlie the symptomatic improvements observed in this short duration study. However, a much longer duration study would be needed in order to assess any potential disease modifying effect that GM1 might have on PD.

Another study was then performed to evaluate long-term safety of GM1 as a prelude to the conduct of a long duration disease modification study. Patients completing the 16-week double-blind, randomized, placebo-controlled study were offered the opportunity to enter an open-label extension of that study [71]. Twenty-six patients (13 from the original placebo group and 13 from the original GM1 group) were enrolled and all received GM1 (200 mg/day by subcutaneous injection) for up to 5 years. Safety was evaluated monthly and efficacy was evaluated every 6 months. Long-term use of GM1 was not associated with any serious adverse events. The gap that existed between the motor scores of the two groups at the end of the randomized phase diminished during the first two years of the open-label study and in later years approximately parallel changes in UPDRS motor scores in the two groups were observed [71]. Patients treated with GM1 throughout both the double blind and the open-label extension study showed only a slight deterioration of UPDRS motor (and ADL scores) over 5 years (Fig. 5), with both scores remaining significantly below those obtained at baseline prior to randomization into the original study. These results suggested that long-term use of GM1 is safe and potentially has disease modifying effects on PD.

These findings led to the design and conduct of a double-blind, randomized, placebo-controlled delayed-start study, designed to try to distinguish between potential symptomatic and disease modifying effects of GM1 in PD [3]. In this study, 76 mild to moderate PD patients were randomized to receive either GM1 (early-start, $N = 38$, mean age 59.7 ± 8.4 years) for 120 weeks or placebo for the first 24 weeks and then GM1 for 96 weeks (delayed start, $N = 38$, mean age 57.7 ± 8.7 years). An additional 17 patients were recruited as a no-treatment comparison group (i.e., received standard-of-care) and were evaluated to provide comparative information regarding disease progression in standard-of-care patients matched with the other participants in the study [3]. There were no statistically significant differences in symptom severity between the

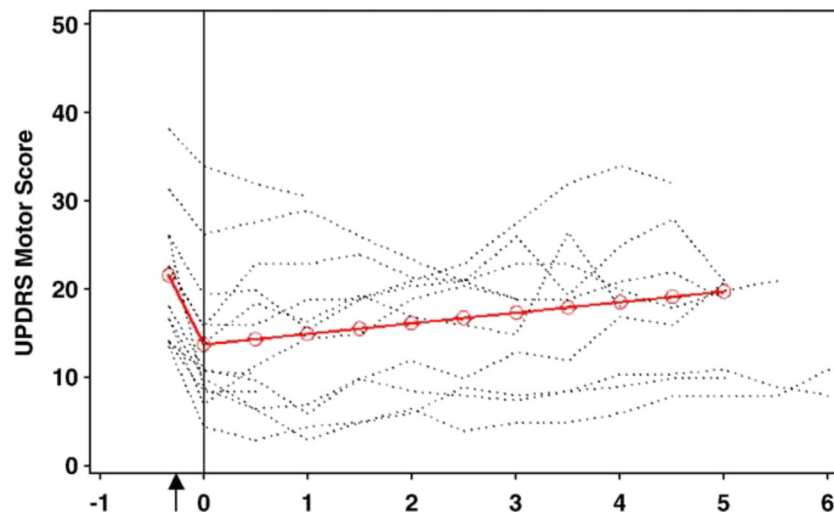


Fig. 5 (a) Mixed-effects linear regression modeling of the UPDRS motor scores over time during the five year open extension period for patients previously randomized to the GM1 group in a brief double blind, placebo-controlled study. The red line depicts the regression modeling; dotted black lines show individual patient raw data. X-axis = years in study,

arrow indicates baseline score at randomization into the main trial; time 0 = the end of the main randomized trial. The regression modeling shows the slow worsening of motor symptoms, assessed by UPDRS motor score, over a five year period of GM1 use. (From: [71], reproduced with permission)

groups at baseline. At week 24, the early-start group showed significant improvement in UPDRS motor scores vs. a worsening of UPDRS motor scores in the delayed-start (placebo) group that paralleled the disease trajectory of the comparison group. After starting GM1 at week 24, the delayed start group began to show improvements in UPDRS scores and their disease trajectory diverged from the comparison group. The early-start group showed a sustained benefit vs. the delayed-start group at week 72 and at end of study week 120, and the trajectory of the two groups remained divergent at the end of the treatment period (Fig. 6). The finding that the delayed-start placebo group remained worse than the early-start treatment group even after receiving GM1 is suggestive of a benefit to the early start use of GM1 and a potential neuroprotection or a disease modifying effect of GM1. Both groups showed significant symptom worsening after 1 and 2 years of washout from GM1, suggesting that the continued use of GM1 is necessary to maintain neuroprotective/disease modifying effects of GM1. As in previous studies, the use of GM1 was safe, with no consistent clinically significant changes in clinical chemistry observed. The most prevalent adverse events were related to injection site reactions [3].

A subset of patients who participated in the delayed start trial discussed above were examined longitudinally with [^{11}C]methylphenidate ([^{11}C]MP) positron emission tomography (PET) as a measure of the concentration of the dopamine transporter (DAT) in the striatum [72]. The decline of the binding potential (BP_{ND}) of [^{11}C]MP in the striatum of PD patients over time has been suggested as a surrogate marker of DA terminal density and disease progression [73]. The purpose of this imaging study was to evaluate potential effects of GM1 treatment on the integrity of striatal DA terminals. The

results of this pilot study, which included 15 early-start, 14 delayed-start, and 11 comparison group patients, showed significant slowing of BP_{ND} loss in several striatal regions in GM1-treated subjects and in some cases, the data suggested an increased BP_{ND} in some striatal regions, compared to baseline (Fig. 7). The clinical results of the delayed start trial of GM1 discussed above as well as the results of the previously discussed five year open extension trial of GM1 in PD suggest that long term use of GM1 may result in a slower than expected symptom progression in PD. The PET imaging findings, although preliminary and in a relatively small number of patients, are consistent with the suggestion that GM1 administration has a potential disease modifying effect on PD.

GM1 for the treatment of Parkinson's disease: issues and future directions

In view of the positive preclinical and clinical data regarding the effects of GM1 on PD, the question often arises as to why this treatment has not been further developed for clinical use. The answers to this question are several-fold. First, there is continuing reluctance regarding the use of an animal brain-derived product, whether from cows or pigs, although the GM1 used to date in humans has been safe and has not been shown to have potential to transmit any prion-like disease. Second, the extraction and purification process for animal brain-derived GM1 is very tedious and costly. Third, GM1 has limited ability to be transported across an intact blood brain barrier [74, 75] and in current formulations, GM1 needs to be administered in relatively high doses by peripheral injection. This is not a highly desirable administration mode for

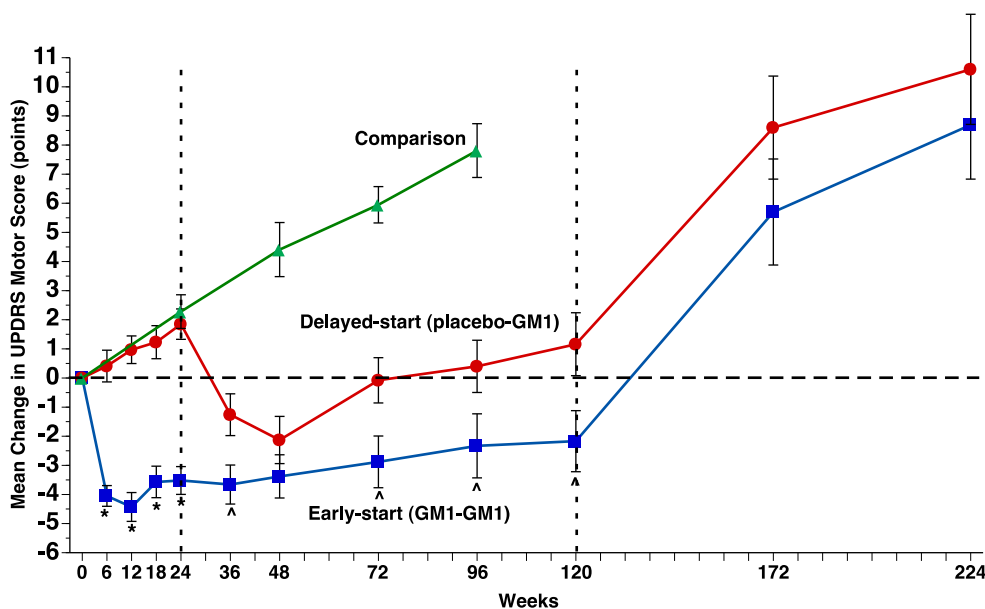


Fig. 6 Early-start (ES) use of GM1 has a potential disease modifying effect on Parkinson’s disease (PD). Mean (\pm SE) change from baseline in ES and Delayed-start (DS) subjects and in a standard-of-care comparison group in a double blind, placebo-controlled, delayed start study of GM1 in PD. Dashed vertical line at week 24 shows end of study Phase I (GM1 vs. placebo). After week 24 (study Phase II), all study subjects received GM1. Dashed vertical line at week 120 shows the end of study Phase II. Horizontal dashed line indicates baseline UPDRS motor scores.

Higher UPDRS motor score = symptom worsening; lower UPDRS motor score = symptom improvement. Note significant improvement in the ES group during the first 24 weeks and the slow progression of symptoms with continued GM1 use. Both treatment groups had slower symptom progression than standard of care comparison patients once all subjects were receiving GM1. * = $p < 0.0001$ ES vs. DS; ^ = $p < 0.05$ ES vs. DS. (Adapted from: [3], reproduced with permission)

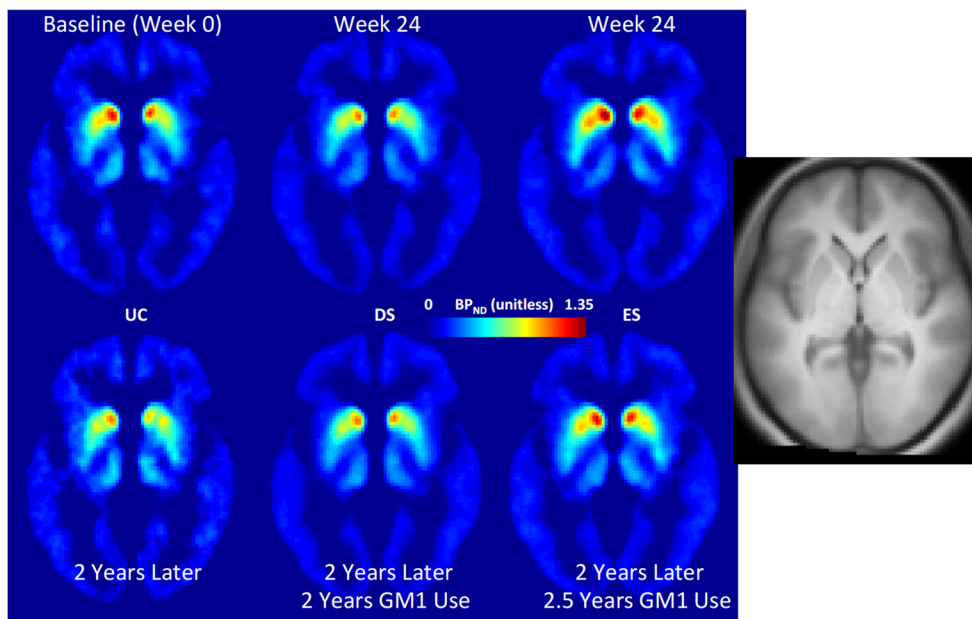


Fig. 7 Positron emission tomography (PET) scanning of striatal dopamine transporter density support a potential disease modifying effect of GM1 on Parkinson’s disease. Averaged striatal binding potential (BP_{ND}) images at baseline (Week 0) for the Comparison group subjects (C, left panel top row, $N = 11$) and at the transition point in the delayed start study (Week 24 for delayed-start (DS: $N=14$) [middle panel] and early-start (ES: $N=15$) [right panel] groups) (top row) and averaged images obtained

2 years later at the end of the second phase of study during which all treatment subjects used GM1 ganglioside. Images show less loss of BP_{ND} over time in ES subjects and DS subjects versus Comparison group subjects. The far right panel shows the averaged images of the MRIs of all participants as an anatomical reference for the PET scans. (From: [72], reproduced with permission)

a drug that would likely need to be given for the life of the patient and in clinical studies has been shown to be associated with a variety of adverse injection site reactions. Despite the positive clinical findings with GM1 in PD, it has recently been suggested that “GM1 did not reach a sufficient amount in the brain target site to perform reparative function”, presumably due to the inability of GM1 to be transported across the blood brain barrier [76] and that the “modest results” obtained in the delayed start clinical trial of GM1 in PD “confirmed that the potency of GM1 is severely limited by the fact that it crosses the BBB in very limited amount” [77]. The clinical findings with GM1 in PD were not “modest” and were actually superior to other attempts at demonstrating disease modification in PD with another agent, rasagiline [78]. The comments regarding access of GM1 to the brain in PD clinical studies are based on suppositions that can neither be supported nor refuted, since it has not been possible to monitor GM1 levels in the brain of human study subjects nor to verify BBB penetrance of GM1 or target engagement in these subjects. However, contrary to what has been suggested [76, 77], based on the clinical and imaging data from PD patients, it is likely that administration of GM1 resulted in a sufficient amount of GM1 gaining access to the brain to enhance functionality of residual DAergic neurons and stabilize and/or protect at-risk neurons. It is not possible to say how much GM1 was delivered to the brain nor is it known how much GM1 needs to be delivered to the brain to achieve a clinical effect. However, it is likely that due to a dysfunctional blood brain barrier in PD [79–81], more GM1 likely entered the brain than would have been expected based on preclinical data obtained from normal animals with an intact BBB, and, a sufficient and clinically significant amount of GM1 was provided to the brains of the subjects in these studies. However, for GM1 to be adopted as a viable therapeutic for PD, new formulations of synthetically produced GM1 with enhanced ability to cross the BBB that can be delivered through non-injection routes of administration will be necessary, as will be development of reliable biomarkers to track the access and effects of GM1 in the brain.

Concluding remarks

The mechanisms underlying the significant clinical effects of GM1 on PD are not entirely clear. However, in view of information discussed in this paper, there is compelling evidence that deficient levels of GM1 (and perhaps other gangliosides) contribute to the pathogenesis of PD and the clinical efficacy of GM1 in PD observed in the limited clinical studies performed to date is likely related to GM1 replacement and the multi-factorial effects that GM1 has on the damaged brain. The future of GM1 as a viable therapeutic for PD will hinge on the development and availability of a non-animal derived product that can be produced in large amounts in a cost-

effective manner and the development of new formulations that allow for delivery through non-injection routes of administration. Derivatives of GM1, such as GM1 oligosaccharide, may also have potential use as a therapeutic alternative to GM1 glycosphingolipid, however, advantages in delivery and efficacy above that obtained with GM1 glycosphingolipid need to be demonstrated. With the enhanced research interest in the role of GM1 and other gangliosides in PD, as well as other neurodegenerative diseases, the hope is that a GM1-related therapeutic may find its way to patients in the not too distant future.

Code availability Not applicable.

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Data availability New original data generated or analysed are included in this published article.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

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