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Role of 12-lipoxygenase derived eicosanoids on epithelial barrier function in intestinal Caco-2 cells

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Eicosanoids are lipid mediators synthesized by the arachidonic acid (AA) cascade from membrane polyunsaturated fatty acids (PUFA) trough three distinct pathways: cyclooxygenase, lipooxygenase (LOX) and cytochrome P450 pathways. Eicosapentaenoic acid, an *n*-3 PUFA, is also substrate of the enzymes of the AA cascade. Thus, 5-LOX gives rise to the 5-series leukotrienes, and 12-LOX generates 12-HEPE, the homologue of 12-HETE when the source is AA. Fish oil supplementation has been used as a preventive measure against a number of diseases including coronary heart disease, cancer, and inflammatory bowel disease (IBD). IBD and other intestinal diseases are associated with the disruption of epithelial barrier function[1]. In this sense, we recently demonstrated that PGE_2, which is increased in the mucosa of IBD patients, induces the disruption of this barrier in differentiated Caco-2 cells[2]. In this line, the present study aims to investigate the potential of 12-LOX derived eicosanoids 12-HETE and 12-HEPE to disrupt paracellular permeability (PP) and if so, the mechanism implicated.

PP permeability was assessed from transepithelial electrical resistance (TER) and apical to basolateral dextran fluxes in Caco-2 cells grown on polycarbonate filters for 21 days. The cultures were maintained for 3 h with the eicosanoids in the apical and basolateral compartments. Intracellular Ca^{2+} concentration was monitored using Fura 2-AM, and cAMP determination and NFκB activation were performed using competitive EIA kits. Tight junction proteins were studied using fluorescent microscopy.

The results indicate that both 12-HETE enantiomers altered PP by decreasing TER and increasing dextran fluxes whereas the *n*-3 derived eicosanoid 12-HEPE did not modify any of these variables. Furthermore, the results indicate that Ca^{2+} and cAMP but not NFκB were the downstream targets of both enantiomers. In addition, tight junction reorganization was observed when studying occludin whereas ZO-1 remained unaltered.

In conclusion, we have demonstrated that 12-(R) and 12-(S)-HETE, eicosanoids derived from *n*-6 PUFA, but not 12-HEPE are able to disrupt epithelial barrier function. This could be, at least in part, an explanation of the beneficial role of fish oil in IBD.

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