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Research report

Plasmalogen precursor analog treatment reduces levodopa-induced dyskinesias in parkinsonian monkeys

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HIGHLIGHTS

- A plasmalogen precursor decreased L-DOPA-induced dyskinesias in parkinsonian monkeys.
- The plasmalogen precursor PPI-1011 reduced dyskinesias earlier in treatment than DHA.
- DHA and PPI-1011 both elevated serum DHA containing ethanolamine plasmalogens levels.
- PPI-1011 and DHA maintained the antiparkinsonian activity of L-DOPA.
- Dyskinesia scores and serum DHA-ethanolamine plasmalogen levels inversely correlated.

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ABSTRACT

L-DOPA-induced dyskinesias (LID) remain a serious obstacle in the treatment of Parkinson's disease (PD). The objective of this study was to test a new target for treatment of dyskinesias, ethanolamine plasmalogens (PlsEtn). PlsEtn play critical roles in membrane structure mediated functions and as a storage depot of polyunsaturated fatty acids such as docosahexaenoic acid (DHA, omega-3) known to reduce dyskinesias. The motor effect of a daily treatment for 12 days of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) *Macaca fascicularis* monkeys with DHA (100 mg/kg) was compared to the DHA-PlsEtn precursor PPI-1011 (50 mg/kg). PPI-1011 and DHA reduced LID while maintaining the antiparkinsonian activity of L-DOPA, however the PPI-1011 effect was observed at the first behavioral time point analyzed following drug administration (day 2) whereas the effect of DHA was not observed until after 10 days of administration. DHA treatment increased plasma DHA levels 2–3× whereas PPI-1011 had no effect. DHA and PPI-1011 increased DHA-PlsEtn levels by 1.5–2× while DHA-phosphatidylethanolamine (PtdEtn) levels remained unaffected. DHA treatment also elevated very long chain fatty acid containing PtdEtn and reduced non-DHA containing PtdEtn and PlsEtn levels. PPI-1011 had no effect on these systems. LID scores were inversely correlated with serum DHA-PlsEtn/total PlsEtn ratios levels in DHA and PPI-1011 treated monkeys. Hence, the antidyskinetic activity of DHA and PPI-1011 in MPTP monkeys appears to be associated with the increase of serum DHA-PlsEtn concentrations. This is the first study reporting an antidyskinetic response to augmentation of DHA-PlsEtn using a plasmalogen precursor thus providing a novel drug target for dyskinesias.

Abbreviations: DA, dopamine; DHA, docosahexaenoic acid; L-DOPA, levodopa; LID, L-DOPA-induced-dyskinesias; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MRM, Multiple reaction monitoring; PD, Parkinson's disease; Pls, Plasmalogen; PlsEtn, Ethanolamine plasmalogens; Ptd, Phosphatidyl; PtdEtn, Phosphatidylethanolamine; Px β-Ox, Peroxisomal β-oxidation.

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1. Introduction

There is yet no cure for Parkinson's disease (PD) and incidence rates are likely to increase with the aging population [1]. Restoring deficient dopamine (DA) with its precursor levodopa (L-DOPA) remains the most effective symptomatic treatment for PD, but a majority of patients develop abnormal involuntary movements called dyskinesias after 5–10 years of treatment; dyskinesias are very difficult to manage pharmacologically and can be debilitating to the patient [2,3].

No drug is yet available for dyskinesias, aside from a modest benefit with amantadine in some PD patients [4]. Hence, alternative drugs are needed that may alleviate dyskinesias. We previously reported that docosahexaenoic acid (DHA, omega-3) has antidyskinetic activity in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model of PD [5].

The present study investigated plasmalogens as a new target to treat dyskinesia. Plasmalogens are a class of glycerophospholipids characterized by a vinyl-ether linkage at the sn-1 position and enriched with polyunsaturated fatty acids at the sn-2 position of the glycerol backbone (Fig. 1).

Plasmalogens play numerous roles in membrane structure mediated functions such as vesicular release of neurotransmitters [6,7], membrane protein activity [8–14], free radical scavenging [15], and serve as storage depots of polyunsaturated fatty acids such as DHA (reviewed:[16]). DHA-containing plasmalogens are critical phospholipids in neuronal membranes and myelin [17,18].

Decreased serum concentrations of plasmalogens in patients suffering from neurodegenerative diseases such as PD [19] and Alzheimer's disease have been reported [20–24] as well as changes during aging [25,26].

Plasmalogen precursors have been shown to effectively increase plasmalogen levels in vitro and in vivo [10–13,20]. PPI-1011 is a specialized ethanolamine plasmalogen (PlsEtn) precursor that contains covalently bonded DHA and lipoic acid at the sn-2 and sn-3 positions of a palmitic ether glycerol. Oral administration of PPI-1011 acts as a sustained release preparation of both DHA and lipoic acid as well as an intermediate in PlsEtn synthesis bypassing the peroxisomal ether lipid synthesis machinery [11]. Since DHA has been reported to increase PlsEtn synthesis [7,10,18], this study compares the relative effects of DHA and DHA-PlsEtn augmentation on L-DOPA behavioral responses and their serum levels in MPTP monkeys.

2. Materials and methods

2.1. Animals

The studies were performed in an animal model of L-DOPA-induced dyskinesia, the MPTP-lesioned dyskinetic macaque monkey. Five to six female ovariectomized cynomolgus MPTP monkeys (*Macaca fascicularis*) aged 5.6–11.3 years and weighing 3.5–5.7 kg (Covance Research Products, Mauritius or Vietnam; Charles River Lab Nevada, China; Primus Bio Resources, China) were used for the behavioral experiments. The number of animals used in the present study was based on our previous experiment with DHA in MPTP monkeys where five MPTP monkeys per group allowed the observation of a significant antidyskinetic effect of DHA [5]. The monkeys chosen had moderate to severe parkinsonian syndrome with scores of 8.2–11.5 on the parkinsonian scales developed at Laval University [5]. The primates were handled in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals. The Laval University committee for the protection of animals approved this study.

2.2. Drugs

PPI-1011 was provided by Phenomenome Discoveries Inc. (Saskatoon, Canada). Docosahexaenoic acid (DHA) was purchased from Nu Chek Prep Inc. (Elysian, MN). PPI-1011 and DHA were diluted with the vehicle Neobee M-5 (Spectrum, New Brunswick, NJ) and concentrations adjusted for each animal to give a fixed volume of administration of 5 ml po. For the dyskinesias experiments, L-DOPA methyl ester (Sigma-Aldrich, Canada) was given subcutaneous (sc) at a fixed dose tailored for each animal (15–35 mg/kg) always in combination with benserazide (sc, 50 mg total) (thereafter called L-DOPA). A ratio of L-DOPA/benserazide of 4:1 (for example 100 mg/25 mg) was used, as is the standard in human treatment of PD [27]. The tailored dose of L-DOPA was determined to elicit an optimal antiparkinsonian response and clear dyskinesias while limiting side effects like stereotypies and hypotension. In a separate experiment, the possibility of antiparkinsonian

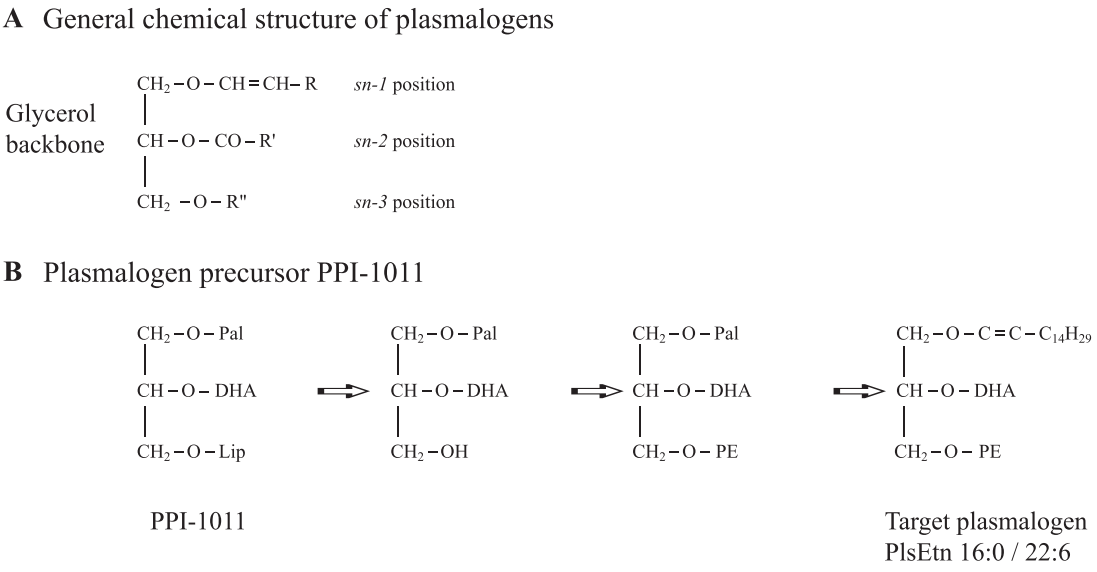

Fig. 1. (A) General chemical structure of plasmalogens and (B) biotransformation of PPI-1011 (plasmalogen precursor) to the target plasmalogen PlsEtn 16:0/22:6 as well as chemical structure of the plasmalogen precursor PPI-1011 and target plasmalogen PlsEtn 16:0/22:6. R & R′: fatty acid or fatty alcohol, R″: phosphoethanolamine or phosphocholine. Pal: palmityl, DHA: docosahexaenoic acid, Lip: lipoic acid, PE: phosphoethanolamine.