A role for DHA and aspirin-acetylated COX-2 metabolites on cancer related angiogenesis

M. Vara-Messler[1], A. Trenti[1], C. Buccellati[2], A. Sala[2,3], M.E. Pasqualini[4], L. Trevisi[1], C. Bolego[1]

[1]Dept. of Pharmaceutical and Pharmacological Sciences, University of Padua, Italy
[2]Dept. of Pharmacological and Biomolecular Sciences, University of Milan, Italy
[3]IBIM, C.N.R., Palermo, Italy
[4]Instituto de Biologia Celular, Faculty of Medicine, University of Cordoba, Argentina

Background: Breast cancer (BC) is the most frequent cancer occurring in women [1]. Epidemiological data have linked n-3 polyunsaturated fatty acid (PUFA) consumption to lower incidence of BC and several experimental studies showed the anti-proliferative effects of n-3 PUFAs in different BC models [2]. Chia oil is rich in α-linolenic acid (ALA 18:3 n-3), while corn oil is rich in linoleic acid (LA 18:2 n-6). Angiogenesis is a tightly regulated process involving endothelial cell (EC) proliferation, migration and tube formation [3]. Docosahexaenoic acid (DHA, 22:6 n-3) a downstream metabolite from ALA, has been demonstrated to regulate cancer-related angiogenesis, while arachidonic acid (AA, 20:4 n-6) promotes angiogenesis [4]. In addition, aspirin (ASA) has antineoplastic effects that are mediated at least in part by metabolites derived from acetylated COX-2 [5]. The study was aimed to determine a possible role of dietary n-3 PUFAs on BC growth and, in particular, the effect of DHA metabolites from ASA-acetylated COX-2 in cancer-related angiogenesis.
Methods: 40 BALB/c mice were fed 1) a Chia Oil (ChO)-rich (n-3), or 2) a Corn Oil (CO)-rich diet (n-6). Afterwards, mice were inoculated with mouse BC cells (LM3) and tumour growth parameters were recorded after 35 days. Mitotic or apoptotic figures were assessed in haematoxylin/eosin-stained tumour sections. Human endothelial cells (ECs) were used at 2nd to 6th passage and treated with DHA or AA (1-50μM) for 24h in the presence or absence of ASA (50 μM). Selected experiments were performed with 17-R HDoHE (100nM-3μM), a DHA metabolite derived from acetylated COX-2. EC viability was analyzed with the MTT assay. The angiogenetic process was evaluated using a) the wound healing assay, b) a micro chemotaxis chamber, and c) matrigel.
Results: After 35 days tumour incidence was higher in CO-fed compared with ChO-fed mice (100 vs 85%, $p<0.05$). Tumour weight and volume as well as metastasis number were lower, whereas tumour latency time was longer in ChO-fed mice. A lower number of mitosis figures and a higher number of apoptotic bodies was observed in ChO with respect to CO group ($p<0.05$). Given these in vivo findings, we next studied whether n-3 PUFAs had direct anti-angiogenic actions on human ECs. Cells were viable when treated with DHA or AA in the presence or absence of ASA for 24 h. Challenging the cells with 17-R HDoHE for 24 h did not affect cell viability. EC migration, as evaluated by wound healing assay, was significantly decreased in cells pretreated for 24 h with 30μM, but not 10μM DHA compared to control. Interestingly, 10μM DHA in the presence of 50μM ASA significantly inhibited ECs migration (64±5.4%, $p<0.001$) after 24 h. By contrast, AA did not affect EC migration at any concentration tested (1-30μM) in the presence or absence of ASA. Pretreatment with 17-R HDoHE (1-3μM, 24 h) decreased EC migration (88%±4.1 and 72%±3.9, $p<0.01$, respectively) evaluated by the wound healing assay. Additionally, 17-R HDoHE (1-3μM, 6h) reduced FBS-induced EC migration as evaluated in a micro chemotaxis chamber (70%±4.5 and 35%±1.8, $p<0.05$ and $p<0.001$, respectively), while DHA did not show any effect. Furthermore, 17-R HDoHE inhibited EC tube formation after a 6 h treatment. In particular, meshes and mesh area were significantly lower in 10 μM DHA-treated cells in the presence of 50μM ASA, as well as in 17-R HDoHE (300nM-3μM)-treated cells.
Conclusion: In-vivo data point to a central role of n-3 PUFAs in BC growth. In vitro data demonstrate that aspirin-acetylated COX-2 enhances the antiangiogenic effects of n-3 PUFAs. Overall, these findings illustrate an opportunity to treat pathological angiogenesis using n-3 PUFAs in combination with ASA.
References:

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