media. Neither fatty acids nor TNFα were cytotoxic. The increase in COX2 gene expression of cultured chondrocytes in response to TNFα was counteracted by DHA (p<0.05) and oleic acid (p<0.05). MMP1 expression was also increased in response to TNFα and this effect was counteracted by oleic acid (p<0.05) and palmitic acid (p<0.05). GAG release by cartilage explants was decreased when cultured in oleic acid (p<0.05). Gene expression of MMP3, MMP13 and ADAMTS4 in cell cultures and NO production by cartilage explants did not significantly change in response to any fatty acid. Exposure of chondrocytes and explants to linoleic acid (n-6) in absence or presence of TNFα did not influence any of the parameters measured.

Conclusions: DHA (n-3), oleic acid (n-9), and palmitic acid (saturated) are able to counteract some of the effects induced by TNFα in cartilage explants or chondrocyte cultures. This was already known for DHA, but reported for the first time for oleic acid and palmitic acid.

Since fatty acids influence inflammation and degradation of cartilage, they can be regarded potential therapeutic targets in OA prevention and treatment.

Figure 1. The effect of fatty acids on COX2 gene expression in chondrocytes in absence or presence of 10 ng/ml TNFα (n=6, from 2 donors).

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230 THROMBIN AND PLASMIN INDUCED PROTEOGLYCAN RELEASE IN HUMAN CARTILAGE IS PAR-DEPENDENT


Purpose: Osteoarthritis (OA) and Rheumatoid Arthritis (RA) are characterized by degradation of the cartilage. Proteases of the coagulation cascade and the fibrinolytic system, such as thrombin and plasmin, are elevated in both plasma and synovial fluid of OA and RA patients and are able to induce cartilage degradation. Cross-talking between coagulation and inflammation is mediated by protease-activated-receptors (PARs), which are expressed at increased level in OA and RA cartilage. These receptors are activated through cleavage by serine proteases, such as thrombin and plasmin. RNA interference is a process in which genes can be silenced sequence-specific. This can be invoked by transfection of tissue/cells with small interfering RNA (siRNA). Our aim was to study whether the thrombin- and plasmin-induced cartilage damage in human cartilage was PAR-dependent.

Methods: Full-thickness OA human articular cartilage tissue was obtained during total knee surgery. Slices of cartilage were cut aseptically from the articular surface. Within 1 hour of dissection the slices were cut into square pieces, weighed aseptically (range, 5.0 to 15.0 mg) and each sample was individually put into culture. Cartilage was cultured for 4 days in the presence of different concentrations thrombin (10, 30, or 100nM), or plasmin (10, 30, or 100nM). In addition, cartilage was transfected with PAR1-4 small interfering RNA (600nM) or control siRNA (600nM), and cultured with thrombin (100nM) or plasmin (100nM). Cartilage matrix turnover, in terms of proteoglycan release, was determined at day 4. To investigate the silencing effect of the siRNA transfection, cartilage RNA was extracted and PAR1-4 mRNA expression was analyzed with RT-PCR.

Results: Thrombin and plasmin increased proteoglycan release in human cartilage in a dose-dependent and statistically significant manner (500% for thrombin at 100nM; 217% for plasmin at 100nM). Thrombin- and plasmin-induced proteoglycan release was statistically significant reduced with PAR1-4 siRNA (60% for thrombin at 100nM; 54% for plasmin at 100nM). Control siRNA failed to reduce thrombin- and plasmin-induced proteoglycan release. Transfection with PAR1-4 siRNA resulted in complete suppression of PAR1-4 mRNA expression, whereas no effect of control siRNA on PAR1-4 mRNA expression was noted.

Conclusions: These results demonstrate for the first time that thrombin and plasmin-induced proteoglycan release in human cartilage is PAR-dependent and offer promise for the use of siRNA as a new strategy for therapeutic intervention in OA and RA.

231 BONE SIALOPROTEIN: A KEY MEDIATOR OF THE ANGIOGENIC ACTIVITY OF HYPERTROPHIC OSTEOARTHRITIC CHONDROCYTES

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Purpose: Hypertrophic differentiation of chondrocytes in osteoarthritis (OA) is a pathological process leading to vascularization and mineralization of the cartilage. The pathogenesis of OA is thought to reiterate changes that occur during endochondral ossification in which angiogenesis is required to initiate chondrocyte hypertrophic differentiation. We previously demonstrated that Bone Sialoprotein (BSP) production is associated with chondrocyte hypertrophy and the severity of osteoarthritic lesions. In this work, we investigated the impact of hypertrophic differentiation on the chondrocytes capacity to promote vascularization. We also speculated that BSP is a key mediator of cartilage vascularization.

Methods: In alginate beads, OA chondrocytes cultured in the presence of serum undergo hypertrophic differentiation in long-term culture. Using this model, we tested the effects of hypertrophic chondrocytes conditioned medium after 24 hours serum deprivation on the invasion and migration of endothelial cells using two different models: the real-time follow-up of the cells performed with the xCELLigence system (Roche) and high-end microscopic analysis of living endothelial cells in a wound healing assay. We also studied BSP gene expression (by RT-PCR) and production (by western blot) during chondrocyte hypertrophic differentiation. The effects of IL-1β (170 pg/ml) and TNFα (25 ng/ml) were tested on the synthesis of BSP by hypertrophic chondrocytes. These cytokines were added to the culture medium before (day 7) or after (day 21) hypertrophic phenotype was reached. Finally, the effect of increased concentration of recombinant BSP (25 ng/ml to 400 ng/ml) on the production of a proangiogenic factor, interleukin-8 (IL8) and an anti-angiogenic factor, thrombospondin-1 (TSP1) was studied by immunoassays.

Results: Hypertrophic OA chondrocytes conditioned medium showed a higher stimulating effect on endothelial cells migration and invasion than serum-containing medium (positive control). We demonstrated that BSP gene expression and protein production were associated with markers of hypertrophy collagen type 10 (COL X), alkaline phosphatase (AP), nucelside triphosphate pyrophosphohydrolase (NTPPHH) in OA but this association was not observed in normal chondrocyte cultures. IL-1β or TNFα in the culture medium decreased gene expression of BSP when added at day 7 or at day 21 (p<0.05). In the same manner, both cytokines...