Docosahexaenoic Acid Modulates Oxidative Stress over PI3K Signaling Pathway Activation in Age Related Macular Degeneration

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Short-Communication

Regular and high consumption of polyunsaturated fatty acids (PUFAs) like DHA (docosahexaenoic acid) prevents the development of age-related macular degeneration (AMD) by acting on oxidative stress. DHA is found in high concentration in the retina. The biochemical properties of DHA influence the fluidity and function of the membrane-photoreceptor [1]. Nutrients from the (Age-related eye disease study 2) (AREDS2) shows that (lutein, zeaxanthin, vitamin C, vitamin E, zinc, copper, eicosapentanoic acid [EPA], and docosahexanoic acid [DHA]) set forth by the National Institutes of Health remain the most proven nutritional therapy for reducing the rate of advanced AMD [2]. Epidemiological studies highlight the benefit of promoting the consumption of long-chain omega-3 PUFAs, including DHA, and lowering the intake of omega-6 fatty acids to reduce the risk for developing AMD [3,4]. The intracellular and the epigenetic mechanism involved in oxidative stress-mediated RPE cell death is partly understood. It is very likely that t-BHP decreased cell proliferation and induced apoptosis via inhibition of histone methyltransferase G9a. Interestingly, as G9a maintains genomic imprinting [5], aberrant expression of imprinted genes leads to severe human disorders [6]. Therefore, DHA may prevent the initiation of aging related diseases via epigenetic associated mechanisms.

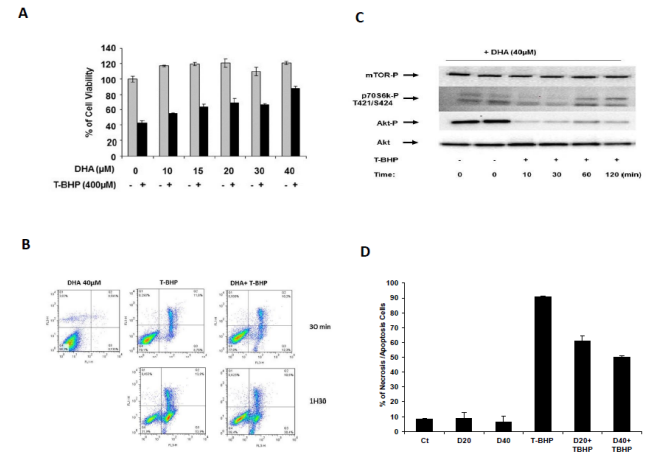

Figure 1: Effect of DHA correlated with activation of cell survival via the signaling pathways involving PI3K/Akt/m-TOR/p70S6K

Otherwise, Better characterization of the signaling pathways involved in the response of RPE cells to oxidative stress should allow the future development of therapeutic strategies against AMD [7]. The PI3K/Akt pathway may be involved in protecting in RPE against harmful effects of oxidative stress [8,9].

The PI3K/Akt pathway stimulates cell growth and is upregulated in cancer cells [10-12]. In addition, this pathway deserves further research into its role in supporting cell survival [13,14]. Activation of PI3K leads to the formation of phosphatidylinositol-3,4,5-triphosphate (PI(3,4,5)P3), which facilitates the phosphorylation and activation of Akt by PDK1 [15]. Phosphorylated Akt induces the association of tuberous sclerosis complex 1 (TSC1) and TSC2 by phosphorylating TSC2 on multiple sites. In their aggregated form, TSC1 and TSC2 are unable to inhibit Rheb, enabling the activation of the mammalian target of rapamycin complex 1 (mTORC1). Activated m-TORC1 phosphorylates p70S6 kinase (p70S6K) [16-18], which has antiapoptotic effects. Silencing or blocking the function of G9a is sufficient for disturbing m-TOR [19].

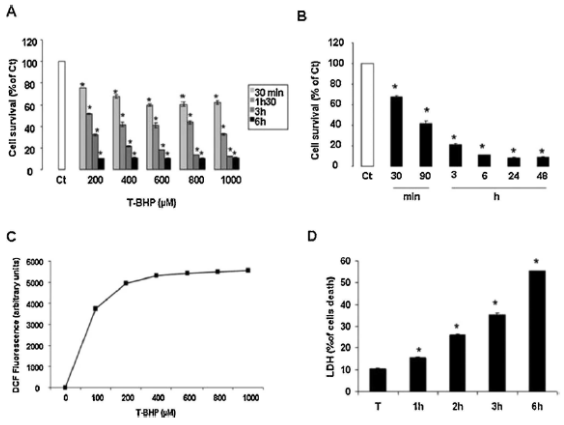

Figure 2: Fluorescent detection of reactive oxygen species

The PI3K/Akt and m-TOR/P70S6k pathways participated in the protection of RPE cells against oxidative stress-induced apoptosis. Moreover, as depletion of G9a inhibits cell proliferation in several cancers [20,21].

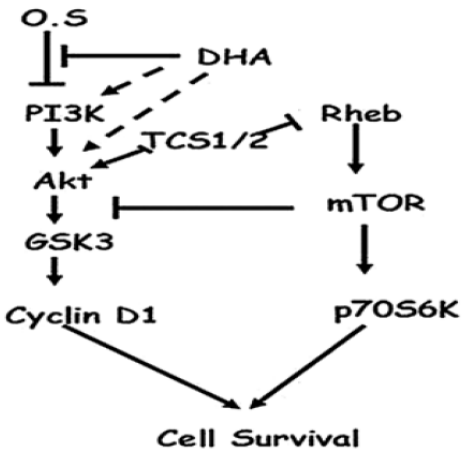

Figure 3: Docosahexaenoic acid protected RPE cells from apoptosis triggered by oxidative stress by enhancing the phosphorylation of Akt and P70S6K.

Here, we suggested that the Akt signaling pathway is an important target for future therapeutic strategies against human RPE cell degeneration diseases such as AMD. Exposure RPE cells to (tert-butyl hydroperoxide) (t-BHP) completely inhibits the phosphorylation of Akt, m-TOR, or p70S6K with more than 80% of apoptotic cells. DHA-enrichment promoted RPE cells against oxidative stress-induced apoptosis by upregulation phosphor-Akt and phosphor-P70S6k .

Results

We studied the protective effect of DHA on PI3K/Akt and m-TOR/p70S6K during oxidative stress-induced apoptosis. In these experiments, RPE cells from (ARPE19 cell line) were treated with (40 μM) DHA for 48 h and then stimulated with 400 μM t-BHP. DHA prevented apoptosis of ARPE-19 cells induced by oxidative stress and significantly reduced the number of apoptotic cells from 54% (Figure 1B) to 30% (Figure 2A-D). DHA (40 Mm) is not toxic dose for ARPE19 cells on the contrary we showed an increase in RPE cells proliferation at this dose (Figure 1A). Here we investigated that the effect of DHA was correlated with activation of cell survival via the signaling pathways involving PI3K/Akt/m-TOR/p70S6K (Figure 1C). RPE cells were enriched with DHA for 48h then exposed to a single exposure of t-BHP; the western blot analysis shows that Akt phosphorylation was increased throughout the kinetics (Figure 1C). Furthermore, both m-TOR and p70S6k phosphorylation were elevated after DHA-enrichment (Figure 1C). The overall results show that DHA prevents RPE cells against oxidative stress over PI3K signaling pathway activation (Figure 1D).

Discussion

In our model of oxidative stress with serum deprivation and treatment with t-BHP, we showed that t-BHP decreased cell proliferation, induced apoptosis and completely inhibited the activity of Akt and P70S6k. Then, we investigate the protective effect of DHA against oxidative stress in human RPE cells, ARPE-19 cells were enriched with DHA (40μM) and then exposed to t-BHP, we showed that DHA reduced apoptotic cells and activate Akt and p70S6k phosphorylation. Here we investigate that Docosahexaenoic acid modulates oxidative stress over PI3K signaling pathway activation in age related macular degeneration model. Zang et al., showed the histone H3 lysine 9 methylases G9a and GIP are required for stable maintenance for imprinted DNA methylation in embryonic stem cells (Figure 3). It is very likely that t-BHP decreased cell proliferation and induced apoptosis via inhibition of histone methyltransferase G9a so it is probable that oxidative stress inhibits G9a in AMD [5]. It will be interesting to show the effect of Oxidative stress on G9a and the response of G9a in RPE cells-enriched with DHA against oxidative stress. Li Kc and al showed that G9a expression was positively correlated to proliferation marker Ki-67 and to poor progression in head and neck squamous cell carcinoma (HNSCC). Moreover, the inhibition of G9a induces autophagic cell death, this finding provides a basis for new therapeutic targets; in this way it will be interesting to show if t-BHP inhibits G9a in ADM, May be T-BHP inhibits G9a in AMD [20]. Yang Q et al., showed that the inhibition of G9a by its inhibitor Bix-01294 reduced proliferation of foetal PASMCs and induced cell cycle arrest in G1 phase demonstrated for the first time that histone lysine methylation is involved in cell proliferation. T-BHP can inhibits G9a too by reducing proliferation of arpe19 and induced cell cycle arrest in G1 so the hypothesis that the oxidative stress can inhibits G9a in AMD [21]. I think that G9a could be an interesting a track study and a good target to explain more the effect of the DHA against oxidative stress in AMD.

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