REVIEW

Role of reactive oxygen species and superoxide dismutase in cartilage aging and pathology

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In the last two decades, there has been an explosive interest in the role of oxidative stress in experimental and clinical medicine. Oxidative stress is strongly correlated with a number of age-related diseases, such as rheumatoid arthritis, osteoarthritis, osteoporosis and atherosclerosis. Repeated intra-articular injections of the antioxidant enzyme superoxide dismutase (SOD) have slowed down progression of rheumatoid arthritis. Unfortunately, none of the native human SODs possess attractive pharmacological properties to make them a clinically useful therapeutic drug, owing to their rapid renal clearance. To overcome these limitations, several synthetic low-molecular-weight compounds that mimic the effects of SOD were developed and have been shown to be efficient *in vivo*. The use of SOD for clinical application still receives great interest and attention. However, the limitation to the injection route of drug administration has not yet been overcome. Current research continues to investigate new strategies to enhance the reactive oxygen species-scavenging systems of the cell, in an attempt to attenuate the damage resulting from oxidative stress.

Keywords: apoptosis, cartilage, inflammation, lipid peroxidation, osteoarthritis, oxidative stress, rheumatoid arthritis, ROS, SOD, telomeres

Aging is one of the main important risk factors involved in the development of degenerative joint diseases [1]. Aging is associated with significant chondrocyte loss and increased vulnerability to reactive oxygen species (ROS)-induced cell death. Chondrocytes are capable of producing ROS, such as superoxide anions ($O_2^{\bullet-}$) [2], hydrogen peroxide (H_2O_2) [3] and hydroxyl radicals ($^{\bullet}OH$) [4]. In addition to these radicals, nitric oxide (NO) synthesized by NO synthase (NOS) enzymes plays an important role in normal cartilage function and pathology. However, the exact role of NO in cartilage is not fully understood. Some investigators have reported a direct cytotoxic effect of NO on human chondrocyte in culture [5,6], other reports have proposed NO to be a physiologic regulator of mitochondrial respiration in chondrocytes [7,8]. Intracellular ROS concentrations are controlled by the balance between ROS production and their elimination by the antioxidant systems (superoxide dismutases [SODs], catalase [CAT] and glutathione peroxidase [GPx]). Low SOD concentrations in joint fluid were reported to be associated with proinflammatory factors, such as cytokines and prostaglandins [9]. Data from clinical studies have shown that intra-articular injections of native SOD (bovine orgotein) produced greater clinical improvement than did intra-articular aspirin in patients with RA involving the knee [10]. Furthermore, SOD overexpression in mouse models of rheumatoid arthritis (RA) have confirmed the ability of SOD to protect against the harmful effects of superoxide [11,12].

It is important that the status of aging cartilage and the mechanisms involved in its degradation are understood in order to develop more powerful therapies.

Reactive oxygen species

Reactive oxygen species can be defined as atoms or molecular fragments containing one or more unpaired electrons in the atomic or molecular orbital. This unpaired electron(s) usually gives a considerable degree of reactivity to the free radical. ROS are normally produced by almost all cells, primarily in the mitochondrial respiratory chain and by certain specialized cells, such as macrophages, via specific cell-surface-bound enzymes that reduce molecular oxygen (O_2) to $O_2^{\bullet-}$. The $O_2^{\bullet-}$ anion is further reduced in the presence of transition metals to H_2O_2, and $^{\bullet}OH$. The production of $O_2^{\bullet-}$ occurs mostly within the mitochondria of a cell [13]. During energy transduction, a small number of electrons 'leak' to oxygen prematurely, forming $O_2^{\bullet-}$. ROS are immediately neutralized by specific intracellular or extracellular antioxidant systems that effectively protect cells from their deleterious action that otherwise may kill them or impair their function [14].

Many environmental stimuli, including ultraviolet radiation, chemotherapeutic agents, hyperthermia and even proinflammatory cytokines and growth factors, generate high levels of ROS that can perturb the normal redox balance or status and shift cells into a state of oxidative stress. The redox status results from a subtle equilibrium

between ROS production and the intracellular antioxidant levels. When the ROS level is high, survival is dependent on the ability of the cell to adapt to or resist the stress, and to repair or replace the damaged molecules.

Oxidative stress and the redox status of the cells can also regulate nuclear histone modifications, such as acetylation, methylation and phosphorylation, leading to chromatin remodeling, and recruitment of basal transcription factors and RNA polymerase II leading to the induction of proinflammatory mediators [15,16]. Within the cell, ROS in excess can cause genetic instability and physiological dysfunction, eventually leading to cell death and progressive aging of the organisms. Thus, inadequate production of ROS plays an important role in joint diseases.

ROS & antioxidant defense mechanisms

Antioxidants are defined as substances that, when present at low concentrations relative to an oxidizable substrate, significantly delay or prevent oxidation of that substrate. In order to protect themselves against oxidative damage, living organisms have developed a variety of antioxidant defense systems that include metal-interacting proteins, vitamin C and E, and specialized antioxidant enzymes. Metal-interacting proteins are important for transporting, chelating and sequestrating metal ions such as copper. For example, proteins such as metallothionein and metallochaperones are responsible for maintaining a metal ion pool in cells, while avoiding metal toxicity. $O_2^{\bullet-}$, either arising through metabolic processes or following oxygen 'activation' by physical irradiation, is depleted, undergoing a dismutation reaction [17]. The burden of ROS production is largely counteracted by an intricate antioxidant defense system that includes SOD, CAT and GPx.

The superoxide dismutases

There are numerous mechanisms involved in cell defense against ROS. SODs are the only enzymes dismuting $O_2^{\bullet-}$ radicals. SOD enzymes speed the conversion of $O_2^{\bullet-}$ to H_2O_2 by approximately four orders of magnitude, whereas CAT and GPx convert H_2O_2 to H_2O [18]. In the presence of transition metals, H_2O_2 can be converted to the highly toxic $^{\bullet}OH$. The GPxs are commonly considered as the most important protective mechanism against ROS, since they have broader substrate specificities and stronger affinity for H_2O_2 than CAT [19]. Three distinct SOD isoforms have been identified in mammals, and their genomic structure, cDNA and proteins have been described. Two isoforms of SOD have copper (Cu) and zinc (Zn) in their catalytic domain and one isoform has manganese (Mn) (Figure 1).

Structure & function of SOD_1

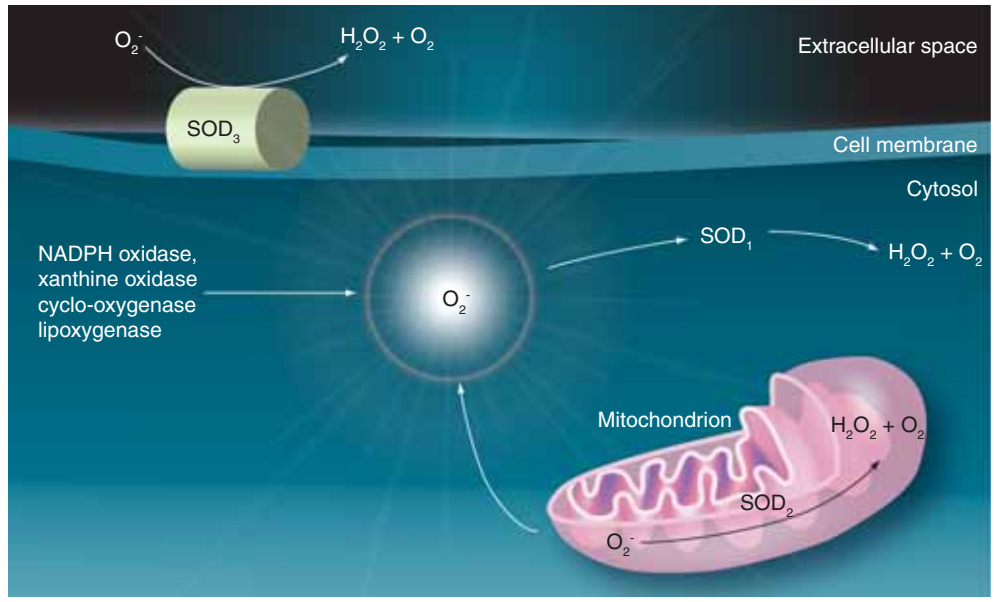
The *SOD_1* gene is located on chromosome 21, and the protein was detected in cellular organelles [20,21]. SOD_1 is a widely distributed ubiquitous enzyme. It comprises 90% of the total cellular SOD. It is a 32-kDa homodimer protein found mainly in the lysosomes, and also in the nucleus of many mammalian cells [22].

Using the human *SOD_1* promoter we [23], and others [24], have identified several binding sites for transcription factors modulated by the redox status of the cell, including AP-1 and NF-κB. *SOD_1* overexpression in mice is associated with protection of cerebral tissues from damage associated with ischemia or Parkinson's disease [25]. *SOD_1* inactivation *in vivo* is not lethal in mice [26] but leads to a marked decrease in cell growth *in vitro* [27], with increased sensitivity to $O_2^{\bullet-}$. In humans, *SOD_1* mutations are responsible for neurodegenerative diseases (e.g., amyotrophic lateral sclerosis) associated with oxidative damage [28]. In mice, *SOD_1* mutations are associated with increased apoptosis and oxidative protein damage [29,30]. Little is known regarding factors that inhibit SOD_1. Our group is analyzing the relationship between proinflammatory cytokines and the cellular antioxidant defense systems. TNF-α overproduction in inflammatory conditions is associated with increased oxidative stress consistent with inhibition of antioxidant enzymes, most notably decreased SOD_1 expression. We have recently demonstrated that TNF-α inhibited *SOD_1* gene and protein through the JNK/AP-1 pro-apoptotic signaling pathway [23]. From these data we can conclude that TNF-α-induced repression of SOD_1 explains the presence of $O_2^{\bullet-}$ and its derivatives in inflammatory conditions.

Structure & function of SOD_2

The second SOD isoform is the MnSOD or SOD_2. It is synthesized in the cytosol as a precursor molecule and is transported to the mitochondria [31]. Being mitochondrial, SOD_2 has an important role in the oxidant resistance and apoptosis of normal cells and rapidly growing cancer cells. The gene encoding *SOD_2* has been mapped to chromosome 6, and the protein exists as a homotetramer with an individual 23-kDa subunit [32]. This isoform has been shown to play an important role in promoting cellular differentiation and tumorigenesis and in protecting against

Figure 1. The subcellular localization of the three SOD isoforms: SOD_1 located primarily in cytosol, manganese SOD_2 localized in mitochondria and extracellular SOD_3.



SOD: Superoxide dismutase.

hyperoxia-induced pulmonary toxicity. *SOD_2* inactivation in mice results in lethal cardiomyopathy and neurodegeneration. In these conditions, the consequence of SOD_2 deficiency is the increased levels of mitochondrial $O_2^{\bullet-}$ and the inhibition of the respiratory chain complexes I and II [33]. In contrast to SOD_1, proinflammatory cytokines such as interleukin (IL)-1β, IL-4, IL-6 and TNF-α are potent SOD_2 activators. Activation of *SOD_2* by these factors is important for cell survival, owing to the key role of the respiratory chain. SOD is efficient to detoxify ROS, only if its enzymatic activity is coupled with that of GPx and/or CAT. Interestingly, using bovine articular chondrocytes, it was recently shown that an IL-1β-stimulating effect on SOD_2 (4 h) activity was associated with a delayed increase of GPx (24 h) and decrease of CAT (96 h) activities, indicating that IL-1β has an uncoupling effect on these antioxidant enzymes [34]. The promoter region involved in *SOD_2* activation contains sites that bind to transcription factors belonging to the NF-κB, C/EBP and NF-1 families.

Structure & function of SOD_3

SOD_3 is the more recently discovered isoform. It is synthesized in the cytosol and has a secretory leader sequence and four heparin-binding domains (one per each of four subunits) that contribute to the binding of SOD_3 to extracellular matrix (ECM) proteins [35]. The *SOD_3* gene is located on chromosome 4. SOD_3 protein exists as a 135-kDa homotetramer and exhibits strong affinity for heparin and other proteoglycans in the ECM and plasma membrane. Heparin-binding seems to be important for SOD_3-mediated antioxidant activity. Loss of the affinity for heparin (which is located on the cell surface) results in SOD_3 release from the cell surfaces into the ECM, thus failing to protect cells from ROS. A recent study also suggests that the heparin-binding domain of SOD_3 can act in certain cell types as a nuclear localization signal, suggesting that this enzyme may also provide antioxidant protection to DNA and nuclear proteins. It is found chiefly in extracellular compartments (plasma, lymph, cerebrospinal fluid and joint fluid) [36]. Inflammatory cells release proteases belonging to the pro-protein convertase family (furin), which cleave the SOD_3 from the ECM, exposing the matrix to damage from ROS and increasing plasma SOD_3 levels [37]. SOD_3 is present in articular cartilage and profoundly decreased in human osteoarthritis (OA) cartilage [38]. Furthermore, transient decreases in SOD_3 in pre-lesional cartilage were shown to be associated with progressive increases in nitrotyrosine residues in a spontaneous mouse model of OA [38]. The only *SOD_3* mutation identified to date affects the heparin-binding site (R213G) and increases the risk of cardiovascular disease [39].

Consequences of oxidative stress on cartilage

Reactive oxygen species are likely to be an initiating or contributing factor in several joint diseases. This may result either from increased production of $O_2^{\bullet-}$ or from decreased protection against ROS molecules. Articular cartilage undergoes substantial structural, molecular and biomechanical changes with aging [40], including surface fibrillation, alteration in proteoglycan structure and composition, increased collagen cross-linking and decreased tensile strength and stiffness (Figure 2).

ROS & chondrocyte apoptosis

Apoptosis can be launched from various cell compartments. Mitochondrial-driven apoptosis is most interesting when ROS-mediated mechanisms are being considered. Typical features in mitochondrial-driven apoptosis include permeabilization of the mitochondrial membrane in a process associated with ROS generation and leakage of cytochrome c from the mitochondria, which then leads to caspase-9 activation. Isolated chondrocytes also express the components of a phagocyte-like NADPH oxidase, which is responsible for the release of oxygen radicals [41]. ROS are capable of inducing apoptotic cell death in chondrocytes [42]. Whilst there is growing support that ROS may contribute to vital cell signaling mechanisms, a large body of evidence has demonstrated the detrimental effects of these highly reactive and labile molecules in cartilage. The important role of apoptosis in cartilage has been demonstrated in *in vitro* [43] and *in vivo* [44] models. *In vitro*, ROS cleave proteoglycan-core protein, hyaluronic acid and collagen [45,46]. *In vivo* ROS inhibit cell division and induce chondrocyte apoptosis through cell signaling and gene activation. In addition, ROS species may stimulate chondrocytes to produce other damaging molecules, such as proinflammatory cytokines (e.g., TNF-α and IL-1β), prostaglandins, matrix metalloproteases and NO. Despite these findings, the *in vivo* role of ROS in cartilage matrix degradation is difficult to evaluate.

The role of apoptosis in the pathogenesis of OA is still difficult to assess because of the chronic nature of the disease process [47]. In human, it has been shown that chondrocytic apoptosis induced by ROS is a pivotal process contributing to the degeneration of cartilage in RA [48]. Critical regulators of apoptosis are the mitochondrial Bcl-2 family of proteins, Bcl-2 and Bcl-Xl being anti-apoptotic and Bax and Bak being pro-apoptotic [49,50]. Intra-articular injection of a pan-caspase inhibitor has been reported to suppress cartilage degradation under OA induction in a rabbit anterior cruciate ligament transection (ACLT) model [51].

Therefore, chondrocyte apoptosis seems to be a potential target for therapeutic interventions in OA.

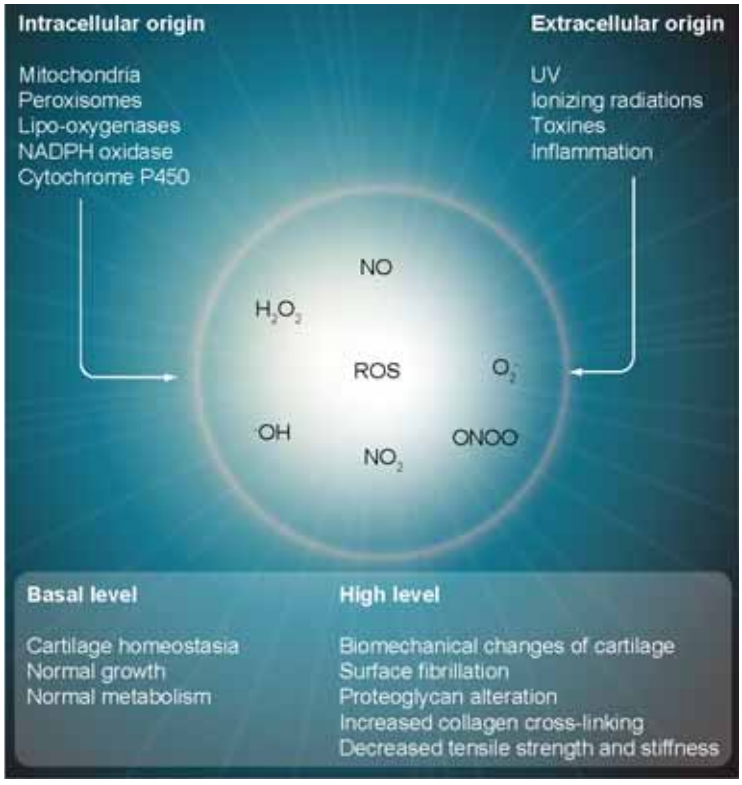
ROS & cell signaling

Beside their deleterious effects, ROS also have physiologic roles as secondary mediators in multiple cell signaling pathways, including those initiated by proinflammatory cytokines and extracellular matrix molecules [52–54].

Current concepts of ROS signaling can be divided into two general mechanisms of action: alterations in intracellular redox state and oxidative modifications of proteins. ROS can alter protein structure and function by modifying critical amino acid residues, inducing protein dimerization, and interacting with Fe–S moieties or other metal complexes. Although a large number of signaling pathways appear to be regulated by ROS, the signaling molecules targeted by ROS are less clear. However, there is growing evidence that redox regulation might occur at multiple levels in the signaling pathways from receptor to nucleus. ROS production has been shown to be coupled with the sustained activation of the ERK signaling pathway for a variety of cellular effects, including apoptosis [55]. ROS generation is required for PKC activation [56], implying its role for amplification of PKC signal. Receptor kinases and phosphatases themselves may be targets of oxidative stress. In cartilage, ROS play an essential role as signal transducers for chondrocyte hypertrophy, involving the MAPK/ERK activating kinase (MEK)–ERK pathway, whereas p38 signaling is required for the transition from a prehypertrophic to a completely hypertrophic chondrocyte phenotype [57].

Activation of c-Jun NH_2-terminal kinase (JNK) signal pathway by IL-1β and TNF-α in chondrocytes has been shown to require ROS as a signaling intermediate [58]. In synovial fibroblasts, ROS have also been shown to be required for signaling initiated through the α5β1 integrin that results in increased production of matrix metalloproteinase (MMP)-1 [59]. Treatment of human articular chondrocytes with either fibronectin fragments or integrin-activating antibodies, resulted in α5β1 integrin activation, associated with increased MMP production [60,61]. In chondrocytes, the integrin signaling pathway, which mediates increased MMP-13 production, includes activation of the three

Figure 2. Origin and effects of reactive oxygen species on cartilage.



NO: Nitric oxide; ROS: Reactive oxygen species; UV: Ultraviolet.

major families of MAP kinases (ERK, JNK and p38) and increased activity of the NF-κB and AP-1 transcription factors [60–62].

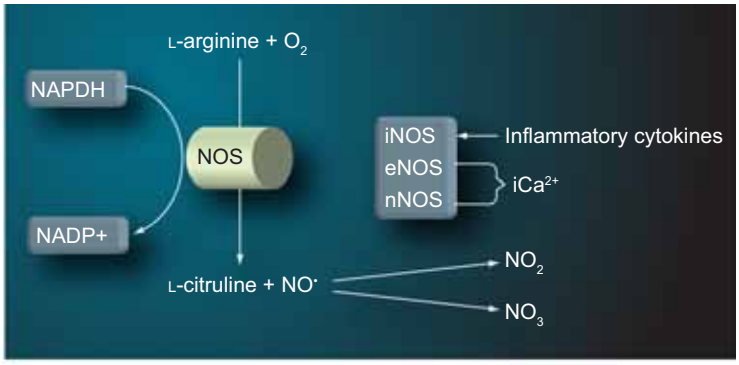
In addition to the activation of different members of signaling cascades involved in cell growth and differentiation, ROS may directly regulate the activity of transcription factors through oxidative modifications of conserved cysteines, for example [63]. Several transcription factors have been shown to be redox-sensitive, including hypoxia-inducible factor-1, specificity protein-1, NF-κB and activating protein (AP)-1. The signal transduction cascades elicited after exposure to IL-1 and TNF-α culminate in a nuclear response characterized by the activation of several transcription factors, including NF-κB and AP-1 [64–66]. Hence, genes with an AP-1 binding site, such as those encoding the metalloproteinases collagenase and stromelysin, are potential targets for the two cytokines [67,68]. ROS production following proinflammatory cytokines and growth factor treatments of bovine articular chondrocytes was shown to be through a NADPH oxidase enzyme complex, resulting in stimulation of c-fos expression [69].

In summary, these data suggest that responses of cells to proinflammatory cytokines and growth factors are dependent on the cell redox status, suggesting that modification of transcription factor-binding activities by ROS appears to be an important signaling mechanism of proinflammatory factors, and ROS may be a therapeutic target in cartilage.

Nitric oxide & cartilage matrix

Numerous reports have suggested that oxidative damage and overproduction of NO may be involved in the pathogenesis of OA [70]. NO is a diatomic molecule with a high affinity for heme iron, sulfhydryl or thiol groups, $O_2^{\bullet-}$ and molecular oxygen. NO is a short-lived molecule with a half-life in the order of seconds. From its small size and neutral charge, NO has the capacity to diffuse freely through cell membranes, acting as a signaling and effector molecule in an autocrine or paracrine manner. The decomposition products of NO are nitrite (NO_2) and nitrate (NO_3). Nitrite is quickly oxidized by oxyhemoglobin in the blood to nitrate, the predominant species found in plasma. *In vitro* nitrite and nitrate are stable metabolites of NO and are used as marker molecules to indirectly determine the presence of NO. NO is synthesized by a family of enzymes termed the NOS, which are transcribed from three distinct NOS genes and are under the control of proinflammatory cytokines. These groups of enzymes catalyze the production of NO and L-citrulline from L-arginine, NADPH and O_2 (Figure 3). NO affects protein, nucleic acid and lipid structures [71]. In addition, NO effects depend on its concentration and the cell redox status. NO alone is not sufficient to cause chondrocyte apoptosis, but it requires an oxidizing environment [72]. NO was shown to suppress proteoglycan synthesis, as determined by the decreased metabolic incorporation of sulphate into glycosaminoglycans, an effect that could be relieved by NOS inhibition [73]. Different markers were used as evidence of oxidative damage in several aging tissues. Nitrotyrosine was shown to be over-expressed in normal cartilage from elderly donors and in OA cartilage, suggesting the presence of oxidative damage in aging and degenerative cartilage [74]. More recently, various type II collagen epitopes have been described as potential biomarkers for OA [75]. These new biomarkers, such as serum C2C neoepitope concentration (a marker of collagen type II degradation), are promising tools for the monitoring of the influence of drug treatment on cartilage metabolism in joint diseases such as OA.

Figure 3. Origin of NO.



eNOS: Endothelial NO synthase; iNOS: Inducible nitric oxide synthase; nNOS: Neural nitric oxide synthase; NO: Nitric oxide; NOS: Nitric oxide synthase.

Lipid peroxidation & cartilage degradation

Lipid peroxidation is one of the major mechanisms of cellular injury in many biological systems. Lipid peroxidation may occur because of an excessive accumulation of abnormal unsaturated lipids, excessive production of ROS and excessive accumulation of transition metals. Once initiated, the reaction is autocatalytic and proceeds as a chain reaction. A decomposition of lipid peroxides generates ROS, lipid hydroperoxides and aldehydic end products such as malondialdehyde, which is used to measure lipid peroxidation. Vitamin E stops this reaction and prevents its propagation. Elderly people who frequently suffer OA are deficient in vitamins. There is growing recognition of the importance of nutritional factors in the maintenance of bone and joint health [76]. Supplementation therapy, particularly with vitamin E and the combination of vitamins B1, B6 and B12, seems to have some protective effects in patients suffering degenerative joint disease [77].

Chondrocyte activation-dependent matrix degradation was shown to be mediated by lipid peroxidation [78]. Lipophilic antioxidants, such as butylated hydroxytoluene and propylgallate potent inhibitors of lipid-free radical reaction [79], were effective in preventing matrix protein oxidation, and support the role of lipid peroxidation in the pathogenesis of cartilage aging and OA. The measurement of advanced glycation end products in skin of patients with RA and OA indicated that lipid accumulation generally precedes local tissue degeneration [80]. The unique distribution of lipids in cartilage, which changes significantly with age and dietary intake of lipids, could offer an abundant substrate to ROS that may initiate lipid peroxidation and induce cartilage damage [78].

These findings indicate that lipid oxidation is involved in collagen matrix degradation. It is clear that targeting this reaction by developing specific inhibitors in order to slow down cartilage degradation will be a good therapeutic strategy.

Oxidative stress & telomere DNA

Aging in articular human cartilage is associated with a decrease in chondrocyte proliferation and accumulation of senescent cells [1,81]. Senescence is readily observed *in vitro* in long-term cultures of continuously dividing somatic cells, which enter growth arrest after a characteristic number of population doublings. This growth arrest is induced by replication-dependent loss of DNA structures found at chromosomal termini, called telomeres, which are necessary for DNA replication [82]. Telomeres are the terminal guanine-rich sequences of chromosomes. They form a 'capped' end structure on the chromosome by allowing cells to distinguish chromosome ends from broken DNA during the replication process by protecting the chromosome ends against exonucleases. ROS can induce irreversible growth arrest, similar to replicative senescence and OA through telomere erosion [83]. It is now recognized that oxidative stress/antioxidative capacity may be prominent among factors that control telomere length. The telomeres may function as a timing mechanism that, when reduced to a critical length, signal a cell to stop dividing and to enter cellular senescence (Figure 4). Recent reports demonstrated that the telomere length of chondrocytes shortened with donor aging, and that decreased mean telomere length was closely related to the increase in senescence-associated β-D-galactosidase expression in human chondrocytes, suggesting that chondrocyte senescence, at least in part, participates in the age-related loss of chondrocyte function that is presumably responsible for deterioration of articular cartilage structure and function [84,85].

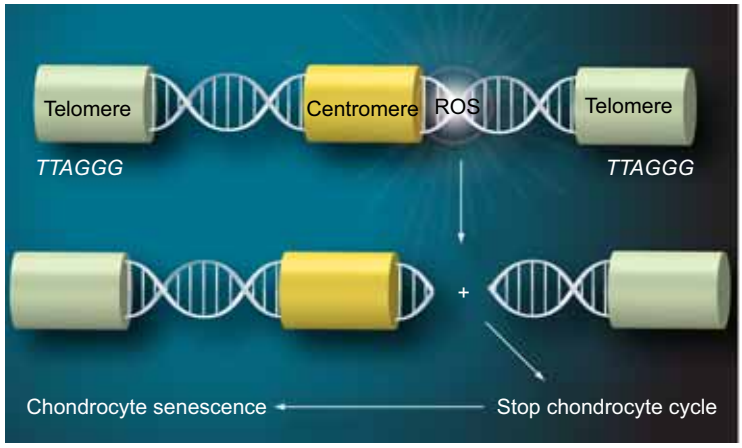
Blockade of signaling pathways regulating ROS production by neutralizing oxygen radicals directly may form new therapeutic methods of preventing severe cartilage destruction.

Beneficial effects of SOD and its mimetics in cartilage

Use of SOD in joint diseases

The use of SOD for clinical application still receives great interest and attention. A protective and beneficial role of superoxide dismutase was demonstrated in a broad range of diseases, both preclinically and clinically. Clinical trials of SOD

Figure 4. Effects of ROS on telomere shortening and chondrocyte senescence.



ROS: Reactive oxygen species.

have been carried out in different pathological conditions. SOD was used very early on to treat bladder inflammation resulting from irradiation [86]. The beneficial effect of SOD observed in this trial is not fully understood, but it appears to have reduced the immunogenicity of the graft. This is particularly intriguing in light of a new hypothesis in which the immune system responds not only to nonself, but also to 'danger signals' [87], perhaps including those generated by oxidative stress.

In mice that are genetically deficient in SOD_3, both the severity of collagen-induced arthritis and the production of proinflammatory cytokines are increased [88]. *SOD_3* gene transfer via the subcutaneous route [12] or into the knee decreased the severity of experimental arthritis in rodents [89]. In humans, serum SOD levels correlated negatively with disease activity [90]. Studies of orgotein in RA yielded conflicting results [10,91]. Native bovine SOD was associated with adverse effects such as immune responses to the enzyme, pain and burning sensations. As a result, native bovine SOD was removed from the market.

The problem of oxidant–antioxidant balance may be the primary reason that SOD-based antioxidant therapy has not made a greater and more rapid impact on clinical medicine. Many studies have been published that appear to be in conflict as to whether SOD is protective or not protective in a particular model system. Furthermore, other limitations for the therapeutic use of SOD is its rapid elimination from the circulation via the kidneys, with a plasma half-life of 6 min. In order to improve its therapeutic activity, attempts were made to prolong the half-life of SOD. For example, it has been covalently conjugated to poly-ethylene glycol, dextran and albumin [92]. Another approach to increase blood half-life is the incorporation of SOD in liposomes [93]. Recent pharmacokinetic studies indicated that liposomal encapsulation of the enzyme increased its terminal half-life in the plasma by five- to ten-fold (depending on the liposome type used) after intravenous administration. The SOD–liposome formulations tested induced a reduction of arthritis indices in a rat model of adjuvant arthritis [94]. In particular, positively charged liposomes containing stearylamine appeared to be promising in this regard. This new encapsulation technique enhanced SOD therapeutic activity in rats with adjuvant arthritis. This novel noninvasive anti-inflammatory treatment of the adjuvant arthritis with SOD for transdermal delivery contributes to an innovative approach in the field of the protein transdermal delivery.

Other approaches to therapy have shown greater promise, in particular the recent construction of a 'super SOD' [95]. This chimeric protein contains SOD_2 primary structure plus the 26 amino acid C terminus of SOD_3. With the chimera being less positively charged than SOD_3C, this means it does not bind as strongly to cell surfaces, allowing its successful intravenous administration [96].

SOD mimetics in joint diseases

To overcome the problems encountered with native bovine SOD, several groups have developed synthetic low-molecular-weight compounds that mimic the effects of SOD. Among SOD mimetics available today, the most promising are nitroxides (tempol) and Mn(II) penta-azamacrocyclic ligand (M40403) [97].

Effects of M40403 on cartilage

M40403 is characterized by excellent stability and a very high level of SOD activity [98]. A recent study demonstrated that M40403 attenuates the degree of chronic inflammation, tissue damage and bone damage associated with collagen-induced arthritis in the rat, and supports the possible use of SOD mimetics as therapeutic agents for the management of chronic diseases such as RA [99]. M40403 increases the rate of $O_2^{\bullet-}$ conversion to O_2 and H_2O_2. It seems specific of $O_2^{\bullet-}$ and does not react with H_2O_2, peroxynitrite or NO. The potent anti-inflammatory effects of M40403 are related to $O_2^{\bullet-}$ elimination, which prevents peroxynitrite formation and therefore nitration of

tyrosine residues in proteins. Furthermore, by diminishing the expression of adhesion molecules such as ICAM or P-selectin, M40403 decreases the influx of neutrophils to inflammatory sites. M40403 potently suppressed the production of $O_2^{\bullet-}$, TNF-α and IL-6 [100].

Nitroxides (tempol) inhibit mitochondrial ROS generation

Oxidative damage to mitochondria is a critical event in oxidative cell damage, and mitochondrial ROS should be a primary target for drug development. Tempol (4-hydroxy-2,2,6,6-tetramethylpiperidine-*N*-oxyl) is a stable piperidine nitroxide of low molecular weight. Unlike recombinant SOD, which is not able to cross biologic membranes, tempol crosses biologic membranes and functions as an intracellular scavenger of superoxide anion and other ROS [101].

Tempol is a soluble derivate of Tempo used in electron-spin-resonance spectroscopy to detect $O_2^{\bullet-}$. Tempol has shown beneficial effects in animal models of inflammation, hypertension, diabetes and endothelial cell dysfunction [102]. Tempol attenuates $O_2^{\bullet-}$ effects *in vitro*, diminishes $^{\bullet}OH$ production and decreases the cytotoxic effects of H_2O_2 and peroxynitrite. Tempol decreased tissue inflammation and damage in a study of rats with collagen-induced arthritis [103].

Conclusion

In summary, these results support the view that small molecules such as SOD mimetics and tempol, which permeate biologic membranes and function as intracellular radical scavengers, may be useful in the treatment of conditions associated with local or systemic inflammation. More comprehensive studies are needed to clarify a possible clinical significance of this topic, and this will be an important area in the future for further research into new therapies.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Executive summary

Reactive oxygen species

- Reactive oxygen species (ROS) are normally produced by almost all cells, primarily in the mitochondrial respiratory chain and by certain specialized cells.

ROS & antioxidant defense mechanisms

- The burden of ROS production is largely counteracted by an intricate antioxidant defense system that includes the enzymatic scavenger superoxide dismutase (SOD), catalase and glutathione peroxidase.

Consequences of oxidative stress on cartilage

- Articular cartilage undergoes substantial structural, molecular and biomechanical changes with aging.
- The *in vivo* role of ROS in cartilage matrix degradation is difficult to evaluate.

ROS & chondrocyte apoptosis

- Apoptosis is potentially a protective mechanism against both exogenous carcinogens and inflammatory states and is regulated in part by the cellular redox state.
- Vitamin E, the free radical scavenger *N*-acetyl cysteine and SOD all showed similar anti-apoptosis activity in human cartilage explants exposed to different compression amplitude, strongly indicating a role for ROS in this process.

ROS & cell signaling

- ROS also have physiologic roles as secondary mediators in multiple cell signaling pathways, including those initiated by proinflammatory cytokines and extracellular matrix molecules.
- In addition to the activation of different members of signaling cascades involved in cell growth and differentiation, ROS may directly regulate the activity of transcription factors through oxidative modifications of conserved cysteines, for example.

Nitric oxide & cartilage matrix

- Nitric oxide (NO) is synthesized by a family of enzymes termed the nitric oxide synthases (NOS), which are transcribed from three distinct NOS genes and are under the control of proinflammatory cytokines.
- Nitrotyrosine was shown to be overproduced in normal cartilage from elderly donors and in osteoarthritis (OA) cartilage, suggesting the presence of oxidative damage in aging and degenerative cartilage.
- More recently, various type II collagen epitopes have been described as potential biomarkers for OA.

Executive summary (cont.)

Lipid peroxidation & cartilage degradation

- Lipid peroxidation may occur because of an excessive accumulation of abnormal unsaturated lipids and excessive production of ROS.
- Some evidence suggests that lipid peroxides may mediate cartilage damage in aging and in age-related diseases such as OA and RA.

Oxidative stress & telomere DNA

- ROS can initiate apoptosis through caspase activation and induce irreversible growth arrest, similar to replicative senescence and OA through telomere erosion and shortening.
- The telomere length of chondrocytes shortened with donor aging, suggesting that chondrocyte senescence participates in the age-related loss of chondrocyte function.

Therapeutic perspectives of SOD in cartilage

- SOD mimetic combined with methotrexate protects rats with collagen-induced arthritis (CIA) from the development of OA.

Beneficial effects of SOD & its mimetics in joint diseases

- The beneficial effect of SOD observed in clinical trials is not fully understood, but it appears to have reduced the immunogenicity of the graft.
- Many studies have been published that appear to be in conflict as to whether SOD is protective, or not protective in a particular model system. Furthermore, another limitation for the therapeutic use of SOD is its rapid elimination from the circulation via the kidneys, with a plasma half-life of 6 min.
- Among SOD mimetics available today, the most promising are nitroxides (tempol) and Mn(II) penta-azamacrocyclic ligand (M40403).
- Tempol decreased tissue inflammation and damage in a study of rats with collagen-induced arthritis.

Future perspective

- The field of oxidative stress and joint diseases will continue to expand in the coming years. The development and availability of new metabolic approaches will, in the next few years, help to develop a more exhaustive portrait of the manifold roles of SOD mimetics in cartilage.
- Indeed, one of the new generation of antioxidants that promises a positive future is the Mn(III) tetrakis (4-benzoic acid)porphyrin (MnTBAP). This molecule targets the signaling pathways that regulate MMP-13 expression, as a potential therapeutic approach for slowing or stopping cartilage destruction.
- As most patients would prefer treatments that are inexpensive, have long-term efficacy, are less painful, less invasive and more easily accessible, with fewer side effects than with existing treatments, the nutritive mixture solution proposed today has a promising future for patients with joint diseases.
- The development of tests for oxidative stress markers will be helpful for physicians to better treat their patients.

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