P38 MITOGEN ACTIVATED PROTEIN KINASE REGULATES MECHANICALLY-INDUCED NITRIC OXIDE AND PROSTAGLANDIN PRODUCTION IN ARTICULAR CARTILAGE

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INTRODUCTION

Biomechanical factors affect both the normal function and the pathophysiology of articular cartilage [1]. For example, dynamic mechanical compression of cartilage explants has been shown to increase the production of the pro-inflammatory mediators nitric oxide (NO) prostaglandin E2 (PGE_2) [2]. These molecules are believed to play important roles in the pathogenesis of osteoarthritis (OA), and NO and PGE_2 can both influence matrix synthesis and degradation in articular cartilage [3-6]. Current pharmacological treatment for OA is limited to steroids and non-steroidal anti-inflammatory agents such as COX2 inhibitors, which reduce pain but do not influence the pathology of the disease process. NOS2 inhibitors, which are not yet used clinically, may prevent the onset of arthritis *in vivo* [3] but can cause a "superinduction" of PGE_2 production *in vitro* in response to mechanical compression or interleukin 1 (IL-1) [2]. P38 inhibitors are a novel class of drugs that target p38 mitogen activated protein kinase (MAPK) and can inhibit both NO and PGE_2 production by IL-1 stimulated chondrocytes [7,8]. They may serve as effective therapeutic agents in animal models of arthritis [9,10]. The effects of mechanical stress on the activity of p38 MAPK, however, are not known. We hypothesized that mechanical compression up-regulates p38 MAPK, leading to increased production of NO and PGE_2. Furthermore, we hypothesized that p38 inhibitors will diminish this effect by blocking p38 MAPK. We also examined any differences in effect between SB203580 (IC50 48 nM) and a more potent p38 inhibitor, compound A (IC50 6 nM).

METHODS

Full thickness explants of articular cartilage (5mm diam.) were obtained from the femoral condyles of 2 yr old female pigs. Samples were cultured in DMEM with 10% heat inactivated serum, 0.1 mM non-essential amino acids, 10 mM HEPES, and 100 U/ml pen/strep. Test and control samples were matched from adjacent sites of the cartilage surface. All experiments were performed at 37º C and 5% CO_2, 95% air. Explants were incubated for 72 hrs prior to testing.

Mechanically loaded samples were subjected to an intermittent compressive load of 0.1 MPa at 0.5 Hz (1 sec on, 1 sec off) for 24 hours using a modified version of the Biopress™ system (Figure 1, Flexcell International). Explants were equilibrated for 1 hr under a 10 gf tare load. All controls were cultured in an unloaded state. Explants were also tested with 20 ng/mL IL-1 (R & D Systems) without compression. For both mechanical stimulation and IL-1 treatment, the effects of two p38 inhibitors were tested: SB203580 and the more potent inhibitor compound A (GlaxoSmithKline, King of Prussia PA). Explants were treated with log doses of the respective inhibitor from 0.01-10 μM.

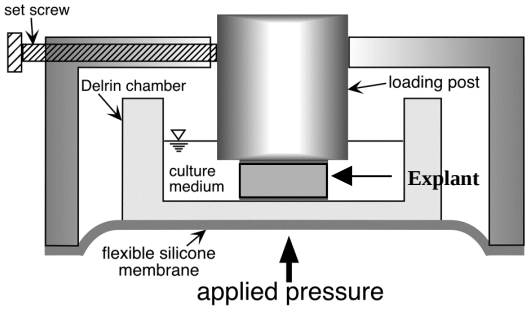

Figure 1. Diagram of the explant compression system. Load was applied to individual cartilage explants by increasing air pressure on a flexible membrane.

PGE_2 production was measured by ELISA (R&D Systems). NO production was measured via the Griess reaction. Results were normalized to the wet weight of the explants determined prior to compression. MAPK activity was determined by Western blot. Cell viability was determined by the live/dead assay (Molecular Probes). Statistical significance was determined by ANOVA with the Duncan *post hoc* test.

RESULTS

Mechanical compression up-regulated p38 MAPK activity by 2.1 fold as measured by Western blot using anti-phospho p38 antibody. Compression significantly increased both NO ($p<0.05$) and PGE_2 ($p<0.01$) production. The more potent p38 inhibitor compound A inhibited NO production at all concentrations (Figure 2, left) and inhibited PGE_2 production at concentrations of 1 μM or greater (Figure 2, right). The p38 inhibitor SB203580, had no conclusive effect on NO production. With no compression, there was a 57% decrease in NO production ($p<0.05$) with 0.1 μM of inhibitor. There were no significant effects at any other concentration. With compression, there were significant decreases in NO production at 0.01 μM and 0.1 μM ($p<0.05$). However, compression with 1 μM SB203580 caused a two-fold increase in NO production compared to control ($p<0.001$). SB203580 also had no definitive effect on mechanically-induced PGE_2 production. SB203580 at 0.01 μM and 0.1 μM significantly inhibited mechanically-induced PGE_2 production ($p<0.05$).

IL-1 significantly increased both NO ($p<0.001$) and PGE_2 ($p<0.01$) production. Compound A inhibited NO production by 25% at concentrations of 0.1 μM and higher ($p<0.05$) and inhibited PGE_2 production by 69% at 1 and 10 μM ($p<0.05$). SB203580 showed few significant effects on NO production. There was a 50% increase in mechanically-induced NO at 0.1 μM ($p<0.05$). SB203580 inhibited PGE_2 production at concentrations of 1 and 10 μM ($p<0.05$).

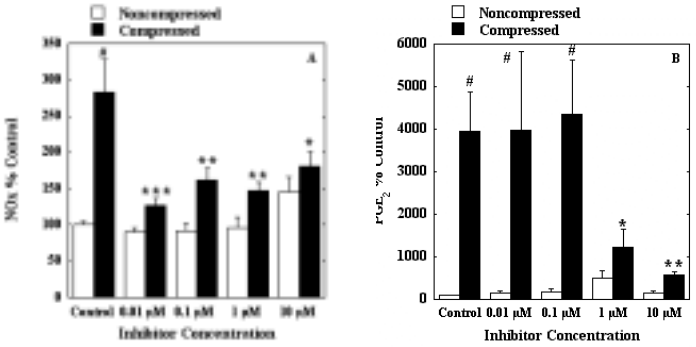

Figure 2. Compound A inhibited mechanically-induced NO (left) and PGE_2 (right) production. #p<0.01 vs paired unloaded group. *p<0.05, **p<0.01, ***p<0.001 vs loaded control.

DISCUSSION

Our findings indicate that dynamic mechanical compression regulates the production of the pro-inflammatory mediators NO and PGE_2 via the induction of p38 MAP kinase. The more potent p38 inhibitor, compound A, inhibited both mechanically-induced NO and PGE_2 production, and inhibited PGE_2 production in response to IL-1. SB203580 had no apparent effect on mechanically-induced NO or PGE_2 production, but inhibited PGE_2 production from IL-1 stimulated explants. Previously SB203580 has been shown to have an inhibitory effect on IL-1 induced NO production in bovine chondrocytes [8]. The inability of SB203580 to have an effect on mechanically-induced NO or PGE_2 could be due to the use of a higher concentration of serum in the present experiments. However, potency, selectivity, and species differences reported for other p38 inhibitors may also account for these results [7].

These findings suggest that inhibitors of p38 MAPK may selectively influence the production of pro-inflammatory mediators induced in cartilage by biomechanical stress or cytokine stimulation. This pathway may therefore represent a potential target for the inhibition of cartilage inflammation and degeneration with OA. Further analysis is currently underway on other downstream effects of p38 MAPK activation in response to mechanical loading.

ACKNOWLEDGMENTS

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REFERENCES

1. Guilak, F., Sah, R., and Setton, L. A., 1997, "Physical Regulation of Cartilage Metabolism," Basic Orthopaedics Biomechanics, V. C. Mow, W. C. Hayes, eds., Lippincott-Raven, Philadelphia, pp. 179-208.
2. Fermor, B., Weinberg, J. B., Pisetsky, D. S., Misukonis, M. A., Fink, C., and Guilak, F., "Induction of Cyclooxygenase-2 by Mechanical Stress through a Nitric Oxide-Regulated Pathway,"
3. Clancy, R. M., Amin, A. R., and Abramson, S. B., 1998, "The role of nitric oxide in inflammation and immunity," Arthritis & Rheumatism, 41, pp. 1141-1151.
4. Abramson, S. B., 1999, "The role of COX-2 produced by cartilage in arthritis," Osteoarthritis & Cartilage, 7, pp. 380-381.
5. Hauselmann, H. J., Stefanovic-Racic, M., Michel, B. A., and Evans, C. H., 1998, "Differences in nitric oxide production by superficial and deep human articular chondrocytes: implications for proteoglycan turnover in inflammatory joint diseases," Journal of Immunology, 160, pp. 1444-1448.
6. Di Battista, J. A., Dore, S., Martel-Pelletier, J., and Pelletier, J. P., 1996, "Prostaglandin E2 stimulates incorporation of proline into collagenase digestible proteins in human articular chondrocytes: identification of an effector autocrine loop involving insulin-like growth factor I," Molecular & Cellular Endocrinology, 123, pp. 27-35.
7. Badger, A. M., Roshak, A. K., Cook, M. N., Newman-Tarr, T. M., Swift, B. A., Carlson, K., Connor, J.R., Lee, J.C., Gowen, M., Lark, M. W., and Kumar, S., 2000, "Differential effects of SB 242235, a selective p38 mitogen-activated protein kinase inhibitor, on IL-1 treated bovine and human cartilage/chondrocyte cultures," Osteoarthritis & Cartilage, 8, pp. 434-443.
8. Badger, A. M., Cook, M. N., Lark, M. W., Newman-Tarr, T. M., Swift, B. A., Nelson, A. H., Barone, F. C., and Kumar, S., 1998, "SB 203580 inhibits p38 mitogen-activated protein kinase, nitric oxide production, and inducible nitric oxide synthase in bovine cartilage-derived chondrocytes," Journal of Immunology, 161, pp. 467-473.
9. Badger, A. M., Bradbeer, J. N., Votta, B., Lee, J. C., Adams, J. L., and Griswold, D. E., 1996, "Pharmacological profile of SB 203580, a selective inhibitor of cytokine suppressive binding protein/p38 kinase, in animal models of arthritis, bone resorption, endotoxin shock and immune function," Journal of Pharmacology & Experimental Therapeutics, 279, pp. 1453-1461.
10. Lee, J. C., Badger, A. M., Griswold, D. E., Dunnington, D., Truneh, A., Votta, B., White, J. R., Young, P. R., and Bender, P. E., 1993, "Bicyclic imidazoles as a novel class of cytokine biosynthesis inhibitors," Annals of the New York Academy of Sciences, 696, pp. 149-170.