Abstract

Title of Thesis: Studies on Functional Role of Docosahexaenoic Acid (DHA) in the Brain.

Docosahexaenoic acid (DHA), an important ω-3 fatty acid, showed opposite effect in two different types of cells, inducing apoptosis in neoplastic cells but promoting differentiation in normal cells. With a view to understand more about the mechanisms of such divergent action of DHA, the present study was conceived. Treatment of C6 glioma and SH-SY5Y cell lines, representing the neoplastic cells, with 100 µM DHA for 24 h caused significant increase in apoptosis, as determined by TUNEL staining, whereas primary astrocytes cultures, representing normal healthy cells were unaffected. Employing proteomic approach, we have identified six proteins which unlike in the astrocytes, were differently altered in the cancer cells upon exposure to DHA, suggesting their putative contribution in causing apoptosis in these cells. Of these, annexin A2, calumenin, pyruvate kinase M2 isoform, 14-3-3ζ were downregulated while aldo keto reductase-1B8 and glutathione–S-transferase P1 subunit showed upregulation by DHA in the cancer cells. Western blot analysis also identified upregulation of PPARα and the MAP kinases, ERK, JNK and p38 as well as increased ROS production selectively in the cell lines. Together, activation of multiple apoptotic pathways in association with excess ROS and activated MAPKs appear to promote cancer cell death by DHA.

Like thyroid hormones (TH), DHA also facilitated astrocytes differentiation in culture, involving a downstream role β-adrenergic receptor (β-AR) system. We explored the detailed signaling mechanisms during the differentiation process induced by TH or DHA. TH, caused an immediate decrease in the specific binding of $^{125}\text{I}$-pindolol to $\beta_2$-AR in cell membranes with a concomitant increase in $\beta_2$-AR levels in the cytosol, which could be blocked by endocytic inhibitors, suggesting endocytosis of $\beta_2$-AR. qRT-PCR and western blot analysis together with knockdown and overexpression experiment demonstrated that β-arrestin-1 is transcriptionally upregulated by TH facilitating increased endocytosis of $\beta_2$-AR, required for endosomal ERK activation to drive the differentiation process. DHA also promoted $\beta_2$-AR internalization and its subsequent down-stream events to induce differentiation of the cells, not by increasing the expression of β-arrestin-1 but by increasing the expression of $\beta_2$-AR levels.