Influence of Oxygen Tension on Interleukin 1-Induced Peroxynitrite Formation and Matrix Turnover in Articular Cartilage

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ABSTRACT. ***Objective.*** Osteoarthritis is characterized by the degradation of articular cartilage. The catabolic activity of chondrocytes is partly regulated by nitric oxide (NO), which with superoxide (O_2^-) leads to the formation of peroxynitrite ($OONO^-$), a potentially damaging reactive species. Cartilage is avascular and functions at reduced oxygen tension. We investigated whether oxygen tension influences the effects of interleukin 1 (IL-1) on peroxynitrite formation and cartilage matrix metabolism.

Methods. Porcine cartilage explants were incubated at either 1% O_2 or 20% O_2 with either 1 ng/ml IL-1α, 25 μM MnTE-2-PyP^{5+} [Mn porphyrin-based catalytic antioxidant, Mn(III) tetrakis(N-ethylpyridinium-2-yl)porphyrin], or 1 ng/ml IL-1 + 25 μM MnTE-2-PyP^{5+} to decrease peroxynitrite formation. Nitrotyrosine, formed by nitration of tyrosine by peroxynitrite, was measured by immunoblot. Proteoglycan and collagen synthesis and proteoglycan degradation were also determined.

Results. IL-1-induced peroxynitrite formation was decreased in 1% O_2 as compared to 20% O_2. MnTE-2-PyP^{5+} inhibited IL-1-induced peroxynitrite formation in either 1% O_2 or 20% O_2. In 1% O_2 (but not in 20% O_2), Mn porphyrin significantly inhibited IL-1-induced proteoglycan degradation. IL-1 decreased both proteoglycan and collagen II synthesis in cartilage explants in 1% O_2 or 20% O_2, but MnTE-2-PyP^{5+} did not prevent these anti-anabolic effects. MnTE-2-PyP^{5+} alone caused a significant decrease in collagen synthesis at 20% O_2 but not at 1% O_2.

Conclusion. Our findings show that oxygen tension alters IL-1-induced peroxynitrite formation, which can influence proteoglycan degradation. Oxygen tension may influence the effects of reactive oxygen and nitrogen species on matrix homeostasis. (First Release Jan 15 2007; J Rheumatol 2007;34:401–7)

Key Indexing Terms:
OXYGEN TENSION NITRIC OXIDE SUPEROXIDE DISMUTASE MIMETIC
INTERLEUKIN 1 ARTICULAR CARTILAGE PROTEOGLYCAN

Osteoarthritis (OA) is a progressive disease characterized by the degradation of articular cartilage, leading to the clinical symptoms of joint pain and inflammation. Although there has been significant debate about whether this disease represents an inflammatory condition, it is now apparent that the pathogenesis of OA involves the upregulation of proinflammatory mediators and cytokines within the joint fluid and cartilage extracellular matrix[1]. For example, arthritis pain and inflammation are currently treated by cyclooxygenase inhibitors, drugs that block prostaglandin production[2]. Recent studies have implicated nitric oxide (NO) as a novel target in the treatment of arthritis[3]. Further, concentrations of proinflammatory cytokines such as interleukin 1 (IL-1) and tumor necrosis factor-α (TNF) are increased in arthritic joints, and may contribute to increased production of NO in arthritic compared to healthy cartilage[4,5]. NO synthesis is catalyzed by the enzyme NO synthase (NOS). NOS2, the inducible form of NOS, is responsible for NO production in cartilage[5]. NOS1 (neuronal NOS) and NOS3 (endothelial NOS) have not been found in cartilage. The exact actions of NO in arthritic joints remain unclear, although *in vivo* studies have shown that inhibition of NOS decreases the occurrence and progression of arthritis in experimental animals[6-8].

NO is a highly reactive radical that is rapidly catabolized after synthesis. It reacts with superoxide (O_2^-) to form peroxynitrite ($OONO^-$), another reactive species that has also been implicated in the pathogenesis of arthritis. Using bovine chondrocytes grown in monolayer, studies have found that exogenous peroxynitrite leads to nuclear translocation of nuclear factor-κB (NF-κB) subunit p65, an event that initiates cata-

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bolic activity in cartilage[9]. Also, inhibition of peroxynitrite formation by superoxide dismutase abrogates IL-1-induced proteoglycan degradation in human cartilage explants[10].

The formation of O_2^-, NO, and peroxynitrite requires oxygen[11]. Therefore oxygen levels in cartilage likely influence production of these inflammatory mediators. Cartilage is an avascular tissue composed of a small population of chondrocytes embedded in a dense matrix of collagen II and proteoglycans. Because cartilage lacks a dedicated blood supply, it functions at a reduced oxygen tension compared to most tissues. Oxygen and other nutrients must diffuse through the synovial fluid and cartilage extracellular matrix, creating a nonuniform oxygen environment throughout the thickness of the tissue. Mathematical models suggest that oxygen levels may range from 5% O_2 on the surface to 1% O_2 in the deep zone[12], while measured oxygen tension in articular cartilage ranges from 7% (53 mm Hg) in the superficial layer to less than 1% (7.6 mm Hg) in the deep zone[13]. The oxygen tension of synovial fluid in humans is 6.5–9.0% (50–70 mm Hg)[14]. At rest, the synovial joint is a relatively hypoxic environment compared with a mobilized joint, and the joint becomes even more hypoxic during stress due to inflammation or mechanical loading[15]. Further, mechanical loading can alter the production of NO and prostaglandins in cartilage[16,17]. In arthritic cartilage, oxygen delivery is compromised as a result of decreased capillary density and deep placement of capillaries within the synovial sac of arthritic joints[18]. Therefore, it is likely that articular cartilage affected by rheumatoid arthritis (RA) and OA is more hypoxic than normal cartilage[19]. Levels of 1% O_2 in articular cartilage therefore may be more representative of pathophysiological levels of oxygen tension.

We previously investigated the effects of low oxygen tension (1% O_2) on NO and prostaglandin production in porcine cartilage explants, and found that low oxygen significantly decreases IL-1-induced NO production[17]. In addition, oxygen tension alters the induction of NO by mechanical stress[20]. Since NO reacts with O_2^- to form peroxynitrite in a diffusion-limited manner, we sought to determine the effect of low oxygen tension on the production of these inflammatory mediators. We used a superoxide dismutase (SOD) mimetic, Mn(III) tetrakis(N-ethylpyridinium-2-yl)porphyrin (MnTE-2-PyP^{5+})[21-23]. Due to its significantly smaller size (965 Da) than the native SOD enzyme (32,000 Da), MnTE-2-PyP^{5+} is expected to localize in tissues and intracellularly. The reaction between NO and O_2^- leading to peroxynitrite formation is diffusion-limited and is reportedly faster than the elimination of the O_2^- by SOD enzyme[24]. Thus some O_2^- may escape the action of SOD and react with NO, forming peroxynitrite. However, in addition to dismuting O_2^-, MnTE-2-PyP^{5+} is nearly as effective in eliminating peroxynitrite and its degradation product the carbonate radical, CO_3^- [25,26]. MnTE-2-PyP^{5+} can also react with NO on a stoichiometric basis[27]. Finally, MnTE-2-PyP^{5+} affects signaling pathways (inactivating apolipoprotein-1, NF-κB, and hypoxia-inducible factor-1) either by reducing reactive species levels or by interacting with signaling proteins directly[28-30].

We hypothesized that in low oxygen tension, MnTE-2-PyP^{5+} would decrease IL-1-induced peroxynitrite production, by either reducing it directly or by decreasing O_2^- levels. Further, we examined the hypothesis that this inhibition of peroxynitrite formation would reverse the catabolic effects of IL-1 on cartilage matrix biosynthesis in a porcine cartilage explant model.

MATERIALS AND METHODS

Tissue collection. Full-thickness explants of articular cartilage were harvested within 3 h of sacrifice from the femoral condyles and patellar grooves of skeletally mature female pigs, using a 4 mm-diameter biopsy punch. Cartilage explants were placed in individual wells of a 48-well culture plate in Dulbecco's modified Eagle's media with 10% fetal bovine serum, 10 μM HEPES buffer, 0.01 mM essential amino acids, 10 units/ml penicillin/streptomycin (all from Gibco, Gaithersburg, MD, USA), and then incubated for 3 days prior to experiments to allow for stabilization of baseline NO levels[16,17].

Experimental design. Cartilage explants were divided into 2 groups: one to be incubated at 1% O_2 (with 5% CO_2, 94% N_2) and one to be incubated at 20% O_2 (with 5% CO_2, 95% air) for 72 h. The explants in these groups were further divided into the following 4 culture media conditions in each oxygen tension: (1) control, (2) recombinant porcine IL-1α (IL-1α), (3) 25 μM MnTE-2-PyP^{5+}, and (4) IL-1 + 25 μM MnTE-2-PyP^{5+} to inhibit peroxynitrite formation. We chose concentrations of IL-1 based on dose-response studies determining the ability of IL-1α to inhibit proteoglycan and collagen II synthesis as measured by radioisotope incorporation. All data reported in this study reflect a concentration of 1 ng/ml IL-1, except where noted. To determine rates of proteoglycan and collagen II synthesis, 37.5 μg/ml ascorbate-2-phosphate, 10 μCi/ml ^{35}S-sulfate, and 20 μCi/ml ^{3}H-proline were added to experimental media. Incorporation of ^{35}S-sulfate into the tissue provides a measure of proteoglycan synthesis in the cartilage matrix, while ^{3}H-proline incorporation provides a measure of collagen synthesis. Explants (3 pigs, 4 explants/pig/experimental group) were then incubated in either 1% or 20% O_2 for 72 h. One explant was taken to determine the viability of the tissue for each group tested. Mn(III) porphyrin was synthesized as described[21].

Hypoxia chamber. We used a modular incubator chamber (Billups-Rothenberg, Del Mar, CA, USA) to culture articular cartilage explants in a low-oxygen environment. Ambient air was evacuated from the chamber and replaced with a mixture of 1% O_2, 5% CO_2, and 94% N_2 (Raleigh Welders Supply, Raleigh, NC, USA) by flushing the chamber with the hypoxic gas via an inlet tube while the outlet tube remained open for 2.5 minutes. The inlet and outlet tubes were then sealed with tubing clips, and the modulator incubator chamber was incubated at 37°C.

Immunoblots for 3-nitrotyrosine. Immunoreactivity for 3-nitrotyrosine was used as a surrogate for peroxynitrite formation. Protein was extracted from cartilage explants from 3 joints, and protein concentrations were determined using the Coomassie blue assay (Calbiochem, San Diego, CA, USA), and stored at –80°C in 20 μg aliquots until further analyses. Twenty micrograms of protein was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a 4–15% Tris-HCl Ready gel (Bio-Rad). The membrane was blocked overnight at 4°C, and the protein reacted with monoclonal antibody to 3-nitrotyrosine (Chemicon, Temecula, CA, USA) at a concentration of 1:2000 overnight at 4°C. The membrane was washed 3 times with TTBS buffer before incubation with goat anti-mouse IgG secondary antibody conjugated to horseradish peroxidase (Pierce) at a concentration of 1:2000 for 1 h at room temperature, and detected with Supersignal West Femto maximum sensitivity substrate (Pierce), according to manufacturer's instructions.

Determination of proteoglycan and collagen II synthesis by radioisotope incorporation. Cartilage explants were removed from experimental media after 72 h of incubation in either 1% O_2 or 20% O_2, weighed, and then washed

4 times in cold 1.0 mM proline and 0.8 mM sodium sulfate to remove any unincorporated radioisotope. Each explant was digested in 1 ml soluene (Perkin-Elmer, Boston, MA, USA) in a glass scintillation vial for 48 h or until tissue was no longer grossly visible. Four milliliters of Hionic-Fluor scintillation fluid (Perkin-Elmer) was then added to each vial, and each sample was counted with a scintillation counter. Data are reported as disintegrations per minute of ^{3}H and ^{35}S normalized to cartilage explant wet weight.

Determination of proteoglycan release using the dimethylmethylene blue (DMB) assay. Media from cartilage explant experiments were stored at –80°C until the DMB assay was performed, as a measure of proteoglycan degradation and release. Briefly, 20 μl of culture media was taken from each individual explant (N = 3 pigs, n = 4 explants/pig/experimental group) and mixed with 125 μl of 1,9 dimethylmethylene blue (pH = 3.0). The proteoglycan concentration in each sample was then determined by absorbance at 540 nm on a Tecan plate reader (Tecan GmbH, Salzburg, Austria) and compared to a standard curve generated with chondroitin-6-sulfate (Sigma, St. Louis, MO, USA). Results were normalized to explant wet weight.

Cell viability. Cell viability was measured by the Live/Dead assay (Molecular Probes, Carlsbad, CA, USA). Briefly, in all experiments an additional articular cartilage explant was taken at the end of the 72 h culture period for viability testing. These explants were washed in phosphate buffered saline (PBS) and incubated for 20 min in PBS containing 2.5 ml calcein-AM as a label of viable cells and 2.5 ml ethidium homodimer-1 as a label for dead cells. The explants were then mounted on a Lab-Tek chambered coverglass (Nunc, Naperville, IL, USA) and imaged using a confocal laser scanning microscope (LSM 510, Zeiss, Thornwood, NY, USA).

Statistical analysis. Statistical significance was determined by analysis of variance and the Newman-Keuls test for post-hoc comparisons in the Statistica software program (Statsoft, Tulsa, OK, USA).

RESULTS

Effects of oxygen tension and MnTE-2-PyP^{5+} on peroxynitrite production. Peroxynitrite production was measured by immunoblots for 3-nitrotyrosine, a marker of peroxynitrite formation (Figure 1A). Quantitation of band density by den-

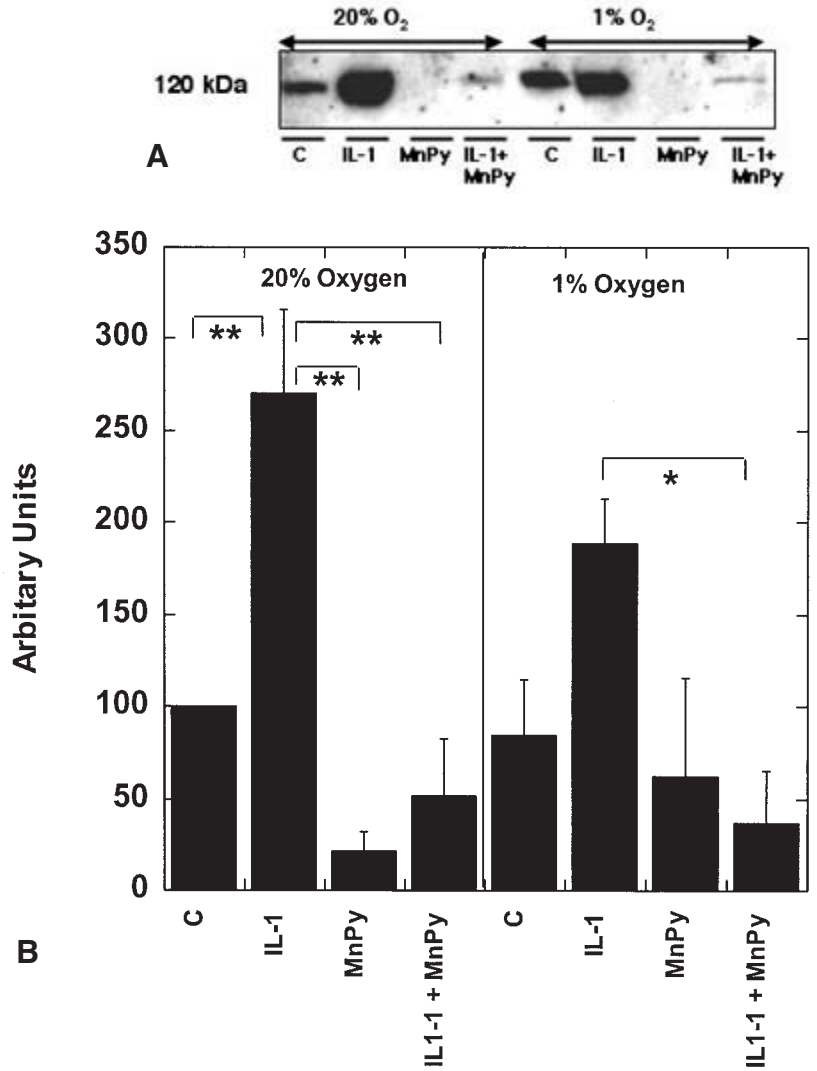

Figure 1. Peroxynitrite formation as measured by 3-nitrotyrosine immunoblot. Peroxynitrite production was measured by 3-nitrotyrosine immunoblot after 72 h in either 1% O_2 or 20% O_2 in the presence of 1 ng/ml IL-1α, 1 ng/ml IL-1α + 25 μM MnTE-2-PyP^{5+}, and MnTE-2-PyP^{5+} alone. (A) Immunoblot and (B) representation of band intensities reported as percentage of control incubated at 20% O_2. Band is at ~120,000 Da. MnPy: MnTE-2-PyP^{5+}. Data are mean + SEM, N = 3 pigs. *p < 0.05; **p < 0.01.

sitometry was carried out on blots from 3 joints and expressed as a percentage of the control incubated at 20% O_2 (Figure 1B). Low amounts of 3-nitrotyrosine were present in control conditions in both oxygen tensions. IL-1 increased levels of 3-nitrotyrosine in both oxygen tensions, which was statistically significant at 20% O_2 but not at 1% O_2. IL-1-induced 3-nitrotyrosine formation was significantly reduced in both oxygen tensions by MnTE-2-PyP^{5+}. MnTE-2-PyP^{5+} alone also caused a trend towards inhibition of peroxynitrite formation.

Effects of oxygen tension and IL-1-induced peroxynitrite on proteoglycan synthesis in cartilage explants. Proteoglycan synthesis was measured by ^{35}S-sulfate incorporation in both 1% and 20% O_2 in the presence of IL-1α and IL-1α + MnTE-2-PyP^{5+}, and with MnTE-2-PyP^{5+} alone. IL-1α decreased proteoglycan synthesis compared to control in both 1% O_2 and 20% O_2, which showed a greater level of significance at 20% O_2. Inhibiting peroxynitrite formation with MnTE-2-PyP^{5+} did not prevent IL-1-induced decreases in proteoglycan synthesis in either oxygen tension. Proteoglycan synthesis with MnTE-2-PyP^{5+} alone did not differ from control in either oxygen tension, although there was a trend towards increased proteoglycan synthesis in cartilage incubated at 1% O_2 with MnTE-2-PyP^{5+} (Figure 2).

Effects of oxygen tension and IL-1-induced peroxynitrite on collagen synthesis in cartilage explants. The synthesis of collagen (predominantly type II) was measured by ^{3}H-proline incorporation in both 1% and 20% O_2 in the presence of IL-1α, IL-1α + MnTE-2-PyP^{5+}, and with MnTE-2-PyP^{5+} alone. IL-1α significantly decreased collagen II synthesis compared to control in both oxygen tensions. Inhibiting peroxynitrite formation with MnTE-2-PyP^{5+} did not prevent IL-1-induced decreases in collagen II synthesis in either oxygen tension (Figure 3). MnTE-2-PyP^{5+} alone caused a significant decrease in collagen II synthesis in 20% O_2, but not in 1% O_2.

Effects of oxygen tension and IL-1-induced peroxynitrite on proteoglycan degradation and release from cartilage explants. Release of proteoglycans was measured in culture media from cartilage explants in both 1% and 20% O_2 in the presence of IL-1α, IL-1α + MnTE-2-PyP^{5+}, and with MnTE-2-PyP^{5+} alone. IL-1α significantly increased proteoglycan release compared to control in both oxygen tensions. Inhibiting peroxynitrite formation with MnTE-2-PyP^{5+} significantly decreased IL-1-induced proteoglycan release in 1% O_2, but not in 20% O_2. Proteoglycan release with MnTE-2-PyP^{5+} alone did not differ from control in either oxygen tension (Figure 4).

Viability studies. Explants were cultured in either 1% or 20% O_2 in the presence of IL-1α, IL-1α + MnTE-2-PyP^{5+}, or with MnTE-2-PyP^{5+} alone for 72 h. The viability of the explants was about 95% or greater.

DISCUSSION

The goals of our study were to determine the role of oxygen tension in regulating production of peroxynitrite by IL-1 and its downstream effects on cartilage matrix turnover. Our data show that low oxygen tension decreases IL-1-induced peroxynitrite production, and that inhibiting superoxide and/or peroxynitrite formation in a low oxygen environment prevents IL-1-induced proteoglycan release. It is widely accepted that under low oxygen tension-hypoxia levels of reactive oxygen species are increased[31]. We have previously observed that IL-1-induced NO levels are decreased under hypoxic conditions[17]. Despite the increase in O_2^-, a decrease in NO levels under low-oxygen, hypoxic conditions would likely be the

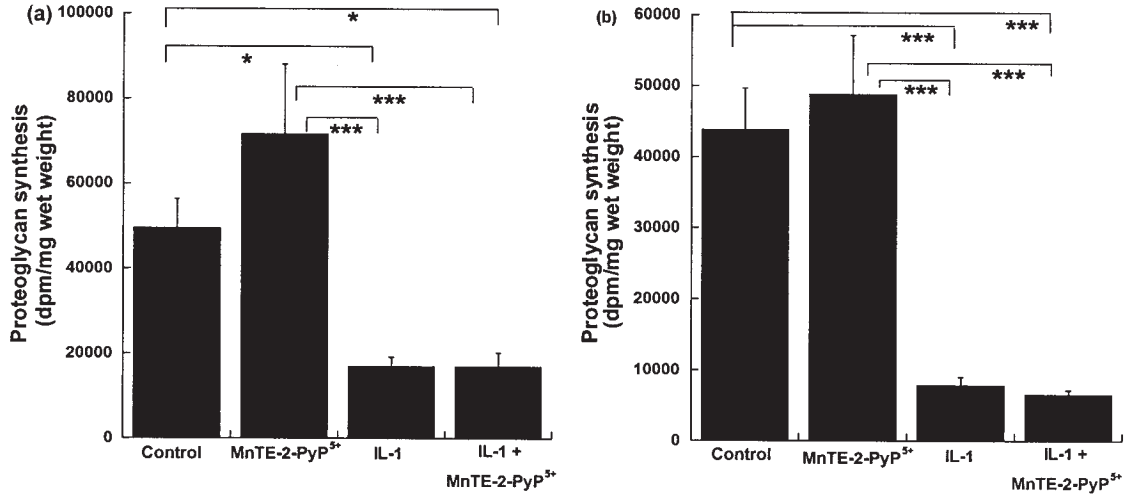

Figure 2. Effect of IL-1-induced peroxynitrite on proteoglycan synthesis in cartilage explants in 1% O_2 or 20% O_2. Proteoglycan synthesis was measured by ^{35}S incorporation into cartilage explants after 72 h in either (a) 1% O_2 or (b) 20% O_2 in the presence of 1 ng/ml IL-1α, 1 ng/ml IL-1α + 25 μM MnTE-2-PyP^{5+}, and MnTE-2-PyP^{5+} alone. Mean ± SEM, N = 3 pigs, n = 11 explants per group. *p < 0.05; ***p < 0.001.

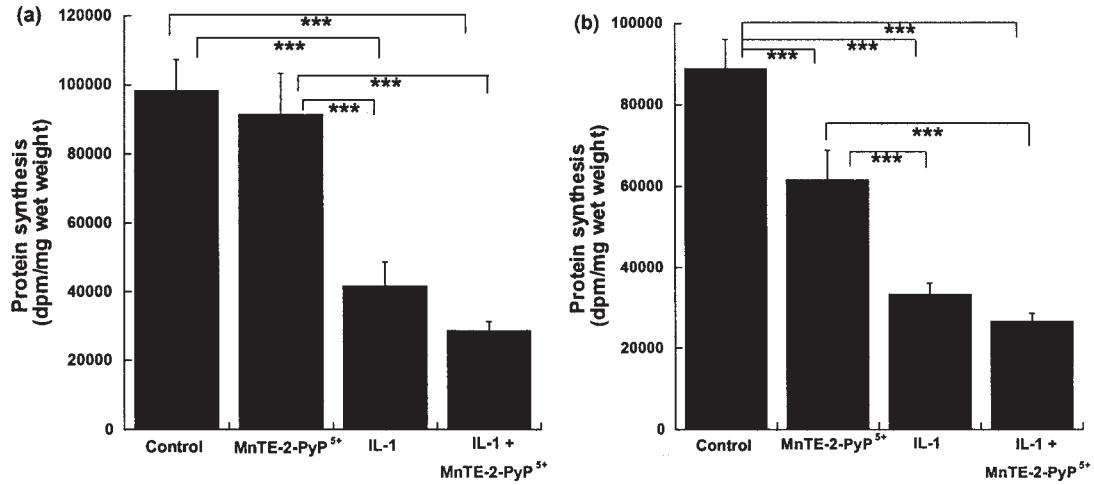

Figure 3. Effect of IL-1-induced peroxynitrite on collagen II synthesis in cartilage explants in 1% O_2 or 20% O_2. Collagen II synthesis was measured by ^3H incorporation into cartilage explants after 72 h in either (a) 1% O_2 or (b) 20% O_2 in the presence of 1 ng/ml IL-1α, 1 ng/ml IL-1α + 25 μM MnTE-2-PyP^{5+}, or MnTE-2-PyP^{5+} alone. Mean ± SEM, N = 3 pigs, n = 11 explants per group. ***p < 0.001.

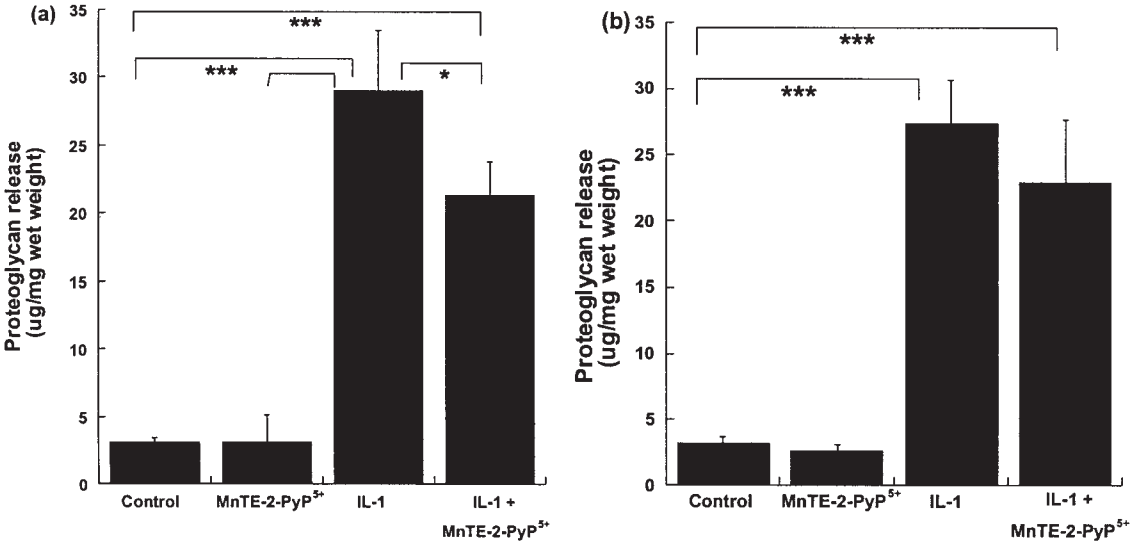

Figure 4. Effect of IL-1-induced peroxynitrite on proteoglycan release from cartilage explants in 1% O_2 or 20% O_2. Proteoglycan release was measured by dimethylmethylene blue assay in the culture media from cartilage explants after 72 h in either (a) 1% O_2 or (b) 20% O_2 in the presence of 1 ng/ml IL-1α, 1 ng/ml IL-1α + 25 μM MnTE-2-PyP^{5+}, or MnTE-2-PyP^{5+} alone. Mean ± SEM, N = 3 pigs, n = 11 explants per group. *p < 0.05; ***p < 0.001.

cause of the decrease in peroxynitrite observed in this work, and is consistent with studies on the effects of fluxes of these 2 radicals on peroxynitrite formation *in vitro* and *in vivo*[32]. Our results provide further evidence that the oxygen tension of cartilage can alter the effects of local reactive oxygen and nitrogen species on matrix homeostasis.

Our current data support a role for superoxide and/or peroxynitrite in the degradation of cartilage at low oxygen tensions. Several studies have shown that NO, a precursor to peroxynitrite, is involved in cartilage degradation. In bovine inflammatory and septic arthritis models, proinflammatory cytokines and endotoxin increase NO production and collagenase activity. Inhibiting NO production in these systems prevents cytokine- and endotoxin-induced increases in collagenase activity[33]. Also, a recent study conducted with human osteoarthritic chondrocytes showed that inhibition of bacterial-induced NO production prevented decreases in cartilage matrix gene expression in a model of septic arthritis[34]. These studies suggest a degradative role for NO itself, but do not consider the role of NO-derived metabolites such as peroxynitrite.

The role of peroxynitrite in the pathogenesis of arthritis is

not fully understood. In our study we utilized the Mn(III) porphyrin (MnTE-2-PyP^{5+}), which can effectively eliminate both O_2^- and its reaction product with NO, peroxynitrite[21-23,25,35]. Reducing peroxynitrite formation with MnTE-2-PyP^{5+} decreased proteoglycan degradation and release by IL-1 in 1% O_2, but not in 20% O_2. The low-oxygen environment and MnTE-2-PyP^{5+} appear to work together to limit IL-1-induced peroxynitrite production and subsequent IL-1-induced increases in proteoglycan release. It is also possible, however, that the inhibition of proteoglycan release by MnTE-2-PyP^{5+} is not due to the inhibition of peroxynitrite formation by MnTE-2-Py^{5+} only, but that the chondrocytes treated with IL-1 respond differently at the 2 oxygen tensions.

While the role of IL-1-induced peroxynitrite in low oxygen is not fully understood, the role of peroxynitrite and other reactive oxygen species in arthritic cartilage has been studied in ambient oxygen tension (20% O_2). IL-1-induced decreases in proteoglycan synthesis are not prevented by inhibition of peroxynitrite formation by the SOD enzyme in a human cartilage explant model, but SOD enzyme does decrease IL-1-stimulated proteoglycan release[10]. It is likely that the SOD enzyme acts extracellularly and so affects degradative but not synthetic processes involved in matrix turnover. One study showed that reducing oxygen-centered reactive species with SOD does not decrease proteoglycan degradation induced by conditioned synovial medium in bovine articular cartilage slices[36]. Conversely, it has been shown in human articular cartilage explants cultured in ambient oxygen tension that inhibiting peroxynitrite formation with SOD decreases IL-1-stimulated proteoglycan release, suggesting a degradative role for peroxynitrite at ambient oxygen levels[10]. However, in our study, SOD mimetic did not ameliorate IL-1-induced proteoglycan degradation at 20% oxygen tension.

We first conducted preliminary experiments using native SOD enzyme at a concentration of 150 μg/ml (data not shown) and found that this high SOD concentration, which was used in Fukuda's study[10], was toxic to chondrocytes. We then utilized the catalytic antioxidant MnTE-2-PyP^{5+}, which is significantly smaller (965 Da) than the native SOD protein (32,000 Da). Previous data indicate that it localizes within mouse heart mitochondria upon single 10 mg/kg intraperitoneal administration at levels sufficient to exert its antioxidant action[37]. While MnTE-2-PyP^{5+} may enter the chondrocytes, due to its cationic nature, it may also associate with negatively charged sulfate groups of cartilage. We found in our present study that MnTE-2-PyP^{5+} reduces peroxynitrite production by chondrocytes. Further, MnTE-2-PyP^{5+} significantly diminished IL-1-induced proteoglycan degradation at physiologically relevant low-oxygen 1% O_2 tensions. A similar trend was observed at ambient oxygen tension, although it was not statistically significant.

In addition to modulating production of inflammatory mediators such as NO[17], or prostaglandin E_2[20], oxygen tension may alter expression of various genes involved in the pathogenesis of arthritis. A recent study showed an increase in IL-1-induced NOS2 gene expression in 5% O_2 as compared to 21% O_2[38]. In light of our previous work showing decreased NO production at 1% O_2, different effects on NOS2 gene expression and NO production in articular cartilage may be exerted at 5% O_2 versus 1% O_2. This effect may be attributed to the fact that 5% O_2 is a physiological oxygen tension for healthy cartilage, but 1% O_2 may represent the pathologic condition in arthritic joints.

In conclusion, our work shows that low oxygen tension decreases IL-1-induced peroxynitrite production. This effect may be due to diminished NO or O_2^- production, with resultant diminished peroxynitrite formation. Our data suggest that peroxynitrite may mediate IL-1-induced changes in proteoglycan degradation and release (but not proteoglycan or collagen II synthesis) in a low-oxygen environment. We show that peroxynitrite does not affect proteoglycan synthesis, collagen II synthesis, or proteoglycan degradation at ambient oxygen tension. Oxygen tension may be an important determinant of inflammation-mediated cartilage degradation.

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