Dexamethasone Inhibits Collagen Degradation Induced by the Combination of Interleukin-1 and Plasminogen in Cartilage Explant Culture

Shigeki Saito, Masao Kato, Mari Masumoto, Shun-ichiro Matsumoto, and Yasuhiro Masuho

Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Ltd., 21 Miyukigaoka, Tsukuba, Ibaraki, 305–8585, Japan. Received January 18, 1999; accepted April 1, 1999

Glucocorticoids ameliorate erosion in animal osteoarthritis (OA) models and suppress synthesis of matrix metalloproteinases (MMP). However, in vitro studies, their inhibitory effects on matrix degradation of cartilage have not been well documented by monitoring aggrecan. Collagen was monitored in this study to examine the effects of dexamethasone in cartilage explant culture. Dexamethasone clearly blocked collagen degradation induced by the combination of interleukin-1 (IL-1) and plasminogen at the concentration of 10^{-9} M, which is much lower than the concentrations reportedly required to inhibit matrix synthesis. In addition, MMP-1 and MMP-3 were suppressed by dexamethasone treatment in a similar range of concentrations. The conversion of plasminogen to plasmin, however, was not blocked by treatment with dexamethasone. These results suggest that the inhibitory effect of dexamethasone on collagen degradation may be due to suppression of MMP production rather than suppression of fibrinolytic cascade. Thus, the ability of glucocorticoids to inhibit matrix degradation in vitro, which could be clearly shown by monitoring collagen degradation, may endorse their efficacy in animal OA models and suggest potential therapeutic effectiveness.

Key words cartilage; collagen; dexamethasone; IL-1; matrix metalloproteinase

Osteoarthritis (OA) is a disease featured by matrix degradation of articular cartilage. This phenomenon is believed to be associated with increased levels of proteolytic enzymes, such as matrix metalloproteinase (MMP). For several decades, intraarticular injections of glucocorticoids have been used successfully to relieve the symptoms of OA and to restore articular functions.1) Their efficacy seems likely to be due not only to their anti-inflammatory properties but also to their protective effects on cartilage matrix, which might be attributable to their suppressive effects on MMP synthesis as shown by many investigators.2) However, there is a skepticism of using steroids for fear of deleterious side-effects on cartilage. Such skepticism has its basis in earlier studies which reported that glucocorticoid injections suppressed cartilage proteoglycan synthesis and caused degenerative lesions in animal models.3–9) However, those experiments were performed using physiologically irrelevant high doses, as much as several mg/kg of body weight for in vivo studies.9) In recent studies which were performed under more clinically relevant conditions, intraarticular injections of low-dose glucocorticoids reduced the progression of cartilage erosion and osteophyte formation in experimental OA models: the rabbit meniscectomy model,7) the guinea pig model8) and the canine anterior cruciate ligament model.9) Contrary to the efficacy of glucocorticoids in animal OA model and their suppressive effects on MMP synthesis, their inhibitory effects on matrix degradation have not been well documented in in vitro studies. Aggrecan degradation has been monitored as a parameter to examine the protective effects in cartilage explant culture as a model for pathogenesis. However, dexamethasone and prednisolone were essentially ineffective in inhibiting interleukin-1 (IL-1) mediated aggrecan degradation,9) inconsistent with in vivo studies. Otherwise, concerning type II collagen which is another major matrix component, the effects of glucocorticoids have not been investigated thus far. Therefore, the present study was focused on the effects of dexamethasone on the degradation of collagen and successfully showed its protective effects on matrix degradation at the cartilage explant level.

MATERIALS AND METHODS

Materials Dexamethasone was obtained from Sigma (St. Louis, MO, U.S.A.). Human tissue inhibitor of metalloproteinases-1 (TIMP-1) was prepared as described previously.11) The cartilage was cut into slices weighing 5 mg, and they were placed in the wells of 24-well plates. Quadruplicate cartilage slices (explants) were used for each point. Explants were cultured in 0.5 ml of Dulbecco’s modified Eagle’s medium (DMEM) containing 1% fetal bovine serum (FBS) with 1 ng/ml human recombinant IL-1α (R&D Systems, Minneapolis, MN, U.S.A.) in the presence of 100 μg/ml plasminogen (TechnoClone GmbH, Vienna, Austria) for 7 d at 37°C in CO₂ incubators. Degradation of the collagen was determined by measuring the release of hydroxyproline, a marker specific for collagen. The culture supernatants and cartilage digested with papain were hydrolyzed separately in 6 N HCl at 105°C for 20 h. Their hydroxyproline contents were then measured using the method of Ellis et al.12) Results are expressed as the percentage of hydroxyproline in the culture supernatant to the sum of hydroxyproline contents in the culture supernatants and cartilage digested with papain.

Quantitation of MMP-1 and MMP-3 by ELISA MMP-1 and MMP-3 in the culture supernatants were measured using double-antibody sandwich ELISA systems, as described previously.11) Mouse polyclonal antibodies generated against rabbit MMP-1 were used as the primary trapping antibody and the secondary biotinylated antibody for the detection.
Fig. 1. Inhibitory Effect of Dexamethasone on Collagen Degradation Induced by the Combination of IL-1 and Plasminogen (Plgn) in Rabbit Cartilage Explants

Rabbit cartilage explants were cultured for 7d with IL-1 (1ng/ml) and Plgn (100μg/ml) in the presence of the indicated concentrations of dexamethasone or TIMP-1 (1μM). Results are expressed as the percentage of hydroxyproline in the culture supernatant to the sum of hydroxyproline contents in the culture supernatants and cartilage digested with papain. Values are expressed as the mean and standard deviation (n=4). This is a representative result of 3 independent experiments. *p<0.005 versus IL-1+Plgn alone (Student’s t-test).

Western Blot Analysis of Plasminogen/Plasmin Conversion Culture supernatants were collected from cartilage explants cultured for 7d in the presence of both plasminogen and IL-1α and/or dexamethasone. The culture supernatants were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions, transferred onto a polyvinylidene difluoride (PVDF) membrane and blocked with Blockace (Snow Brand, Sapporo, Japan). The membrane was then incubated with goat anti-human plasminogen affinity purified IgG-peroxidase conjugate (Affinity Biologicals Inc., South Bend, IN, U.S.A.) at a dilution rate of 1:1000, followed by staining by enhanced chemiluminescent detection reagents (NEN, Boston, MA, U.S.A.) according to the manufacturer’s instructions.

RESULTS

The Inhibitory Effect of Dexamethasone on Collagen Degradation Induced by the Combination of IL-1 and Plasminogen in Rabbit Cartilage Explant Culture Rabbit cartilage explants were cultured for 7d with IL-1 and plasminogen in the presence of dexamethasone to examine the effects of dexamethasone on collagen degradation. The combination of IL-1 and plasminogen induced 80% release of collagen from 5% in unstimulated explants (plasminogen alone). Dexamethasone blocked this collagen degradation in a dose-dependent manner (Fig. 1). At a relatively low concentration, 10^{-8} M, dexamethasone inhibited collagen degradation. Approximately 20% of collagen release remained at a maximum concentration of dexamethasone, while TIMP-1 (1μM) inhibited collagen degradation almost completely.

Suppressive Effect of Dexamethasone on the Production of MMP-1 and MMP-3 in Rabbit Cartilage Explant Culture To examine the mechanism by which dexamethasone blocked collagen degradation, MMP production was examined using ELISA. Dexamethasone inhibited IL-1-induced MMP-1 production (Fig. 2A) and MMP-3 production (Fig. 2B) in a dose-dependent manner; the concentrations required for this inhibition were 10^{-10} M and 10^{-9} M, respectively, similar to those required to inhibit collagen degradation. These results suggest that the suppression of MMP by dexamethasone resulted in the inhibition of collagen degradation.

Effects of Dexamethasone on Fibrinolytic Cascade There remains a possibility that the inhibitory effect of dexamethasone on collagen degradation is in part due to the sup-
pression of fibrinolytic cascade. Inhibition of fibrinolytic cascade may result in failure of MMP activation as reported previously, since the present cartilage explant system contains plasminogen which is the primary factor activating proMMP. Conversion of plasminogen to plasmin was examined by Western blotting to determine whether dexamethasone affects the fibrinolytic cascade. As shown in Fig. 3, plasminogen was converted to the low molecular-weight form in the presence (lane 4) or absence of IL-1 (lane 3), suggesting that cartilage explants spontaneously activated plasminogen. Low levels of plasminogen activators in cartilage might spontaneously convert plasminogen to plasmin without stimulation, similar to the study in vascular smooth muscle cells. The conversion of plasminogen to plasmin was not blocked by the treatment of dexamethasone ranging from 100 nm to 10 μM (lane 5—7), but by aprotinin (lane 8). In addition, it seems likely that IL-1 accelerated the further processing of plasmin to the smaller species (lane 4). The appearance of the smaller species may be caused by MMPs induced by IL-1.

**DISCUSSION**

The present study clearly demonstrated that dexamethasone blocked collagen degradation induced by the combination of IL-1 and plasminogen in rabbit cartilage explant culture. Inhibitory effects of glucocorticoids on matrix degradation have not been well described in cartilage explants since aggregan degradation, which has been preferentially monitored so far, is not blocked by glucocorticoids. Thus, instead of aggregan, collagen degradation was measured in this study. The previous study established a rapid collagen degradation system in cartilage explants stimulated by IL-1 and plasminogen. This collagen degradation was found to be mediated by MMPs, consistent with other collagen degradation systems. Using this system, the inhibitory effect of dexamethasone on matrix degradation was successfully demonstrated in vitro. Inhibition of collagen degradation by dexamethasone is in good agreement with the amelioration of cartilage lesion by intraarticular injections of glucocorticoids in animal OA models. Thus, the present findings provide the basis for the efficacy of glucocorticoids shown in animal OA models.

Suppressive effects of dexamethasone on the production of MMP-1 and MMP-3 were observed at similar concentrations required to inhibit collagen degradation, consistent with previous research. Except for MMP-1, the present explant system may contain MMP-13, which is a candidate that cleaves collagen in OA. Its synthesis might have also been reduced by dexamethasone as described in the study using equine chondrocytes, although that was not monitored in this study. The previous studies showed that MMP inhibitors, such as TIMP-1 and neutralizing antibodies against either MMP-1 or MMP-3, blocked collagen degradation in the same explant system, suggesting that MMPs are responsible for this degradation. Therefore, the reduction of MMPs, including MMP-1, MMP-3 and possibly MMP-13, can be the reason why dexamethasone inhibited collagen degradation.

There remains another possibility that the inhibitory effect of dexamethasone on collagen degradation is due to the suppression of fibrinolytic cascade, since the present cartilage explant system contains plasminogen which is the primary factor activating proMMP. Inhibition of fibrinolytic cascade may result in failure of MMP activation, as reported previously. Actually, Augustine and Oleksyszyn proposed a hypothesis that the inhibition of matrix degradation by glucocorticoids is due to the suppression on fibrinolytic cascade. However, that hypothesis is unlikely in this system since dexamethasone did not suppress the conversion of plasminogen to plasmin in cartilage explants (Fig. 3). It seems inconsistent with the report that dexamethasone reduced the production of urokinase plasminogen activator (u-PA) and tissue plasminogen activator (t-PA) in synovial cells, which may result in decrease of plasminogen activation. This discrepancy may be explained by the possible different susceptibility to dexamethasone between cartilage and synovial cells. Thus, it seems likely that the inhibitory effect of dexamethasone on collagen degradation is due to suppression of MMP-synthesis rather than to suppression of fibrinolytic cascade.

Collagen release was not completely inhibited by dexamethasone even at the maximum concentration, while it was nearly completely inhibited by TIMP-1. TIMP-1 neutralizes activity of all MMPs by forming 1:1 stoichiometric complexes with MMPs, while dexamethasone reduces gene expression of MMPs. Incomplete inhibition of MMP expression by dexamethasone (Fig. 2) may result in incomplete inhibition of collagen release. There also remains the possibility that expression of MMP which cleaves minor types of collagen may not be blocked by dexamethasone, leading to incomplete inhibition of collagen release.

Dexamethasone suppresses matrix biosynthesis and modulates cartilage collagen composition from type II to type I predominance, leading to degeneration of the articular cartilage. The concentration of dexamethasone required to inhibit collagen degradation in the present study (10−9 M) was lower than that required to significantly suppress matrix biosynthesis (10−7 M). This may indicate that glucocorticoids have potential therapeutic implications, since maintaining a proper concentration of glucocorticoids may bring out their therapeutic effects and minimize their degenerative side-effects on the chondrocyte metabolism.
In summary, by monitoring collagen degradation this study clearly showed the inhibitory effect of dexamethasone on matrix degradation in cartilage explant culture. These findings may endorse the efficacy of glucocorticoids that was shown in animal OA model and suggest their potential therapeutic effectiveness.

REFERENCES