Cyclic Compression Loaded on Cartilage Explants Enhances the Production of Reactive Oxygen Species

TAKASHI TOMIYAMA, KANJI FUKUDA, KENJI YAMAZAKI, KAZUKI HASHIMOTO, HIRONOBU UEDA, SHIGESHI MORI, and CHIAKI HAMANISHI

ABSTRACT. ***Objective.*** Although mechanical forces are an essential factor in the regulation of cartilage metabolism, the precise mechanisms involved have not yet been determined. We previously demonstrated that mechanical forces on chondrocytes inhibited proteoglycan (PG) synthesis. We also demonstrated the induction of reactive oxygen species (ROS) is loaded on the chondrocytes. Our purpose was to determine the ROS induction with mechanical compression and its involvement in PG synthesis of cartilage slices.

Methods. Bovine articular cartilage slices were subjected to cyclic compression loading. Synthesis of PG and ROS was measured using $Na_2[^{35}S]$-SO_4 and a chemiluminescent probe, respectively. The induction of nitrotyrosine was determined using immunohistochemistry.

Results. The synthesis of PG was significantly inhibited with 2.0 MPa of compression stress; 1 h of compression was sufficient to inhibit PG synthesis. The ROS inhibitor ebselen reversed the compression-inhibited synthesis of PG. Compression on the cartilage induced synthesis of ROS and the expression of nitrotyrosine.

Conclusion. Mechanical compression at 2.0 MPa inhibited PG synthesis by cartilage explants. ROS were involved in this action. (First Release Feb 15 2007; J Rheumatol 2007;34:556–62)

Key Indexing Terms:
COMPRESSION CARTILAGE REACTIVE OXYGEN SPECIES

Osteoarthritis (OA) is a degenerative disease of diarthrodial joints in which degrading and reparative processes in articular cartilage, subchondral bone, and synovium occur concurrently. The pathological features of cartilage in OA, including changes in matrix composition, chondrocyte clustering, and cartilage fissuring and flaking, are well recognized. At present, it is believed that these changes are induced by a common final pathway, as a result of mechanical forces acting on cartilage. These mechanical forces are an essential factor in the regulation of cartilage metabolism, and the precise mechanisms involved remain to be determined[1]. Recent observations demonstrated that mechanical stress loaded on the chondrocytes enhanced matrix-degrading enzymes, e.g., matrix metalloproteinase expression[2] and proinflammatory mediators, such as prostaglandin E_2[3].

Reactive oxygen species (ROS), such as the superoxide anion radical (O_2_), hydrogen peroxide (H_2O_2), peroxynitrite (ONOO–), and the hydroxyl radical (–OH), are formed in cells as a consequence of normal aerobic respiration. Previous studies demonstrated that ROS are involved in the cartilage degradation associated with inflammatory joint disease[4,5]. In addition, the induction of ROS was found in cultured chondrocytes undergoing loading during cyclic tensile stretch[6].

We previously applied cyclic tensile stretch on cultured chondrocytes. We found that a low magnitude (5% elongation) increased proteoglycan (PG) synthesis and a high magnitude (17% elongation), in turn, decreased the PG synthesis of the chondrocytes[7]. However, chondrocytes in normal cartilage are rounded in shape and surrounded with a matrix. Accordingly, the physiological significance of our results is questionable, and may not be extrapolated to cells maintained in native cartilage tissue with respect to the complex interactions between the chondrocytes and the extracellular matrix. With this in mind, cartilage slices were used to determine the effects of mechanical forces, specifically intermittent compression, on cartilage metabolism *in vitro*[8,9]. In our study, we focused on the effect of intermittent compression loading on the cartilage slices and the alterations in synthesis of PG.

Our purpose was to demonstrate the induction of ROS in cartilage slices and its involvement in PG synthesis.

MATERIALS AND METHODS

Drugs and chemicals. Alpha-minimum essential medium (MEM) and fetal bovine serum (FBS) were obtained from Gibco (Grand Island, NY, USA) and

From the Department of Orthopaedic Surgery, Kinki University School of Medicine, Osaka, Japan.

Supported by a research grant from the Scientific Research Fund of the Ministry of Education, Science and Culture of Japan.

T. Tomiyama, MD, PhD; K. Fukuda, MD, PhD; K. Yamazaki, MD, PhD; K. Hashimoto, MD, PhD; H. Ueda, MD, PhD; S. Mori, MD, PhD; C. Hamanishi, MD, PhD, Department of Orthopaedic Surgery, Kinki University School of Medicine.

Address reprint requests to Dr. K. Fukuda, Department of Orthopaedic Surgery, Kinki University School of Medicine, 377-2 Ohno-higashi, Osaka-sayama, Osaka 589-8511, Japan.
E-mail: k-fukuda@med.kindai.ac.jp

Accepted for publication November 6, 2006.

HyClone (Logan, UT, USA), respectively. L-012 (8-amino-5-chloro-7-phenylpyridol [3,4-d] pyridazine-1, 4(2H,3H) dione), cetylpyridinium chloride, ebselen (2-phenyl-1,2-benzisoselenazol-3(2H)-one), and papain were purchased from Sigma (St. Louis, MO, USA). The $Na_2[^{35}S]$-SO_4 was obtained from New England Nuclear (Boston, MA, USA). Anti-nitrotyrosine (mouse monoclonal IgG) was purchased from Upstate Biotechnology, Inc. (Lake Placid, NY, USA).

Explant culture. Cartilage slices were obtained from the metacarpophalangeal joint of 6- to 9-month-old cows. Cartilage slices obtained from 2 to 3 animals were pooled for these studies. Briefly, 5 mm-diameter cylindrical discs of cartilage and underlying bone were punched from the condylar ridge of the metacarpophalangeal joints. The first 100–300 µm of tissue was then removed to obtain a flat surface, and 2 mm-thick cartilage slices were cut. The cartilage was detached from the bone for study. The explants were cultured in MEM with 10% heat inactivated FBS, 0.1 mM nonessential amino acids, 10 mM HEPES, 100 U/ml penicillin and streptomycin (Gibco, Gaithersburg, MD, USA). The test and control explants were removed from adjacent sites on the joint surfaces and paired at harvest to account for site-dependent variations in the tissue. To determine if ROS production was cell-mediated, cartilage slices were immersed 3 times in liquid nitrogen to kill the cells just prior to compression loading.

Compression experiments. All compression experiments were performed after allowing explants to equilibrate in culture for 72 h after harvest. For each experiment, 6 pairs of control and test explants were placed into individual compression wells in 1 ml of culture medium and allowed to equilibrate for 1 h. All experiments were performed at 37°C in 5% CO_2 and 95% air. When used, the ROS inhibitor ebselen, a synthetic heterocyclic seleno-organic compound that has been shown to act as a scavenger of ROS, was added to the culture medium 1 h before beginning the loading regimen to permit it to diffuse into the explants. Compressive loads were applied to individual explants using the Biopress system: the Flexercell® Compression Plus™ System, FX-4000C™ (Flexcell International, Hillsborough, NC, USA; Figure 1). This is a computer-driven instrument that simulates biological compression conditions using pressure to deform tissue samples with unconfined loading. Application of air pressure to the silicone membrane causes the chamber to rise and exert a force on the cartilage slice by compressing it between a piston and a stationary plate in the Biopress™ culture plates. Mechanical loads were applied as a square waveform at 0.5 Hz (1 s on, 1 s off) corresponding to stress magnitudes of 0.05, 0.1, 0.5, or 2.0 MPa. The compressive stress was determined from the applied load and the initial cross-sectional area of the cartilage slices. The relationship between the air pressure and resulting load on the cartilage slices was linear[10,11]. All control specimens were cultured in an unloaded state.

Cell viability assay. Cartilage slices were loaded with 2.0 MPa of cyclic compression for 1 h and 24 h, respectively. A cell death detection kit (Takara, Kyoto, Japan) was used to detect and quantify apoptosis at the single-cell level by TUNEL staining. In brief, slides were deparaffinized, rehydrated, and washed in Tris-buffered saline (TBS). Following 2 × 5 min TBS washes, TUNEL reaction mixture was added, and the section covered with a glass cover slip and incubated 60 min at 37°C. The slides were then rinsed with distilled water, counterstained with Mayer's hematoxylin for 5 min, mounted with water-soluble mountant, and analyzed under light microscopy. TUNEL staining was performed in at least 3 sections from each specimen. All sections were examined by 400× light microscopy.

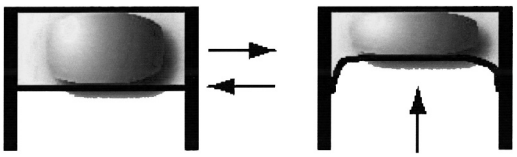

Figure 1. A compression system. Each compression well contains a flexible silicone membrane to which air pressure is applied under the center of each well (left). Vertical movement was allowed because of the flexible bottom of each well. Positive pressure (right, arrow), controlled by a microcomputer, drives the well upward, applying force to the cartilage slice. Compression experiments were performed at a frequency of 0.5 Hz (square wave with 1 s on, 1 s off), determined from the applied load and the initial cross-sectional area of the cartilage slices. The relationship between the air pressure and resulting load on the cartilage slices was linear[11].

Measurement of ROS. Cartilage slices were compressed at different magnitudes for 1 h and were then washed with 1 ml of ice-cold PBS. ROS levels in cartilage slices were measured as described[6]. Briefly, cartilage slices, which are transparent, allow the measurement of chemiluminescent intensity. The cartilage slices were placed into a black 96-well plate. A reaction mixture containing the chemiluminescent probe (L-012, 0.15 mg/ml), which reacts with ROS in the cells[12], was added and incubated 1 min. Chemiluminescence from the reaction mixture was detected.

PG synthesis. Each disc was transferred to a tissue culture well within 2 min after the loading session and incubated in fresh medium containing $Na_2[^{35}S]$-SO_4 at 10 µCi/ml for the next 4 h. The slices were then rinsed for 15 min (4 times) in 0.5 ml ice-cold saline to remove any free isotope, weighed, and frozen at –20°C until analyzed. The slices were digested with 1 mg/ml papain and aliquots in a scintillation cocktail (Ecosint, National Diagnostics, Atlanta, GA, USA) were counted using a Beckman LS68 scintillation counter by assessing the incorporation of [^{35}S]-sulfate into cetylpyridinium chloride precipitable material[7].

Nitrotyrosine immunohistochemistry. Cartilage slices that were cultured in the presence or absence of compression loading for the times indicated were fixed in Zamboni solution at 4°C for 8 h. After cryoprotection, the slices were embedded in OCT compound, frozen in liquid nitrogen, cut in 8- or 10-µm sections on a cryostat, and mounted on glass slides. Cryosections were incubated with anti-nitrotyrosine antibody (1:10) for 24 h, biotinylated anti-mouse IgG antibody (1:200) for 2 h, 3% H_2O_2 in 30% methanol for 15 min to remove endogenous peroxidase, and avidin-biotinylated horseradish peroxidase complex (ABC, Vector) for 1 h. All sections were counterstained with hematoxylin. All incubation procedures were done at room temperature[4].

Statistical analyses. The results are presented as the mean ± standard deviation (SD). To determine whether significant differences existed between untreated cartilage, cartilage slices subjected to compression loading, and ebselen-treated cartilage simultaneously subjected to compression loading, one-way analysis of variance (ANOVA) was applied. All other comparisons between the paired test and control specimens were made using the Student t test. A level of $p < 0.05$ was considered significant.

RESULTS

When different magnitudes of mechanical compression were loaded on the cartilage slices for 24 h at 0.5 Hz, no significant changes in PG synthesis, expressed as the percentage of the static, noncompressed control, were observed at 0.05 MPa of mechanical compression. However, PG synthesis was significantly stimulated with 0.1 and 0.5 MPa, but was inhibited with 2.0 MPa of mechanical compression (Figure 2).

To determine the required time of compression with 2.0 MPa to inhibit PG synthesis, we measured PG synthesis under different durations of compression. Compression for 1 h with 2.0 MPa, followed by recovery for 23 h, resulted in a significant inhibition of PG synthesis in the articular cartilage explants, relative to the uncompressed controls (Figure 3). The significant inhibition of PG synthesis relative to the uncompressed controls was unchanged by longer durations of compression (Figure 3). On the other hand, 10 min compression at 2.0 MPa in 24 h did not alter the PG synthesis of the

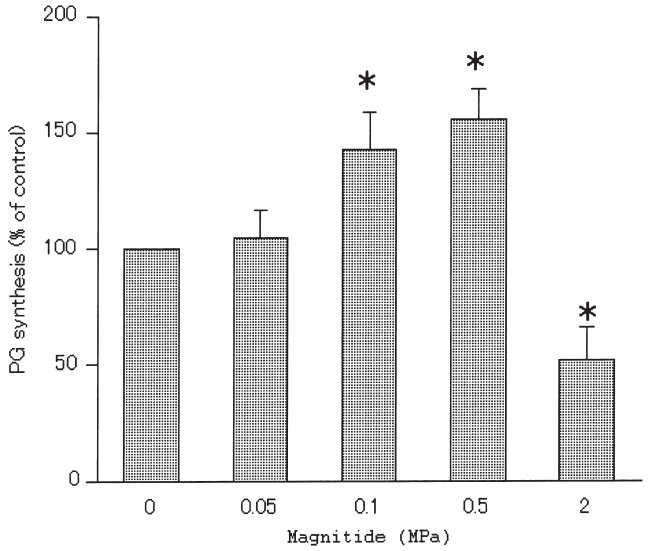

Figure 2. Effects of different magnitudes of mechanical compression on proteoglycan (PG) synthesis. Bovine cartilage slices were exposed to mechanical compression for 24 h at 0.5 Hz. Different magnitudes of compression were used: 0, 0.05, 0.1, 0.5, and 2.0 MPa. After mechanical loading, slices were incubated in the presence of $Na_2[^{35}S]$-SO_4 at 10 μCi/ml for the next 4 h and PG synthesis was then measured. Data are expressed as percentage of the static (noncompression) control. Columns and bars represent the mean and SD of 6 samples. *$p < 0.01$.

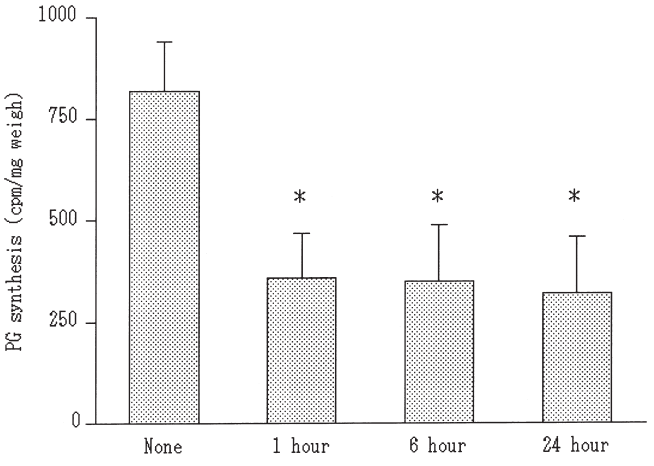

Figure 3. Effects of different compression durations on proteoglycan (PG) synthesis. Bovine cartilage slices were exposed to 2.0 MPa of mechanical compression for the indicated time periods at 0.5 Hz as follows: None (control), no compression; 1 h, compression for 1 h with 23 h of recovery; 6 h, compression for 6 h with 18 h recovery; 24 h, compression for 24 h, no recovery. Then the slices were incubated in the presence of $Na_2[^{35}S]$-SO_4 at 10 μCi/ml for the next 4 h and PG synthesis was measured. Columns and bars represent mean and SD of 6 samples. *$p < 0.01$.

articular cartilage explants (data not shown). Therefore, all compression experiments were performed on explants cultured with compression for 1 h at 2.0 MPa, followed by recovery for 23 h.

To determine whether inhibition of PG synthesis was based on the cell damage, we performed TUNEL labeling to determine the presence of chondrocyte apoptosis. No TUNEL labeling was found in the cartilage slices with compression for 1 h at 2.0 MPa, followed by recovery for 23 h. On the other hand, few TUNEL-positive cells were found in the cartilage slices with compression for 24 h with 2.0 MPa (Figure 4).

To determine the induction of ROS with 1 h of compression loaded on the cartilage, we measured ROS levels in the cartilage 3 and 6 h after different magnitudes of compression, and found that compression loading on the cartilage enhanced induction of ROS in a magnitude-dependent fashion (Figure 5). We then determined the involvement of ROS in the compression-inhibition of PG synthesis; the effects of the antioxidant, ebselen, were examined in cartilage slices exposed to 2.0 MPa of mechanical compression for 1 h at 0.5 Hz. The ROS inhibitor ebselen reversed the mechanical inhibition of PG synthesis (Figure 6). Further, to examine the kinetics of this effect, different concentrations of ebselen were added to this system and we found that ebselen reversed the compression-inhibited synthesis of PG in a dose-dependent fashion (Figure 7).

To determine whether compression of the cartilage explants induced ROS synthesis, cartilage slices, compressed (2.0 MPa) for 1 h at 0.5 Hz in the presence or absence of ebselen (100 μM), were then incubated with a reaction mixture containing the chemiluminescent probe (L-012, 0.15 mg/ml). The compression of cartilage clearly enhanced chemiluminescence when compared to the static control; ebselen inhibited this compression-enhanced chemiluminescence (Figure 8). Levels of ROS were also measured at different times (0, 1, 3, 12, and 24 h) after cartilage slices were exposed to mechanical compression at 2.0 MPa for 1 h at 0.5 Hz in the presence or absence of ebselen (100 μM). Mechanical compression for 1 h at 2 MPa increased ROS production, which peaked at 3 h. This increase was abolished in the presence of the ROS selective inhibitor, ebselen (Figure 9). When chondrocytes were killed with liquid nitrogen, there was no increase in ROS (data not shown).

The expression of nitrotyrosine in cartilage slices exposed to mechanical compression (2.0 MPa) for 1 h at 0.5 Hz in the presence or absence of ebselen (100 μM) was investigated. In the absence of mechanical compression, there was no evidence of immunoreactive nitrotyrosine (Figure 10A). However, extensive immunoreactivity to the nitrotyrosine antibody was observed in and around chondrocytes 23 h after compression (Figure 10B). Ebselen abolished this compression-induced immunoreactivity (Figure 10C).

DISCUSSION

Recent studies have clearly demonstrated that ROS are not only deleterious agents involved in cartilage degradation, but they also act as integral factors in intracellular signaling mechanisms[13]. For the maintenance of normal articular cartilage integrity, the magnitude of mechanical loading is essentially important. When mechanical forces are excessive or abnormally concentrated through regions of the articular car-

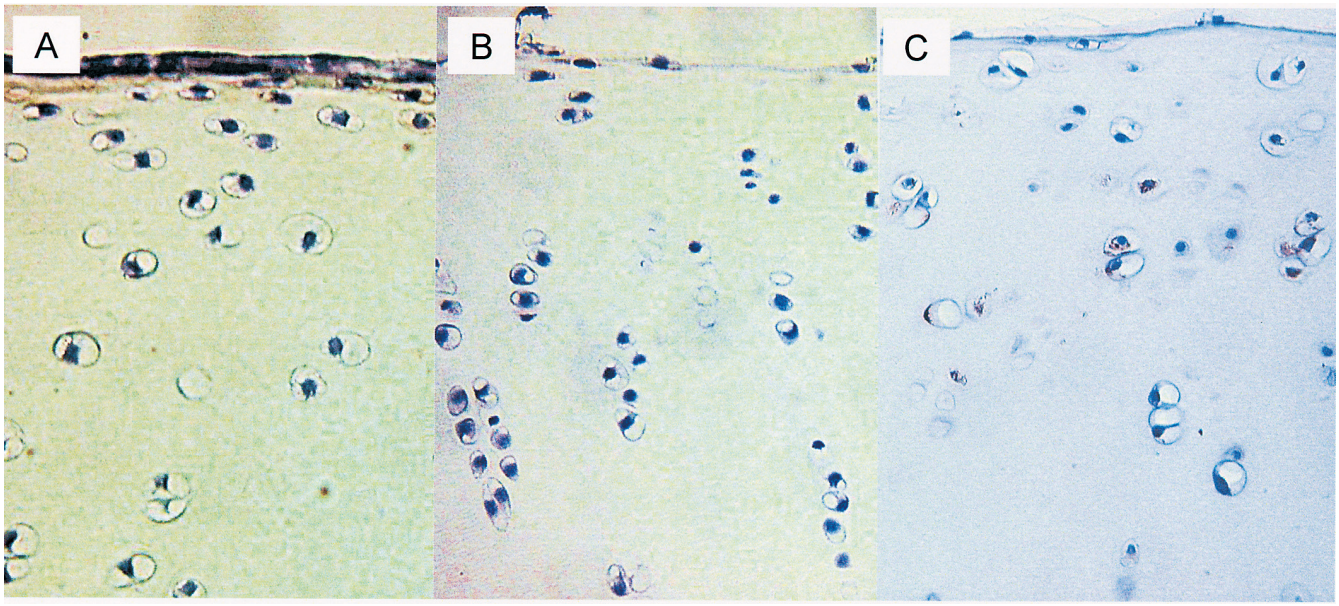

Figure 4. Representative views of TUNEL staining reveal cell viability in mechanically loaded articular cartilage. Cartilage slices were loaded with 2.0 MPa of cyclic compression for 1 h (B) and 24 h (C). A cell death detection kit was used to detect and quantify apoptosis at the single-cell level by TUNEL staining. Slides were counterstained with Mayer's hematoxylin. (A) Static control.

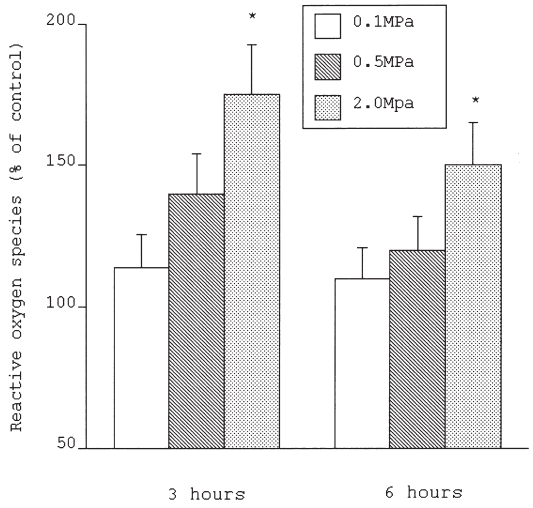

Figure 5. Effects of different magnitudes of compression on induction of reactive oxygen species (ROS). Bovine cartilage slices were exposed to 2.0 MPa of mechanical compression for 1 h at 0.5 Hz. ROS induction in the cartilage was measured after 3 and 6 h. Columns and bars represent mean and SD of 6 samples. *$p < 0.01$.

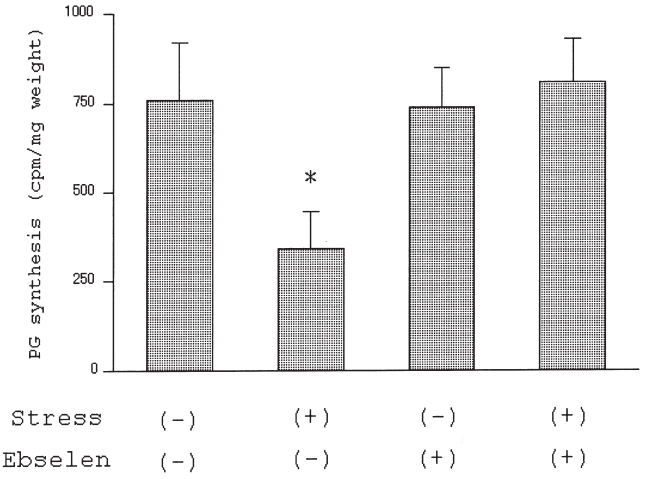

Figure 6. Effects of the antioxidant, ebselen, on mechanical stress-inhibited PG synthesis. Cartilage slices were exposed to 2.0 MPa of mechanical compression (stress) at 0.5 Hz in the absence or presence of ebselen (100 µM). The duration of loading was 1 h followed by a 23-h recovery period. Slices were incubated in the presence of $Na_2[^{35}S]$-SO_4 at 10 µCi/ml for the next 4 h. Columns and bars represent mean and SD of 6 samples in each group. Statistical analysis by one-way ANOVA. *Significantly different from all other groups including control ($p < 0.05$).

tilage, increased tissue degradation occurs. The relationship between mechanical loading and ROS synthesis by chondrocytes is not fully understood. We previously described enhanced ROS synthesis in cultured chondrocytes undergoing cyclic tensile strain[6]. Because chondrocytes in cartilage are protected by the presence of a collagen- and PG-rich matrix *in vivo*, mechanical stress must also be investigated not only with cells, but also with cartilage explants. In addition, the effect of subchondral bone should be elucidated because a particular cartilage is sandwiched between materials that are less compliant. In this context, cartilage-on-bone specimens were used[14].

Direct mechanical forces have been applied to cartilage explants, either as a dynamic shear[15], a mechanical compression[16], or both[17]. *Ex vivo* compressive loading conditions, including unconfined compression, semiconfined compression, and hydrostatic pressure, differ quite significantly from *in vivo* conditions. The most frequently used *in vitro* testing

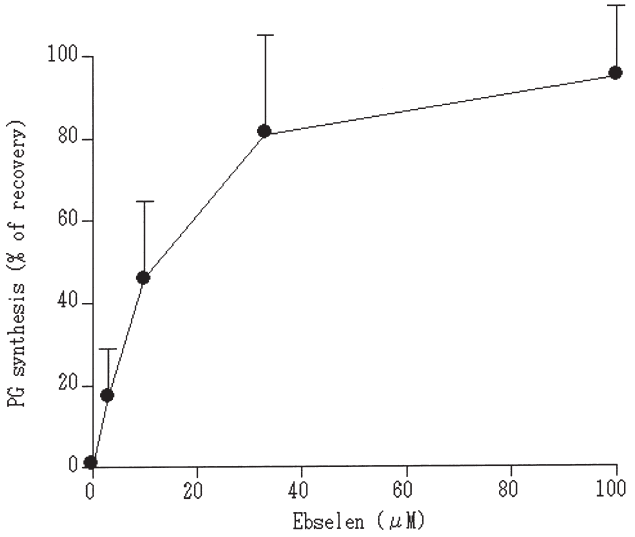

Figure 7. Dose-dependent effects of ebselen on mechanical stress-inhibited proteoglycan (PG) synthesis. Cartilage slices were exposed to 2.0 MPa of mechanical compression (stress) for 1 h at 0.5 Hz in the presence of different concentrations of ebselen. After 23 h of recovery in the presence of ebselen, PG synthesis was measured. Data are expressed as percentage of recovery. Circles and bars represent mean and SD of 6 samples. *$p < 0.01$.

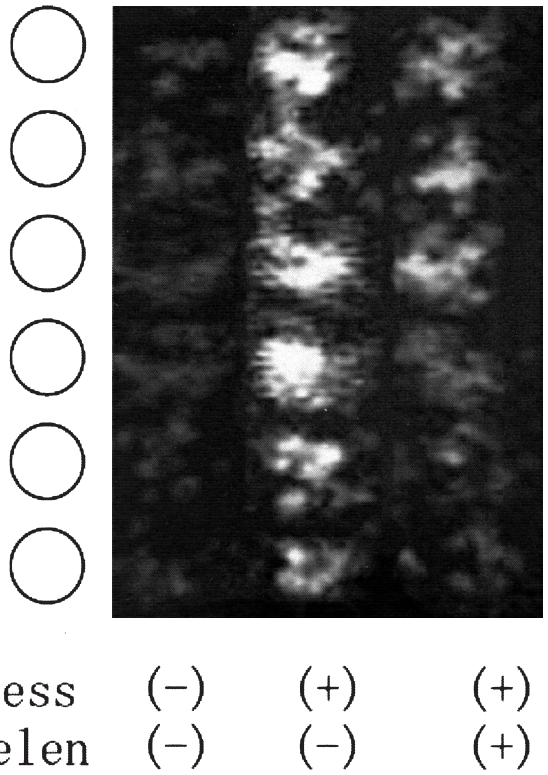

Figure 8. ROS generation with mechanical compression. Cartilage slices were exposed to 2.0 MPa of mechanical compression (stress) for 1 h at 0.5 Hz in the absence or presence of ebselen (100 μM). Cartilage slices were transferred into the wells of a black 96-well plate. A reaction mixture containing the chemiluminescent probe L-012 (0.15 mg/ml), which reacts with ROS in the cells[12], was added and incubated 1 min. These dotted scintillating photonic images were digitally processed to determine ROS-generating sources.

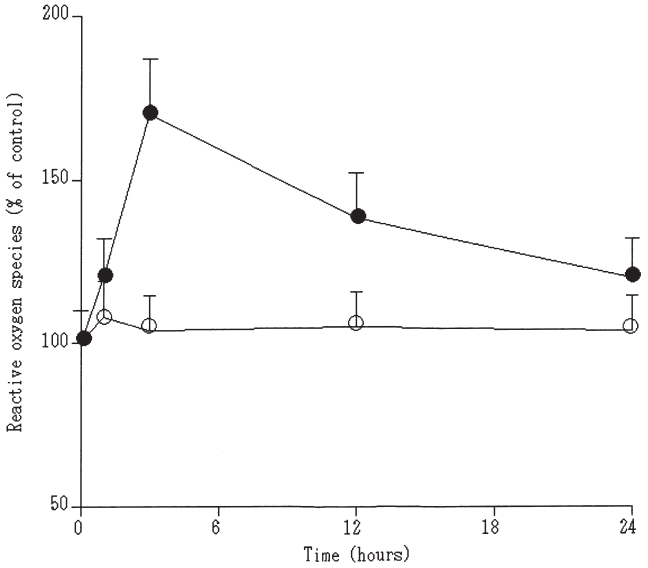

Figure 9. Effects of mechanical compression on ROS synthesis. Cartilage slices were exposed to 2.0 MPa of mechanical compression for 1 h at 0.5 Hz in the absence (●) or presence (○) of ebselen (100 μM). ROS levels were measured at the indicated times. Circles and bars represent mean and SD of 6 samples.

protocol has been the cyclic unconfined compression of articular cartilage in a bath of culture medium[18]. We used the unconfined compression system, which is technically the easiest *ex vivo* experiment to perform. Here the upper and lower surfaces of the cartilage explant are compressed between 2 impermeable or permeable platens and the lateral expansion of the disk is unrestricted[16].

We found significantly enhanced PG synthesis with 0.1 and 0.5 MPa and significantly inhibited PG synthesis with 2.0 MPa of compression. Human intraarticular pressures fluctuate between 0.2 MPa and 3–4 MPa during the normal walking cycle, but pressures can rise to 10–20 MPa in hip cartilage during standing[19]. Therefore, the range of forces used in this study system was not as extensive as those to which cartilage is exposed *in vivo*. In addition, we loaded the compression force on some cartilage slices for only 1 h, followed by a 23-h recovery period. In other studies, the duration of some of the mechanical regimens of stress exceeded those encountered during normal activities. For example, continuous static loading of cartilage for 24 h would be highly unusual[11]. Therefore, in our study, the stress conditions (2 MPa for 1 h at 0.5 Hz followed by a 23-h recovery period) that led to the inhibition of PG synthesis would not be considered to be nonphysiological.

One possible explanation of how PG synthesis was inhibited at 2.0 MPa of cyclic compression was enhanced cell death. Several recent studies suggested that mechanical loading can trigger apoptosis in cartilage, and that excessive and extensive mechanical loads can cause cell necrosis as well as structural damage[20]. In an extremely large magnitude of impact (> 20 MPa), the extent of chondrocyte death is expand-

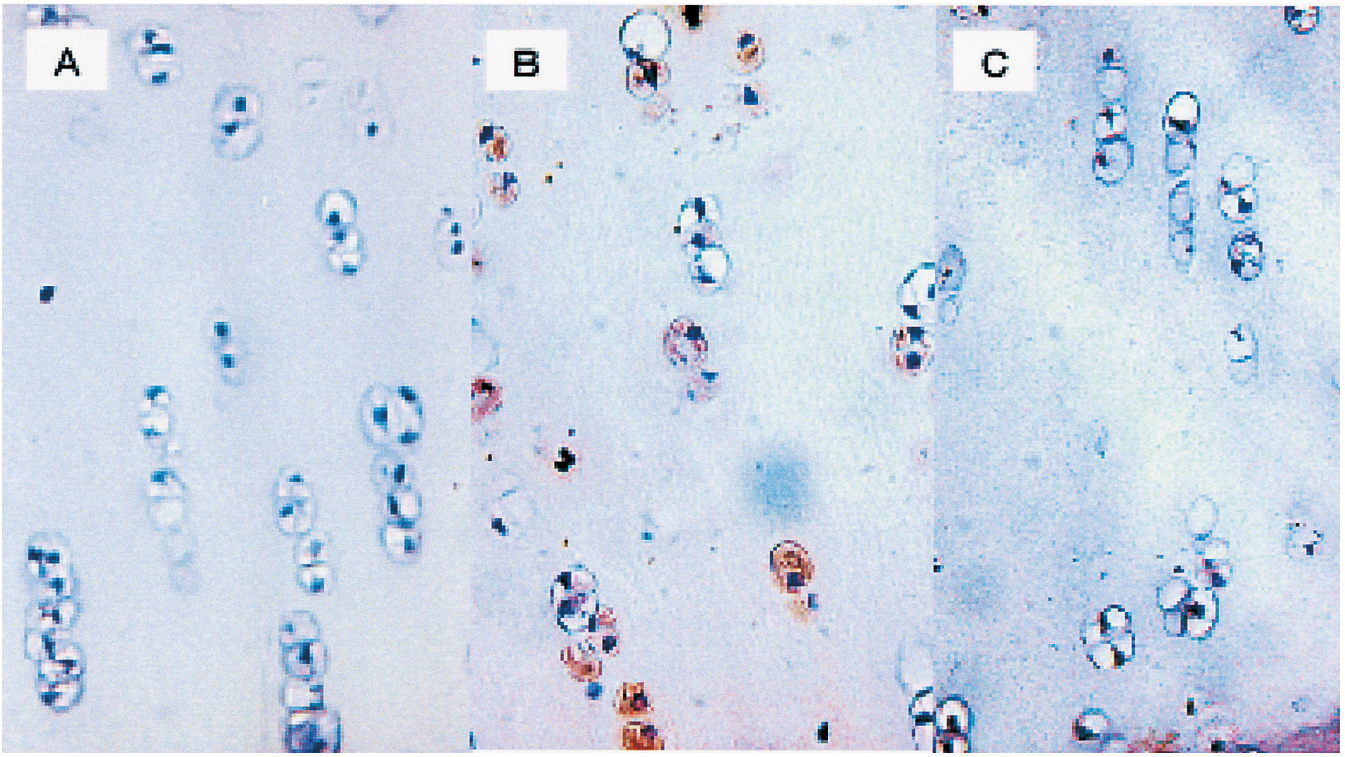

Figure 10. Immunohistochemical demonstration of the expression of nitrotyrosine in cartilage. A, B, and C are at the same magnification. Cartilage slices were exposed to 2.0 MPa of mechanical compression (stress) for 1 h at 0.5 Hz in the absence or presence of ebselen (100 µM). A: static control, B: compression in the absence of ebselen (a positive-reaction product of nitrotyrosine is evident in cartilage cells and extracellular matrix), C: compression in the presence of ebselen.

ed and associated with increased damage of the cartilage matrix. Chondrocyte death in cartilage was also observed using repeated compression with subimpacts and intermittent loads. However, the threshold of magnitude to induce cell death was over 5 MPa[21]. In our study, we loaded cyclic compression at 2 MPa for 1 h and found no increase in cell death. However, further precise experiments will be required, since we did not observe the cell necrosis. On the other hand, cyclic compression during 24 h induced chondrocyte apoptosis, consistent with another report[22].

The other possible explanation of inhibited PG synthesis was an enhanced nitric oxide (NO) synthesis, which was believed to inhibit PG synthesis. We previously reported the induction of NO from chondrocytes undergoing cyclic tensile stretch[23]. Further, increased NO production from cartilage slices with mechanical compression at 0.1–1.0 MPa has been reported[11]. Further experiments using a NO synthesis inhibitor will be necessary to determine the role of NO in this system. As well, NO has long been considered to be the primary inducer of chondrocyte apoptosis[24]; it has become clear that NO by itself cannot initiate apoptosis[13,25].

We demonstrated that the ROS scavenger, ebselen, reversed compression-inhibited synthesis of PG, suggesting the involvement of ROS in inhibition of PG synthesis. Because ROS elicit several important signaling pathways in chondrocytes[13], it is possible that ebselen reversed the cell signaling effects of ROS compared to its nonspecific free-radical effects on molecules. We also directly demonstrated ROS induction with mechanical compression. Because there were no increases in ROS levels in cartilage slices exposed to liquid nitrogen (data not shown), ROS induction must be chondrocyte-dependent. Together, these data suggest that the induction of ROS in chondrocytes undergoing mechanical stress leads to the inhibition of PG synthesis.

One important question remained. How do ROS suppress PG synthesis in the cartilage compression model? The antioxidant system against oxidative stress neutralizes the toxic effect of ROS. Therefore, a reduction in the antioxidant capacity can also have a deleterious effect on the extracellular matrix of articular cartilage. The enhanced production of endogenous ROS resulting from decreased levels of glutathione, one of the important antioxidant components, can reduce the synthesis of PG[26] and hyaluronic acid[27], which are both components of the extracellular matrix of the cartilage. Of these ROS, we focused on peroxynitrite, because we previously found it had a significant effect on cytokine-induced PG synthesis[4]. Recently, the significant role of peroxynitrite in cartilage degradation has been described[28,29]. Peroxynitrite is generated from the biradical reaction of NO and superoxide anion as follows: O_{2-} + NO → ONOO– + H^+. Because of its high reactivity, peroxynitrite was not measured directly *in vivo*. However, peroxynitrite modifies tyrosine residues in

proteins to form nitrotyrosine, which can be detected immunohistochemically. The immunoblot analyses of the articular cartilage explants demonstrated that mechanical compression induced the expression of nitrotyrosine. The concurrent generation of NO and ROS could occur in compressed cartilage and lead to the inhibition of PG synthesis[4].

We conclude that mechanical compression at 2.0 MPa inhibits the PG synthesis of cartilage and that ROS are involved in this action.

REFERENCES

1. Tanaka S, Hamanishi C, Kikuchi H, Fukuda K. Factors related to degradation of articular cartilage in osteoarthritis: a review. Semin Arthritis Rheum 1998;27:392-9.
2. Deschner J, Rath-Deschner B, Agarwal S. Regulation of matrix metalloproteinase expression by dynamic tensile strain in rat fibrochondrocytes. Osteoarthritis Cartilage 2006;14:264-72.
3. Guilak F, Fermor B, Keefe FJ, et al. The role of biomechanics and inflammation in cartilage injury and repair. Clin Orthop Relat Res 2004;423:17-26.
4. Oh M, Fukuda K, Asada S, Yasuda Y, Tanaka S. Concurrent generation of nitric oxide and superoxide inhibits proteoglycan synthesis in bovine articular chondrocytes: involvement of peroxynitrite. J Rheumatol 1998;25:2169-74.
5. Asada S, Fukuda K, Nishisaka F, Hara F, Hamanishi C. Hydrogen peroxide induces apoptosis of chondrocytes: involvement of calcium ion and extracellular signal-regulated protein kinase. Inflamm Res 2001;50:19-23.
6. Yamazaki K, Fukuda K, Matsukawa M, et al. Cyclic tensile stretch loaded on bovine chondrocytes causes depolymerization of hyaluronan: involvement of reactive oxygen species. Arthritis Rheum 2003;48:3151-8.
7. Fukuda K, Asada S, Kumano F, Saitoh M, Otani K, Tanaka S. Cyclic tensile stretch on bovine articular chondrocytes inhibits protein kinase C activity. J Lab Clin Med 1997;130:209-15.
8. Jones IL, Klamfeldt A, Sandstrom T. The effect of continuous mechanical pressure upon the turnover of articular cartilage proteoglycans in vitro. Clin Orthop Relat Res 1982;165:283-9.
9. Palmoski MJ, Brandt KD. Effects of static and cyclic compressive loading on articular cartilage plugs in vitro. Arthritis Rheum 1984;27:675-81.
10. Kuroki K, Cook JL, Stoker AM, Turnquist SE, Kreeger JM, Tomlinson JL. Characterizing osteochondrosis in the dog: potential roles for matrix metalloproteinases and mechanical load in pathogenesis and disease progression. Osteoarthritis Cartilage 2005;13:225-34.
11. Fermor B, Weinberg JB, Pisetsky DS, Misukonis MA, Banes AB, Guilak F. The effects of static and intermittent compression on nitric oxide production in articular cartilage explants. J Orthop Res 2001;19:729–37.
12. Nishinaka Y, Aramaki Y, Yoshida H, Masuya H, Sugawara T, Ichimori Y. A new sensitive chemiluminescence probe, L-012, for measuring the production of superoxide anion by cells. Biochem Biophys Res Commun 1993;193:554-9.
13. Henrotin YE, Bruckner P, Pujol JP. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthritis Cartilage 2003;11:747-55.
14. Clements KM, Bee ZC, Crossingham GV, Adams MA, Sharif M. How severe must repetitive loading be to kill chondrocytes in articular cartilage? Osteoarthritis Cartilage 2001;9:499-507.
15. Nugent GE, Aneloski NM, Schmidt TA, Schumacher BL, Voegtline MS, Sah RL. Dynamic shear stimulation of bovine cartilage biosynthesis of proteoglycan 4. Arthritis Rheum 2006;54:1888-96.
16. Wong M, Carter DR. Articular cartilage functional histomorphology and mechanobiology: a research perspective. Bone 2003;33:1-13.
17. Fitzgerald JB, Jin M, Grodzinsky AJ. Shear and compression differentially regulate clusters of functionally-related temporal transcription patterns in cartilage tissue. J Biol Chem 2006;281: 24095-103. Epub 2006 Jun 16.
18. Suh JK. Dynamic unconfined compression of articular cartilage under a cyclic compressive load. Biorheology 1996;33:289-304.
19. Hodge WA, Carlson KL, Fijan RS, et al. Contact pressures from an instrumented hip endoprosthesis. J Bone Joint Surg Am 1989; 71:1378-86.
20. Lucchinetti E, Adams CS, Horton WE Jr, Torzilli PA. Cartilage viability after repetitive loading: a preliminary report. Osteoarthritis Cartilage 2002;10:71-81.
21. Loening AM, James IE, Levenston ME, et al. Injurious mechanical compression of bovine articular cartilage induces chondrocyte apoptosis. Arch Biochem Biophys 2000;381:205-12.
22. Lin PM, Chen CT, Torzilli PA. Increased stromelysin-1 (MMP-3), proteoglycan degradation (3B3- and 7D4) and collagen damage in cyclically load-injured articular cartilage. Osteoarthritis Cartilage 2004;12:485-96.
23. Matsukawa M, Fukuda K, Yamasaki K, Yoshida K, Munakata H, Hamanishi C. Enhancement of nitric oxide and proteoglycan synthesis due to cyclic tensile strain loaded on chondrocytes attached to fibronectin. Inflamm Res 2004;53:239-44.
24. Hashimoto S, Ochs RL, Komiya S, Lotz M. Linkage of chondrocyte apoptosis and cartilage degradation in human osteoarthritis. Arthritis Rheum 1998;41:1632-8.
25. Matsushita T, Fukuda K, Yamazaki K, et al. Hypoxia-induced nitric oxide protects chondrocytes from damage by hydrogen peroxide. Inflamm Res 2004;53:344-50.
26. Baker MS, Feigan J, Lowther DA. Chondrocyte antioxidant defenses: the roles of catalase and glutathione peroxidase in protection against H_2O_2 dependent inhibition of proteoglycan biosynthesis. J Rheumatol 1988;15:670-7.
27. Bates EJ, Lowther DA, Johnson CC. Hyaluronic acid synthesis in articular cartilage: an inhibition by hydrogen peroxide. Biochem Biophys Res Commun 1985;132:714-20.
28. Carlo MD Jr, Loeser RF. Increased oxidative stress with aging reduces chondrocyte survival: correlation with intracellular glutathione levels. Arthritis Rheum 2003;48:3419-30.
29. Bezerra MM, Brain SD, Greenacre S, et al. Reactive nitrogen species scavenging, rather than nitric oxide inhibition, protects from articular cartilage damage in rat zymosan-induced arthritis. Br J Pharmacol 2004;141:172-82.