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Inflammation Research

Dose-dependent effects of corticosteroids on the expression of matrix-related genes in normal and cytokine-treated articular chondrocytes

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Abstract. *Objective and Design:* To assess the effects of glucocorticoids on the expression of multiple matrix-related genes in normal and cytokine-treated cultured equine articular chondrocytes in a phenotypically correct suspension culture.
Material or Subjects: Articular cartilage harvested from the joints of 15 foals, 7 yearling horses, and 16 adult horses.
Treatment: Glucocorticoids (dexamethasone, prednisolone, triamcinolone) at 10^{-10} to 10^{-4} M.
Methods: Equine articular chondrocytes maintained in suspension cultures were treated with glucocorticoids with and without human recombinant interleukin 1-β (IL1-β) and tumor necrosis factor-α (TNF-α). Northern blots of total RNA from the treated cells were probed with equine specific cDNA probes for a number of cartilage matrix-related genes. Zymography, Western blotting, and fluorography were also performed to study the effects on protein synthesis.
Results: The glucocorticoids, dexamethasone, triamcinolone, and prednisolone, markedly decreased MMP1, MMP3, MMP13, TIMP1, and ferritin steady-state mRNA levels. There were no qualitative differences seen among the tested corticosteroids although dexamethasone and triamcinolone appeared to be slightly more potent than prednisolone. The effects of the glucocorticoids on MMP transcription occurred consistently at lower doses than those required to similarly downregulate type II collagen and aggrecan. Link protein and fibronectin mRNA were increased by the glucocorticoids, and biglycan and decorin were minimally affected. Fluorography of [14-C] proline-labeled media demonstrated that the decrease in type II collagen transcription (mRNA levels) was paralleled at the protein level. Zymography and Western blotting confirmed the decrease in functional metalloproteinases found in chondrocyte cultures following glucocorticoid treatment.
Conclusions: The effects of glucocorticoids are complex inasmuch as they differentially affect numerous genes involved in the composition of cartilage matrix and the degradation of that matrix. This study provides new insight into the effects of glucocorticoids on the regulation of extracellular matrix and matrix-related genes by demonstrating that low doses of glucocorticoids can inhibit the degradative metalloproteinases with minimal negative effects on the transcription of extracellular matrix genes.

Key words: Corticosteroid – Chondrocyte – Cytokine – Metalloproteinase

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Introduction

Corticosteroids are among the most widely used drugs for the management of arthritis in both horses and humans [1–3]. They are potent anti-inflammatory agents, capable of inhibiting phospholipase A2 thereby blocking the arachidonic acid cascade and the generation of prostaglandins, kinins, and other inflammatory mediators. Because the drugs are such efficient anti-inflammatory compounds, they usually improve the clinical signs of swelling and pain. Despite the clinical success seen following administration of intra-articular corticosteroids, there is major concern that the beneficial effects are outweighed by deleterious effects on articular cartilage [4]. Most in vitro studies [5–7] and in vivo experiments [8–12] have shown matrix loss and/or decreased proteoglycan synthesis in treated articular cartilage, but other study designs have resulted in the conclusion that glucocorticoids are chondroprotective under specific conditions [13–15]. It has also been previously stated that glucocorticoids have a generalized inhibitory effect on the synthesis of type II collagen [16–18]. Although there are previous reports of glucocorticoid effects on the expression of some matrix-related genes by chondrocytes both in vitro [16, 19–21] and in vivo [22], no studies of which we are aware have examined the dose-related effects of these drugs on the simultaneous expression of multiple matrix-related genes in the same experimental system.

Although there are multiple pathways that lead to articular cartilage damage, there is general agreement that inflam-

matory cytokines such as interleukin-1(IL-1) and tumor necrosis factor-α (TNF-α) play pre-eminent roles in chronic destructive arthritis [23–26]. Both cytokines have been shown to be increased in inflammatory conditions and both clearly increase the production of degradative enzymes such as matrix metalloproteinases and aggrecanases [27, 28].

Here we explore the effects of glucocorticoid dose on the differential expression of constitutive matrix genes such as type II collagen, aggrecan, and link protein as well as other matrix-related genes such as the matrix metalloproteinases. These data clearly demonstrate that glucocorticoids affect genes involved in both the degradative and biosynthetic pathways of genes related to cartilage pathology and repair. More importantly, it appears that the balance of glucocorticoid effects on chondrocytes may be balanced by dose.

Materials and methods

Cell culture

Articular cartilage was harvested from the grossly normal joints of 38 horses (16 adults {≥ 2 years old}, 15 foals {< 12 months old} and 7 yearlings {12–24 months old}). The cartilage was minced with a scalpel and digested overnight in 0.5% collagenase (Collagenase D, Boehringer Mannheim, Indianapolis IN) [29]. After collagenase digestion, isolated chondrocytes were rinsed twice in phosphate-buffered saline and counted in a hemocytometer. Viability was estimated by trypan blue exclusion; experiments were continued only if the viability of isolated chondrocytes was > 95%. Isolated chondrocytes (10×10^6) were distributed into 30 mm culture dishes treated with poly(2-hydroxy ethyl methacrylate) (Sigma Chemical, St. Louis MO) to prevent cell adhesion and maintain chondrocyte phenotype [30, 31]. Comparisons were only made between dishes of cells from the same horse. The suspension cultures were maintained at 37 °C, 5% CO_2 at saturated humidity in DMEM, 10% FCS, and 50 µg/ml ascorbic acid for 2 days prior to treatment. The media were changed, and the cells were treated with doses of glucocorticoids ranging from 10^{-10} M to 10^{-4} M with or without either 25.0 ng/ml human recombinant TNF-α or 10.0 ng/ml human recombinant IL1-β (Collaborative Biomedical Products, Becton Dickinson, Franklin Lakes NJ). Pilot experiments and previously published work [28] had demonstrated maximal effects of these cytokines at these doses under these culture conditions. Cells were treated for 24 h prior to harvesting cells and spent media. After early experiments demonstrated a narrower range of dose effects and no qualitative differences between the glucocorticoids, the majority of experiments used dexamethasone in dose ranges from 10^{-8} M to 10^{-6} M concentrations. The short-term effects of the agents were expected to be evident after 24 h incubation. In selected experiments, 3.0 µCi/ml [14-C] labeled proline was included in the treatment media.

Northerns

Cells were solubilized in a monophasic acid phenol (TRIzol-GIBCO BRL Life Technologies, Gaithersburg MD) and aliquots saved for scintillation counting. Total RNA was extracted from the remaining cells and separated in denaturing 1% agarose gels (10 µg/ lane). The gels were capillary blotted to charged nylon membranes (Hybond N^+- Amersham Pharmacia Biotech, Piscataway, NJ). Probes were prepared by [α-^{32}P] dCTP random primed labeling of equine cDNA inserts for type II collagen, aggrecan, biglycan, decorin, link protein, fibronectin, ferritin, tissue inhibitor of metalloproteinase-1 (TIMP-1), and matrix metalloproteinases 1, 3, and 13 (interstitial collagenase [MMP1], stromelysin [MMP3], and collagenase-3 [MMP13] respectively). Prehybridization and hybridization were performed at 65 °C for 30 min and 90 min respectively using a commercial hybridization buffer (RapidHyb, Amersham Pharmacia Biotech, Piscataway, NJ). Northerns were washed three times for 20 min at 55 °C with 0.5X, 0.25X and 0.1X SSC and 0.1% SDS. The membranes were exposed to radiographic film (Reflection-NEN Life Sciences, Boston MA) for 6–24 h at –70 °C or were exposed in storage phosphor cassettes (Storm PhosphorImager-Amersham Pharmacia Biotech, Piscataway, NJ). The resulting images were quantified using computer integrated densitometry (NIH Image-*http://rsb.info.nih.gov/nih-image*). Variations in gel loading were corrected by densitometric measurement of the ribosomal bands in photo negatives of the ethidium-stained gels. Ninety-seven northern blots were performed.

Fluorography

Media were collected from the [^{14}C]-Proline-labeled media and acidified with glacial acetic acid (2.9% volume : volume). Pepsin (Sigma Chemical, St. Louis MO) (10 mg/ml) was added and digestion continued for at least 48 h at 4 °C. The samples were exhaustively dialyzed (12,000– 14,000 molecular weight cutoff), precipitated in 2 M NaCl, and centrifuged (30 min at 12,000 g). The pellet was resuspended in 0.5 M acetic acid and an equal volume of 2X denaturing sample buffer (50 mM Tris [pH 6.8], 2% SDS, 15% glycerol, 1% mercaptoethanol) and separated in 7.5% SDS-PAGE. The gels were enhanced (EN^{3}HANCE-NEN Life Sciences, Boston MA) dried, and exposed to radiographic film for 7–14 days. The resulting images were scanned, digitized, and quantified with NIH Image software.

MMP-3 Western Blotting

Protein in equal aliquots of spent media was precipitated by adding 2X volume of ice-cold acetone and freezing in –70 °C for 1 h. The samples were then centrifuged at 10,000g and 4 °C for 30 min. The pellet was dissolved in 0.5 M acetic acid and added to denaturing sample buffer and separated in 10% SDS-PAGE. The gel was soaked for 10–15 min in TRIS-glycine buffer with 5% methanol to partially remove the SDS and transferred to nitrocellulose membranes (NitroPure-Micron Separations Inc, Westborough MA) using an electroblotting apparatus (Hoefer Scientific, San Francisco CA) After transfer, the filters were washed at room temperature for 30 min in 0.05% polyoxyethylene-20-sorbitan monolaurate (Tween 20-Fisher Scientific, Pittsburgh PA) in Tris buffered saline (TBS/TW20). Blocking was done for at least one hour in TBS/TW20 with 3% nonfat milk at room temperature. The filters were incubated overnight at 4 °C with the primary antibody (1:500 polyclonal ovine anti-human MMP-3 (E. Arner, DuPont Merck, Wilmington DE) in TBS/TW20 + 0.5% milk. After two 10 min washes in TBS/TW20, the second antibody (1:5000 alkaline phosphatase conjugated goat anti-sheep antibody (Sigma Chemical, St. Louis MO)) was incubated for 60 min at room temperature. The filters were then washed 3 times 10 min in TBS/TW20 and 2 times 10 min in TBS before adding the development substrate solution (BCIP/NBT Color Development Substrate-Promega, Madison WI).

Zymography

One µl of 100 mM p-aminophenyl mercuric acetate (APMA) (Sigma Chemical, St. Louis MO) was added to 100 µl spent media and incubated at 37 °C for 30 min. Matching samples were prepared without APMA activation. Proteins in all samples were precipitated with the addition of 2 volumes of acetone followed by cooling at –70 °C for 1 h. The samples were centrifuged (12,000 g, 30 min, 4 °C) and the pellet dried in a vacuum centrifuge. The pellet was resuspended in water, an aliquot mixed 1:1 with a loading buffer (0.125 M Tris HCl [pH 6.8], 20% glycerol, 4% SDS, 0.05% bromophenol blue) and incubated at room temperature for 10 min. Aliquots were separated in gels containing either 0.1% gelatin or 0.05% casein (Novex, San Diego, CA) The gels were incubated in a renaturing buffer (2.5% Triton X-100) for

30 min at room temperature followed by another 30 min in a developing buffer (10 mM Tris base, 40 mM Tris HCl, 0.2 M NaCl, 5 mM $CaCl_2$, 0.02% [w/v] Brij 35). The gels were then incubated overnight at 37°C in fresh developing buffer, stained with 0.5% Coomassie blue R250 for 30 min, destained for 15 min in 45% methanol/7% acetic acid solution and washed twice for 20 min in water before drying. The dried gels were digitized and the cleared areas of proteinase activity were quantified with NIH image software.

Data Analysis

Statistical comparisons were made by two-way analysis of variance. Normality of the data was assessed by a Shapiro-Wilks test before natural log transformation of the data. Differences were considered significant if $p^3/_4$ 0.01.

Results

Northern Blot analysis

As previously reported [28], both IL1-β and TNF-α increased steady-state mRNA of MMP1, MMP3, and MMP13 dramatically (4–14-fold). One endogenous inhibitor of MMPs, TIMP1, was not increased by TNF-α and only slightly increased (1.5X) by IL1-β. Messenger RNA for the acute phase reacting iron binding protein subunit, ferritin (heavy chain), also was increased 2–3-fold by the cytokines (Fig. 1). Interleukin 1-β and TNF-α both decreased mRNA levels of type II collagen (~50%), link protein, and aggrecan (>75%) with smaller effects on biglycan, decorin, and fibronectin (Fig. 1).

Differences in changes in transcription of the studied genes were seen primarily between 10^{-8} M and 10^{-6} M doses of steroids. There were no qualitative differences seen between the three studied steroids although prednisolone was less potent on a molar basis. Dexamethasone significantly ($P \leq 0.01$) decreased MMP1, MMP3, MMP13, TIMP1 and ferritin steady-state mRNA in a dose-dependent manner at concentrations of $\geq 10^{-8}$ M. These decreases ranged over the tested dose range from 10%–80% (Fig. 2A, B). Over the same dose range, dexamethasone decreased type II collagen and aggrecan steady state mRNA significantly (~60%) only at doses greater than 10^{-6} M. The tested doses had an insignificant effect on biglycan and decorin. The glucocorticoids significantly increased link protein mRNA 1.5-2.5-fold and fibronectin mRNA 1.5-2-fold at 10^{-8}–10^{-6} M (Fig. 2A, C).

Dexamethasone at a concentration of $\geq 10^{-8}$ M effectively reversed the transcriptional effects of the cytokines on MMP, TIMP1, and link protein but did not appear to have an additive effect on the other measured matrix components. Both link protein and fibronectin mRNA increased with increasing doses of dexamethasone even in the presence of the cytokines (Fig. 3A–E).

Zymography and Western blot analysis

All three of the studied glucocorticoids had similar effects at a functional protein level, inhibiting caseinase activity at doses of $\geq 10^{-8}$ M (Fig. 4). Gelatin zymography revealed two major bands concluded to be MMP2 and MMP9 based on size [32, 33]. There was a dose-dependent decrease in the active gelatinases in the media following glucocorticoid treatment of the cells, but the effect was less marked than that seen in the casein gels, presumably reflecting MMP3 activity. Western blot analysis of MMP3 further confirmed the transcriptional effects carried through to the protein level. Western blot analysis with human TIMP1 antibodies was not successful.

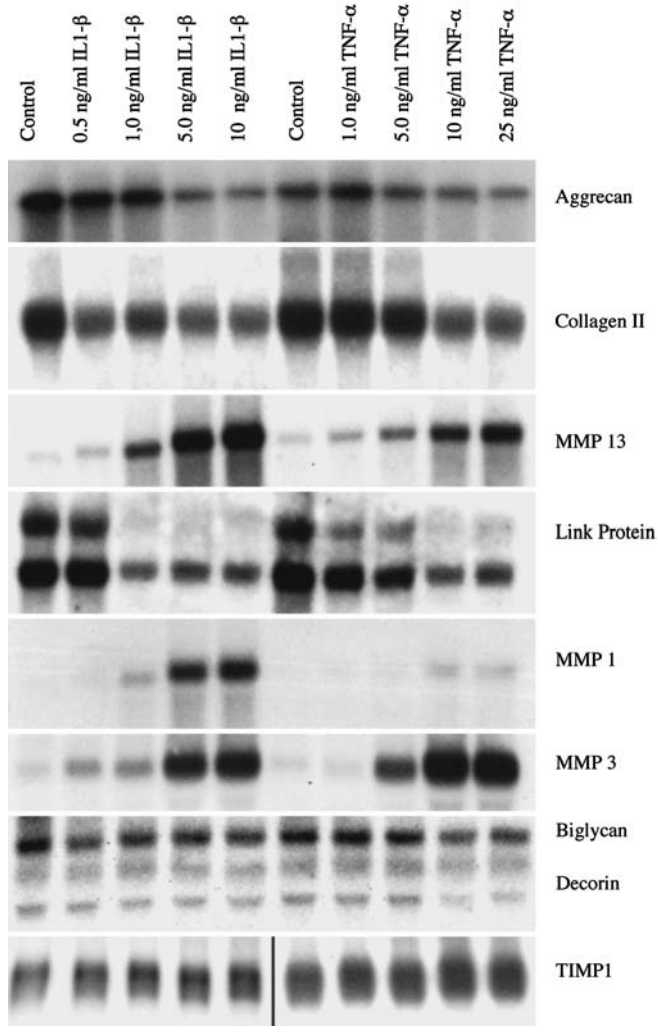

Fig. 1. Northern blot from chondrocytes exposed to different concentrations of IL1-β and TNF-α. Steady-state mRNA for aggrecan, type II collagen, and link protein are decreased by the cytokines. The metalloproteinases 1, 3, and 13 are increased. The tissue inhibitor of metalloproteinase-1 (TIMP1) and the small proteoglycans, biglycan and decorin, are less affected.

Fluorography

Dexamethasone decreased the synthesis of pepsin-resistant [14-C] proline-labeled protein at doses $>10^{-6}$ M. This effect appeared to be dose dependent (Fig. 5).

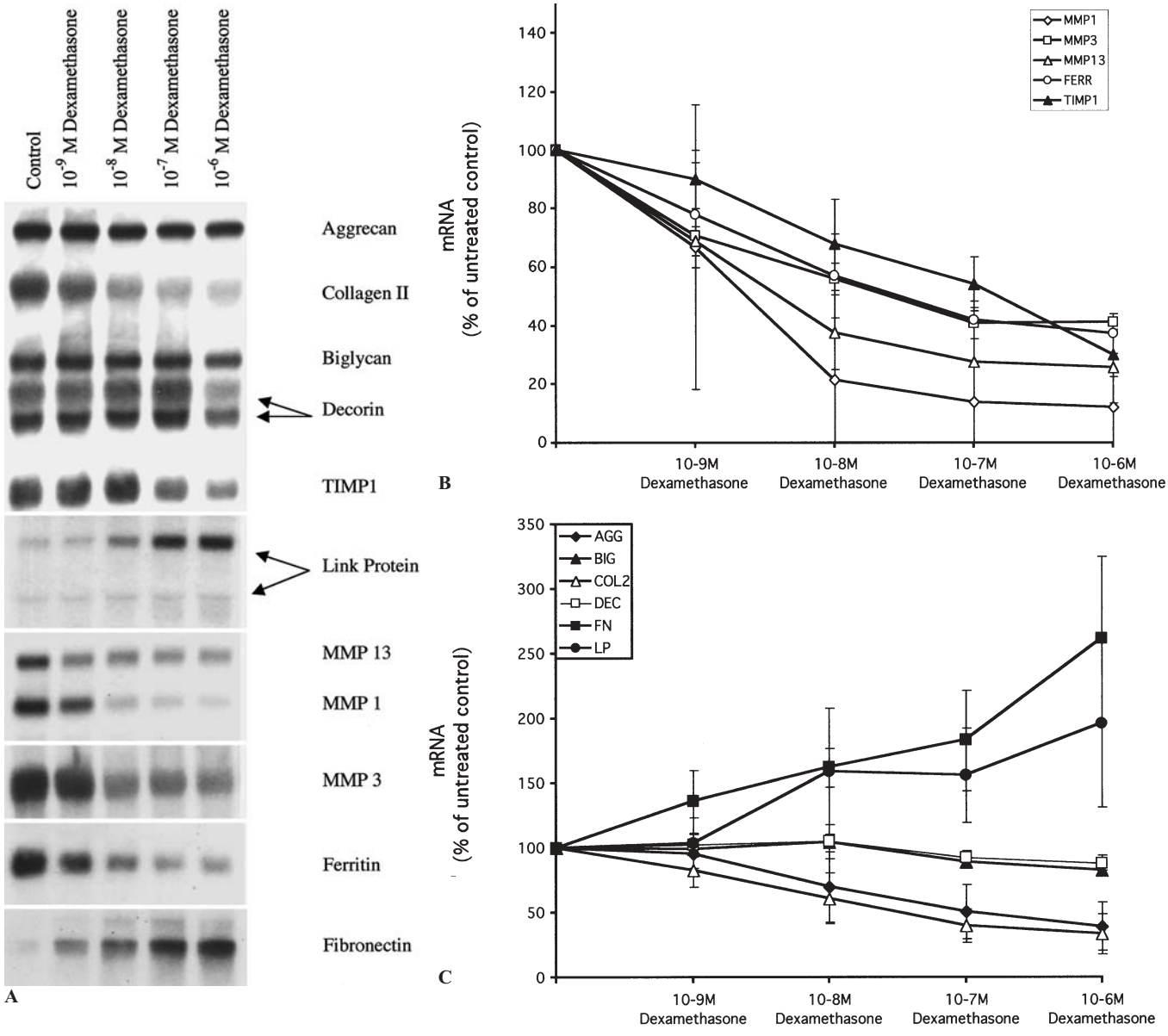

Fig. 2A–C. Dexamethasone decreased MMP1, MMP3, MMP13, TIMP1 and ferritin at concentrations of $\geq 10^{-8}$ M, diminished type II collagen and aggrecan at $\geq 10^{-6}$ M and had insignificant effect at any dose on biglycan and decorin. The glucocorticoids increased link protein and fibronectin mRNA at 10^{-8}–10^{-7} M. (Means ± SEM)

Discussion

Type II collagen is the major collagen of articular cartilage and is an essential component of its architecture and biomechanical performance. In light of its important role in articular cartilage, it is important to understand how drugs such as glucocorticoids affect its synthesis. This is particularly important because intra-articular glucocorticoids are often used to alleviate pain and inflammation in arthritis. In this culture system, whereby chondrocytes are phenotypically maintained [31], high doses ($\geq 10^{-6}$ M) of glucocorticoids significantly decreased the expression of type II collagen by isolated articular chondrocytes. This finding is consistent with previous claims that glucocorticoids may be deleterious to articular cartilage. In our study, however, the effects of glucocorticoids at high doses did not significantly affect the levels of biglycan and decorin mRNA. Perhaps, the most significant finding of this study is that the effect of glucocorticoids on decreasing MMP mRNA was seen at lower concentrations than those concentrations that inhibit the gene expression of structural matrix molecules. When the glucocorticoids were combined with cytokines, they effectively reversed this upregulation of MMP expression induced by IL-1 or TNF-α. Most studies examining the effects of glucocorticoids on articular cartilage have focused on the net synthesis/loss of proteoglycans in the articular cartilage matrix, and often

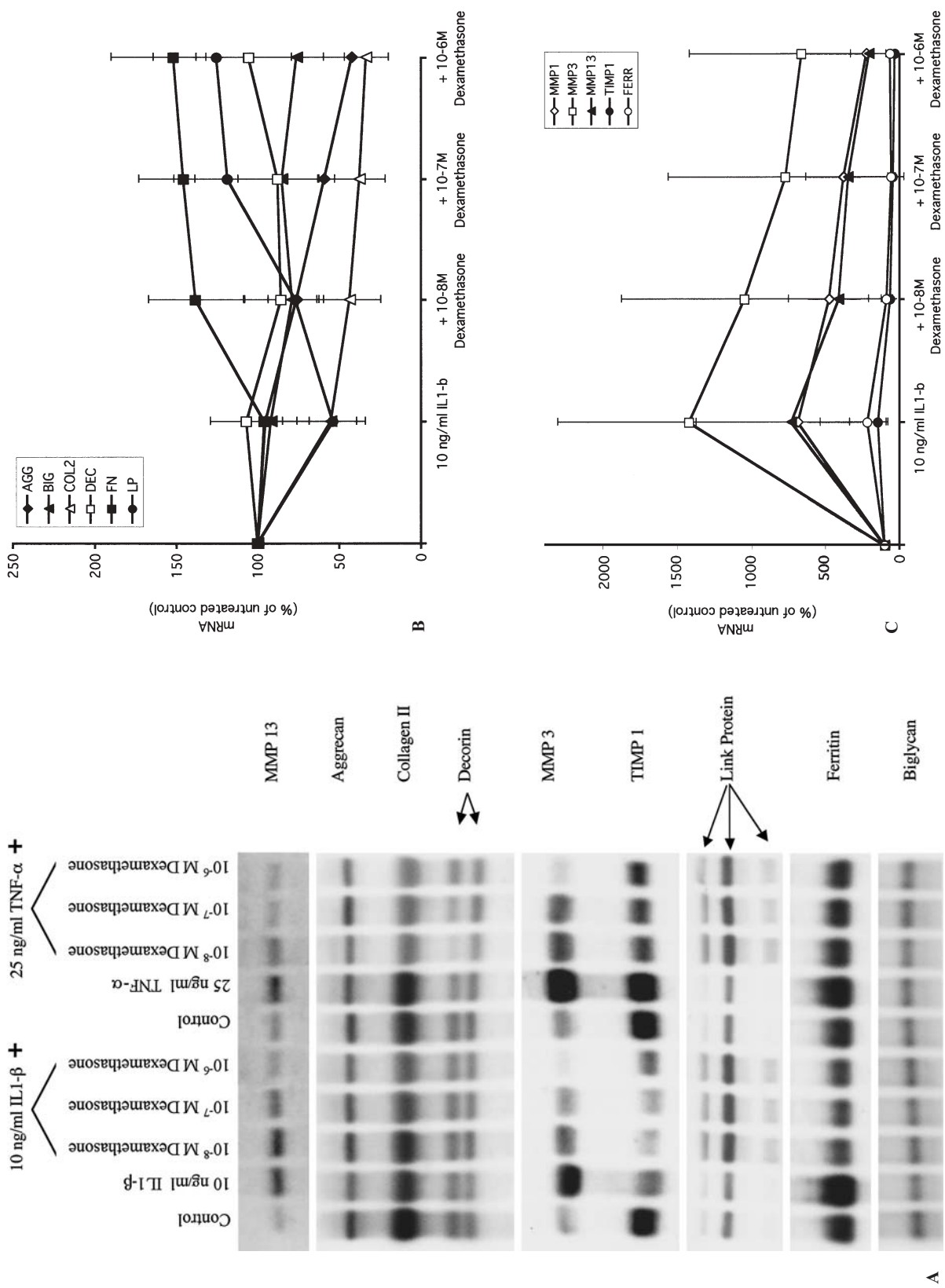

Fig. 3A–E. Steady-state mRNA for type II collagen, aggrecan, and link protein were decreased by IL1-β. The effect on link protein was reversed by increasing doses of dexamethasone, but the decrease in type II collagen and aggrecan was enhanced. (**A, B**) Steady-state mRNA for metalloproteinases 1, 3, and 13 were increased by IL1-β and the effect reversed by increasing doses of dexamethasone. The effects on ferritin and TIMP-1 were similar but less dramatic. (**A, C**) TNF-α diminished steady-state mRNA levels of several matrix elements, especially type II collagen. Only the effect on link protein was effectively reversed by the addition of increasing doses of dexamethasone. (**A, D**) Steady-state mRNA for metalloproteinases 1, 3, and 13 were increased by TNF-α and the effect reversed by increasing doses of dexamethasone. The effects on ferritin and TIMP-1 were similar but less dramatic. (**A, E**) (Means ± SEM)

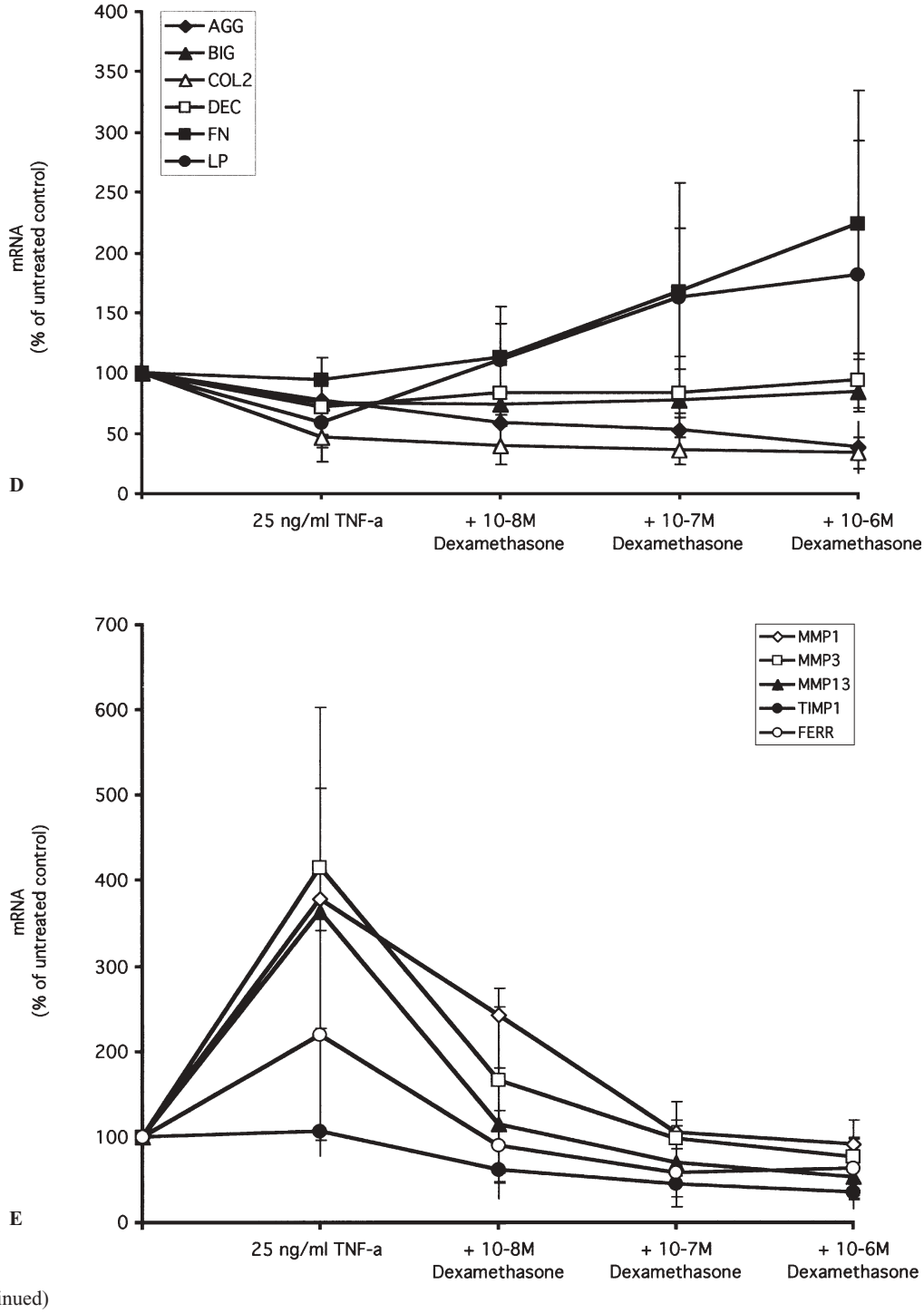

Fig. 3A–E (continued)

more specifically on the incorporation of radiolabeled sulfate in the glycosaminoglycans of the matrix. In general, there has been an increase in the incorporation of radiolabeled sulfate seen in early osteoarthritic cartilage that is believed to reflect a reparative response to injury/disease. MacLeod et al reported a doubling of aggrecan steady-state mRNA levels in cartilage in inflamed equine joints and a decrease to control levels in joints treated with methylprednisolone acetate [22]. In contrast with our experiments in cultures of normal equine chondrocytes, steady-state mRNA levels of aggrecan and versican were not consistently decreased by glucocorticoids except at high concentrations. The small leucine-rich proteoglycans, biglycan and decorin, were not significantly altered by glucocorticoids. This is in contrast to results in other cells, such as osteoblasts and bone marrow stromal cells in which dexamethasone (10^{-7} M) increased decorin and decreased biglycan expression [34] and dermal fibroblasts where biglycan was unaffected by

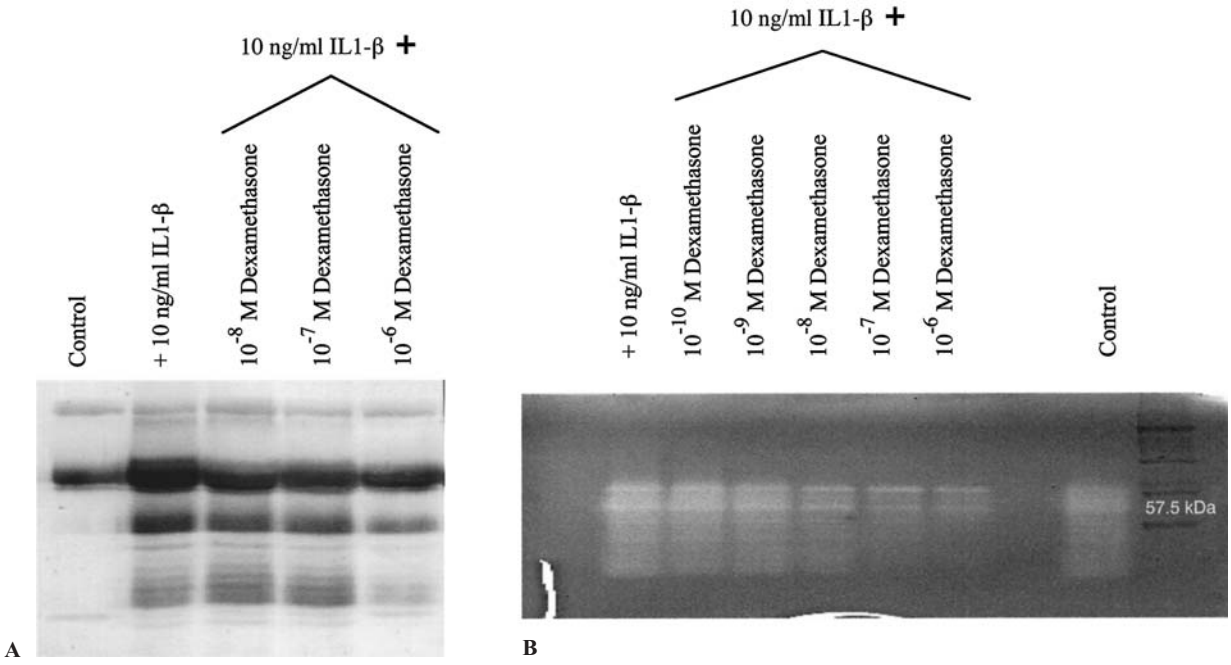

Fig. 4. Western Blot with MMP3 antibody (**A**) and casein zymography (**B**) both show an increase MMP3 following IL1-β exposure that was diminished in a dose-dependent manner by dexamethasone. Both the immunostained band and the zone of greatest caseinase activity were present at the predicted size of active MMP3.

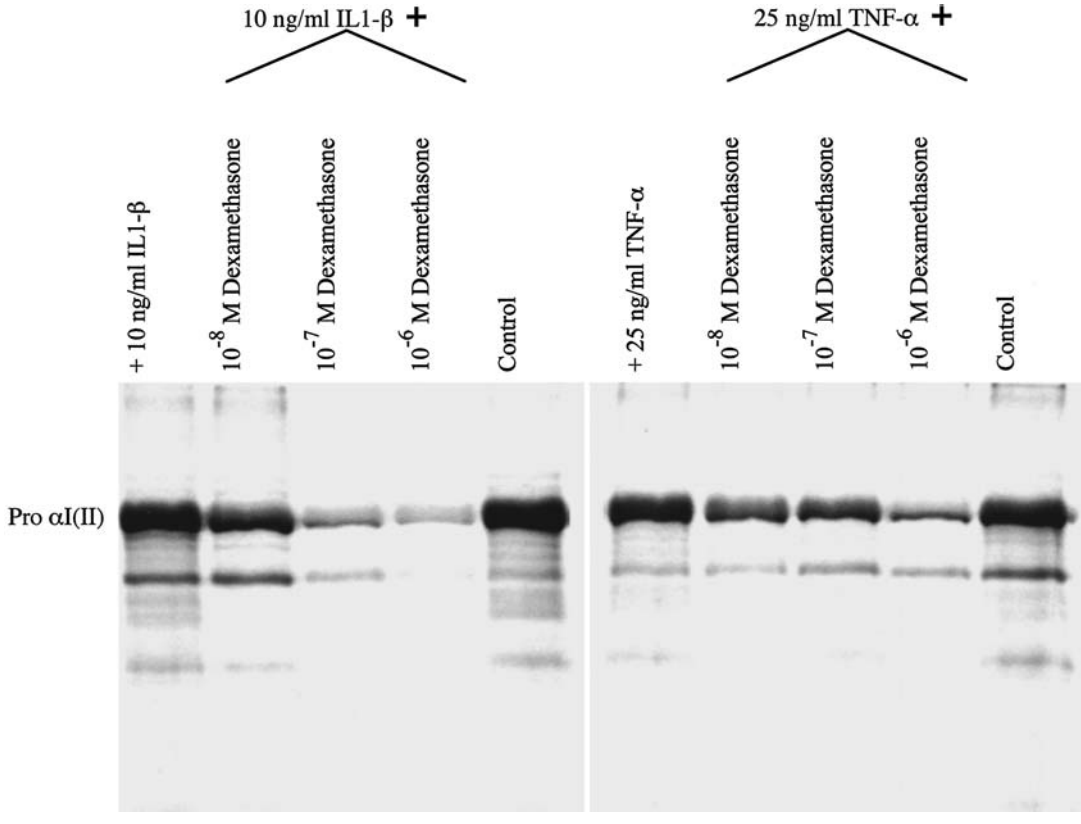

Fig. 5. Fluorogram of pepsin digested media from chondrocytes cultured in the presence of cytokines and different doses of dexamethasone. The slight decrease in the collagen II synthesis following exposure to IL1-β and TNF-α was worsened by increasing doses of the glucocorticoid.

dexamethasone (10^{-9} M – 10^{-6} M) while decorin was increased [35]. The role of these molecules in normal and diseased cartilage is not completely understood although it is suggested that in some cells they can be modulated by cytokines known to be involved in cartilage and joint tissue pathology [36]. Biglycan is believed to be involved in cell-cell interactions through its interactions with other matrix components such as type VI collagen [37]. Biglycan also appears to play an important role in skeletal growth and bone formation [38–40]. Decorin is believed to play a role in collagen fibrillogenesis and possibly collagen-matrix interactions [37, 41]. Decorin has been reported to increase in exercised cartilage [42, 43] and increase with age [44, 45], but to be little affected by the cytokines IL1-β and TNF-α. Glucocorticoids could have additional post-transcriptional and translational effects on any or all of these proteoglycans that might affect glycosaminoglycan content, sulfation patterns, and net protein synthesis.

Fibronectin is a large (> 220,000 MW), nearly ubiquitous connective tissue protein that is involved in organization of extracellular matrices [46, 47]. In our study, fibronectin appeared to be minimally affected by cytokines but increased by $\geq 10^{-8}$ M dexamethasone. This finding of glucocorticoids increasing fibronectin expression has been previously reported in chick hepatocytes [48] and some human fibroblastic cell lines [49]. This increase in fibronectin steady-state mRNA levels was not seen by MacLeod following glucocorticoid treatment of inflamed equine joints [22]. Fibronectin has been shown to increase in osteoarthritic cartilage [50] but in MacLeod's study was not increased in cartilage from inflamed joints before structural changes in the cartilage were manifest [22]. The cartilage-specific isoform of fibronectin was seen to decrease in cartilage following glucocorticoid exposure [21]. Fibronectin fragments appear to play an important role in degradative processes of joints [51, 52] but it is not clear how the synthesis of fibronectin in one or more forms is directly related to the pathogenesis of arthritis.

Link protein is a 41,000–46,000 MW glycoprotein that functions in cartilage matrix to attach the core aggregating protein, aggrecan, to a long polymeric backbone of hyaluronan thus forming the supramolecular aggregate that is essential for normal biomechanical function of articular cartilage [53]. The protein and cDNA have been previously characterized in the horse [54]. Expression of and maintenance of link protein has been shown to be altered in arthritic cartilage [55, 56] and its transcription decreased in a dose-dependent manner by both IL1-β and TNF-α [28, 57]. As evident in our present study, this decrease in link protein mRNA levels seen following cytokine treatment was reversed by exposure to $\geq 10^{-8}$ M dexamethasone. The apparent upregulation of link protein by glucocorticoids has been previously reported [58].

Ferritin is a large (~450,000 MW) iron-binding protein comprising 24 assembled subunits, termed "H" (heavy or heart) and "L" (light or liver). Neither H-ferritin nor L-ferritin has a known function in articular cartilage but the mRNA for H-ferritin is increased in many different cell types by exposure to various cytokines [59–62]. It appears that ferritin is a relatively non-specific acute phase reactant to various inflammatory stimuli in a number of tissues [59–61, 63]. There is considerable evidence that ferritin levels in synovial fluid and serum are increased in patients with rheumatoid arthritis [64–66]. Glucocorticoids decreased baseline steady-state mRNA levels of ferritin and definitely reversed the upregulation of ferritin seen following exposure to cytokines. The significance of ferritin and regulation of its expression in articular cartilage is still unknown.

The hallmark of osteoarthritis is loss of the normal structure and function of articular cartilage. As mentioned previously, synthesis of multiple structural proteins of the matrix is actually increased in disease [22] but the net outcome is progressive loss of matrix integrity. Clearly the balance of synthetic and degradative pathways is tipped towards net loss of matrix in diseased joints. Among the most active elements in the degradative side of the equation are the matrix metalloproteinases (MMP). MMP are zymogens and, in addition to depending on prior activation, require zinc for catabolic activity. Although there are marked similarities among the MMP, each MMP has a preferred substrate that presumably is somehow related to its role in development, normal tissue remodeling, as well as disease processes. MMP3 (stromelysin 1) is well characterized in horse cartilage and believed to have the proteoglycans as preferred substrates [67–69]. MMP13 (collagenase 3) has type II collagen as a preferred substrate and has been studied in equine chondrocytes [20, 28]. MMP1 or interstitial collagenase is another MMP capable of cleaving fibrillar collagens that is secreted by multiple cells of fibroblastic lineage including chondrocytes. It is generally accepted that MMP increases in arthritis and that it specifically increases in response to many cytokines. It is clear from our results and those of others [13, 20, 70] that glucocorticoids are capable of dramatically decreasing MMP in articular tissues. In this study, it appears that an obvious decrease in steady-state mRNA levels is seen following doses of glucocorticoids that do not appear to have a major effect on other matrix components. There were differences in baseline MMP expression in chondrocytes cultured from different age animals with older horses being generally higher. The techniques used in this study did not allow us to separate topographically distinct subpopulations and assess their response to the cytokines or the steroids. Others [71] have demonstrated subsets of chondrocytes in cartilage with differing baseline MMP expression.

The balance of synthesis and degradation in cartilage is not only linked to the expression of MMP but also to the naturally occurring inhibitors termed tissue inhibitors of metalloproteinases or TIMP. We have cloned and described TIMP 1 [68] but at least two other TIMP have been described in other species' cartilage. TIMP bind to and inactivate metalloproteinases so the relative balance of MMP and TIMP synthesis is believed to be an important factor in the development of arthritis [72–75]. We have previously shown that cytokines cause an increase in MMP expression to a far greater extent than the small increase (1.5–2X) in TIMP mRNA levels induced by the same cytokines [28]. The net result of this imbalance is presumably an excess of degradative activity. In the present study, TIMP1 also was significantly decreased by increasing doses of corticosteroids. It is intuitive that a decrease in TIMP1 expression would not be beneficial in a diseased joint. Nevertheless, the overwhelming effect of the glucocorticoids on the cytokine-induced chondrocytes' expression of MMP may still yield a net beneficial effect. Such net protective effects of glucocor-

ticoids on articular cartilage integrity have been seen in a number of controlled studies [13, 14, 76, 77]. The more pertinent finding to the therapeutic use of glucocorticoids may be the dose or concentration of the drugs in the tissue. It also will be important to determine if other TIMPs found in joint tissues are regulated similarly.

In 2 separate studies, [78, 79] synovial fluid levels of methylprednisolone acetate and methylprednisolone were measured in equine joints following their intra-articular administration. Although it is impossible to directly relate synovial fluid concentrations with those actually affecting the chondrocytes within articular cartilage, the molar concentrations were approximately 10^{-8} M to 10^{-6} M following intrasynovial injection of 100 mg of the steroid. This corresponds to the range of concentrations studied in our experiments. Although concentrations of glucocorticoids that we studied in vitro were within the range of expected tissue concentrations seen in vivo, the actual concentrations exposed to the chondrocytes are not known. It also must be acknowledged that the complexity of glucocorticoids' effects on other cells in the joint may be important in the net therapeutic result.

Glucocorticoids clearly have complex effects on many genes important in the synthesis and maintenance of articular cartilage. The mechanism of corticosteroid inhibition of matrix metalloproteinase and TIMP1 synthesis is probably related to the consistent presence of a consensus AP1 element in the promoter region of these genes that is inhibited by the glucocorticoids [80–82]. We have also demonstrated this element in the equine MMP3 and MMP1 genes. In contrast, link protein and type II collagen have similar AT-rich glucocorticoid-responsive enhancer elements in the first intron [58, 83]. Glucocorticoid regulation of collagen I transcription is mediated by a specific glucocorticoid-responsive element and a transforming growth factor beta (TGF-β) element. Because the effects of corticosteroids on matrix-related genes are dose-dependent, specific to certain genes and occur through various mechanisms, it appears that beneficial effects of steroids may be obtained at doses that do not adversely affect matrix protein synthesis. The independent mechanisms also offer the future possibility of independently regulating these genes.

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