Original

Influence of Dexamethasone on Matrix Metalloproteinase Production from Human Synovial Fibroblasts in vitro

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Abstract: The effects of dexamethasone on the production of matrix metalloproteinase (MMP) by synovial fibroblasts (SFs) were examined in vitro. Cultured SFs originating from patients with osteoarthritis were stimulated with either lipopolysaccharide or transforming growth factor-β (TGF-β) in the presence of various concentrations of dexamethasone. MMP and tissue inhibitor of metalloproteinase (TIMP) concentrations in 24-hour culture supernatants were determined by ELISA. Although lower doses of dexamethasone (10 and 25 µg/mL) did not suppress the production of MMP-1, -2, or -3 in response to lipopolysaccharide stimulation, dexamethasone at concentrations greater than 50 µg/mL significantly suppressed the production of those MMP types. In contrast, dexamethasone did not inhibit TIMP-1 and -2 production by SF, even at a concentration of 250 µg/mL. Dexamethasone also did not inhibit the production of MMPs and TIMPs by SF stimulated with TGF-β. These results suggest that dexamethasone suppresses the production of MMPs by inhibiting signaling pathways that are activated by inflammatory stimulation. These findings also indicate that the suppressive activity of dexamethasone on MMP production may be partially responsible for the favorable effect of this drug on the clinical status of inflammatory diseases of the knee such as osteoarthritis.

Key words: matrix metalloproteinase, dexamethasone, fibroblasts, osteoarthritis, in vitro

Introduction

Osteoarthritis (OA) is characterized by loss of articular cartilage and secondary bone as well as changes to the synovium, including marginal osteophyte formation and synovitis. The 2 main macromolecules of articular cartilage extracellular matrix are the large aggregating proteoglycans, aggrecan and type II collagen. Type II collagen fibrillar network forms the backbone of cartilage and provides it with stability and tensile strength, while the proteoglycan component is highly hydrated, absorbs loads, and provides cartilage with compressive stiffness. Destruction of these components occurs in OA cartilage.

Both biochemical and mechanical changes may contribute to the development of OA disease. Chondrocytes respond to excessive loading by elaboration of degradative enzymes

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and inappropriate repair responses\(^4,5\)). In addition, functional changes in the chondrocytes, together with inflammatory cytokines such as interleukin-1 and tumor necrosis factor, may promote deterioration by inducing the expression of proteinases\(^6-8\)). These proteinases include the matrix metalloproteinases (MMPs), members of a unique family of zinc-binding endopeptidases that are responsible for degradation of collagen, elastin, and other macromolecules in the extracellular matrix\(^3,9\)). As expected from their degradative properties, the uncontrolled expression of MMPs has been reported to be associated with many pathological conditions involving the articular cartilage\(^3,10\)). Further, an important mechanism for the regulation of the activity of MMPs is via binding to a family of homologous proteins referred to as the tissue inhibitors of metalloproteinases (TIMPs). The two-domain TIMPs are of relatively small size, and yet have been found to exhibit several biochemical and physiological/biological functions, including inhibition of active MMPs, pro MMP activation, cell growth promotion, matrix binding, inhibition of angiogenesis and the induction of apoptosis, whereas their controlled expression is essential for normal tissue remodeling and wound healing\(^11\)).

Glucocorticoids are frequently used for treatment of OA. Despite their therapeutic efficacy in modulating the clinical status of OA (especially in reducing pain), the influence of glucocorticoids on cartilage metabolism is not well defined\(^9\)). Inflammation is a prominent clinical feature of OA, and its manifestations at affected joints include intense infiltration by inflammatory cells, swelling, and synovial effusion\(^12-14\)). These inflammatory responses require alterations in the composition of the basement membrane, a process in which MMPs are involved. It has been established that synovial fibroblasts (SFs), in addition to chondrocytes, are important producers of MMPs\(^3,9,10,15\)). Therefore, the present study was undertaken to examine the effects of the glucocorticoid dexamethasone (DEX) on MMP production by SFs in response to inflammatory stimulation.

Materials and Methods

Reagents

DEX was purchased from Sigma-Aldrich (St. Louis, MO) and was dissolved in RPMI-1640 medium (Sigma-Aldrich) supplemented with heat-inactivated 10% fetal calf serum (RPMI-FCS; Irvine Scientific, Santa Ana, CA) at appropriate concentrations just before use. Lipopolysaccharide (LPS) extracted from Klebsiella pneumoniae was purchased from Sigma-Aldrich and dissolved in RPMI-FCS. Recombinant transforming growth factor-\(\beta\) (TGF-\(\beta\); R & D Systems, Dinarello, CA) was also dissolved in RPMI-FCS.

Source of cells

Synovial tissues were obtained from 5 OA patients during surgery for joint replacement. Informed consent was obtained for the procedures, which was approved by the Ethics Committee of Showa University. The donors were all female, aged between 70 and 78 years (mean, 75 years). They did not receive any medication, including non-steroidal anti-inflammatory drugs, in the 2 weeks prior to surgery. The tissues obtained were cut into small pieces (approximately 1.0 mm square) and then rinsed several times with normal saline containing with 500 U penicillin, 500 \(\mu\)g/mL streptomycin, and 5.0 \(\mu\)g/mL amphotericin B. The tissues were then plated at a density of 10 pieces per dish in 100-mm tissue culture dishes, covered with microscope slides, which were stuck to the dish with
sterile vaseline and then stored at 37°C in a humidified atmosphere containing 5% CO₂. When a monolayer of fibroblast-like cells was found to be confluent, the explanted tissues were removed. The cells were then trypsinized and replated at a concentration of 5 × 10⁵ cells/mL. The medium was changed every 3 days for 2 to 3 weeks until confluence was attained. Subsequently, the cells were split 1:2 at confluency and passaged. The cells were then characterized and used as SFs.

Cell culture

SFs at generation 5 to 7 were introduced into each well of 24-well culture plates at a concentration of 1.0 × 10⁵ cells/mL and treated with either LPS or TGF-β in the presence of various concentrations of DEX. After 24 hours the culture supernatants were removed and stored at −40°C until required.

Assays for MMP and tissue inhibitors of metalloproteinases (TIMP)

MMP and TIMP concentrations in culture supernatants were determined by commercially available human MMP-1, -2, -3, -9, -13, TIMP-1 and TIMP-2 assay kits (Amersham Biosciences, Piscataway, NJ) according to the manufacturer’s directions. The sensitivity of these ELISA kits was 1.7 ng/mL for MMP-1, 0.37 ng/mL for MMP-2, 0.6 ng/mL for MMP-3, 0.6 ng/mL for MMP-9, 0.094 ng/mL for MMP-13, 51.0 ng/mL for TIMP-1 and 3.0 ng/mL for TIMP-2.

Statistical analysis

Differences between the control and experimental data were analyzed using ANOVA followed by Fisher’s PLSD test. P<.05 was considered significant. Data are means±SE of individual synovial tissues obtained from 5 different patients.

Results

Effects of LPS and TGF-β treatment on SF production of MMP

We first determined the concentration of LPS that stimulated maximum production of MMP from SFs, by measuring MMP-1 released into culture supernatants. Although treatment of cells with 0.1 µg/mL LPS caused a slight (nonsignificant) increase in MMP-1 levels in culture supernatants, LPS at greater than 0.5 µg/mL significantly increased the levels of MMP-1 (Fig. 1A). Treatment of SFs with TGF-β also increased MMP-1 levels. The minimum concentration of TGF-β that caused maximum production of MMP-1 was 1.0 ng/mL (Fig. 1B). We then examined the types of MMP produced by SFs after LPS or TGF-β treatment. SFs produced significant amounts of MMP-1, -2 and -3, but not MMP-9 and MMP-13, after LPS application at 0.5 µg/mL (Fig. 2). Treatment of SF with TGF-β also caused significant production of MMP-1, -2 and -3. However, TGF-β application had a minimal effect on the production of MMP-9, and -13 (data not shown).

Effects of DEX on SF production of MMP and TIMP

The second set of experiments was designed to examine the influence of DEX on MMP and TIMP production by SFs in response to LPS and TGF-β stimulation. To do this, we first examined the effect of DEX on LPS-induced MMP production by measuring MMP-1, -2, and -3. Treatment of SF with lower concentrations (10 to 25 µg/mL) of DEX did
Fig. 1. Changes in synovial fibroblast (SF) production of MMP-1 induced by treatment with either LPS or TGF-β in vitro. Data in this and the following figures are means±SE of individual synovial tissues obtained from 5 different patients. SFs at $1.0 \times 10^5$ cells/mL were treated with various concentrations of either LPS (A) or TGF-β (B) for 24 hours. MMP-1 concentrations in the culture supernatants were determined by ELISA. *$P < .05$.

Discussion

MMPs are essential factors for cleaving components of the extracellular matrix in cartilage. High levels of MMPs and imbalances between MMP and TIMP have been found in synovial fluid from patients with OA. Furthermore, when chondrocytes and SFs are stimulated with inflammatory cytokines in vitro, those from OA patients produce higher levels of MMPs than those from healthy controls. These findings implicate MMPs...
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Fig. 2. Types of MMP produced by SFs in response to LPS and TGF-β treatment in vitro. SFs at 1.0 × 10⁵ cells / mL were treated with either 0.5 μg / mL LPS or 1.0 ng / mL TGF-β for 24 hours. MMP concentrations in culture supernatants were determined by ELISA.

In the development and maintenance of OA and other diseases that are destructive to cartilage. Modulation of the ability of cells in joints to produce MMPs could therefore be of great value for treatment of OA. At present, little is known about the effects of glucocorticoids on MMP production, even though these drugs are frequently used in OA treatment⁹,¹⁰).

In the present study we found that DEX suppressed the LPS-induced production of MMPs by SF. The effect of DEX was dose-dependent, with 50 μg / mL being the minimum concentration of DEX showing suppressive activity against MMP production. For treatment of OA, DEX at a concentration of 10 mM (393 mg / mL) is injected directly into the intra-articular space. After injection, DEX remains in the knee for 1 to 2 weeks without being metabolized²⁰). Therefore, the concentrations of DEX found to be effective in our study would typically be exceeded in vivo during corticosteroid treatment for OA. This suggests that at least some of the therapeutic effect of corticosteroids in OA may occur via an action on SF to suppress MMP production.

MMPs comprise an enzyme superfamily of at least 21 members, which can be classified into subgroups of collagenases, stromelysins, gelatinases, membrane-type MMPs and other MMPs²,²¹. MMP-1 cleaves type I and II collagens, the most abundant proteins present in the osteoid layer, into characteristic 3 : 1 length fragments, which are subsequently dena-
The digestion of type I and II collagens by MMP-1 thus appears to be the initial step of the entire bone resorption process. Subsequently, denatured collagen fragments can be degraded further by 2 gelatinases, MMP-2 and MMP-9. In contrast, MMP-3 has broad substrate specificity and can cleave other bone matrix proteins such as proteoglycans, glycoproteins and native collagen types IV, VII and IX in the osteoid layer. Together with these known actions of MMPs, the present results suggest that DEX decreases the ability of SF to produce MMPs under inflammatory stimulation, which thereby reduces degeneration of the osteoid layer covering the mineralized bone matrix. This should act to limit the destruction of joint cartilage and subchondral bone.

Apart from a pivotal role in cartilage degradation, MMPs enhance microvascular permeability, which is responsible for edema and cell migration into the diseased area. The attenuating effect of DEX on MMP production could therefore inhibit inflammatory cell infiltration into the synovial fluid, and reduce swelling or synovial effusion in the affected joints.

In addition to their inhibitory actions against MMP, TIMPs exert an inhibitory effect on the growth of fibroblasts. Histological observation of OA joints has shown that fibrosis...
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Fig. 4. Effects of DEX on TGF-β-induced MMP and TIMP production by SFs in vitro. SFs at 1.0 × 10^5 cells/mL were treated with 1.0 ng/mL TGF-β in the presence or absence of dexamethasone (DEX) for 24 hours. MMP and TIMP concentrations in culture supernatants were determined by ELISA. *P<.05; NS, not significant.

develops on the surface of cartilage\(^27\). Our results showed a lack of effect of DEX on TIMP production, suggesting that administration of DEX into patients would not impair the actions of TIMP in suppressing the growth of fibroblasts in OA joints. Together with the inhibition of MMP by TIMP, this would favorably modify the clinical condition of the affected joints.

In various cell types, LPS exerts its action by binding to a membrane receptor, with subsequent activation of the transcriptional factor NF-κB\(^28\). In unstimulated cells, NF-κB is present in the cytoplasm and is bound to IκB, which prevents NF-κB from entering the nucleus. Stimulation of LPS activates IκB kinases, which specifically phosphorylate IκB, resulting in degradation of IκB. The NF-κB released from IκB then translocates into the nucleus, binds to the regulatory element of the target genes, and controls their transcription. On the other hand, TGF-β acts via its receptors TβRI and TβRII to enhance activation of the transcriptional factor Smad\(^29,30\). Activated Smad, especially Smad2 and Smad3, enters the nucleus and controls transcription of mRNA responsible for the production of specific proteins\(^29,30\). In the present study we found that DEX suppressed LPS-induced, but not TGF-β-induced MMP production, suggesting that inhibitory action of DEX may therefore be partially due to a suppressive effect on NF-κB activation.
In conclusion, this study has clearly demonstrated that DEX acts not only against the degenerative joint disease symptoms of pain and inflammation but can also interfere with the collagenase activity that underlies the destruction of articular cartilage during OA. These effects may contribute, in part, to a favorable modification of the clinical status and quality of life for patients with OA.

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