CYTOKINES INCREASE PRODUCTION OF NITRIC OXIDE AND PROSTAGLANDIN E2 FROM HUMAN MENISCUS

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Introduction:
The mechanisms for the onset and disease progression in osteoarthritis (OA) are not fully understood, but seem to involve a combination of biochemical and biomechanical factors. In the knee, these factors involve both menisci and articular cartilage. Human chondrocytes release nitric oxide (NO) and prostaglandin E2 (PGE2) both associated with OA, in response to a variety of stimuli including catabolic cytokines such as IL-1β, TNF-α and IL-17 [1]. Presently, however, no studies have demonstrated NO or PGE2 production by the fibrochondrocytes of the human menisci, either spontaneously or in response to pro-inflammatory cytokines. The goal of this study was to (a) determine whether human menisci from osteoarthritic knees are capable of producing NO and PGE2 spontaneously and (b) analyze the effect of various cytokines and combinations of cytokines on NO and PGE2 release.

Materials and Methods:
Menisci were obtained from 17 patients (19 knees) undergoing total knee replacement for OA (age range 47-75, mean age 66.2). Explants were allowed to equilibrate in standard media for 72 hours. The meniscal explants were then cultured for 24 hours in the presence and absence of 0.1mg/ml IL-1, 50ng/ml IL-17 or 1.0ng/ml TNF. Cytokine doses were sub-maximal as determined by NO dose response curves on human meniscus. The effects of two NOS inhibitors (the non-selective inhibitor L-NMMA, and the NOS2 selective inhibitor 1400W) and a COX2 selective inhibitor (NS398) were also tested. Total NO production was measured by enzymatically reducing nitrate to nitrite, and then nitrate levels were determined via the Griess reaction. PGE2 levels were measured by ELISA (R & D Systems).

Results:
Meniscal explants spontaneously produced NO and PGE2. NO production was significantly (p<0.05) increased over baseline by IL-1, TNF and IL-17. IL-1 stimulated a significantly (p<0.0005) greater production of NO than TNF, and TNF stimulated a significantly (p<0.0005) greater response than IL-17 (Figure 1). The effect was reversed by 1400W and L-NMMA. Furthermore, the combination of IL-17 and TNF significantly increased NO production compared to TNF or IL-17 alone (p<0.0005).

Figure 1: Cytokine induced NO production, Mean ± SEM, n=32
* p < 0.05 compared to Control
** p < 0.005 compared to IL-17
*** p < 0.0005 compared to TNF or IL-17

PGE2 production was significantly increased by IL-1 (p<0.05). Neither TNF nor IL-17 significantly increased PGE2 production over baseline (Figure 2). Each combination of two cytokines tested resulted in significantly greater PGE2 production than any cytokine alone. The addition of IL-1 to TNF additively increased PGE2 production. The addition of IL-17 to TNF or IL-1 resulted in PGE2 production greater than the predicted additive effect of these cytokines, suggesting a synergistic response. Inhibition of NO production with 1400W or L-NMMA led to significant (p<0.0005) increases in IL-1 stimulated PGE2 production while inhibition of PGE2 production with NS398 significantly increased IL-17 stimulated NO production (p<0.05).

Figure 2: Cytokine induced PGE2 production, Mean ± SEM, n=16
* p < 0.05 compared to Control
** p < 0.05 compared to IL-1
*** p < 0.0005 compared to IL-1 + TNF or TNF + IL-17
**** p < 0.0005 compared to TNF or IL-17

Discussion:
Our data suggest that human menisci from osteoarthritic knees produce NO and PGE2 spontaneously, and that this production can be enhanced by inflammatory cytokines. This study shows that the meniscus can respond to cytokines that have important pro-inflammatory or catabolic capacity. This suggests knee menisci may play a biochemical (in addition to their established biomechanical) role, in the pathogenesis of osteoarthritis in the knee. NO and PGE2 appear to be produced through the NOS2 and COX2 pathways, since selective NOS2 and COX2 inhibitors blocked the effects of the cytokines. The levels of cytokine induced NO and PGE2 production differed among cytokines, as well as among different combinations of cytokines.

Of the cytokines tested, IL-1 had the single greatest effect on both NO and PGE2 production. NO appeared inhibit PGE2 production by explants stimulated by IL-1, while NO production by explants stimulated by IL-17 was inhibited by PGE2. This type of complex interaction between the COX and NOS pathways has been demonstrated in several tissues in the body, including articular cartilage and bone. The different responses of IL-17 and IL-1 stimulated explants indicate that these cytokines may operate through different pathways.

Since NO has a modulating effect on matrix synthesis [2], cytokine induced NO production might represent a mediator of meniscus degeneration and may play an important role in the progression of OA in human knee joints. Further understanding of the relationship between the NOS and COX pathways will aid in the development of new pharmacologic therapies for arthritis.


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