Effects of n-3 Highly Unsaturated Fatty Acids on Human Platelet Mitochondria

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Background: It has been proposed that the n-3 highly unsaturated fatty acids (HUFA) have beneficial effects on cardiovascular function, affecting platelet aggregation, cyclo-oxygenase and lipoxygenase metabolism, and platelet interactions with vascular endothelium. Recent studies of platelet metabolism have implicated mitochondria in intrinsic signalling of platelet cell death and showed that this signalling pathway is highly sensitive to increased intracellular calcium ion concentrations¹, but less is known about the influence of specific n-3 polyunsaturated fatty acids on mitochondrial function in platelets.

Methods: In this study, human platelets, prepared from 64 healthy volunteers, were incubated with the n-3 HUFA eicosapentaenoic acid (EPA), dihomogamalinolenic acid (DHA) or with vehicle (ethanol), in a closed sterile system under blood bank conditions for 2-19 days (²). Treatment groups were randomised by using pooled platelet preparations from 4 volunteers, each pool being docked to 4-6 40-50ml gas permeable apheresis packs, which were incubated at 20-24°C, with continuous agitation. HUFA (10nM-100µM) or ethanol (0.1%) were added on day 2. Small samples (1-2ml) of platelet suspensions were withdrawn under sterile conditions on days 2, 5, 7, 9, 12, 14, 16 and 19, and Mitochondrial membrane potential, Caspase 3 (²) and intracellular peroxidative activity (³) were analysed within 2 hours of sampling of platelet suspensions.

Results: In platelets incubated for 2-10 days under blood bank conditions, EPA 10nM-100µM was associated with a dose dependent increase in intracellular peroxidation. However, under these conditions, neither EPA nor DHA, even at the highest concentration (100 µM) were associated with increased intrinsic cell death signalling, detected by caspase 3 and mitochondrial membrane potential, which increased and decreased respectively in control and n-3 treated platelet preparations, with similar kinetics to those previously observed in stored human platelets¹. Calcium sensitive cell death signalling (¹) was attenuated in preparations incubated with 100nM-100 µM of n-3 HUFA. This attenuation was most marked over longer incubation periods (5-19 days). The identity of the protective agents in calcium challenged platelets were investigated, and preliminary experiments using cyclooxygenase and lipoxygenase inhibitors suggested that lipoxygenase products were involved.

Conclusions: the n-3 HUFA, EPA and DHA, incubated with human platelet concentrates under blood bank conditions, exerted a protective effect on platelet calcium sensitive cell death signalling via the intrinsic mitochondrial pathway. This study provides further evidence of platelet effects of the n-3 polyunsaturated fatty acids, which may have beneficial effects on cardiovascular function and preserve the function of stored platelets for clinical use.