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Lipid molecules and signaling cascades during oxidative stress in the developing brain and neural cell cultures

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Docosahexaenoic acid abundance in the brain: A biodevice to combat oxidative stress

Membrane lipids, while being essential in defining the outer from the inner cellular environment and additionally, in playing important regulatory roles as signaling molecules and second messengers, may occasionally undergo oxidative stress and thus, accelerate pathological processes. The resultant free radicals (FR) and lipid peroxides (LPO) can produce major disturbances in cell function by damaging cellular macromolecules and ultimately induce cell death. Therefore, the ability to detoxify reactive oxygen species (ROS) and prevent signaling cascades precipitating cell death, are key elements in understanding the link between oxidative stress, aging and neurodegenerative disorders such as Parkinson and Alzheimer disease (AD).

Docosahexaenoic acid (DHA, 22:6 n-3), a ubiquitous brain polyunsaturated fatty acid (PUFA), is believed to be a particularly vulnerable lipid to oxidative stress. This vulnerability is more pronounced in brain because of its extremely high level of oxygen consumption and generation of harmful ROS. Under steady state conditions, ROS and their byproducts are maintained at low levels, due to appropriate antioxidant defense mechanisms. Nevertheless, supplements of DHA or related n-3 fatty acids indicate no change or even decreased LPO in spite of the expected high oxidability of DHA. We are investigating the molecular basis for this seemingly paradox, in a fetal ischemia/oxidative stress model, using dietary DHA or n-3 deprivation of the mothers during pregnancy. Our data suggest that DHA lipid hydroperoxides formed act as antioxidants by preventing propagation of free radicals.

Expression of genes in stressed brain tissue and cell cultures using a self-designed c-DNA microarray

Ischemic stress is a cause for many neurological disorders and aging and is considered as a most potent stimuli for gene expression. Some genes, may be involved in exacerbation of the ischemic damage, while others may be neuroprotective and presumably promote recovery. The functional significance of the timed-appearance of these genes, early or late after cerebral ischemia, and the interplay among them remains still a great puzzle. One approach to clarify the network of combinatorial gene interactions is to identify novel genes associated with ischemic stress. A new approach has been undertaken by us to examine multiple patterns of gene expression (MPGE), consequent to oxidative stress in selected regions of the rat brain, after maternal dietary changes or following supplements of DHA to cultured cells. We have prepared a custom-made platform, currently based on 49 known genes encoding for signal transduction, apoptosis and oxidative stress events, and are studying their relative expression and possible functional expression clustering. To this end, we could identify a number of clusters based on unaffected, over-and under-expressed gene families. Work in progress using subtraction libraries is addressed at characterizing MPGE profiles in the hippocampus and in related structures of the adult and fetal rat brain under normal and after oxidative stress conditions.

Signaling cascades in oxidative stress-induced cell death in neuronal cells: The role of metal ions

Neurodegeneration and cell death in AD may result from damaging ROS, a fraction of which may arise from the interaction of β-amyloid (Ab) protein with Fe^{2+}. The signaling cascades concordant with this possible scenario have been studied in primary rat neuronal cultures. Co-addition of Ab1-40 and Fe^{2+} caused a rapid and sustained elevation of free FR as detected by dichlorofluorescein staining and a 6 fold increase in LPO products. Unlike Ab1-40, Fe^{2+} addition enhanced FR formation after 30 min, while a combination of both was effective after 5 min. The iron chelator, deferoxamine (DFX), blocked FR formation. Impaired mitochondria activity, caspase 3 activation and positive TUNEL stain, all indicative of cell death, were found. The extracellular signal-regulated kinases (ERK, 42 kDa and 44 kDa isoforms) were increased rapidly after either Ab1-40 or Fe^{2+} addition. Activation of ERKs by Ab1-40 was associated with a rapid nuclear translocation which was blocked by the addition of Fe^{2+}. A combination of Fe^{2+}/Ab1-40 caused a delay in ERKs activation in contrast to a rapid p38 kinase activation. Addition of DFX prevented the above effects. The kinetics of ERK activation and nuclear translocation via Ab1-40/Fe^{2+}-induced toxicity may constitute an important element in AD pathophysiology.

Early ethanolamine phospholipid translocation marks stress-induced apoptotic cell death in oligodendroglial cells. The consequences of H_2O_2/Fe^{2+}-induced oxidative stress on translocation of ethanolamine phosphoglyceride (EPG) was studied in oligodendroglia-like cells (OLN 93) following three days of supplementation with 0.05-0.1 mM DHA and a series of aminoethanol bases, including monomethylethanolamine (mEa) and dimethylethanolamine (dEa) at mM concentrations. Added DHA was predominantly esterified into EPG species and those cells enriched in DHA showed an enhanced sensitivity to oxidative stress and died eventually by apoptosis. A rapid but transient EPG translocation from the inner to the outer plasma membrane, with a maximum at 30 min following the addition of H_2O_2, was noticed using trinitrobenzenesulfonic acid reagent. EPG acted as a signaling molecule with biphasic kinetic characteristics. mEa and dEa supplements reduced EPG synthesis, prevented its externalization and rescued cells from apoptotic death. Following stress, the fatty acid profile of the externalized EPG showed marked losses in PUFA and aldehydes compared to the remaining intracellular EPG. Prevention of EPG species selective translocation to the outer membrane leaflet by altering phospholipid asymmetry may be important in the mechanism of rescue from cell death. Under these conditions, activation and nuclear translocation of ERK, notable at very early times after stress, persisted up to 24 h, at the time apoptotic death was noticed by FACS, DNA ladder and TUNEL techniques. Upstream inhibition of the ERK cascade, inhibited EPG outward flip, impaired ERK translocation and prevented cell death indicating a linkage between these events. dEa/DHA-treated cells showed neither EPG outward flip, nor significant ERK nuclear translocation, and were completely rescued following H_2O_2 stress. Since SPG showed little or no translocation during these early times after stress, EPG translocation is a major consequence and a necessary signal to cell death cascade that acts most likely via ERK activation. The unique fatty acid profile of the externalized EPG after DHA enrichment, suggests an essential role of the membrane lipid composition in promoting or preventing apoptotic cell death.

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