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Aggrecan Degradation in Chondrocytes is Mediated by Reactive Oxygen Species and Protected by Antioxidants

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Reactive oxygen species (ROS) are implicated in aging of cartilage and in the pathogenesis of osteoarthritis. However, the biological role of chondrocytes-derived ROS has not been elucidated. An *in-vitro* model was developed to study the role of chondrocyte-derived ROS in cartilage matrix degradation. The primary articular chondrocytes were cultured and the aggrecan matrix was radiolabeled with 35-sulfate. The labeled aggrecan matrix was washed to remove unincorporated label and chondrocytes were returned to serum free balanced salt solution. The cell-monolayer-matrix sensitivity to oxidative damage due to either hydrogen peroxide or glucose oxidase was established by monitoring the release of labeled aggrecan into the medium. Lipopolysaccharide (LPS) treatment of chondrocyte-monolayer enhanced the release of labeled aggrecan. Catalase significantly prevented the release of labeled aggrecan in LPS-chondrocyte cultures, suggesting a role for chondrocyte-derived hydrogen peroxide in aggrecan degradation. Superoxide dismutase or boiled catalase had no such inhibitory effect. The effect of several antioxidants on LPS-chondrocyte-dependent aggrecan degradation was examined. Hydroxyl radical scavengers (mannitol and thiourea) significantly decreased aggrecan degradation. A spin trapping agent N-*tert*-butyl-phenylnitrone (but not its inactive analog *tert*-butyl-phenylcarbonate) significantly decreased aggrecan degradation. Butylated hydroxytoluene also inhibited aggrecan degradation, whereas the other lipophilic antioxidant tested, propyl gallate, had a marked dose-dependent inhibitory effect. These data indicate that general antioxidants, hydroxyl radical scavengers, antioxidant vitamins, iron chelating agents, lipophilic antioxidants, and spin trapping agents can influence chondrocyte-dependent aggrecan degradation. These studies support the role of a chondrocyte-dependent oxidative mechanism in aggrecan degradation and indicate that antioxidants can prevent matrix degradation and therefore may have a preventive or therapeutic value in arthritis. The enhancement of oxidative activity in chondrocytes and its damaging effect on matrix may be an important mechanism of matrix degradation in osteoarthritis.

Keywords: Cartilage, chondrocytes, reactive oxygen species, antioxidants, matrix degradation, osteoarthritis

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INTRODUCTION

Cartilage degeneration is a hallmark of osteoarthritis, a disease of chondrocytes.[1] The degeneration of the articular cartilage is associated with chronic pain and loss of joint function, which causes significant disability. The arthritic process results in a selective and irreversible degradation of bone and cartilage matrix.[1] In a series of reports,[2–7] we and others have documented that chondrocytes produce reactive oxygen species (ROS). The production of ROS by chondrocytes can affect the preservation of cartilage matrix. For example, ROS can mediate intracellular signaling and concurrent gene activation in chondrocytes.[8,9] Articular chondrocytes express cell-specific components of NADPH-oxidase complex, such as p22-phox, p40-phox, p47-phox and p67-phox.[10] In activated neutrophils and monocytes/macrophages the cell-specific gene products of NADPH-oxidase complex physically come together and initiate single electron reduction of oxygen and the release of ROS outside the cells. Phagocytes use toxic properties of ROS to eliminate pathogens. In contrast, the biological role of secreted ROS in cartilage is not known.

The observation that *in-vitro* exposure to ROS damages cartilage matrix suggests that chondrocyte-derived ROS may mediate matrix degradation *in-vivo*.[11–17] However, the role of ROS in cartilage matrix degradation is difficult to evaluate *in-vivo* because cartilage is avascular and ROS are extremely labile. We have developed a lipopolysaccharide (LPS) model of chondrocyte-dependent matrix degradation to decipher the role of ROS in matrix degradation. The model is simple, sensitive, and reproducible. Using this model, we provide evidence that production of hydrogen peroxide by chondrocytes is linked to aggrecan degradation. In addition, we tested a number of antioxidant compounds that can inhibit chondrocyte-dependent matrix degradation.

MATERIALS AND METHODS

Reagents

LPS from *E. coli* 0127:B8, superoxide dismutase, catalase, N-*tert*-butyl-phenylnitrone (BPN), and inactive analog of BPN (*tert*-butyl-phenylcarbonate (BPC)), desferrioxamine, ascorbic acid, propyl gallate, mannitol, taurine, thiourea, N-acetyl-cysteine (NAC), acetylcarnitine, butylated hydroxytoluene (BHT), vitamin E, 2-mercaptoethanol, glucose oxidase, and dimethylsulfoxide (DMSO) were purchased from Sigma Chem. Co. (St. Louis, MO). Hydrogen peroxide was of reagent grade from Fisher Scientific (New Jersey). Dulbecco's minimum essential medium (DMEM), fetal bovine serum (FBS), Hank's balanced salt solution (HBSS), Earl's balanced salt solution (EBSS), L-glutamine, gentamicin, HEPES buffer, penicillin, and streptomycin were purchased from GIBCO (Grand Island, NY). 35-sulfate and 51-chromium were from DuPont-NEN Research Products (Wilmington, DE).

Isolation of Rabbit Articular Chondrocytes

NZW rabbits (6–8 pounds) of either sex were killed by i.v. injection of Beuthanasia-D Special (Schering Corp., Kenilworth, NJ). This protocol has been approved by the Institutional Animal Care and Use Committee. The chondrocytes were prepared as described previously.[18] The viability of chondrocytes was confirmed by trypan blue exclusion method. Primary chondrocytes were resuspended in 10% FBS in DMEM containing antibiotics (1%) and HEPES buffer (10 mM, pH 7.4) (complete media).

Experimental Design

Primary rabbit articular chondrocytes were distributed into microtitre plates at a concentration of $2–4 \times 10^4$ cells/well in 0.2 ml of complete media. Chondrocytes were allowed to attach

and deposit matrix for 3–5 days and media was changed every 3 days. Confluent cells in microtitre plate were labeled with 1–2 μCi/well 35-sulfate during the last 24–48 h of cell culture. The unincorporated sulfate in the matrix was removed by washing the cell monolayer with warm HBSS 4–5 times by flipping the plates. Cells were returned to the albumin or serum-free EBSS. Experiments were run in triplicate or quadruplicate wells. The test reagents were added at a volume of 20 μl/well and total volume was adjusted to 200 μl with EBSS. The cultures were incubated at 37°C in a humidified 5% CO_2 incubator for 4–24 h. One hundred microliter aliquot was removed and scintillation counted. The total plastic-bound 35-sulfate labeled matrix (i.e. residuum) in control sets of wells was solubilized with 0.5 M NaOH and counted.

Effects of Antioxidants

LPS-induced aggrecan degradation was used as a background to test the effect of various antioxidants. Chondrocytes were stimulated with LPS (100 μg/ml). As a general strategy, we tested three concentrations of antioxidants, i.e., low, intermediate, and high, in a quadruplicate set of wells. The duration of culture was 4–8 h. Aggrecan degradation induced by hydrogen peroxide was used as a positive control. The cultures were processed as described above. The data are expressed as percent inhibition of aggrecan release, which was calculated assuming LPS-induced aggrecan release as 100%. The effect of antioxidants was tested in at least three independent experiments.

Statistical Analysis

Results are expressed as mean ± SEM. Total counts in residuum ± SEM are reported in individual figures or tables. There was a 10% coefficient of variation between the mean and highest and lowest counts in random wells of each experiment. The differences of the means between groups were evaluated by Student's-*t* test (Statview® program); $p < 0.05$ were considered statistically significant.

RESULTS

Hydrogen Peroxide Treatment of 35-sulfate Labeled Chondrocyte-monolayer-matrix Results in Aggrecan Degradation

Initially, the susceptibility of 35-sulfate labeled-chondrocyte-matrix to oxidative damage was investigated. Labeled chondrocyte monolayers were exposed to 1 and 10 mM hydrogen peroxide. Chondrocytes were also treated with glucose oxidase to produce a flux of hydrogen peroxide. The release of 35-sulfate was significantly enhanced at 4 and 10 h in both hydrogen peroxide and glucose oxidase exposed cultures, as compared with background release of labeled material by untreated chondrocytes (Table I). The release of 35-sulfate labeled material in the medium indicates aggrecan degradation because the label is selectively incorporated in the aggrecan.

Lipopolysaccharide Treatment of Chondrocytes Results in Release of Labeled Aggrecan

LPS treatment of chondrocytes resulted in the release of labeled aggrecan in a dose-dependent manner (Figure 1). At high LPS concentrations, aggrecan release was observed at 2 h. A time-dependent increase in aggrecan release was observed at low as well as high concentrations of LPS. The data suggest that LPS activates chondrocyte-dependent aggrecan release.

Chondrocyte Lysis does not Contribute to Aggrecan Release

Preliminary studies indicated that treatment of chondrocyte monolayers with hydrogen peroxide and glucose oxidase results in chondrocyte lysis

TABLE I Effect of hydrogen peroxide and glucose oxidase on the release of 35-S labeled aggrecan

Treatment	35-S labeled aggrecan released (Mean CPM ± SEM)			
	4 h	*p* value	10 h	*p* value
Background	751 ± 50		1170 ± 37	
Hydrogen peroxide (1 mM)	1202 ± 86	0.002*	1409 ± 141	0.34
Hydrogen peroxide (10 mM)	1452 ± 58	0.004*	1811 ± 51	0.006*
Glucose oxidase (1 U/ml)	982 ± 44	0.079	1448 ± 32	0.021*
Glucose oxidase (10 U/ml)	1020 ± 42	0.005*	1876 ± 129	0.021*

Residuum mean CPM 8572 ± 314.
*Statistically significant as compared with background CPM at respective time points.

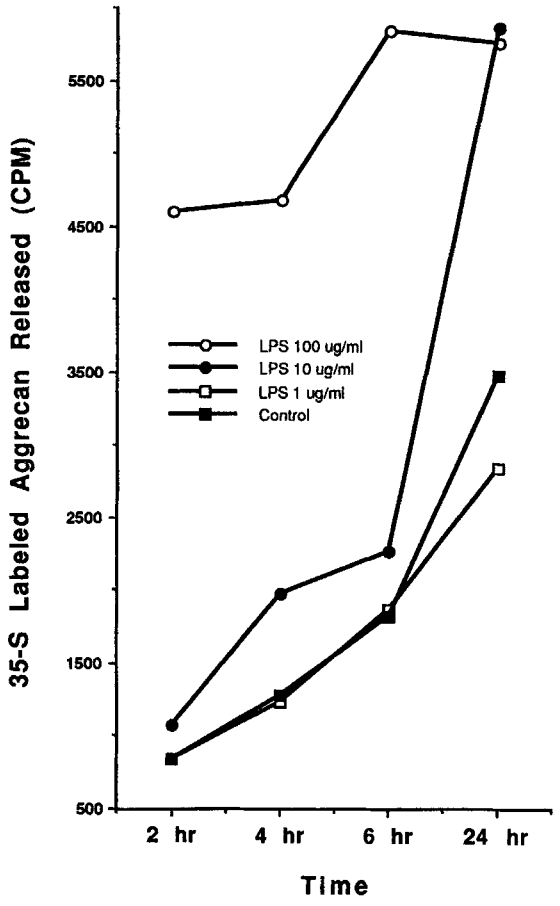

FIGURE 1 Effect of LPS on the release of 35-S labeled aggrecan by the articular chondrocytes. A monolayer of primary articular chondrocytes in microtitre plate was labeled with 35-sulfate for 24 h. Quadruplicate sets of wells were stimulated with LPS in EBSS. Replicate sets of control (untreated) and LPS-stimulated chondrocytes were harvested at specified time points and processed for scintillation counting. Total residuum for 35-S labeled aggrecan, 13120 ± 548 CPM.

as well as matrix release (data not shown). Thus it is possible that LPS treatment of chondrocytes could result in cell lysis and secondary matrix release. To investigate whether cell lysis induced by LPS could be the cause of 35-sulfate release, a set of chondrocytes was labeled with 51-chromium, and an identical set of cells of the same origin were labeled with 35-sulfate. The cells were processed in an identical fashion and stimulated with LPS (100 μg/ml). Chondrocyte lysis was monitored by quantitating the release of 51-chromium into the media and aggrecan degradation was monitored by quantitating release of 35-sulfate. LPS treatment did not cause an increase in chromium release (Figure 2), indicating that LPS treatment does not induce chondrocyte lysis. Since LPS does not have aggrecanase activity, the likely mechanism of 35-sulfate release may be LPS-induced activation and release of chondrocyte-derived products.[19] Because chondrocytes produce ROS in a burst-like fashion,[2–4] it is possible that ROS may be involved in the process of LPS-induced matrix degradation.

Catalase Inhibits Labeled Aggrecan Release by LPS-stimulated Articular Chondrocytes

Catalase treatment caused a significant decrease in the release of 35-sulfate due to either glucose oxidase or LPS (Table II). Because catalase specifically detoxifies hydrogen peroxide, the data indicate that the hydrogen peroxide produced in

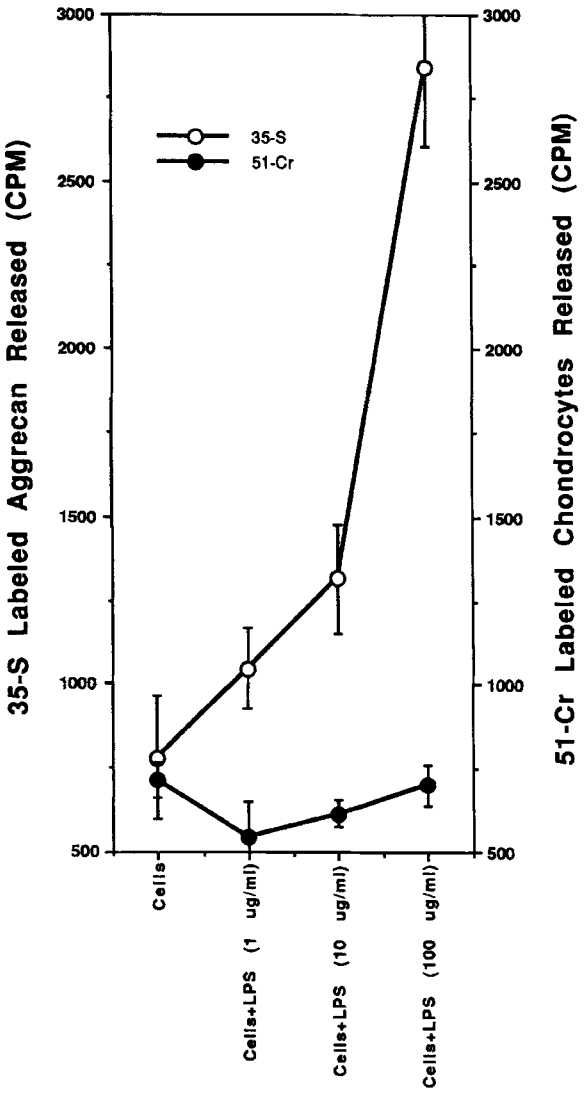

FIGURE 2 Effect of LPS on aggrecan release and cell lysis. Replicate sets of microtitre plates of primary articular chondrocytes of a single origin were labeled with 35-sulfate (1 μCi/well) or 51-chromium (1 μCi/well) for 24 h. The plates were washed and handled identically and quadruplicate sets of wells were untreated or exposed to LPS, in the final concentrations shown. The radioactive-labeled material released after 4 h incubation was monitored. Samples of 35-S labeled aggrecan were processed for scintillation counting and 51-Cr samples were counted by gamma counter. Shown are mean CPM ± SEM. Total residuum for 35-S labeled aggrecan was 6672 ± 351 CPM and for 51-Cr labeled chondrocytes 8320 ± 484 CPM.

the LPS-chondrocyte-matrix cultures is involved in matrix degradation and 35-sulfate release. This conclusion is further supported by the fact that neither boiled catalase nor SOD were effective in preventing the release of 35-sulfate labeled aggrecan. Figure 3 shows the dose-dependent effect of catalase on LPS-chondrocyte-dependent release of 35-sulfate labeled-aggrecan.

Antioxidants Inhibit the Release of 35-sulfate Labeled Aggrecan by LPS-stimulated Articular Chondrocytes

The effect of various antioxidants was tested in order to obtain additional evidence for the role of chondrocyte-derived ROS in aggrecan degradation (Table III). Vitamin E did not affect 35-sulfate release by LPS-stimulated chondrocytes, whereas vitamin C inhibited the release of 35-sulfate in LPS-stimulated chondrocytes. PBN, a spin trapping agent, significantly inhibited 35-sulfate release induced by LPS-chondrocytes; conversely, BPC, an inactive analog of PBN, did not inhibit LPS-dependent 35-sulfate release (Table III). Hydroxyl radical scavengers such as mannitol and thiourea inhibited LPS-induced 35-sulfate release. The percent inhibition with mannitol and thiourea varied between 40% and 50%; in contrast, another hydroxyl radical scavenger, DMSO, did not inhibit LPS-induced 35-sulfate release (Table III).

Lipophilic antioxidants propyl gallate and butylated hydroxytoluene, which inhibit free radical reaction, had significant inhibitory effect on LPS-induced 35-sulfate release. There was a dose-dependent inhibition of 35-sulfate release by propyl gallate. The percent inhibition with BHT varied between 39% and 51%. Desferrioxamine, an iron chelating agent, has *in-vivo* and *in-vitro* antiinflammatory activity, probably mediated by chelation of iron and prevention of hydroxyl radical formation. In our assays, only low concentrations of deferrioxamine inhibited LPS-dependent 35-sulfate release, whereas intermediate and high concentrations (10 μM)

TABLE II Inhibitory effect of catalase on aggrecan degradation.

Treatment	Concentration	35-S labeled aggrecan released CPM ± SEM	*p* value*
Background		1763 ± 180	
Glucose oxidase (GO)	10 U/ml	2814 ± 275	
GO + catalase	300 U/ml	1635 ± 196	0.02*
GO + boiled catalase		2424 ± 211	0.33
GO + SOD	50 U/ml	2537 ± 220	0.27
LPS	100 μg/ml	6885 ± 547	
LPS + catalase	300 U/ml	4614 ± 562	0.02*
LPS + boiled catalase		6240 ± 162	0.21
LPS + SOD	50 U/ml	7206 ± 230	0.33

Mean CPM ± SE at 8 h of quadruplicate cultures. Residuum CPM 12399 ± 815. Statistical analysis was derived between GO and GO + antioxidants, and between LPS and LPS + antioxidants.
*Statistically significant.

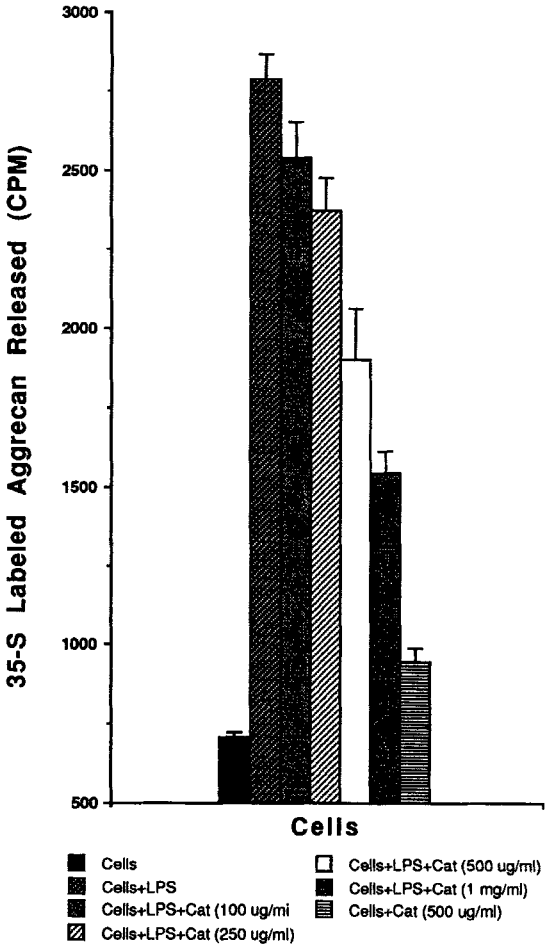

FIGURE 3 Effect of catalase on LPS-chondrocyte-dependent aggrecan release. Chondrocytes were stimulated with LPS (100 μg/ml) in presence or absence of increasing concentration of catalase (100, 250, 500 and 1000 μg/ml) in EBSS. The results are mean CPM of quadruplicate sets of wells ± SEM.

appear to be toxic to chondrocyte-matrix, because these concentrations of the agent produced higher than background release of 35-sulfate (Table III).

Taurine, which scavenges hypochlorous acid, had no effect on LPS-induced 35-sulfate release, indicating that hypochlorous acid may not play a role in matrix degradation. High concentrations of histidine, caused significant decrease of LPS-induced 35-sulfate release, while low and intermediate concentrations of histidine resulted in an insignificant effect on LPS-induced 35-sulfate release (Table III). The thiol-containing antioxidant NAC at low and intermediate concentrations had no inhibitory effect on LPS-induced 35-sulfate release; by contrast, high concentrations of NAC resulted in a significant decrease in LPS-induced 35-sulfate release. Non-specific reducing agent 2-mercaptoethanol, at final concentrations between 0.01% and 0.1% had no inhibitory effect on LPS-induced 35-sulfate release. Acetylcarnitine showed an inhibitory effect only at high concentrations (Table III).

DISCUSSION

Aggrecan combined with hyaluronan forms enormous link-protein-stabilized aggregates that are restrained within a three-dimensional latticework of Type-II collagen-containing fibrils.[20] The aggrecan core protein has a proteolytically

TABLE III Effect of antioxidants on aggrecan degradation by LPS-stimulated articular chondrocytes

Treatment	Concentration	% Inhibition	Significant
Vitamin C	0.1, 0.5, 5 mM	44, 44, 47	+, +, +
Vitamin E	50, 100, 250 μM	17, 24, 10	−, −, −
PBN	5, 50, 500 μM	43, 52, 42	+, +, +
Inactive analog of PBN	5, 50, 500 μM	0, 0, 0	−, −, −
Mannitol	2, 20, 200 mM	40, 50, 42	+, +, +
Thiourea	0.7, 7.5, 75 mM	39, 51, 40	+, +, +
DMSO	0.01, 0.1, 1%	0, 0, 0	−, −, −
Propyl gallate	0.5, 5, 50 μM	37, 55, 72	+, +, +
BHT	5, 50, 500 μM	39, 51, 48	+, +, +
Desferrioxamine	0.1, 1, 10 μM	49, 20, 13	+, −, −
Taurine	0.1, 1, 10 mM	2, 8, 23	−, −, −
Histidine	0.1, 1, 10 mM	15, 24, 37	−, −, +
NAC	0.1, 1, 10 μM	22, 21, 33	−, −, +
2-Mercaptoethanol	0.001, 0.01, 0.1%	3, 2, 0	−, −, −
Acetylcarnitine	1, 10, 100 μM	2, 8, 29	−, −, +

The meaning of symbols is as follows: +, significant and −, not significant.

sensitive segment (called aggrecanase site) at an interglobular domain between G1 and G2 domains near the amino terminus. The proteinase (aggrecanase) responsible for core protein catabolism at the "aggrecanase site" (Glu^{373}-Ala^{374}) has not yet been conclusively identified but is speculated to belong to a family of matrix metalloproteinases.[19,21] In addition to the aggrecanase site, matrix metalloproteinases cleave the aggrecan at its interglobular domain at Asn^{341}-Phe^{342} site.[22]

Accelerated catabolism of matrix components in articular cartilage is a hallmark of cartilage loss in osteoarthritis.[1] The degradation of proteoglycan has been extensively studied and can be acute, transient, reversible and of minor consequence to the overall integrity of cartilage. In contrast, sustained degradation of proteoglycan can result in irreversible damage when cartilage integrity is compromised and can thus progress to osteoarthritis.

Demonstration of *in-vivo* role of ROS in cartilage degradation is difficult because cartilage is avascular. The production of ROS is difficult to detect in tissues because ROS are extremely labile.[23] To overcome these difficulties, we developed an *in-vitro* LPS model, which is simple, sensitive, and detects direct participation of chondrocyte-dependent aggrecan degradation. The model has a high number of cells to matrix ratio, tilting the system toward the production of larger amounts of chondrocyte products and thus concentrating the damaging effect on matrix. The temporal relationship between the production of ROS and rapid release of aggrecan helps to establish the cause and effect relationship between the burst-like production of ROS by chondrocytes and matrix catabolism.

LPS has been shown to produce cartilage matrix degradation.[24,25] Therefore, the release of labeled sulfate in the medium appears to be an active degradation. In our model, only catalase significantly decreased the release of aggrecan by LPS-stimulated chondrocytes, suggesting that chondrocyte-derived hydrogen peroxide play a role in aggrecan catabolism. Inactive (boiled) catalase or SOD had no effect on LPS-induced enhancement of aggrecan release, indicating specificity of the effect of catalase, and further suggesting chondrocyte-derived superoxide anions has little role in aggrecan damage/release. In control experiments, catalase diminished the effect of glucose oxidase, lending further support to the specific effect of catalase. It should be

noted that activated chondrocytes produce both superoxide anion and hydrogen peroxide;[3,4] the selective role of hydrogen peroxide in aggrecan catabolism as compared to lack of effect of superoxide anion suggests aggrecan is exclusively sensitive to hydrogen peroxide and its products. Furthermore, catalase resulted in a dose-dependent inhibition of the release of 35-S labeled aggrecan. Almost 50% inhibition of aggrecan release by catalase in LPS-stimulated chondrocytes indicated that hydrogen peroxide-induced mechanism of aggrecan degradation contributes to about half of the total aggrecan release by LPS-stimulated chondrocytes. The non-oxidative proteolytic mechanism is probably responsible for the remainder of aggrecan release.

It is also possible that LPS-dependent aggrecan release may have been due to the conversion of inactive proMMPs to active MMPs by chondrocyte-derived ROS. ROS have been shown to result in activation of proMMPs.[26] Although our results do not exclude this possibility, the temporal relationship between the production of ROS and rapid release of aggrecan strongly indicates the association of aggrecan degradation with respiratory burst activity of chondrocytes. Furthermore, MMP production by activated chondrocytes occurs relatively much later than the observed aggrecan release. The fact that catalase did not completely inhibit aggrecan release suggests that, in the assay, chondrocyte-derived proteases are also involved in the aggrecan degradation, a process which is independent of chondrocyte ROS production. Furthermore, it should be noted that LPS did not result in chondrocyte cytotoxicity as indicated by chromium labeled method, an established method to measure cell cytotoxicity[27] suggesting that aggrecan release (degradation) is not as a result of cell death.

An additional goal for this study was to identify the antioxidants that can block the LPS-induced matrix degradation, which may be of value for testing the *in-vivo* role of chondrocyte-derived ROS in arthritis. Antioxidants have been used to decipher and validate the role of ROS in various *in-vitro* and *in-vivo* model systems.[28] Each antioxidant has a unique mechanism of scavenging ROS. For example, mannitol, a hydroxyl radical scavenger, has been used to confirm the involvement of hydroxyl radicals. Similarly, *in-vivo* interventional therapies with antioxidants have unraveled the role of free radicals in the pathogenesis of atherosclerosis and degenerative diseases of the brain.[29,30]

In-vivo hydrogen peroxide is detoxified and metabolized by the antioxidant enzymes catalase and glutathione peroxidase. However, in the presence of transition metals, hydrogen peroxide can be further degraded to powerful oxidant hydroxyl radicals. Recently we have documented a transition metal-dependent formation of hydroxyl radical in chondrocytes and cartilage.[7] In the present study, the hydroxyl radical scavenger mannitol inhibited LPS-induced 35-sulfate release, suggesting the role of hydroxyl radicals in the LPS-induced aggrecan degradation. Hydroxyl radicals are powerful oxidants that can oxidize a range of cellular and extracellular targets.[23] Thiourea, in addition to being a scavenger of hydroxyl radicals, also removes hydrogen peroxide, hypochlorite and peroxynitrite, and had therefore a significant effect in our assay system. It is interesting to note that desferrioxamine, an iron chelating agent, significantly decreased 35-sulfate release by LPS-stimulated chondrocytes. The effect of a low concentration of desferrioxamine may be due to the decreased formation of hydroxyl radicals via transition metal-catalysed Haber Weiss reaction. On the other hand, higher concentrations of desferroxamine enhanced the release of chondrocyte matrix, perhaps by causing pro-oxidant activity in chondrocytes.[31] A number of clinical observations have suggested a close correlation between iron availability and arthritis.[32]

Hydrogen peroxide results in fragmentation of link proteins, loss of ability of proteoglycan monomers to associate with hyaluronic acid, fragmentation of hyaluronic acid, chemical

modification of link proteins, and other changes.[14,17] Our observations indicate that exposure to small concentrations of hydrogen peroxide for a period of 2 h was sufficient to damage the aggrecan. In previous studies, exposure to a large concentration of hydrogen peroxide (15–240 mM) for a period of 24 h was used to demonstrate the damaging effect on proteoglycan aggregate.[17] This observation has resulted in the notion that proteoglycans are relatively resistant to oxidative damage by hydrogen peroxide. In contrast, our data suggest that the concentration of hydrogen peroxide accumulated in our *in-vitro* cultures was in fact sufficient to cause aggrecan damage.

In a model of experimental osteoarthritis, guinea pigs maintained on high levels of vitamin C (150 mg/day) consistently showed decreased joint damage as compared to animals on a low level of vitamin C.[33] Vitamin C increases the synthesis of collagen and sulfated proteoglycans, inhibits the activity of lysosomal enzymes involved in the degradation of cartilage matrix components, and functions as an antioxidant. In the present study the physiological concentration of vitamin C had a significant effect on LPS-chondrocyte-dependent aggrecan degradation. Epidemiological studies have suggested that increased intake of vitamin E may be associated with reduced incidence of knee osteoarthritis.[34] In our study, vitamin E (which has lipid peroxidation chain-breaking antioxidant activity) had an insignificant effect on 35-sulfate release, suggesting that the lipid peroxidation process plays only a marginal role in aggrecan degradation. In contrast, lipophilic antioxidants such as BHT and propyl gallate, potent inhibitors of lipid free radical reaction, were quite effective in our model system. There was a dose-dependent inhibitory effect of propyl gallate. The discrepancy between the effects of lipophilic antioxidants and vitamin E is not discerned from present studies.

PBN, a spin trapping agent, has been shown to be effective in preventing ischemia-reperfusion injury and in reversing age-related changes in old animals.[30] In the present study, PBN inhibited 35-sulfate release by LPS-activated chondrocytes, whereas an inactive analog of PBN was ineffective. It is of interest to note the *in-vitro* concentrations of PBN and BHT that resulted in significant decrease in 35-sulfate are pharmacologically relevant. Both BHT and PBN have received extensive investigation in various animal models of diseases thought to be linked to free radical damage,[29,35] and BHT has been shown to prevent the development of atherosclerosis in rabbit models.[35] Collectively, the findings with catalase and antioxidants indicate that production of hydrogen peroxide by chondrocytes is a central event and formation of hydroxyl radical can further indiscriminately damage cartilage matrix.

Cartilage derives nutrients from synovial fluid which contains a number of antioxidant enzymes such as SOD, catalase, and peroxidase, as well as the antioxidants hyaluronic acid, D-glucuronic acid, albumin, α-tocopherol, ascorbic acid, polyphenols, tannins, and other less defined antioxidants.[36] Of these agents, intraarticular injection of purified and recombinant SOD has been studied in the prevention of osteoarthritis.[37]

In conclusion, our study provides direct evidence that chondrocyte-derived ROS mediate aggrecan catabolism. These findings support the hypothesis that free radicals play a free damaging role in aging and osteoarthritis.[38] From a number of antioxidants tested, we identified two antioxidants, BHT and PBN, which in micromolar concentrations were effective in inhibiting LPS-dependent aggrecan degradation. Since it is possible to defect micromolar blood levels of these two antioxidants by pharmocologial means it therefore should be practical to test their usefulness in the prevention or treatment of osteoarthritis.

Acknowledgments

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