Oligomerization of SOD1 Familial ALS Mutant by Docosahexaenoic Acid and Docosahexaenoic Acid Hydroperoxides *in vitro*

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Docosahexaenoic acid (C22:6, ?-3, DHA) is a fatty acid highly unsaturated, therefore susceptible to oxidation and is present in large concentrations in the brain. Studies suggest that the reactions of lipid peroxidation in the brain may be associated with the progression of neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS). ALS is a progressive and fatal disease caused by selective degeneration of motor neurons in the brain, brainstem, and spinal cord. Twenty percent of familial ALS (fALS) cases are caused mainly by point mutations in the *sod1* gene. The objective of this study was to evaluate the effect of DHA and DHA hydroperoxides (DHA-OOH) on oligomerization of SOD1 fALS mutants *in vitro*. The effect of DHA and DHA-OOH on oligomerization of holo- and apo-SOD1 was examined by SDS-PAGE under non-reducing and reducing conditions and by bis-ANS and thioflavin S dye binding fluorescence assays. Thioflavin S binding revealed that apo-SOD1 is prone to aggregation when treated with DHA due to the formation of β-amyloid structures. In parallel, bis-ANS fluorescence showed that β-amyloid formation is associated to the increased hydrophobicity of apo-SOD1 after exposition to DHA. SDS-PAGE analysis detected high-molecular weight (>50 kDa) species under non-reducing conditions. Surprisingly, DHA-OOH had a minor effect compared to DHA. This appears to be related to cysteine residues oxidation by the hydroperoxide moiety, which prevents the still unknown pathway of apo-SOD1 oligomerization promoted by DHA. Overall, the results reported above point toward a mechanism of SOD1 fALS mutants aggregation dependent on the direct reaction between the unsaturated fatty acid (DHA) and the protein. The mechanism and the relevance of DHA induced SOD1 aggregation is now being investigated.

Keywords: Docosahexaenoic acid, hydroperoxides, SOD1 FALS mutant, oligomerization.

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