Osteoarthritis and Cartilage (1999) **7,** 377–379

Article No. joca.1998.0216, available online at http://www.idealibrary.com on IDEAL®

1063–4584/99/040377+03 $12.00/0

Osteoarthritis and Cartilage

Journal of the OsteoArthritis Research Society International

Nitric oxide in osteoarthritis

R. Studer*, D. Jaffurs†, M. Stefanovic-Racic*, P. D. Robbins† and C. H. Evans*†

**Department of Orthopaedic Surgery, †Department of Molecular Genetics & Biochemistry, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA*

Summary

Activated articular chondrocytes produce large amounts of nitric oxide (NO), and there is increasing evidence that this is involved in the etiopathogenesis of osteoarthritis (OA). Because of its short half-life, the biological effects of endogenously produced NO are likely to occur locally within the cartilage. We have observed that inhibitors of NO synthases relieve the inhibition of matrix synthesis that otherwise occurs in response to IL-1. To avoid the use of inhibitors, we have recently transduced chondrocytes with the iNOS (NOS-2) gene and confirmed the ability of the endogenously produced NO to inhibit matrix synthesis. Despite the high levels of NO made by these cells, there was no evidence of apoptosis or other forms of cell death. NO was also shown to inhibit the production of TGF-β_1 by cells treated with IL-1, as well as to decrease matrix production in response to IGF-1. The hypothesis that NO inhibits matrix production by interfering with important autocrine and paracrine factors should be entertained.

Key words: NO, Osteoarthritis, Cartilage.

Introduction

Much evidence implicates nitric oxide (NO) in the pathophysiology of osteoarthritis (OA), including the elevations in NO^-_x existing in the serum and synovial fluid of patients with OA.[1,2] Although the origin of the NO is unknown, articular chondrocytes are likely to be the major intra-articular source. Activated articular chondrocytes produce as much, if not more, NO than any other cell in the body.[3] Unlike chondrocytes recovered from normal human articular cartilage, chondrocytes recovered from patients with OA produce NO spontaneously[4] and express NO synthase (NOS).[5] When stimulated in culture, articular chondrocytes express the inducible isoform of NOS (iNOS or NOS II).[6] However, chondrocytes within human OA cartilage may express an unusual species of NOS, possibly a dysregulated neuronal NOS (nNOS or NOS I).[5] This matter awaits clarification.

Endogenously produced NO suppresses the synthesis of the cartilaginous matrix;[7] its possible role as a catabolic mediator remains controversial.[8–11] There is also evidence that NO induces apoptosis in articular chondrocytes, while protecting them from necrosis in response to other reactive oxygen species.[12] According to Hashimoto *et al.*,[13] the remnants remaining after apoptotic death of the chondrocytes become matrix vesicles and thus participate in the mineralization that occurs during OA.

Our studies have focused on the mechanisms through which NO inhibits matrix synthesis by chondrocytes. Here we review recent progress.

Corresponding author: C. H. Evans, Ph.D., D.Sc., University of Pittsburgh School of Medicine, 200 Lothrop Street, Rm. C-313 PUH, Pittsburgh, PA 15213. Tel: (412) 648-1092; Fax: (412) 648-8412; E-mail: cevans@vms.cis.pitt.edu

Methods

Primary, monolayer cultures of lapine articular chondrocytes were established by standard methods. Certain confirmatory experiments were conducted using organ cultures of cartilage fragments sliced form the femoral condyles of rabbits' knees.

High levels of NO synthesis were produced by incubation with 2 ng/ml human, recombinant interleukin-1β (hrIL-1β) or by infecting cells with an adenovirus carrying the human NOS-II gene under the control of a cytomegalovirus early promoter (ad-iNOS). A similar vector carrying the lac Z gene (ad-LacZ) was used as a control in certain experiments; sepiapterin was added as a precursor of tetrahydrobiopterin. NO production was estimated from the accumulation of nitrite in conditioned medium. N-methyl-L-arginine (NMA) was used to inhibit NOS activity.

Synthesis of proteoglycan was measured by the incorporation of $^{35}SO_4^{2-}$ into macromolecular material. Collagen synthesis was measured by the incorporation of [^{3}H-proline into collagenase-sensitive proteins. Transforming growth factor-β (TGF-β), insulin-like growth factor-1 (IGF-1) and isoform specific antibodies against TGF-β were purchased from commercial sources.

Cell death was assessed both visually and by TUNEL staining.

Results

NO PRODUCTION BY TRANSDUCED CHONDROCYTES

Cells infected with the ad-iNOS vector, but not the ad-lac Z vector, produced copious amounts of NO for the entire 10-day culture period (data not shown). Sepiapterin increased these levels even further. Unlike the case with untransduced cells stimulated with hrIL-1β,[8] NO production did not decline rapidly after 24–48 h of culture (data not shown).

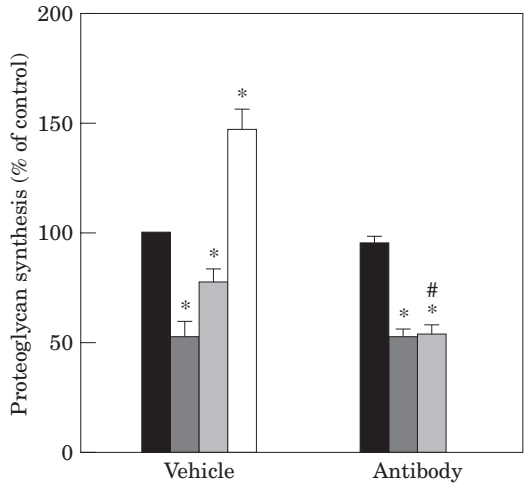

Fig. 1. Antibody to TGF-β_1 blocks the ability of NMA to restore proteoglycan synthesis by chondrocytes treated with IL-1β. Values are means±SE (N=6). *=P<0.05 vs vehicle control. #=P<0.05 vs cells treated with IL-1β and NMA. ■, Control; ▩, IL-1; ⬚, IL-1+NMA; □, TGF-beta.

Introduction of the iNOS gene into the chondrocytes markedly inhibited the synthesis of both proteoglycan and collagen; NO production was suppressed, and inhibition of matrix synthesis relieved, by addition of 0.5 mM NMA.

These data confirm that NO is capable of inhibiting matrix production in the absence of the other metabolic changes provoked by IL-1.

Even though the ad-iNOS transduced cells were producing supraphysiological amounts of NO, there was no evidence of apoptosis or other modes of cell death.

NO AND GROWTH FACTORS

NO has been reported to inhibit the production of, and response to, TGF-β by mesangial cells.[14] In chondrocytes, NO was shown to inhibit the production of TGF-β_1, but not TGF-β_2 or β_3, by cells treated with hrIL-1β. Moreover, NO inhibited the autoinduction of TGF-β. There was no effect of NO on the activation of latent TGF-β_1.[15]

Experiments using neutralizing antibodies to TGF-β_1 confirmed the importance of this autocrine factor in maintaining proteoglycan synthesis in response to IL-1β. In brief, these antibodies completely blocked the ability of NMA to antagonize the inhibition of proteoglycan synthesis by IL-1 (figure). This strongly suggests that NMA's ability to reverse this inhibition is mediated via TGF-β_1.[15]

Under the experimental conditions used here, NO blocked the synthesis of TGF-β_1 but not responses to TGF-β.

In preliminary experiments, we have shown that NO also antagonizes the ability of IGF-1 to stimulate proteoglycan synthesis (data not shown). Of interest in the present context is the observation that matrix synthesis by chondrocytes retrieved from osteoarthritic joints is resistant to the stimulatory effects of IGF-1.[16]

Discussion

These data suggest that the inhibitory effects of NO upon matrix synthesis reflect its ability to interfere with the production of, and responses to, the autocrine factors TGF-β and IGF-1. They do not, however, shed light on the mechanisms through which NO interferes with these autocrine loops.

These findings are of additional interest in light of the altered responses to TGF-β[17] and IGF-1[16] of chondrocytes retrieved from osteoarthritic joints. The refractory behavior of OA chondrocytes to IGF-1 is thought to reflect the high production of IGF binding proteins by these cells.[16] The latter may reflect alterations in PGE_2 production occurring in response to NO. The involvement of NO in insensitivity to IGF-1 is strongly suggested from the data obtained recently using $iNOS^{-/-}$ mice.[18]

The responses of chondrocytes to TGF-β, in contrast, are increased in OA.[17] Our data suggest that production of TGF-β by chondrocytes is reduced by NO, but they do not directly address possible effects on responses to TGF-β. Collectively, these data strengthen the case for the involvement of NO in OA and other circumstances of altered cartilage metabolism.

References

1. Farrell AJ, Blake DR, Palmer RMJ, Moncada S. Increased concentrations of nitrite in synovial fluid and serum samples suggest increased nitric oxide synthesis in rheumatic diseases. Ann Rheum Dis 1992;51:1219–22.
2. Renoux M, Hiliquin P, Galoppin L, et al. Release of mast cell mediators and nitrites into knee joint fluid in osteoarthritis—comparison with articular chondrocalcinosis and rheumatoid arthritis. Osteoarthritis Cartilage 1996;4:175–9.
3. Stadler J, Stefanovic-Racic M, Billiar TR, et al. Articular chondrocytes synthesize nitric oxide in response to cytokines and lipopolysaccharide. J Immunol 1991;147:3915–20.
4. Amin AR, Attur M, Patel RN, et al. Superinduction of cyclooxygenase-2 activity in human osteoarthritis-affected cartilage. J Clin Invest 1997;99:1231–7.
5. Amin AR, DiCesare P, Vyas P, et al. The expression and regulation of nitric oxide synthase in human osteoarthritis-affected chondrocytes: evidence for upregulated neuronal nitric oxide synthase. J Exp Med 1995;182:2097–102.
6. Charles IG, Palmer RMJ, Hickey MS, et al. Cloning, characterization and expression of a cDNA encoding an inducible nitric oxide synthase from the human chondrocyte. Proc Natl Acad Sci USA 1993; 90:11 419–23.
7. Taskiran D, Stefanovic-Racic M, Georgescu HI, Evans CH. Nitric oxide mediates suppression of cartilage proteoglycan synthesis by interleukin-1. Biochem Biophys Res Commun 1994;200:142–8.
8. Stefanovic-Racic M, Morales TI, Taskiran D, et al. The role of nitric oxide in proteoglycan turnover by bovine articular cartilage organ cultures. J Immunol 1996;156:1213–20.
9. Murrell GAC, Jang D, Williams RJ. Nitric oxide activates metalloproteinase enzymes in articular cartilage. Biochem Biophys Res Commun 1995;206: 15–21.
10. Bird JL, Wells T, Platt D, Bayliss MT. IL-1β induces the degradation of equine articular cartilage by a mechanism that is not mediated by nitric oxide. Biochem Biophys Res Commun 1997;238:81–5.

11. Sasaki K, Hattori T, Fujisawa T, et al. Nitric oxide mediates interleukin-1-induced gene expression of matrix metalloproteinases and basic fibroblast growth factor in cultured rabbit articular chondrocytes. J Biochem 1998;123:431–9.
12. Blanco FJ, Ochs RL, Schwarz H, Lotz M. Chondrocyte apoptosis induced by nitric oxide. Am J Pathol 1995;146:75–85.
13. Hashimoto S, Ochs RL, Rosen F, et al. Chondrocyte-derived apoptotic bodies and calcification of articular cartilage. Proc Natl Acad Sci USA 1998;95:3094–9.
14. Craven PA, Studer RK, Felder J, et al. Nitric oxide inhibition of transforming growth factor-β and collagen synthesis in mesangial cells. Diabetes 1997; 46:671–81.
15. Studer RK, Georgescu HI, Miller LA, Evans CH. Nitric oxide inhibits TGF-β production by chondrocytes: implications for matrix synthesis. Arthritis Rheum 1999;42:248–257.
16. Martel-Pelletier J, Di Battista JA, Lajeunesse D, Pelletier JP. IGF/IGF BP axis in cartilage and bone in osteoarthritis pathogenesis. Inflamm Res 1998; 47:90–100.
17. Lafeber FPJG, Van der Kraan PM, Huber-Brunig O, et al. Osteoarthritic human cartilage is more sensitive to transforming growth factor β than is normal cartilage. Br J Rheum 1993;32:281–6.
18. Van de Loo FAJ, Arntz OJ, Van Enckevort FHJ, et al. Reduced cartilage proteoglycan loss during zymosan-induced gonarthritis in NO2-deficient mice and in anti-interleukin-1 treated wild-type mice with unabated joint inflammation. Arthritis Rheum 1998;41:634–46.