

PP9

Docosahexaenoic acid inhibits oxidative stress and cytokine expression in pancreatic stellate cells exposed to double-stranded RNA or TNF-alpha

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Activated pancreatic stellate cells (PSC) play a pivotal role in the pathogenesis of pancreatic fibrosis and inflammation. Reactive oxygen species (ROS) levels are increased in the serum of the patients with pancreatitis. ROS mediates expression of inflammatory cytokines in various tissues. Docosahexaenoic acid (DHA), omega 3-polyunsaturated fatty acid, shows anti-inflammatory effects in inflammatory diseases. In the present study, we investigated whether DHA inhibited ROS production and cytokine expression in PSC exposed to double-stranded RNA (Poly (I:C)), as a model of viral infection, or TNF-alpha. PSC were isolated from rats and treated with poly (I:C) or TNF-alpha in the presence or absence of DHA. mRNA expressions of MCP-1 and CX3CL1 were assessed using real-time PCR. DCFDA (2',7' -dichlorofluorescein diacetate), MitoSox red and JC-1(5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide) were used to determine intracellular ROS production, mitochondrial ROS production, and mitochondrial membrane potential (MMP), respectively. As a result, TNF-alpha and Poly (I:C) induced increases in intracellular and mitochondrial ROS as well as expression of MCP-1 and CX3CL1, but reduced MMP in PSC. DHA inhibited intracellular and mitochondrial levels of ROS, disruption of MMP, and cytokine expression in PSC treated with Poly (I:C) or TNF-alpha. In conclusion, supplementation of DHA may be beneficial for preventing pancreatic inflammation/fibrosis by inhibiting cytokine expression in PSC.

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PP10

Protective role of HSP70 against oxidative stress-mediated IL-8 expression in ataxia telangiectasia fibroblasts received glutamine deficiency

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Ataxia telangiectasia (AT) is a neurodegenerative and inherited disease caused by a mutation in the ataxia telangiectasia mutated (*ATM*) gene. *ATM* gene is associated with cell cycle arrest, DNA repair, or apoptosis. Reactive oxygen species (ROS) levels were higher in AT cells lacking *ATM* gene than in normal cells. HSP70 is a molecular chaperone which protects the cells from oxidative stress. Glutamine, a conditionally essential amino acid, is converted to glutamic acid which is a component of glutathione (GSH). Previously, we showed that low GSH level and high levels of ROS and IL-8 in AT cells which was cultured in glutamine deficient medium. In the present study, we investigate the mechanism of ROS production in AT cells received glutamine deficiency in related to HSP 70. As a result, glutamine deficiency increased ROS levels and induced NF- κ B activation and IL-8 expression in AT cells. HSP70 levels were decreased by glutamine

deficiency. Glutamine deficiency-induced increase in ROS and IL-8 expression are inhibited in AT cells transfected with *HSP70*. In conclusion, *HSP70* may protect AT cells from oxidative damage by suppressing ROS production and IL-8 expression.

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PP11

Antioxidant screening in hydrogen peroxide-induced oxidative damage in human somatic and embryonic stem cells

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Oxidative stress, defined as the imbalance between reactive oxygen species production and cellular antioxidant systems, is implicated in a wide range of diseases, and has been extensively studied as a potential target for therapeutic intervention by antioxidants. In order to induce long-term oxidative stress, Hs27 (human fibroblasts) and HUES3 (human embryonic stem cells) were exposed to increasing concentrations of hydrogen peroxide (H_2O_2) by bolus addition every 24 hours during a total of 72 hours. Cell viability, determined by alamarBlue[®] assay, sharply decreased at 64 μ M H_2O_2 in Hs27 and at 128 μ M H_2O_2 in HUES3. Following the same long-term exposure experimental design, different antioxidants were tested for cytotoxicity in Hs27 cells at increasing concentrations: glycine (GLY), sodium pyruvate (PYR), N-acetylcysteine (NAC), ascorbic acid (ASC), Trolox (TRO), sodium selenite (SEL) and zinc chloride (ZN). Three non-toxic concentrations were selected for each antioxidant to analyze their protective effect in the presence of increasing concentrations of H_2O_2 . GLY, ASC, TRO, SEL and ZN showed no protective effect, while PYR and NAC showed dose-dependent protective effect to up to 16 mM H_2O_2 in the presence of 25 mM PYR and up to 256 μ M H_2O_2 in the presence of 2.5mM NAC. Then, PYR and NAC were selected to analyze their cytotoxicity and antioxidant effect in HUES3. Cytotoxicity appeared at lower concentrations in HUES3 than in Hs27. One single concentration was selected for each antioxidant. PYR showed protective effect to up to 512 μ M H_2O_2 at 2.5mM, although NAC showed no protective effect in HUES3. Consequently, PYR is a powerful antioxidant against H_2O_2 -induced oxidative stress in both somatic and embryonic stem cells, while NAC is protective only for somatic cells, and other well-known antioxidants like ascorbic acid were not able to prevent cytotoxicity.

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PP12

γ -Glutamyl cysteine modulates the inflammatory response via protein phosphatases

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