EFFECTS OF N-3 FATTY ACIDS ON CHONDROCYTE SIGNALLING PATHWAYS

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Introduction
Previous studies within our laboratory have shown that supplementation of polyunsaturated n-3 fatty acids (PUFAs), but not other fatty acids have a beneficial effect on reducing the expression and activity of degradative and inflammatory factors known to cause damage and destruction of cartilage in arthritic diseases (1). Data has shown that the expression and activity of degradative enzymes such as the aggrecanases and matrix metalloproteinases and cytokine-induced inflammatory mediators such as cyclooxygenase-2 (COX-2), but not the constitutive COX-1, 5-lipoxygenase (5-LOX), 5-LOX-activating protein (FLAP), IL-1α and IL-1β, and TNF-α are all reduced in a dose-dependent manner after supplementation of n-3 fatty acids (2). As yet the molecular mechanisms leading to these observations are still to be elucidated.

Dietary fats are known to have profound effects on gene expression as well as their role as an energy source and their effect on membrane lipid composition. In mammals several transcription factors (e.g. peroxisome proliferator activated receptors [PPARs], sterol regulatory element binding proteins [SREBP] and NFkB) have been identified as either direct or indirect targets of fatty acids (3).

This study was aimed at using microarray technology to help elucidate the second messenger signal pathways involved in the inhibition of mRNA expression of degradative enzymes and inflammatory mediators.

Methods

Human articular cartilage was obtained from patients undergoing total knee replacement surgery for osteoarthritis. Explants were precultured for 72 hours at 37°C, 5% CO₂ in DMEM plus 50μg/ml gentamicin, 0.5% antibiotic/antimycotic solution and 10% FCS. Explants were then washed three times and placed in individual wells of a 24 well plate with 1ml serum-free DMEM with or without 300μg/ml of n-3 polyunsaturated fat (PUFA) (e.g. eicosapentaenoic acid) or 20:5 (eicosapentaenonic acid), or n-6 PUFA (18:2 linoleic acid) or 20:4 (arachidonic acid) for 8 hours at the same conditions. The media was then removed, explants washed 2 and fresh media (without fatty acid supplementation) replaced with or without 10ng/ml recombinant human IL-1β and cultured for a further 4 days. Explants were harvested and total RNA extracted and isolated using a dismembranator and Qiagen RNeasy Kit following the manufacturers protocol.

RT-PCR was carried out using an RNA PCR kit (Perkin-Elmer, UK) as described previously (4), using oligonucleotide primers (Invitrogen, UK) corresponding to complementary DNA sequences for marker genes from several signalling pathways associated with inflammation and arthritic disease (such as the mitogen-activated protein kinase [MAPK] pathway, the NFκB pathway, IL-1 pathway, NFAT pathway TGFβ/anti-proliferation pathway and the stress pathway).

The expression profiles from different RNA populations were then compared using the commercially available Human Pathway Finder -1

Results and Discussion

RT-PCR was carried out on total RNA extracted as described above. So far, 11 of 17 sets of primers designed have produced correctly amplified DNA, sequencing has proved this (see Fig. 1). The cartilage was cultured for 8 hrs with fatty acids and a further 4 days with IL-1. Although under these conditions COX-2 is abolished (data not shown) with n-3 fatty acid, the other signalling genes are as yet to show any differences. It is possible that altering these conditions, especially the time course, may bring about some interesting observations, although it may be that the message for these genes doesn’t change at all. Activation of many of these factors relies on phosphorylation of the protein so it could be that this is affected or abolished with n-3 fatty acid supplementation.

Human RNA samples from control and IL-1β (10ng/ml, 4days) treated cultures were compared using microarray technology. Marker genes from the MAPK pathway, NFκB pathway and TGFβ/anti-proliferation pathway and IL-2 were seen to increase in the presence of IL-1. In contrast RNA samples from IL-1 treated cultures supplemented with n-3 fatty acid (EPA, 300μg/ml, 24 hr) showed decreases in (i) the MAPK pathway marker genes (egr-1 and c-fos), (ii) the NFκB pathway genes (iNOS, NFκB, IκB-β and c-myc), as well as genes from the TGFβ/anti-proliferation pathway (p19, p21 and p57). Interestingly IL-2 expression was also significantly decreased after supplementation with n-3 fatty acid (see Fig. 2).

Antibodies to the active forms of the MAPK enzymes were used in western blotting. IL-1β has shown to up regulate the active phosphorylated ERK, JNK and p38 proteins compared to control cultures. It seems that with increased IL-1 stimulation, p38 remains constitutive (data not shown).

Overall, these results suggest that signalling pathways involved in fatty acid metabolism include the MAPK pathways, the NFκB pathway and the anti-proliferation pathway. Results suggest that addition of n-3 fatty acids to our chondrocyte cultures can inhibit a number of interesting signalling pathways involved in stress, inflammation and cellular proliferation which are all manifested in the pathogenesis of arthritic disease.

References