Differential Influence of DHA on hPASMC Proliferation

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Background: Hypertrophy and hyperproliferation of the vascular pulmonary artery smooth muscle cells represent a hallmark of pulmonary arterial hypertension (PAH), often associated with markers of inflammation. Docosahexaenoic acid (DHA) is a member of omega-3 fatty acids with established anti-inflammatory and anti-proliferative effects. We investigated the effect of DHA on proliferation of primary human pulmonary artery smooth muscle cells (hPASMC).

Method: Primary SMC were isolated from human pulmonary arteries from patients undergoing lung surgery. The study protocol was approved by the Institutional Review Board of the Medical University of Graz in accordance with national law. hPASMC were grown in a CO2 incubator under standard normoxic (21% O2) or hypoxic (1% O2) conditions. Proliferation of hPASMC was investigated using [3H]thymidine incorporation, cell viability and flow cytometry. Apoptosis was assessed by active caspase-3 for flow cytometry. Results: hPASMC incubated with DHA at 50 µM for 3 days showed significantly inhibited cell proliferation. This effect was more pronounced in hypoxic compared to normoxic cells. Furthermore, a differential DHA effect with respect to oxygenation was supported by flow-cytometric cell cycle analysis and active caspase-3 measurements. In normoxic cells, DHA treatment resulted in a slightly decreased percentage of the G1 subpopulation with concomitant increase in the G2/M subpopulation and a slight induction of apoptosis. In contrast, DHA-treated hypoxic cells were blocked in the G1 phase with a significant induction of apoptosis. In accordance, cyclin D1 mRNA levels were significantly decreased in hypoxic cells and slightly increased in normoxic cells upon 24- and 48- hour incubation with DHA compared with untreated controls.

Conclusion: The inhibitory effect of DHA on hPASMC proliferation is more pronounced in hypoxia. The underlying mechanisms include down-regulation of cyclin D1 expression, attenuation of cell cycle progression, and induction of apoptosis.