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Protective effects of PUFA in neuronal apoptosis

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Background

Mammalian brain is rich in long chain polyunsaturated fatty acids (LCPUFA). Docosahexaenoic acid (22:6n-3), the major n-3 fatty acid found in brain, is highly enriched in neuronal cells (1). Growing evidences support the essential role of 22:6n-3 in neuronal function. In animal models n-3 fatty acid deficiency caused memory deficit (2), learningdisability (3,4), and visual acuity loss (5). In humans, various neurological disease states have been shown to be associated with a deficient 22:6n-3 status, implying the influence of this fatty acid in neuronal function (6,7). In the case of preterm-infants with underdeveloped brains, the inclusion of 22:6n-3 fatty acid in infant formula has been shown to improve visual attention (8). More recently, it has been shown that 22:6n-3 is required for the survival of rat retinal photoreceptors (9) and exerts a protective effect on apoptosis of retinal photoreceptors during development (10).

Neuronal apoptosis normally occurs during the development and maturation period (11-13). However, it has been shown that various neurodegenerative conditions are also associated with apoptotic neuronal cell death (14-16). Neuronal cell survival is critically dependent on the supply of trophic factors which influences downstream signaling pathways (17). For example, in many cells PI_3 kinase dependent Akt serine/ threonine kinase transduces a survival signal through phosphorylating proapoptotic protein BAD which in turn associates with 14-3-3, preventing the interaction of BAD with Bcl-2 and Bcl-X_L (18-20). Deprivation of trophic factors inhibits PI_3 kinase/Akt and subsequently BAD phosphorylation, which enables binding of BAD to Bcl-X_L resulting in mitochondrial damage. Subsequent release of cytochrome c activates caspases, ultimately leading to apoptotic cell death (21).

Growing evidence indicates that Raf-1 activation, which is known to be essential for transducing signals of many growth factors, can play an important role in the regulation of apoptotic processes (22-24). Activation of Raf-1 kinase has been shown to prevent apoptosis in hematopoietic cells (22). Ithas been also shown that inhibition of Raf-1 in cells expressing BCR/ABL, which protects these cells from apoptosis induced by growth factor deprivation, can induce apoptosis (23). In addition, expression of constituitively active mitochondrial Raf-1 hasbeen shown to restore antiapoptotic potential of a transformation-deficient BCR/ABL mutant (24). Recently, it has been reported that activation of mitochondrial Raf-1 is involved in the antiapoptotic effect of Akt (25). Although mechanisms of Raf-1

activation is complex and still remains controversial, translocation of Raf-1 to the membrane and subsequent phosphorylation are considered to be important steps for its activation (26-29). It has been shown that Raf-1 kinase contains distinct binding domains for acidic phospholipids, phosphatidylserine and phosphatidic acid (30), and therefore the membrane localization of Raf-1 may be dependent on the concentration of these phospholipids.

Phosphatidylserine is the major acidic phospholipid in mammalian cell membranes and is particularly enriched with 22:6n-3 fatty acid (1). We have previously demonstrated that 22:6n-3, which is abundantly present in neuronal cells, promotes the accumulation of phosphatidylserine in cell membranes (31,32).

Summary of Study

In the present study, we explored the biological significance of 22:6n-3 by examining its effect on apoptotic behavior upon trophic factor removal in relation to its capacity to modulate phosphatidylserine accumulation. We found that enrichment of neuronal cells with 22:6n-3 increased the accumulation of PS and the membrane localization of Raf-1, down-regulated Caspase-3 activity and prevented apoptotic cell death under serum free conditions. Its protective potential was sensitive to the extent of PS accumulation, suggesting that the observed antiapoptotic effect of 22:6n-3 may be mediated at least in part through the enhanced PS accumulation in neuronal membranes. Enrichment of Neuro 2A cells with docosahexaenoic acid (22:6n-3) decreased apoptotic cell death induced by serum starvation as evidenced by the reduced DNA fragmentation and Caspase-3 activity. The protective effect of 22:6n-3 became evident only after at least 24h of enrichment prior to serum starvation and was potentiated as a function of enrichment period. During enrichment 22:6n-3 incorporated into PS steadily, resulting in a significant increase in the total PS content. Similar treatment with oleic acid (18:1n-9) neither altered PS content nor resulted in protective effect. Hindering PS accumulation by enriching cells in a serine-free medium diminished the protective effect of 22:6n-3. Membrane translocation of Raf-1 was significantly enhanced by 22:6n-3 enrichment in Neuro 2A cells. Consistently, *in vitro* biomolecular interaction between PS/PE/PC liposomes and Raf-1 increased in a PS concentration-dependent manner. Collectively, enrichment of neuronal cells with 22:6n-3 increases the PS content and Raf-1 translocation, downregulates Caspase-3 activity, and prevents apoptotic cell death. Both the antiapoptotic effect of 22:6n-3 and Raf-1 translocation are sensitive to 22:6n-3 enrichment-induced PS accumulation, strongly suggesting that the protective effect of 22:6n-3 may be mediated at least in part through the promoted accumulation of PS in neuronal membranes.

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