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Modulation of amyloid precursor protein processing by synthetic ceramide analogues

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ABSTRACT

Previous studies suggest that membrane lipids may regulate proteolytic processing of the amyloid precursor protein (APP) to generate amyloid-beta peptide (Abeta). In the present study, we have assessed the capacity for a series of structurally related synthetic ceramide analogues to modulate APP processing in vitro. The compounds tested are established glucosylceramide synthase (GS) inhibitors based on the D-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) structure. PDMP and related compounds PPMP and EtDO-P4 inhibited Abeta secretion from Chinese hamster ovary cells expressing human APP (CHO-APP) with approximate IC_{50} values of 15, 5, and 1 μM, respectively. A trend for reduced secretion of the APP alpha-secretase product, sAPPalpha, was also observed in PDMP-treated cells but not in PPMP- or ETDO-P4-treated cells, whereas levels of the cellular beta-secretase product APP C-terminal fragment, CTFbeta, were increased by both PDMP and PPMP but unaltered with EtDO-P4 treatment. Our data also revealed that EtDO-P4 inhibits endogenous Abeta production by human neurons. In conclusion, this study provides novel information regarding the regulation of APP processing by synthetic ceramide analogues and reveals that the most potent of these compounds is EtDO-P4.

1. Introduction

1.1. Role of amyloid-β peptide (Aβ) in Alzheimer's disease (AD)

A prominent feature of AD is the presence of amyloid plaques in brain regions associated with memory and learning. Amyloid plaques contain Aβ peptides as a major constituent, and it is established that Aβ is derived from the amyloid precursor protein (APP) which undergoes two major pathways of enzymatic cleavage in the neuron [1]. The α-secretase pathway, which represents the major pathway for APP processing, does not generate Aβ as the metalloproteases (such as ADAM-10) responsible cleave in the middle of the Aβ sequence. In the second pathway, however, sequential cleavage of APP by β-secretase (BACE-1) and γ-secretase (a complex containing presenilins PS-1 or PS-2 as the catalytic subunit) generates Aβ peptide predominantly of 40 or 42 amino acids [2,3]. Once formed, Aβ peptides may assemble as soluble oligomeric species that lead to protofibrils (Fig. 1), which are neurotoxic at submicromolar concentrations [4,5]. It is clear that different macromolecular forms of Aβ regulate inflammation, oxidative stress, and lipid metabolism; all processes that are implicated in AD neurodegeneration [6–8]. In addition to factors that regulate the net production of Aβ in the brain, the ratio of Aβ1–40 to Aβ1–42 species generated, their propensity to form macromolecular complexes, and their clearance from the central nervous system (CNS) are all potential therapeutic targets for AD [2,9]. Despite the recognised role for Aβ in AD neurodegeneration, the factors that modify Aβ production and deposition are not completely understood.

1.2. Glycosphingolipids (GSLs), Aβ, and AD

The brain is a rich source of GSLs that represent a large family of complex lipids derived from the sphingolipid biosynthetic pathway (Fig. 2). The initial rate-limiting enzyme for GSL synthesis is glucosylceramide synthase (GS), an enzyme that catalyses the conversion of ceramide to glucosylceramide (GlcCer). Through the action of glycosyl transferases, GlcCer may be further acted upon to form more complex GSLs that may contain sialic acid residues in which case the GSLs become negatively charged and are referred to as gangliosides (e.g., monosialylated gangliosides GM1, GM2, and GM3). Nonsialylated "neutral" GSLs such as lactosyl ceramide (LacCer) and ceramide trihexoside (CTH) are also present in the brain [10].

More than 30 years ago, it was reported that reductions in the levels of specific gangliosides were associated with AD; however, it

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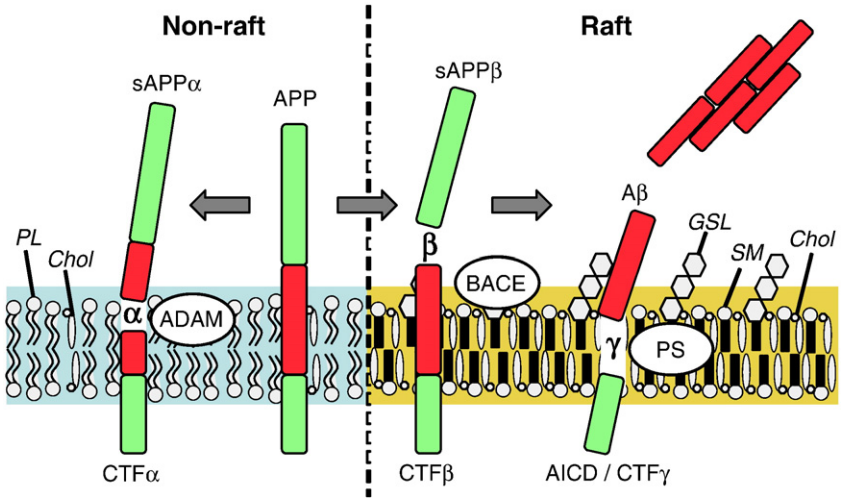

Fig. 1. Schematic representation of amyloid precursor protein (APP) processing. Amyloidogenic processing by β-secretase and γ-secretase generates Aβ peptides in the cholesterol- and GSL-enriched lipid raft microdomains within cell membranes. Nonamyloidogenic processing by α-secretase occurs predominantly in nonraft microdomains. Abbreviations are explained in the text.

was concluded that this was a "phenomenon accompanying extensive degradation of brain tissue rather than a factor in the aetiology of dementia" [11]. Similar studies performed almost a decade later also reported ganglioside reductions in AD brains and suggested this was due to "reduced density of nerve endings in the demented brains" [12]. These data suggest that the reduction of gangliosides observed in the AD brain may be a consequence of the disease rather than a cause.

Subsequent *in vitro* and *in vivo* studies indicated that ganglioside (particularly GM1) administration could potentiate the trophic effects of nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) [13–15]. Perhaps prematurely, these observations were set against the background data indicating "reduced" GSL levels in AD brains, and this led to the proposal that intracerebroventricular administration of GM1 could be used to treat AD [16,17]. Overall, this approach appeared to be unsuccessful as a treatment for AD and concerns were raised regarding immunological responses to GM1 administration [18–21].

Separate studies suggested that GM1 and other GSLs may in fact *promote* Aβ production and its assembly into neurotoxic complexes. It is known that GSLs are colocalised with Aβ in amyloid plaques and it has been proposed that GM1 may interact with Aβ to form a seed for amyloid plaque formation [22,23]. In addition, when GD3 synthase gene knockout mice (phenotype characterised by reductions in the levels of several brain gangliosides) were crossed with APPswe+PSEN1DE9 amyloidogenic mice, both soluble Aβ and plaque load were reduced (85–95%), and this was associated with improved performance in cognitive tests [24]. Other recent studies have shown that GM1 also resolubilises mature Aβ fibrils to regenerate neurotoxic Aβ protofibrils from amyloid plaque [25]. Finally, *in vitro* studies indicate that GSLs may stimulate both BACE and γ-secretase activity to promote Aβ generation [26,27].

Together, these findings suggest that therapeutic intervention to reduce GSL synthesis may be worth investigating as a novel strategy to reduce Aβ-associated neurodegeneration *in vivo*. Related to this, the synthetic ceramide analogue D-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP) is an established GSL synthesis inhibitor that has been shown to inhibit Aβ secretion from SH-SY5Y neuroblastoma cells [28]. Although PDMP is not suitable for long-term animal studies due to its high hepatic metabolism (plasma $t_{1/2}$ ~1 h), PDMP derivatives (Fig. 3) including D-threo-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol (PPMP) and D-threo-ethylenedioxy-1-phenyl-2-palmitoylamino-3-pyrrolidino-1-propanol (EtDO-P4) may provide viable alternatives [29,30]. EtDO-P4, in particular, has been successfully used in long-term studies in mice [31,32].

The aim of the present study was to investigate the impact that synthetic ceramide analogues PDMP, PPMP, and EtDO-P4 have on APP processing and Aβ production using CHO cells that stably express human APP695.

2. Materials and methods

2.1. Materials

Synthetic ceramide analogues D-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), D-threo-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol (D-PPMP), and L-threo-1-phenyl-2-hexadecanoylamino-3-morpholino-1-propanol (L-PPMP) were purchased from Matreya (Pleasant Gap, PA, USA). D/L-Threo-

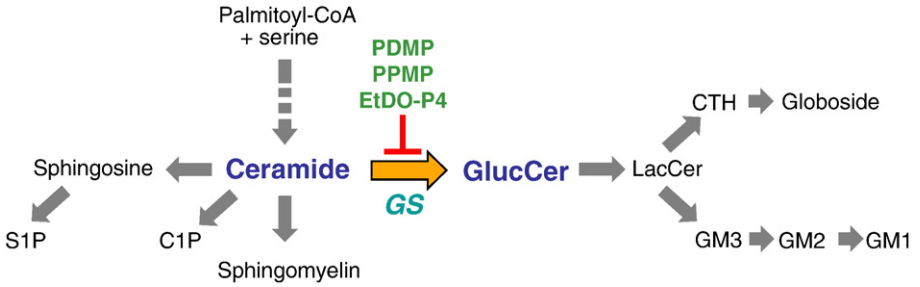

Fig. 2. Simplified scheme of sphingolipid biosynthesis. PDMP, PPMP and EtDO-P4 inhibit glucosylceramide synthase (GS) which catalyses the first step in glycosphingolipid synthesis. S1P, sphingosine-1-phosphate; C1P, ceramide-1-phosphate; Other abbreviations are explained in the text.