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(54) **MIXTURES OF AND METHODS OF USE FOR POLYUNSATURATED FATTY ACID-CONTAINING PHOSPHOLIPIDS AND ALKYL ETHER PHOSPHOLIPIDS SPECIES**

(76) Inventors: **Su Chen**, Malta, NY (US); **Hung Kwong**, Malta, NY (US)

Correspondence Address:
LAW OFFICE OF MICHAEL A. BLAKE
112 BROAD STREET
MILFORD, CT 06460

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(57) **ABSTRACT**

Mixtures of natural phosphatidylcholine species, natural lysophosphatidylcholine species, phosphatidylserine species, phosphatidylethanolamine species, 1-hydroxy-2-acyl-phosphatidylcholine species, 1-hydroxy-2-acyl-phosphatidylserine molecular species, 1-hydroxy-2-acyl-phosphatidylethanolamine molecular species, 1-O-alkyl-2-hydroxy phosphatidylcholine species, 1-O-alkyl-2-docosaheaxnoyl phosphatidylcholine species 1-O-alkyl-2-docosahexaenoyl phosphatidylserine species, and 1-O-alkyl-2-docosahexaenoyl phosphatidylethanolamine species, Methods using the above disclosed mixtures in mammals to treat various conditions.

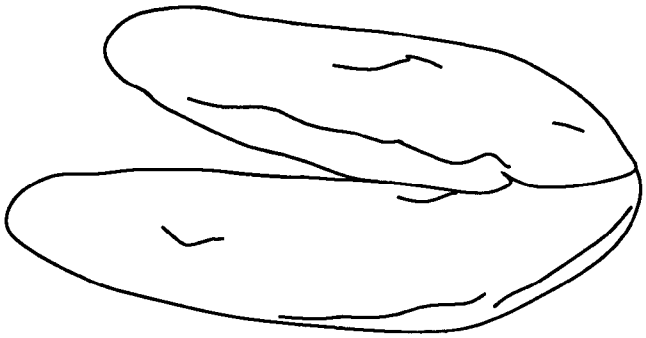

Fig. 1

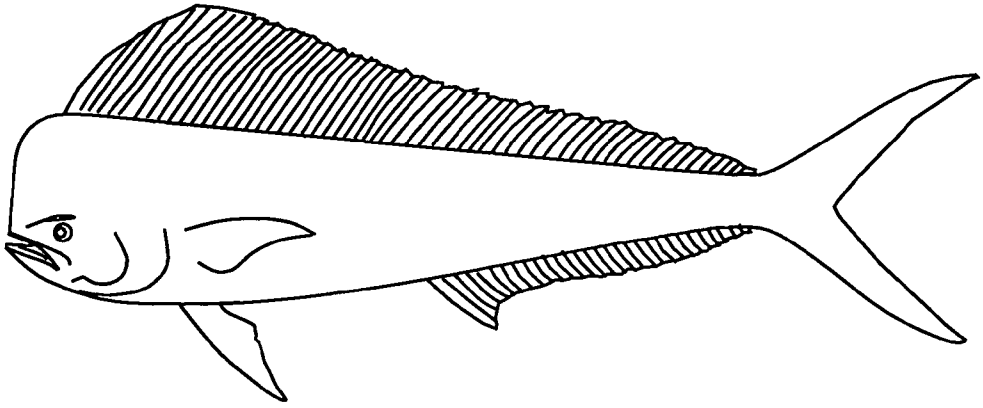

Fig. 2

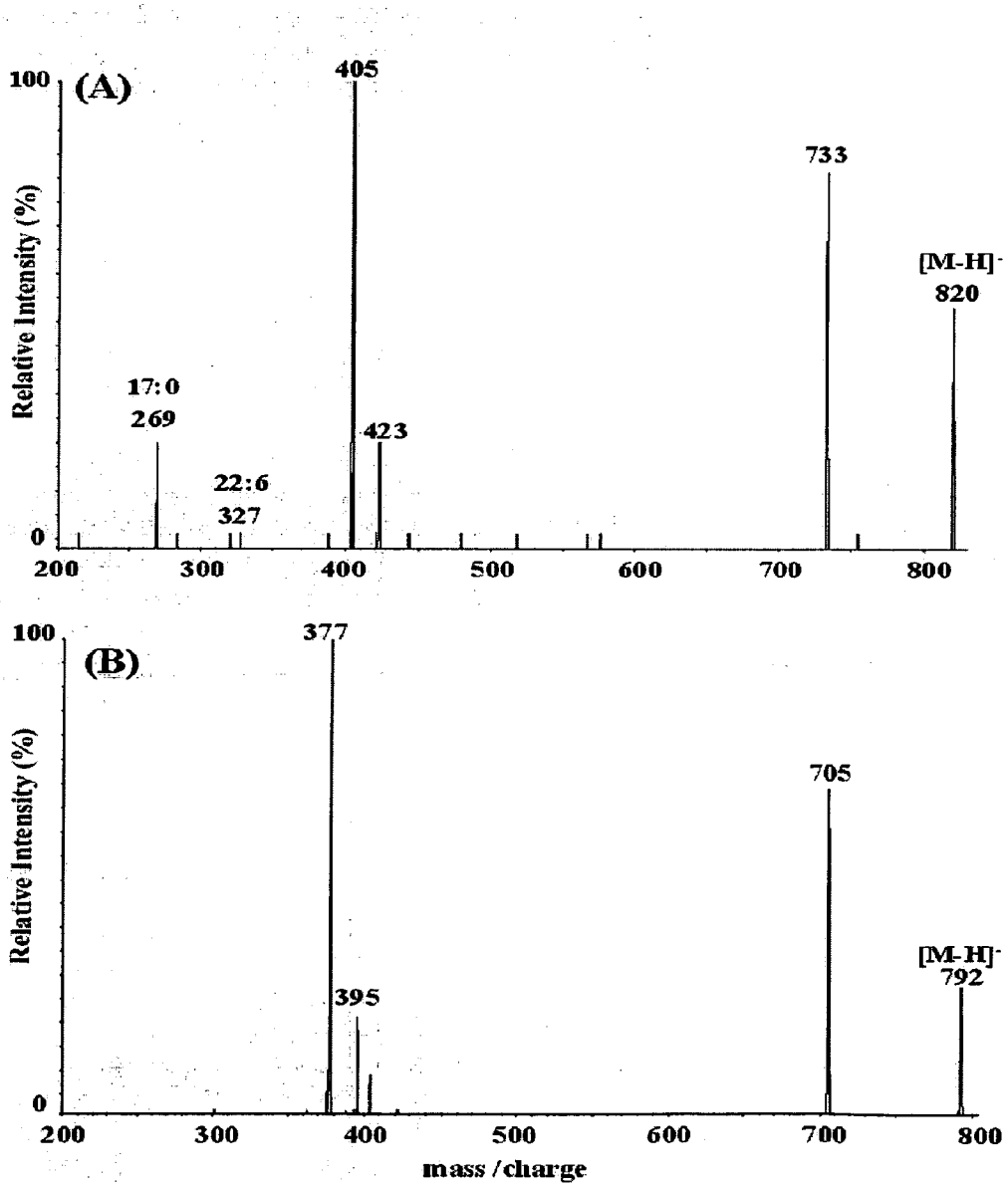

Fig. 3

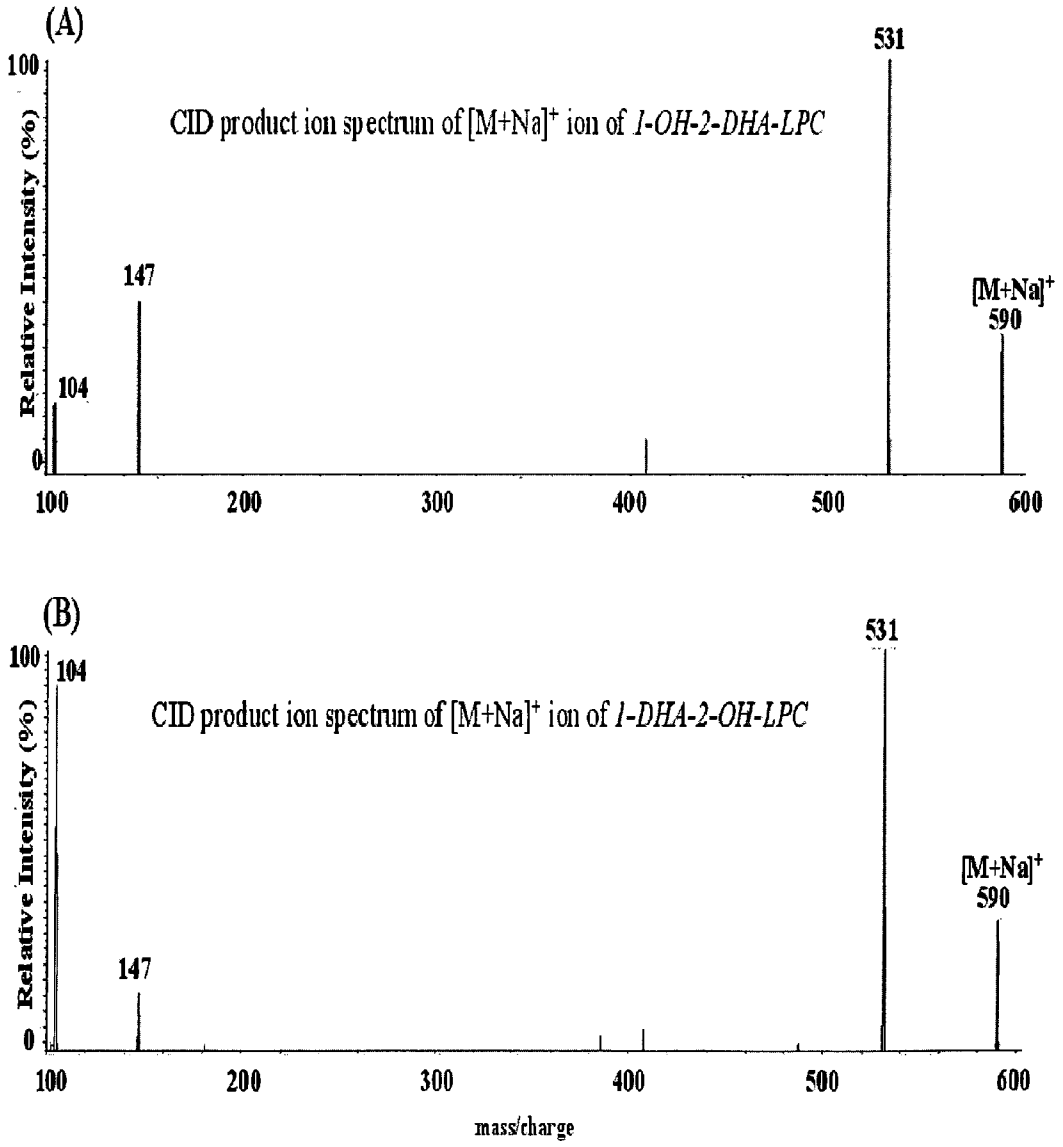

Fig. 4

MIXTURES OF AND METHODS OF USE FOR POLYUNSATURATED FATTY ACID-CONTAINING PHOSPHOLIPIDS AND ALKYL ETHER PHOSPHOLIPIDS SPECIES

FIELD OF THE INVENTION

[0001] The present invention relates to (1) the extraction and purification of new mixtures of phosphatidylcholine and alkyl ether phosphatidylcholine species as well as lysophosphatidylcholine species, which are obtained from the liver of saltwater fishes, with the structural characterization of (i) having a mixture of acyl and enriched alkyl fatty chains linked to the sn-1 position of the glycerol backbone, and (ii) having enriched ω-3 polyunsaturated fatty acid chains, in particular docosahexaenoic acid (DHA), linked to the sn-2 position or the sn-1 position of the glycerol backbone; (2) the preparation of disclosed mixtures of phosphatidylcholine, phosphatidylserine and phosphatidylethanolamine species as well as lysophosphatidylcholine, lysophosphatidylserine and lysophosphatidylethanolamine species from the phospholipid species mixtures extracted from the liver of saltwater fishes by enzymatic reactions; and (3) their use as carriers of ω-3-polyunsaturated fatty acids to the brain for the prevention and alleviation of neurodegenerative and neurological diseases which may be caused by the deficiency of ω-3 polyunsaturated fatty acids, in particular DHA.

BACKGROUND OF THE INVENTION

[0002] Omega-3 (or ω-3) polyunsaturated fatty acids, in particular docosahexaenoic acid (DHA), are especially important during prenatal brain development and maintenance of human brain function. Compared with saturated fatty acid, polyunsaturated fatty acid with multiple double bonds within molecule, in particular DHA, causes carbon-carbon chains to become more curved. The more kinked the fatty acid is, the more space it will take up, when it is built into cell membrane phospholipids, in order to keep neuronal membrane functions. This is the main reason that why the brain requires a large amount of nutritionally essential polyunsaturated fatty acids, especially DHA, because DHA and DHA-containing molecular species of phospholipids may contribute to important brain functions including signal transduction and information processing [Akbar et al., Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. *Proc. Natl. Acad. Sci. U.S.A.* 102: 10858 (2005)]. Alteration of neuronal membrane DHA-containing phospholipid species can not only influence crucial intracellular and intercellular signaling but also alter many membrane physical properties such as fluidity, phase transition temperature and bilayer thickness. The deficiency of DHA markedly affects neurotransmission, membrane-bound enzyme and ion channel activities leading to brain aging, Alzheimer's disease, Parkinson's disease, schizophrenia and peroxisomal disorders. For example, a study indicated that the concentration of DHA in patients with Alzheimer's disease is significantly decreased [Conquer, et al., Fatty acid analysis of blood plasma of patients with Alzheimer's disease, other type of dementia, and cognitive impairment, *Lipids,* 35:1305 (2000)]. The studies of Garcia et al. [Garcia et al., Effect of docosahexaenoic acid on the

al., Inhibition of neuronal apoptosis by docosahexaenoic acid (22:6n-3): Role of phosphatidylserine in anti-apoptotic effect. *J. Biol. Chem.* 275:35215 (2000)] found out the new role of DHA and phosphatidylserine in neuronal apoptosis, indicating that exogenous DHA may enhance phosphatidylserine accumulation in apoptotic Neuro-2A cells leading to the protection of neuronal cells from apoptotic death. The studies strongly suggest that one of supporting roles for anti-apoptosis of neurons is supplying DHA to the brain.

[0003] Because the human body cannot synthesize ω-3 polyunsaturated fatty acids, in particular DHA, exogenous introduction of DHA to human has been applied. There are a few products available for use as brain nutrients, such as fish oils (DHA-containing neutral lipids) and similar products.

[0004] Although these products contain DHA and other omega-3 polyunsaturated fatty acids, experiments have demonstrated that only a very small amount of DHA can be found in the brain after administering a large amount of these products. But an early study showed that DHA-containing lysophospholipid in albumin, rather than the forms of free DHA and other esterified DHA, is preferred in the uptake of DHA in the brain of young rats when an in vitro model of blood-brain barrier is used [Thies et al., Unsaturated fatty acids esterified in 2-acyl-1-lysophosphatidylcholine bound to albumin are more efficiently taken up by the young rat brain than unesterified form. *J. Neurochem.* 59: 1110 (1992)].

[0005] Interestingly, a study reported that dietary phospholipid with DHA-containing molecular species as supplementation is much more efficient than soybean phospholipid for ensuring a normal level DHA in the brain during the period of brain development in rats [Bourre and Dumont, *Neurosci. Lett.,* 335:129 (2002)] because DHA species are absent in the latter. The result suggests that DHA-containing phospholipid species are effective forms to be used as DHA carriers to brain.

[0006] Phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylethanolamine (PE) as well as lysophosphatidylcholine (Lyso PC), lysophosphatidylserine (Lyso PS) and lysophosphatidylethanolamine (Lyso PE) are naturally occurring phospholipid classes, existing in mixture forms of the molecular species. The structural diversity of the molecular species of phospholipids has been described in detail [Chen, *Lipids,* 28, 85 (1997); Chen et, al. *Biomed. Mass Spectrom.* 21, 655 (1992)]. Biochemical and biophysical functions of phospholipids are well documented and appear to be determined by the fatty acid composition of the lipids.

[0007] Ether phospholipids are usually found in animal tissues and human cells as minor components, existing together with molecular species of diacyl phospholipids carrying the same polar head group. It is well known that there are two predominant types of ether bonds in the phospholipid. One form is represented by the plasmalogens (with 1-alk-1'-enyl fatty chain linked to the sn-1 position of the glycerol backbone), which is the most abundant subclass of phospholipids in most tissues. The other form is alkyl phospholipids that contain 1-O-alkyl fatty chain(s) linked to the sn-1 position of the glycerol backbone. Although mixtures of phospholipids and ether phospholipids have been found in animals and humans [Diagne, et. al., Studies on

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