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Original Contribution

Nonenzymatic oxygenated metabolites of α-linolenic acid B_1- and L_1-phytoprostanes protect immature neurons from oxidant injury and promote differentiation of oligodendrocyte progenitors through PPAR-γ activation

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ABSTRACT

Phytoprostanes (PhytoP's) are formed in higher plants from α-linolenic acid via a nonenzymatic free radical-catalyzed pathway and act as endogenous mediators capable of protecting cells from damage under various conditions related to oxidative stress. Humans are exposed to PhytoP's, as they are present in relevant quantities in vegetable food and pollen. The uptake of PhytoP's through the olfactory epithelium of the nasal mucosa, upon pollen grain inhalation, is of interest as the intranasal pathway is regarded as a direct route of communication between the environment and the brain. On this basis, we sought to investigate the potential activities of PhytoP's on immature cells of the central nervous system, which are particularly susceptible to oxidative stress. In neuroblastoma SH-SY5Y cells, used as a model for undifferentiated neurons, B_1-PhytoP's, but not F_1-PhytoP's, increased cell metabolic activity and protected them from oxidant damage caused by H_2O_2. Moreover, B_1-PhytoP's induced a moderate depolarization of the mitochondrial inner membrane potential. These effects were prevented by the PPAR-γ antagonist GW9662. When SH-SY5Y cells were induced to differentiate toward a more mature phenotype, they became resistant to B_1-PhytoP activities. B_1-PhytoP's also influenced immature cells of an oligodendroglial line, as they increased the metabolic activity of oligodendrocyte progenitors and strongly accelerated their differentiation to immature oligodendrocytes, through mechanisms at least partially dependent on PPAR-γ activity. However, B_1-PhytoP's did not protect oligodendrocyte progenitors against oxidant injury. Taken together, these data suggest that B_1-PhytoP's, through novel mechanisms involving PPAR-γ, can specifically affect immature brain cells, such as neuroblasts and oligodendrocyte progenitors, thereby conferring neuroprotection against oxidant injury and promoting myelination.

Free radical-catalyzed oxidation of polyunsaturated fatty acids (PUFAs)[2] is a hallmark of oxidative stress. Arachidonic acid (AA; C20:4ω6) and docosahexaenoic acid (DHA; C22:6ω3) are the most abundant PUFAs in mammals, and their peroxidated products—isoprostanes (IsoP's) and neuroprostanes (NeuroP's), respectively—are considered among the most sensitive and reliable biomarkers of oxidative stress [1,2]. IsoP's and NeuroP's are large families of regio- and stereoisomeric prostaglandin-like molecules; some of them have been shown to display significant biological activities that could contribute to as well as protect from oxidant injury.

A third biologically active lipid family is represented by phytoprostanes (PhytoP's), which are formed in plants via a nonenzymatic

Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; bFGF, basic fibroblast growth factor; CV, crystal violet; DHA, docosahexaenoic acid; IsoP, isoprostane; MAP2, microtubule-associated protein 2; mMP, mitochondrial inner membrane potential; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NeuroP, neuroprostane; OL, oligodendrocyte; OP, oligodendrocyte progenitor; PDGF, platelet growth factor; PhytoP, phytoprostane; PPAR-γ, peroxisome proliferator-activated receptor γ; PUFA, polyunsaturated fatty acid; RA, retinoic acid; TMRE, tetramethylrhodamine ethyl ester perchlorate

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free radical-catalyzed pathway analogous to that leading to IsoP and NeuroP formation [3]. The PhytoP precursor is α-linolenic acid (ALA; C18:3ω3), a predominant PUFA in higher plants, generally lacking the enzymatic capacity to form longer chain PUFAs such as AA and DHA. Several classes of PhytoP's are constitutively present in plants and their levels rise in response to oxidative stress [4]. As their counterparts in the animal kingdom, PhytoP's occur in several classes, named according the prostaglandin classification, each of which can be generated as two racemic regioisomers (for further details see [5] and Fig. 1). The development of a new chemical strategy, based on a furan approach, has led to the synthesis of enantiomerically pure B_1-, F_1-, and E_1-PhytoP's [6,7], thus allowing the full assessment of the biological activities of each of these compounds.

Among the best studied PhytoP's are those of the A and B classes. Several lines of evidence indicate that A_1- and B_1-PhytoP's in plant cells influence the expression of numerous genes, most of which are involved in the detoxification of xenobiotics and in cytoprotective responses, suggesting that B_1-PhytoP's represent endogenous mediators capable of counteracting cell damage (see [8] and references therein). Much less is known of the potential biological activities of PhytoP's in mammalian systems.

Humans are potentially exposed to PhytoP's. Vegetable foods, in particular vegetable oils, contain high levels of ALA, which could be converted into PhytoP's by autoxidation during cooking and/or storage or after oral consumption in the gastrointestinal tract. Karg et al. [9] have shown that PhytoP's of the A_1, B_1, E_1, and F_1 classes are present in up to milligram quantities per 100 ml in fresh vegetable oil, with the highest levels found in linseed and soybean oils. These levels may further increase by more than an order of magnitude with storage, reaching submillimolar concentrations. After oral ingestion, PhytoP's are adsorbed and found in plasma and urine in conjugate and free form, respectively. Plasma and urinary levels of F_1-PhytoP's were found increased in healthy men after 4 weeks of flaxseed oil supplementation compared to a control group receiving olive oil supplementation [10]. PhytoP's have also been detected in parenteral lipid nutrition used in intensive care medicine, containing lipid fractions from vegetable oils such as soybean and olive oils [10].

In addition to oral ingestion, humans can be exposed to PhytoP's through inhalation of pollen, rich in both ALA and PhytoP's. Upon contact with the respiratory mucosa, pollen grains release allergens in conjunction with many other substances, including bioactive lipids, collectively called pollen-associated lipid mediators. Aqueous pollen extracts contain significant levels of E_1-, F_1-, A_1-, and B_1-PhytoP's. Of these, E_1-PhytoP is among the most prominent and it has been identified as one of the pollen-associated lipid mediators, capable of modulating dendritic cell functions and favoring a type 2-dominated proallergenic immune response [11,12]. The fact that PhytoP's can be released from pollen grains upon contact with the airway mucosa, and in particular nasal mucosa, is intriguing as the intranasal pathway is regarded as a direct route of communication between the environment and the brain.

In addition to the above-mentioned immunomodulatory activity of E_1-PhytoP on human dendritic cells, few other PhytoP biological

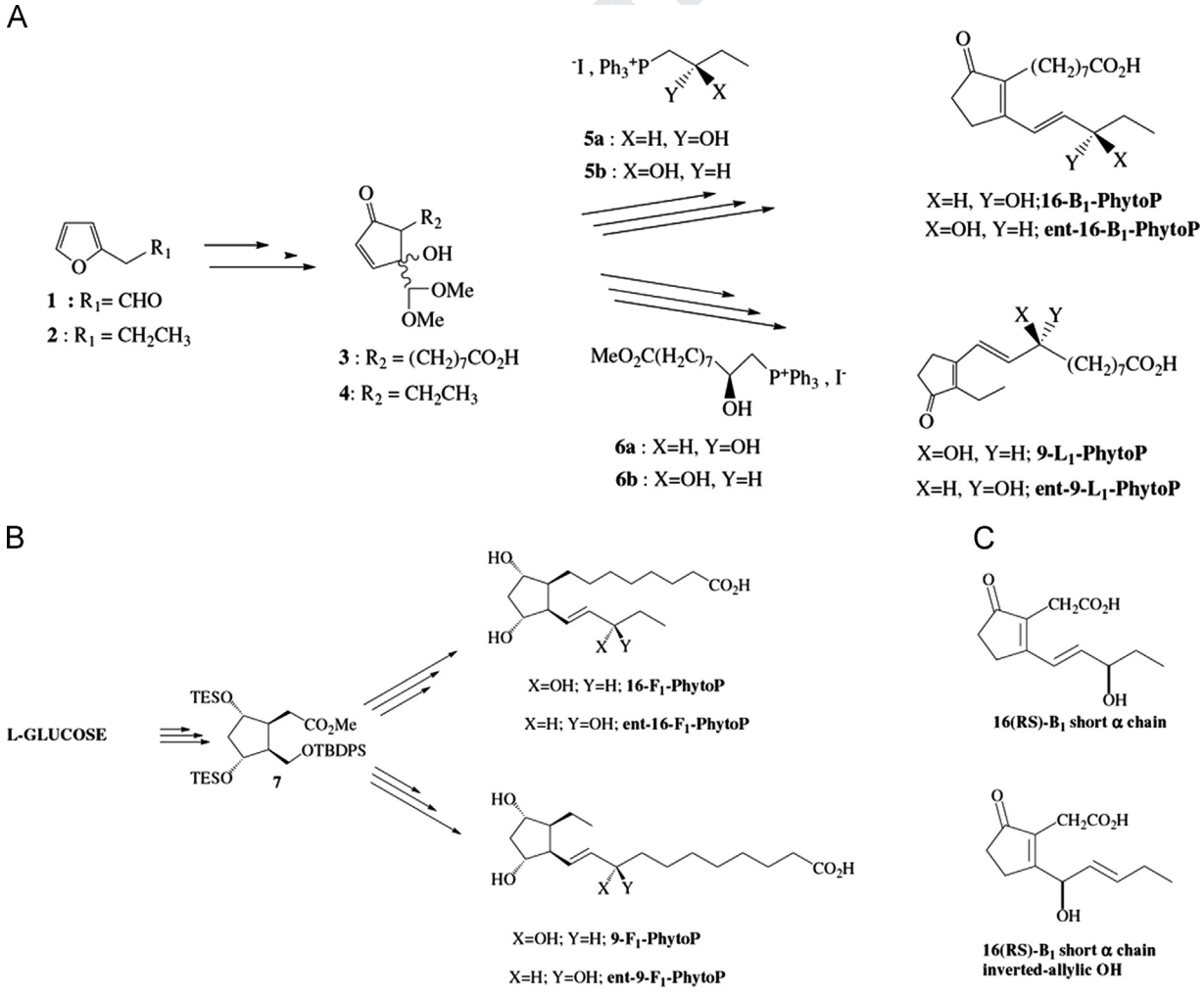

Fig. 1. Total synthesis of F_1-, B_1-, and L_1-PhytoP's and analogs. See Materials and methods for details.