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Effects of arachidonic and eicosapentaenoic acid derived eicosanoids on polymorphonuclear transmigration

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The *n*–3 polyunsaturated fatty acids contained in fish oil provide it with anti-inflammatory effects on different inflammatory diseases[1,2]. Several mechanisms can be involved in the influence of the degree of unsaturation of dietary fatty acids on the development of inflammatory diseases[3]. The beneficial effects of fish oil on inflammatory diseases have been attributed to the EPA/docosahexaenoic acid (DHA) content. EPA is also substrate for AA cascade enzymes, but induced the production of alternative eicosanoids such as 3-serie prostanoids and 5-serie leukotrienes that are considered to be less pro-inflammatory compared with AA metabolites. Thus, fish oil diet reduced AA mobilization and the subsequent prostaglandin (PG)E_2 synthesis[4]. However, the molecular basis of beneficial effect of EPA supplementation is poorly understood as well as the comparative biological effects of AA and EPA metabolites.

Leucocyte recruitment to inflamed areas is a pivotal event in the development of the inflammatory processes[5]. In this work, we studied the effects of PGE_2 and PGE_3 on endothelium permeability, the effects of leukotriene B_4 (LTB_4) and LTB_5 on endothelium permeability as well as mononuclear adhesion and migration.

Endothelial monolayer permeability to albumin was measured in ECV304 cell cultures using the Casnocha *et al*. methodology[6]. Polymorphonuclear (PMN) granulocytes were isolated from human blood samples using Histopaque-1077. ECV304 cell confluent cultures were plated with PMN in the presence of eicosanoids and allowed to attach at 37°C for 3 h. Non-adherent cells were removed, cultures were fixed and PMN adhered to ECV304 monolayer were counted under a phase-contrast microscope. LFA-1 and MAC-1, and E-selectin and ICAM-1 expression on PMN and ECV304 surface, respectively, were analysed with a fluorescein-activated cell sorter analyser using the corresponding antibodies to each adhesion molecule. PMN chemotaxis was measured using the modified Boyden chamber technique[7] and a locomotion index was calculated using Maderazo and Woronick method[8]. Results are means $\pm$ SE of three independent experiments performed in duplicate. Student's *t* test was used to determine the significance.

Our results show that both prostaglandins (PGE_2 and PGE_3, 0.1–100 nM) increased *trans*-endothelial Evans blue-albumin (EBA) permeability in a concentration-dependent manner, reaching a maximum plateau effect at 100 nM. Interestingly, the effect of PGE_3 (increased 115 ± 3% *v.* control) ($P<0.001$) was slight higher than PGE_2 (90 ± 4%) ($P<0.001$) action and both were significantly antagonised by EP_1 (SC19200, 1 μM) and EP_2 (AH6809, 1 μM) antagonist, but not by EP_3 and EP_4 antagonist. LTB_4 and LTB_5 presented a slight effect on EBA extravasation (32 ± 2% and 21 ± 1.5%, respectively.

LTB_4 (1–100 nM) caused significant increases in the number of PMN cell adhering to endothelial cells (280 ± 13%–405 ± 21%) ($P<0.001$ in all concentrations), whereas LTB_5 was not able to induce an appreciable effect. This effect of LTB_4 was mediated through the enhancement of adhesion molecules such as LFA-1 and MAC-1 on PMN surface and E-selectin and ICAM-1 expression on surface of endothelial cells. Finally, we observed that LTB_4 (10–100 nM) is a highly potent chemoattractant (migration index 1.25 ± 0.05 and 1.68 ± 0.1 *v.* migration index of 1 in control condition) ($P<0.05$ and $P<0.001$, respectively), whereas LTB_5 presents a weak effect (migration index of 1.15 ± 0.06 at 100 nM).

In conclusion, the summation of these differences in the LTB_4/LTB_5 effect on PMN transmigration may contribute to explain the beneficial impact of omega-3 in inflammatory processes.

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