Intermittent Hydrostatic Pressure Inhibits Shear Stress-Induced Nitric Oxide Release in Human Osteoarthritic Chondrocytes *in Vitro*

MEL S. LEE, MICHAEL C.D. TRINDADE, TAKASHI IKENOUE, DAVID J. SCHURMAN, STUART B. GOODMAN, and R. LANE SMITH

ABSTRACT. ***Objective.*** To test the effects of intermittent hydrostatic pressure (IHP) on nitric oxide (NO) release induced by shear stress and matrix macromolecule gene expression in human osteoarthritic chondrocytes *in vitro*.

Methods. Chondrocytes isolated from cartilage samples from 9 patients with osteoarthritis were cultured and exposed to either shear stress or an NO donor. Nitrite concentration was measured using the Griess reaction. Matrix macromolecule mRNA signal levels were determined using reverse-transcriptase polymerase chain reaction and quantified by imaging analysis software.

Results. Exposure to shear stress upregulated NO release in a dose and time-dependent manner. Application of IHP inhibited shear stress induced NO release but did not alter NO release from chondrocytes not exposed to shear stress. Shear stress induced NO or addition of an NO donor (sodium nitroprusside) was associated with decreased mRNA signal levels for the cartilage matrix proteins, aggrecan, and type II collagen. Intermittent hydrostatic pressure blocked the inhibitory effects of sodium nitroprusside but did not alter the inhibitory effects of shear stress on cartilage macromolecule gene expression.

Conclusion. Our data show that shear stress and IHP differentially alter chondrocyte metabolism and suggest that a balance of effects between different loading forces preserve cartilage extracellular matrix *in vivo*. (J Rheumatol 2003;30:326–8)

Key Indexing Terms:

NITRIC OXIDE | MECHANICAL LOADS | OSTEOARTHRITIS
CHONDROCYTES | AGGRECAN | COLLAGEN

Articular chondrocytes respond to mechanical stimuli by altered matrix macromolecule expression and release of soluble mediators, including nitric oxide (NO)[1-4]. NO serves as multifunctional cellular messenger for an array of biological processes and is implicated in rheumatological diseases[5-7]. In bovine articular chondrocytes, induction of NO by shear stress modulates matrix metabolism[8]. Other types of mechanical loads may exert differential effects on articular chondrocyte metabolism through NO[9,10]. We tested effects of intermittent hydrostatic pressure (IHP) on NO release by human osteoarthritic chondrocytes induced by shear stress and matrix macromolecule gene expression following exposure to shear stress or an NO donor.

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Supported by NIH Grant AR45788.

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Submitted March 14, 2002; revision accepted June 4, 2002.

MATERIALS AND METHODS

Cell culture. Human osteoarthritic chondrocytes were isolated from cartilage samples of 9 patients receiving total joint replacements as described[2]. Chondrocytes were plated at a density of $5 \times 10^4/cm^2$, maintained in DMEM/F12 supplemented with fetal bovine serum and antibiotics and placed in serum-free medium 24 h prior to loading, as described[11]. The cells were then exposed to mechanical loading in the presence of fresh serum-free medium. Each experiment was carried out in duplicate and repeated with 6 individual samples. To test the response of chondrocytes to IHP after preconditioning with an NO donor, sodium nitroprusside (SNP) (Sigma Chemical, St. Louis, MO, USA) was added to the cultures at concentrations of 20 µM or 2000 µM, prior to mechanical loading. To test the response of chondrocytes to IHP after treatment with shear stress, the cells were exposed to shear stress for 2 h (200 rpm; 1.6 Pa) followed by transfer to a pressure vessel for application of IHP.

Mechanical loading. IHP (10 MPa at 1 Hz) was applied to confluent cell monolayers by placing the culture plates in heat-sealed bags filled with serum-free medium, as described[2]. The loading of IHP included test periods of 4 h per day for 1, 2 or 4 days.

NO release. Nitrite, the stable end product of NO oxidation, was used as an indicator of NO synthesis. Nitrite concentration in the culture medium was measured using the Griess reaction with sodium nitrite as the standard, as described[8].

Analysis of mRNA expression. Following loading, the cells were maintained

in culture for 24 h and then lysed for extraction of RNA (Tri-Reagent, Sigma). Total RNA was isolated and signal levels for matrix macromolecule mRNA were determined using reverse transcriptase polymerase chain reaction (RT-PCR). Primer sequences used for transcript detection were as follows: type II collagen (sense), 5′-CTGGCTCCCAACACTGCCA-ACGTC-3′, and type II collagen (antisense), 5′- TCCTTTGGGTTTG-CAACGGATTGT-3′; aggrecan (sense), 5′-CACTGTTACCGCCAC-TTCCC-3′, and aggrecan (antisense), 5′-GAGAT-CGTTCCACTCGCCCT-3′; actin (sense), 5′-CAGGTCATCACYATYGGCAATGAGC-3′, and actin (antisense), 5′-CGGATGTCMACGTC-ACACTTCATGA-3′. PCR products were visualized on ethidium bromide-stained 1.2% agarose gels and the signals were quantified by imaging analysis software (ImageQuant, Molecular Dynamics, Sunnyvale, CA, USA) and normalized to ß-actin expression.

Statistical analysis. One-way analysis of variance with *post-hoc* Newman-Keuls multiple comparisons of means was used for statistical analysis with $p < 0.05$ considered to be significant.

RESULTS

Differential effects of shear stress and intermittent hydrostatic pressure on NO release. Exposure to increasing levels of shear stress from 0.4 to 1.6 Pa over 2 h increased nitrite levels in the culture medium from 8 to 15 µM when assayed after a subsequent 22 h ($p < 0.001$). The nitrite level in the culture medium was 5 µM in the absence of applied shear stress. Application of shear stress at 1.6 Pa for 2, 6, or 24 h increased nitrite levels to 14, 18, and 30 µM ($p < 0.05$), respectively, when assayed at 24 h. Application of IHP (10 MPa at 1 Hz) to chondrocyte cultures for test periods of 4 h per day for 1, 2, or 4 days did not alter nitrite levels.

IHP inhibition of shear stress induced NO release in osteoarthritic chondrocytes. To determine if IHP alters induced chondrocyte NO release, cultures were exposed to shear stress at 1.6 Pa for 2 h followed by IHP for 4 h. NO release was decreased by 35% ($p < 0.05$) from chondrocytes exposed to shear stress and then treated with IHP, when compared to chondrocytes exposed to shear stress alone (Figure 1).

IHP inhibition of sodium nitroprusside induced NO release in osteoarthritic chondrocytes. Sodium nitroprusside (SNP) preconditioned chondrocytes were subjected to IHP for 4 h and subsequently maintained for 24 h to allow post-loading assessment of cellular metabolism. The nitrite levels in the culture medium of chondrocytes maintained in the absence IHP were 11 and 46 µM with 20 and 2000 µM of SNP, respectively. Application of IHP to the SNP treated chondrocytes decreased nitrite levels by 20 to 25% ($p < 0.01$) (data not shown).

Differential effects of shear stress and intermittent hydrostatic pressure on matrix macromolecule gene expression. Application of shear stress to chondrocytes down-regulated type II collagen mRNA from 18 to 25% ($p < 0.05$) and aggrecan mRNA from 30 to 40% ($p < 0.05$), respectively, when compared to chondrocytes maintained in the absence of shear stress (Figure 2A). In contrast, IHP up-regulated type II collagen mRNA by 24, 53, and 112% ($p < 0.05$) with

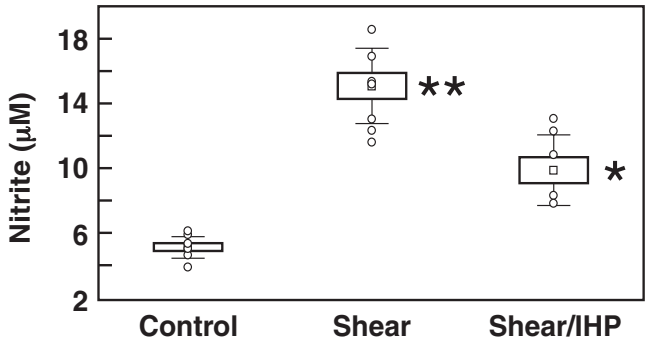

Figure 1. Effect of intermittent hydrostatic pressure (IHP) on the nitric oxide release in chondrocytes preconditioned with shear stress. *$p < 0.05$ compared to the control. **$p < 0.01$ compared to control cultures or culture exposed to shear stress followed by application of IHP (Shear/IHP).

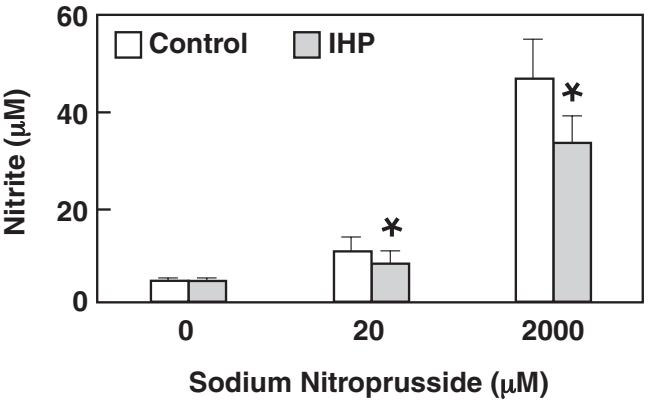

Figure 2. Effects of intermittent hydrostatic pressure (IHP) on nitric oxide release in osteoarthritic chondrocytes preconditioned with sodium nitroprusside (SNP). *$p < 0.01$ compared to the control.

1, 2, and 4 days of interval-based mechanical loading, respectively (Figure 2B). Expression of aggrecan mRNA was also up-regulated by 35, 59, and 126% ($p < 0.05$) by application of IHP for 1, 2, or 4 days (Figure 2B).

Effects of intermittent hydrostatic pressure on matrix macromolecule gene expression in NO donor-preconditioned chondrocytes. Addition of SNP at 20 µM and at 2000 µM to chondrocytes increased the NO levels to 11 and 46 µM, respectively, and decreased collagen and aggrecan mRNA expression by 14% and 24% (ANOVA $p < 0.05$), respectively (Figure 3). Application of IHP to chondrocytes exposed to 20 µM SNP increased aggrecan and type II collagen mRNA levels (ANOVA $p < 0.005$) (Figure 3), when compared to untreated cells. However, with 2000 µM SNP, IHP counteracted the downregulating effect of the NO donor on aggrecan but not type II collagen mRNA expression. IHP reduced nitrite levels observed with SNP by 70 to 80% (data not shown). In contrast to effects observed with SNP, application of IHP failed to reverse inhibitory effects of shear stress on type II collagen and aggrecan mRNA.

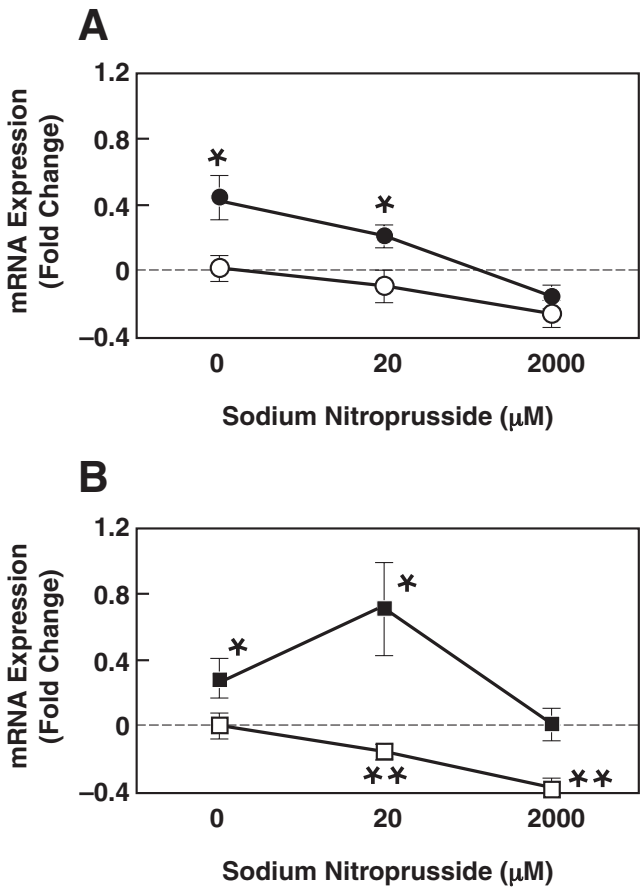

Figure 3. Effects of intermittent hydrostatic pressure (IHP) on matrix macromolecule gene expression in osteoarthritic chondrocytes preconditioned with sodium nitroprusside (SNP). A: Type II collagen expression with IHP (closed circles) and without IHP (open circles) loading. *$p < 0.01$ compared to samples without IHP exposure. B: Aggrecan expression with IHP (closed boxes) and without IHP (open boxes) loading. *$p < 0.005$ compared to cultures maintained in the absence of IHP. **$p < 0.05$ compared to cultures without SNP preconditioning and maintained in the absence of IHP.

DISCUSSION

Articular chondrocytes release NO in response to inflammatory factors and shear stress[3,4,8,10,12]. NO levels are elevated in serum and synovial fluid of patients with arthritis and in animals with experimentally induced and autoimmune arthritis[6,7]. In chondrocytes, NO release down-regulates proteoglycan synthesis, enhances cartilage matrix protein degradation through matrix metalloproteinases, and promotes apoptosis[7,12,13]. The importance of NO in the pathogenesis and treatment of osteoarthritis is reinforced by the fact that inhibitors of NOS activity ameliorate severity of arthritis in animal models[7,14].

The role of mechanical forces, such as IHP and shear stress, in maintenance and degeneration of cartilage manifests in effects on matrix synthesis and alterations in chondrocyte morphology and proinflammatory mediator expression[1-4,15]. In our study, NO release from human osteoarthritic chondrocytes was differentially regulated by different mechanical loads. NO release from chondrocytes was upregulated by shear stress and inhibited by IHP. NO donor-mediated down-regulation of matrix protein mRNA in chondrocytes exposed to SNP was counteracted by IHP. In contrast, IHP failed to reverse shear stress-mediated down-regulation of matrix protein mRNA. These differences may be due to the fact that shear stress not only increases NO but also increases expression of interleukin-6, monocyte chemotactic protein-1, and other soluble factors[3,15]. Our results show that shear stress-induced NO release is counteracted by IHP and suggest that the balance between different types of mechanical loading preserve cartilage extracellular matrix.

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