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OSTEOARTHRITIS and CARTILAGE

Acute synovitis and intra-articular methylprednisolone acetate in ponies

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Summary

Objective: To determine how acute synovitis, with and without intra-articular methylprednisolone acetate (MPA), affect synthesis of proteoglycan, total protein, and collagen in articular cartilage and total protein synthesis in synovial membrane.

Design: Synovitis was induced in 10 ponies by the injection of 0.5 ng lipopolysaccharide (LPS) into the left radiocarpal and midcarpal joints every 2 days for a total of four treatments. Synovitis was documented by clinical examination and synovial fluid analyses. Two days before euthanasia, MPA (0.1 mg/kg) was injected with the last dose of LPS into both the left and right radiocarpal and midcarpal joints of five of these ponies. Proteoglycan synthesis in articular cartilage explants from these joints was measured by incorporation of sodium [^{35}S]sulfate. The size of the proteoglycan monomers and their aggregation with hyaluronan was assessed by size-exclusion chromatography. Protein synthesis in articular cartilage was measured by incorporation of [^{3}H]proline and collagen synthesis by conversion of [^{3}H]proline into [^{3}H]hydroxyproline. Protein synthesis was measured in synovial membrane explants by incorporation of [^{35}S]methionine.

Results: Ponies developed carpal effusion and mild lameness accompanied by increased total nucleated cell count and total solids in synovial fluid in response to the LPS injections. Moderate to severe synovial membrane proliferation and inflammation were observed histopathologically in joints injected with LPS but no consistent light-microscopical changes were observed in the articular cartilage from these joints. Intra-articular MPA alone was associated with decreased proteoglycan synthesis and increased protein and collagen synthesis in the cartilage explants. Total protein synthesis by synovial membrane was also increased by MPA alone. In contrast, no differences in protein or proteoglycan synthesis were observed in explants from the joints with synovitis, with or without intra-articular MPA. Treatment with MPA, LPS, and LPS/MPA did not alter proteoglycan aggregate size, but LPS-induced synovitis resulted in an increase in the second largest population of monomers. MPA increased the synthesis of small proteoglycan monomers.

Conclusion: Based on the methods used, acute synovitis prevented changes induced by intra-articular MPA alone. Results suggested that the effect of intra-articular MPA on joint metabolism was different between inflamed and normal joints. Experimental studies must consider the effect of inflammation, as well as the potential to introduce *in vitro* culture artifacts when investigating the effect of intra-articular corticosteroids on chondrocyte function.

Key words: Synovitis, Corticosteroids, Cartilage, Metabolism.

Introduction

Synovitis has been implicated in the initiation of osteoarthritis in racehorses [1–4]. Although the role of synovitis as a primary initiator of osteoarthritis in humans is controversial, it has, nevertheless, been associated with osteoarthritis [5, 6] and with the intermittent exacerbations that occur in this disease [7, 8]. Synovitis results in release of cytokines like interleukin-1 and tumor necrosis factor α [4, 9, 10], which down-regulate matrix synthesis in isolated chondrocytes and cartilage explants [11–14]. However, articular cartilage matrix synthesis was increased in models of osteoarthritis in dogs [15–17] and there was a trend ($P = 0.06$) toward an increase in equine joints with synovitis [18]. Steady-state mRNA levels of aggrecan and type II procollagen were increased

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significantly in cartilage from equine joints with lipopolysaccharide (LPS)-induced synovitis [19].

Corticosteroids are potent anti-inflammatory drugs for treatment of inflamed joints and are widely used in humans [20] and horses [21]. Although helpful in relieving symptoms of joint inflammation, experimental evidence indicates that intra-articular corticosteroids like methylprednisolone acetate (MPA) (Depomedrol®, Upjohn) cause loss of basophilia and decreased intensity of safranin 0 staining of articular cartilage, chondrocyte necrosis, decreased proteoglycan content, decreased collagen synthesis, increased water content and delayed healing of experimentally-induced osteochondral defects [22–31]. A similar effect of corticosteroids on articular cartilage in horses [32] and other species [26–28, 33] occurs *in vitro*. Keratan sulfate, a marker of articular cartilage degradation [34–36], was elevated significantly in the synovial fluid of ponies treated 21 days previously with intra-articular MPA [21]. It has been shown in rats [37], horses [38], and *in vitro* in dogs [39], that the degenerative processes induced in articular cartilage by intra-articular corticosteroids ('steroid-induced arthropathy') render the tissue more susceptible to mechanical injury. Despite the experimental data that intra-articular corticosteroids damage articular cartilage, the controversy over the relationship between their therapeutic benefits as anti-inflammatory agents and the occurrence of 'steroid-induced arthropathy' continues [20]. Part of the problem in explaining the difference between clinical response and results of experimental data may reside in the experimental model used and the dose of corticosteroid administered [20, 29–31, 39–41].

We propose that the effect of intra-articular corticosteroids on joint metabolism is influenced by dose [32], joint use [38] and the local joint environment. We recently demonstrated that dynamic load counteracted the effect of moderate doses of methylprednisolone sodium succinate (Solumedrol®, Upjohn) *in vitro* [38] and that the response of articular cartilage to cytokine-induced injury *in vitro* depended on the dose of corticosteroid used as well as whether the cartilage had been treated with cytokines before treatment with corticosteroids [41]. The objective of the current study was to compare the effects of synovitis and intra-articular MPA on cartilage and synovial membrane gene expression and metabolism in the carpal joints of rested ponies. Here, we present the protein synthesis data and compare it to transcriptional data that has been reported elsewhere [19].

Materials and Methods

Ten ponies were used ranging in age from 2–3 years and in weight from 200–300 kg. The 20 carpi from these 10 ponies were assigned to one of four experimental groups. All ponies received 0.5 ng LPS (Sigma, St. Louis, MO, U.S.A.) into the left radiocarpal and midcarpal joints every other day for a total of four injections (Fig. 1). Preliminary studies showed that 0.5 ng LPS produced a consistent inflammatory response in ponies compared with the 0.125 ng described by Palmer and Bertone in horses [42]. In five of these ponies, 0.1 mg/kg of MPA was administered into the left radiocarpal and midcarpal joints (with the last dose of LPS) and into the contralateral joints on the right limb. We considered this a moderate dose of intra-articular MPA (about half of that often injected into carpal joints of horses clinically), but we injected this into both carpal joints of each forelimb (Fig. 1). The other five ponies received no MPA in either carpus. Therefore, there were four different joint treatments, but the joint treatments were confounded within pony in that each pony received only two of the four treatments. Venous blood for complete hemograms was collected on days 0, 4, 7, and 8 during the experiment. Carpal synovial fluid was collected into sodium EDTA on the same days. Total solid concentration and nucleated cell count were measured on synovial fluid and the distribution of these cells was assessed. Ponies were euthanized on day 8 with an overdose of pentobarbital sodium.

HISTOPATHOLOGY

The carpal joints were opened aseptically, examined and photographed. Synovial membrane was collected from the dorsolateral aspect of the carpal joints (Fig. 1), fixed in 10% phosphate-buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin. Histopathological evaluation of the synovial membrane was performed by a pathologist (K.P.F.) unaware of treatment assignments. The degree of inflammation as judged by the cellular infiltrate, the proliferation of synovial lining cells, and the amount of fibrous tissue was graded from the least inflamed (0) to the most severely inflamed (4) [43, 44].

Articular cartilage and attached subchondral bone was collected from the dorsomedial radial carpal bone (Fig. 1), decalcified in 10% formic acid, dehydrated, embedded in paraffin, sectioned at 6 μm, and stained with safranin O/fast green or hematoxylin and eosin. Cartilage was evaluated

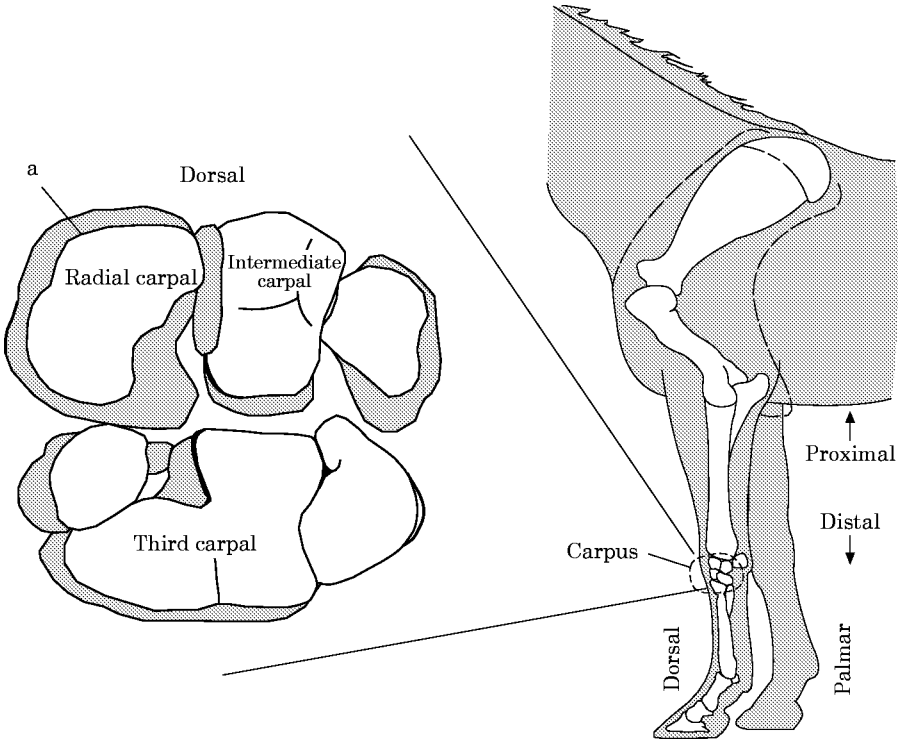


FIG. 1. Diagram shows the location of the carpal joint in the horse and the insert shows the distal surface of the proximal row and the proximal surface of the distal row of carpal bones. The location of the articular cartilage sample taken from the radial carpal bone for histopathology is shown (a). Synovial membrane was sampled from the dorsal (anterior) surface of this joint.

for surface architecture, chondrocyte morphology, and pericellular, territorial, and interterritorial matrix metachromasia [45] without knowledge of treatment assignments.

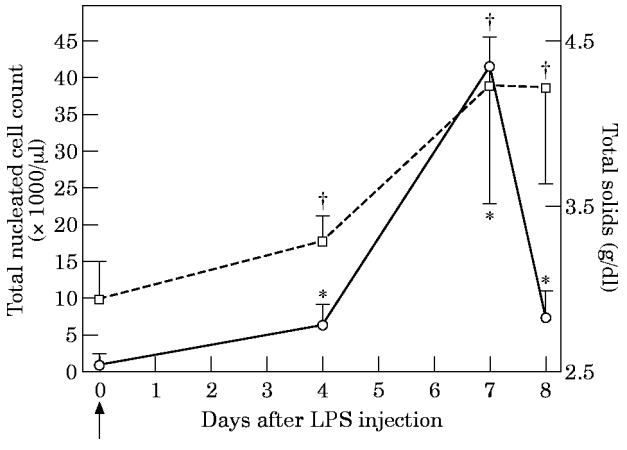


FIG. 2. Graph showing changes in the total nucleated cell count (TNCC; – – –) and total solids (TS; ——) of synovial fluid from equine carpal joints with LPS-induced acute synovitis. Values are the least squares mean ± S.E.M. of measurements on synovial fluid taken from the radiocarpal and midcarpal joints of five ponies on the days indicated. The ponies were injected with LPS on days 0 (after collection of the initial synovial fluid sample), 2, 4, and 6.

BIOCHEMISTRY

Proteoglycan synthesis

Eight-millimeter-diameter cartilage discs (excluding calcified cartilage) were excised aseptically from the proximal and distal intermediate carpal bone (Fig. 1) with a dermal biopsy punch and scalpel blade. The discs were pooled, washed, allocated randomly into individual wells and incubated in defined, serum-free, medium based on Ham's F_{12} (Gibco, Grand Island, NY, U.S.A.) which contained a supplement with insulin (ITSCR+, Collaborative Research, Bedford, MD, U.S.A.) and included ascorbate and $CaCl_2$ [46]. Cartilage was incubated at 10 mg wet wt/ml medium. Explants were incubated at 37°C, 90% humidity, 5% CO_2/95% air, in quadruplicate, with 20 μCi/ml of sodium [^{35}S]sulfate for 6 h. The average time between euthanasia and initiation of radiolabelling was 7 h. Following radiolabelling, the explants were washed in ice-cold Gey's balanced salt solution (Gibco) and frozen at –70°C.

Explants were digested with papain (1.0 mg/ml) (Sigma) at 0.1 ml per 10 mg wet wt at 65°C for 8 h [47]. Radioisotope incorporation was measured with scintillation counting. Sulfated glycosaminoglycan content of the explants was determined

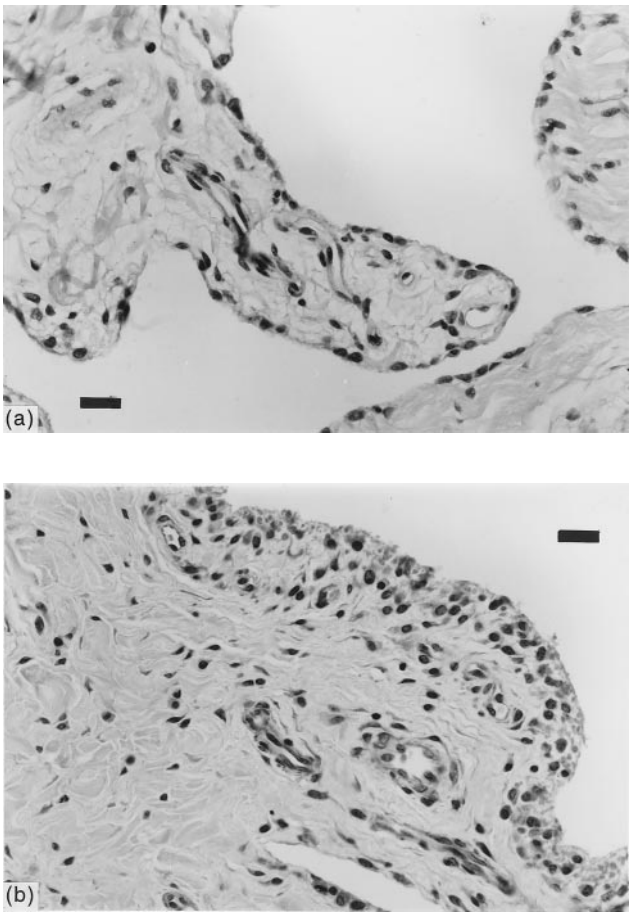


FIG. 3. Photomicrographs of synovial membrane from normal (a) and inflamed (b) midcarpal joints of ponies. (a) Normal synovial membrane consisting of a layer of lining cells (1–2 cells thick) adherent to the subintima. (b) The synovial membrane following four injections of 0.5 ng of LPS is hypertrophic and hyperplastic accompanied by cellular infiltration and fibrovascular subintimal proliferation. Bar = 20 nm. Hematoxylin and eosin stain.

with the dimethylmethylene blue dye-binding (DMMB) assay [47]. Proteoglycan synthesis was expressed as counts per minute/μg glycosaminoglycan.

Size-exclusion chromatography

Cartilage explants from two ponies (four carpi, representing each treatment group) were radiolabelled with sodium [^{35}S]sulfate (20 μCi/ml) for 6 h and washed to remove free radioactive sulfate. Proteoglycans were extracted with 4 M guanidinium chloride containing protease inhibitors, concentrated (Amicon Concentrators, Amicon Inc., Beverly, MA, U.S.A.; 30 000 molecular weight cut-off) by centrifugation (100 000 counts/min concentrated to 1.0 ml), and proteoglycan monomers were chromatographed dissociatively on a Sepharose CL2B (Pharmacia, Piscataway, NJ, U.S.A.) column. The columns were calibrated with ferritin, dextran blue, and bromphenol blue. All samples were chromatographed with bromphenol blue to ensure that chromatographic conditions remained constant. For associative chromatography, extracts containing 100 000 counts/min were concentrated to 100 μl and diluted to 0.4 M guandinium chloride by adding 700 μl of 0.1 M Na_2SO_4 and 0.05 M NaAc in 0.1% Triton X-100 at pH 6.1. Hyaluronan (Healon®, Pharmacia) was prepared at 2.0 mg/ml in the same buffer and added to the extract to a final concentration of 0.4 mg/ml. The extract was then chromatographed associatively on a Sepharose CL2B column. Column eluant was collected in fractions, radioactivity was measured with scintillation counting and sulfated glycosaminoglycans in each fraction were measured with the DMMB assay [47].

PROTEIN ANALYSIS

Cartilage

Separate cartilage discs were incubated in Dulbecco's modified Eagle's medium (Gibco) which contained a supplement with insulin (ITSCR+, Collaborative Research), ascorbate and $CaCl_2$ [46]. Cartilage was incubated for 6 h in quadruplicate at 10 mg wet wt/ml medium containing 40 μCi/ml of [^{3}H]proline at 37°C, 90% humidity, 5% CO_2/95% air. Following radiolabelling, the explants were placed in fresh medium without radiolabel and chased for 8 h under the same conditions to maximize incorporation of [^{3}H]proline. The explants were washed in ice-cold 50% ethanol and frozen at 70°C. Collagen synthesis was measured as [^{3}H]proline incorporation into [^{3}H]hydroxyproline as described [32]. Collagen synthesis was expressed as counts per minute. [^{3}H]hydroxyproline per microgram dry weight cartilage and total protein synthesis as total counts per minute in the combined [^{3}H]proline and [^{3}H]hydroxyproline peaks per microgram dry weight cartilage.

PROTEIN SYNTHESIS

Synovial membrane

Synovial membrane was collected aseptically from the dorsolateral aspect of the midcarpal joint (Fig. 1) and incubated in quadruplicate in Dulbecco's Modified Eagle's Medium sup-

plemented with insulin (ITSCR+), ascorbate, and $CaCl_2$. Explants were incubated at 20 mg wet wt/ml medium at 37°C, 90% humidity, 5% CO_2/95% air, with 10 μCi/ml [^{35}S]methionine for 6 h. Following radiolabelling, the explants were washed gently in ice-cold Gey's balanced salt solution (Gibco) and frozen at –70°C. Proteins were extracted from explants with 4 M guanidinium chloride followed by 1 M sodium hydroxide. Proteins were precipitated on filters with perchloracetic acid and unincorporated radiolabel was removed by washing. Radioactivity in the protein precipitate was counted in scintillation fluid and expressed per miocrogram protein in the membrane which was estimated by the Coomassie blue assay (Peirce Chemical Co., Rockford, IL, U.S.A.).

STATISTICAL METHODS

Measurements on synovial fluid were $\log_e$ transformed and analyzed with a mixed model analysis of variance for repeated measures in which the pony was random and the joint treatment was fixed within pony. Synthesis measurements were expressed as a percent of the control joint, $\log_e$ transformed, and analyzed with a mixed model analysis of variance with $P < 0.05$ considered significant. Linear contrasts were constructed to determine differences between control and treated joints and correlation analysis was based on Pearson's correlation coefficient.

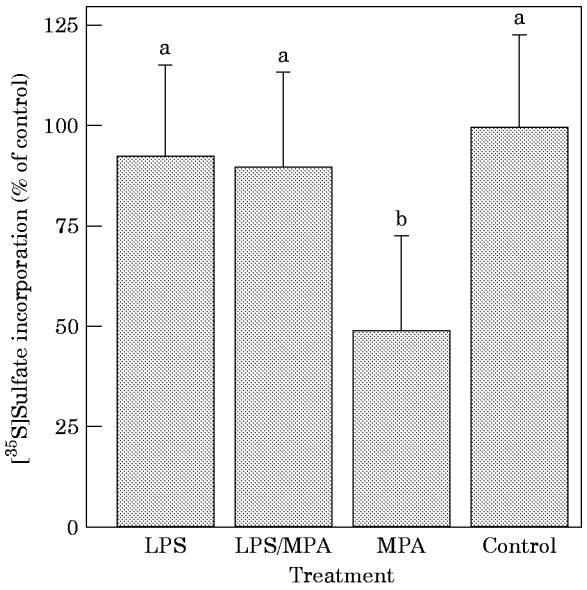


FIG. 4. Histogram showing corticosteroid- and synovitis-induced changes in proteoglycan synthesis in equine articular cartilage. Carpal cartilage explants were harvested from ponies in the four experimental groups described in the Materials and Methods. Quadruplicate explants were radiolabelled with [^{35}S]sulfate to measure proteoglycan synthesis. Data are expressed as least squares mean ± S.E.M. and as a percent of control joint synthesis. MPA, methylprednisolone acetate; LPS, lipopolysaccharide. Different letters indicate $P < 0.05$ between treatment groups.

Results

CLINICAL

Ponies remained normothermic with no changes in hemograms. LPS-treated carpi had carpal effusion and ponies showed minimal lameness accompanied by a positive response to flexion test. Synovitis was accompanied by a significant increase in total nucleated cell count and total solids over the course of the experiment except that total solids fell between day 7 and day 8 (Fig. 2). There was no significant effect of MPA on total cell count or total solids in synovial fluid of joints with, or without, LPS-induced synovitis.

HISTOPATHOLOGY

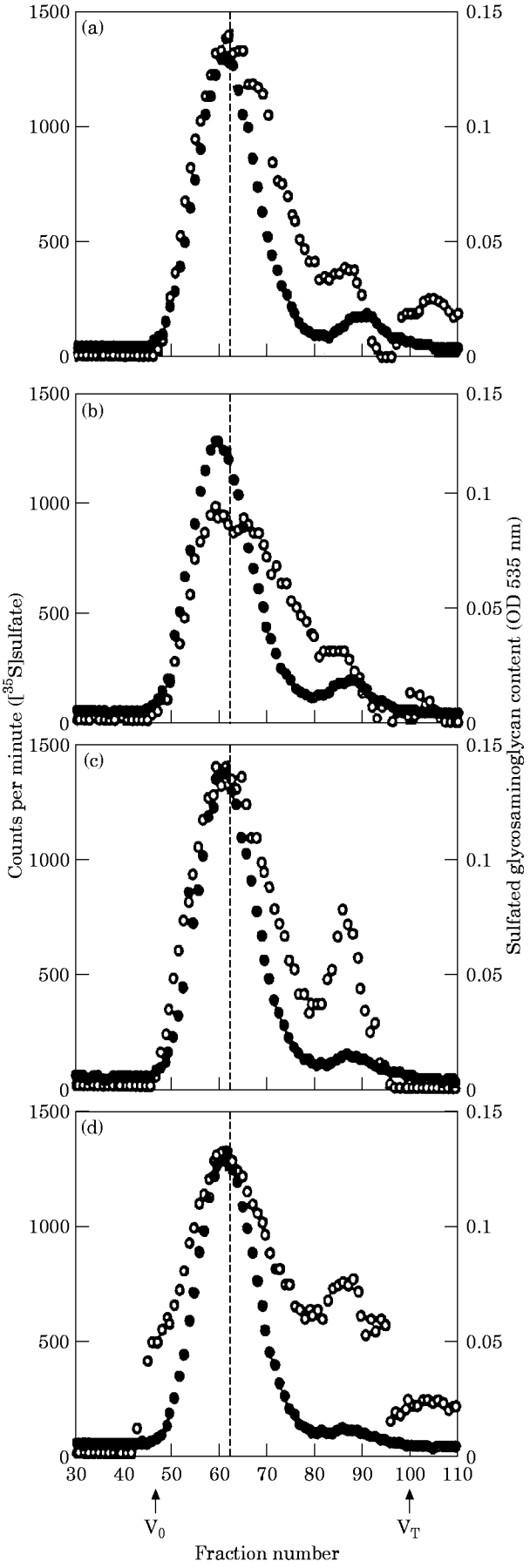
Both the LPS- and LPS/MPA-treated joints had moderate to severe inflammatory changes (mean score 3.5) while the MPA-treated and control joints had mild to no inflammatory changes (mean score 0.5) (Fig. 3). There were no significant differences between groups based on histopathologic examination of articular cartilage.

PROTEOGLYCAN SYNTHESIS

MPA caused a significant decrease in proteoglycan synthesis in carpal articular cartilage compared with all other treatment groups (Fig. 4). There was no difference between proteoglycan synthesis in cartilage from control joints or those treated with LPS or LPS/MPA (Fig. 4).

SIZE-EXCLUSION CHROMATOGRAPHY

When extractable proteoglycans were chromatographed dissociatively, most of the radioactive sulfate was incorporated into aggrecan monomers that eluted first from the column and not into the smaller (later-eluting) proteoglycans when compared to the proportion of small proteoglycans in



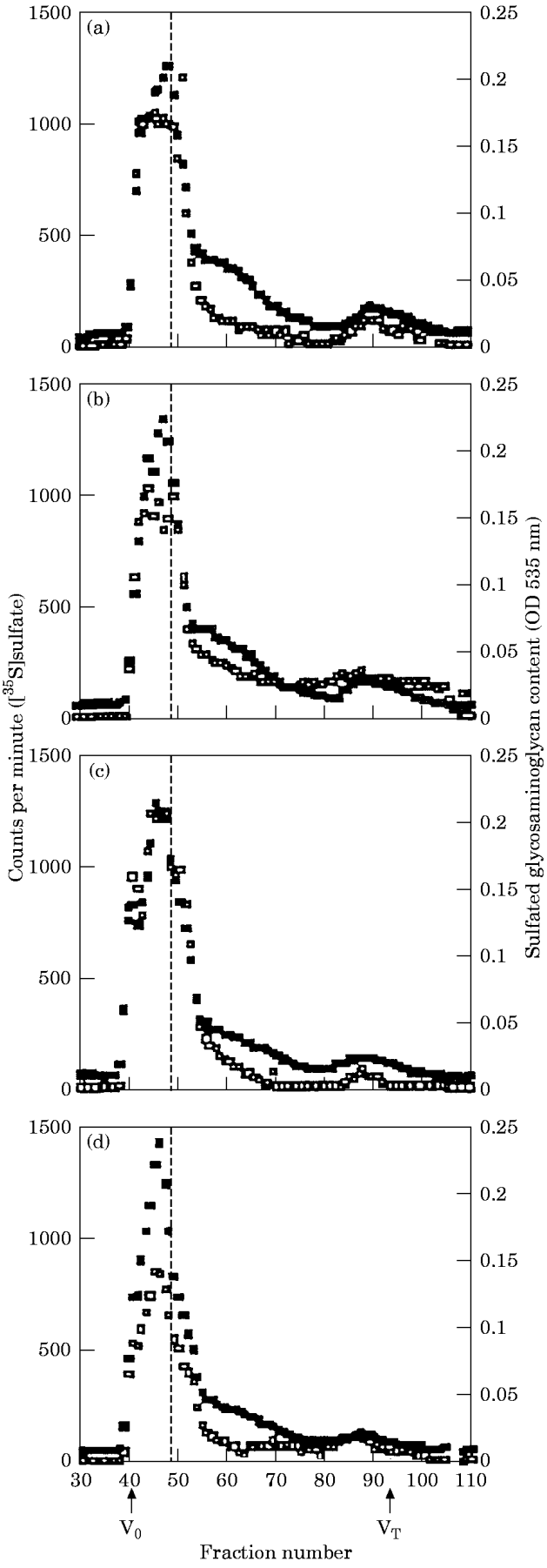
total extractable proteoglycans (Fig. 5). Total proteoglycans, as measured by the DMMB assay, eluted in three populations; two major overlapping peaks eluting initially and one peak of small proteoglycans eluting later. Treatment with MPA [Fig. 5(c)] increased the synthesis of a population of small proteoglycan monomers compared to the proportion of small proteoglycans in total proteoglycans extracted from control cartilage. LPS induced an increase in the second largest population of monomers based on an increase in the size of the second largest peak in the elution profile relative to the size of the first peak and a decrease in the slope of this peak between fractions 65 and 80 [Fig. 5(b)]. The combined effects of both of these treatments were apparent in extracts of cartilage from joints treated with LPS/MPA [Fig. 5(d)].

When extractable proteoglycans were chromatographed associatively, little change was observed in elution location (Fig. 6).

PROTEIN SYNTHESIS IN CARTILAGE

Collagen [Fig. 7(a)] and total protein [Fig. 7(b)] synthesis in explants from joints treated with MPA were significantly higher than synthesis in the explants from the control joints. Collagen and total protein synthesis in explants from LPS- or LPS/MPA-treated joints were not significantly different to synthesis in explants from control joints, although synthesis in these joints tended to be higher than synthesis in control joints. There was a strong positive correlation ($r = 0.88$, $P < 0.0001$) between collagen and total protein synthesis, but no significant correlation between synthesis of proteoglycan and collagen by cartilage.

FIG. 5. Elution profiles of size-exclusion dissociative chromatography. Sepharose CL2B columns were calibrated with blue dextran, ferritin and bromphenol blue. Bromphenol blue was added to all samples to check for consistent elution location. Proteoglycans in cartilage explants were labelled with sodium [^{35}S]sulfate and extracted with 4 M GuHCl. Proteoglycans were chromatographed dissociatively in 4 M GuHCl. Fractions were collected and scintillation counted (closed circles) and sulfated glycosaminoglycans (open circles) were measured with the dimethylmethylene blue dye-binding assay. (a)–(d) elution profile of dissociative chromatography of proteoglycans extracted from cartilage of control (a), LPS-injected (b), MPA-injected (c), and LPS/MPA-injected (d) joints.



PROTEIN SYNTHESIS IN SYNOVIAL MEMBRANE

Total protein synthesis in the synovial membrane was significantly higher in MPA-treated joints than in control joints (Fig. 8). There was no difference in protein synthesis in synovial membrane from joints treated with LPS or LPS/MPA compared with control joints.

Discussion

Several main points arise from our study: the synovitis we induced in the carpal joints of ponies did not progress to histologic evidence of osteoarthritis within the time frame of the study. However, our transcriptional data reported elsewhere [19] clearly showed that synovitis significantly increased steady-state levels of type II procollagen mRNA (a six- to sevenfold elevation) and, to a lesser extent, aggrecan core protein mRNA (a twofold elevation). Our synthesis data did not corroborate this effect and we shall offer a possible explanation for this dichotomy. Nevertheless, the effect of intra-articular MPA on synthesis of both collagen and proteoglycan by cartilage explants was affected by the presence of synovitis. Finally, intra-articular MPA caused the synthesis of a population of small proteoglycans which may affect cartilage function.

The experimental model of intra-articular injection of subnanogram amounts of LPS to induce synovitis in horses was described originally by Palmer and Bertone [42]. Intra-articular injection of LPS induces an acute phase response [48]. Significant increases in interleukin-1, interleukin-6, and tumor necrosis factor-α have also been measured in naturally-occurring acute and severe chronic synovitis in horses [10]. The dose of LPS used in the current study induced negligible lameness with no systemic effects or clinical signs of endotoxemia. Therefore, the contralateral joint

FIG. 6. Elution profiles of size-exclusion, associative chromatography. Sepharose CL2B columns were calibrated with blue dextran, ferritin and bromphenol blue. Bromphenol blue was added to all samples to check for consistent elution location. Proteoglycans in cartilage explants were labelled with sodium [35S]sulfate and extracted with 4 M GuHCl. Proteoglycans were chromatographed associatively in 0.4 M GuHCl after addition of exogenous hyaluronan. Fractions were collected and scintillation counted (closed squares) and sulfated glycosaminoglycans (open squares) were measured with the dimethylmethylene blue dye-binding assay. (a)–(d) elution profile of associative chromatography of proteoglycans extracted from cartilage of control (a), LPS-injected (b), MPA-injected (c) and LPS/MPA-injected (d) joints.

can be used as an untreated control. At the dose of 0.5 ng LPS/joint, we did not observe significant changes in articular cartilage morphology as described by Todhunter *et al*. [45] who used 3 μg LPS per injection. They observed decreased uptake of the histochemical stain toluidine blue, chondrocyte hypertrophy, and expression of glycosaminoglycans containing the 3-B-3 epitope which is considered to be a marker of cartilage injury and repair [49]. These findings are consistent with induction of the hypertrophic phase of osteoarthritis. We presume that the lack of effect that we observed on cartilage morphology in the current study was due to the very low dose of LPS and the short (8-day duration) experiment. Although we observed no systemic effects of LPS, any amount of intra-articular LPS might affect the metabolism in the contralateral joint. If there was any undetectable effect, it was also accounted for by the mixed model analysis of variance in which the least squares means adjust for the baseline level of synthesis in each pony. Nevertheless, the model did permit us to show an interaction between synovitis and intra-articular MPA. Different amounts of either LPS or MPA may have produced more dramatic results, but the negligible pain induced by our model was advantageous and we chose a moderate dose of intra-articular MPA that approximates amounts used in clinical situations.

Proteoglycan synthesis in cartilage was depressed by MPA and this effect was abrogated in the presence of synovitis. However, there was no increase in proteoglycan synthesis in response to acute synovitis as reported by others [15–17, 50, 51]. The *P*-value in Palmer *et al*.'s study [18] on the effect of LPS-induced synovitis in equine joints did not reach significance at an α level of 0.05. Steady-state levels of aggrecan mRNA isolated directly from articular cartilage in these same carpal joints was increased twofold by synovitis [19]. Although we expected transcriptional changes in the expression of aggrecan and other matrix macromolecules in cartilage to be followed by parallel translational changes in the synthesis of the corresponding macromolecules, this was not observed. For the translational experiments, we performed our radiolabeling as soon as possible after tissue explantation. Intra-articular injection of radiolabel would be preferable but difficult to control radioactive contamination in a large animal. Another option would

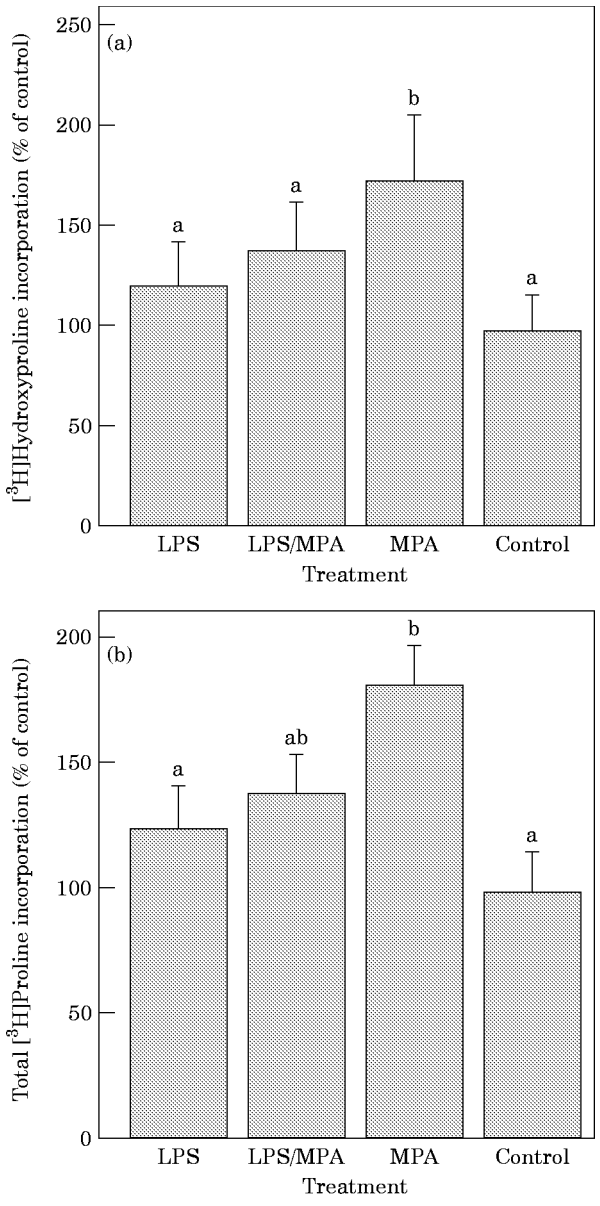


FIG. 7. (a) Histogram showing corticosteroid- and synovitis-induced changes in total collagen synthesis in equine articular cartilage. Carpal cartilage explants were collected from ponies in the four experimental groups described in Materials and Methods. Explants were radiolabelled in quadruplicate with [^{3}H]proline and incorporation into [^{3}H]hydroxyproline was used as a measure of collagen synthesis. Data are expressed as least square means ± S.E.M. and as a percent of control joint synthesis. MPA, methylprednisolone acetate; LPS, lipopolysaccharide. Different letters indicate $P < 0.05$ between treatment groups. (b) Histogram showing corticosteroid- and synovitis-induced changes in total protein synthesis in equine articular cartilage. Carpal cartilage explants were collected from ponies in the four experimental groups described in Materials and Methods. Explants were radiolabelled with [^{3}H]proline and total incorporation of [^{3}H]proline was used as a measure of total protein synthesis. Data are expressed as least square mean ± S.E.M. and as a percent of control joint synthesis. MPA, methylprednisolone acetate; LPS, lipopolysaccharide. Different letters indicate $P < 0.05$ between treatment groups.

have been to radiolabel the whole bone immediately upon excision. However, this presented logistical problems and increased the risk of radioactive contamination and cost. In the 7 h that elapsed between cartilage collection and initiation of radioactive labelling and the 6 h of the pulse, the anabolic response to inflammation may have faded. Another point to consider, however, is that transcriptional and translational events are not necessarily tightly correlated. Translational regulation, post-translational modifications, cytoplasmic transport and secretion rates can affect protein synthesis.

MPA induced the synthesis of the smallest proteoglycan eluting between fraction 85–90 [Figure 5(c) and (d)]. This small proteoglycan may affect cartilage function or integrity. We have observed this effect previously *in vitro* following treatment of cartilage with MPA [32]. This change in proteoglycan synthetic profile may reflect a partial 'de-differentiation' of the chondrocytes toward a more fibroblastic phenotype and warrants further investigation.

In contrast to MPA-induced suppression of aggrecan synthesis, explants from joints treated with MPA had increased collagen and total protein synthesis compared with control joints. Again, these translational data did not support the transcriptional results in which decreased steady-state COL2A1 mRNA levels were observed in these same joints from cartilage processed immediately after euthanasia and without an *in vitro* culture period [19]. Therefore, the increased synthesis of collagen and protein by cartilage explants as a result of MPA treatment is probably due to either a residual protective effect of MPA on chondrocyte function following explantation or to a rebound phenomenon that occurs when cartilage explants are cultured following earlier *in vivo* suppression of synthesis caused by MPA [41]. We have previously interpreted similar results from *in vitro* experiments as evidence that low doses of MPA stimulated collagen and protein synthesis in cartilage explants [32]. However, it is now apparent that we should consider the possibility of a rebound effect on synthesis during the radiolabelling and chase period based on the more recent data. A rebound phenomenon may also explain the effect of MPA on protein synthesis observed in the synovial membrane explants.

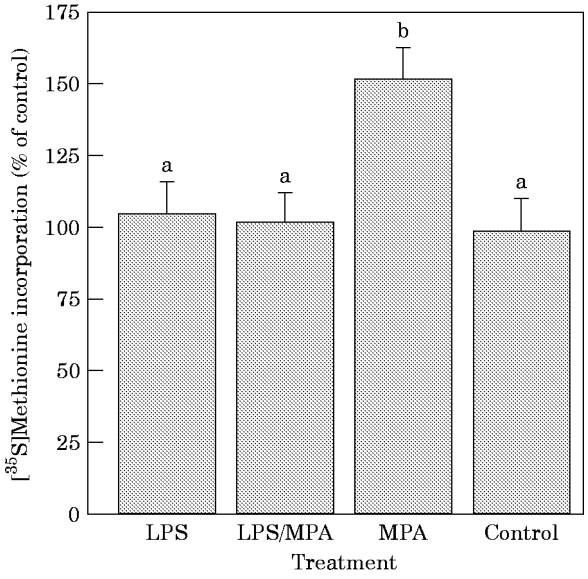


FIG. 8. Histogram showing corticosteroid and synovitis-induced changes in total protein synthesis of equine synovial membrane. Carpal synovial membrane explants were collected from ponies in the four experimental groups described in Materials and Methods. Explants were radiolabelled with [^{35}S]methionine and incorporation into total protein was measured following extraction. Data are expressed as least squares mean $\pm$ S.E.M. and as percent of control joint synthesis. MPA, methylprednisolone acetate; LPS, lipopolysaccharide. Different letters indicate $P < 0.05$ between treatment groups.

Synovial inflammation prevented the depression of chondrocyte proteoglycan synthesis and the increase in collagen synthesis observed with treatment by MPA alone. The effect of corticosteroids on proteoglycan synthesis in equine cartilage explants [41] and isolated chondrocytes [52] is partially abrogated by prior exposure to cytokines. Intra-articular MPA may be removed rapidly from an inflamed joint, dilutional effects from the synovial effusion on the concentration of MPA may play a role, inflammatory cytokines and growth factors in synovial fluid may alter chondrocyte gene expression [19], and glucocorticoid receptor levels in synovial tissue may have been decreased in the inflamed joint [53].

Molecular mechanisms of corticosteroid action and interactions between corticosteroids and cytokines may partly explain our findings and the sometimes contradictory results of studies involving normal [29–31] and diseased [39, 40, 62–66] joints. Binding of the activated steroid–receptor complex to DNA modulates the transcription of genes that have functional steroid–response elements [54, 56]. Thus, depending on the gene, transcriptional modulation can be positive or negative [55]. Our data reported here indicate that the duration of the effect appears to vary for the aggrecan core protein gene compared to the COL2A1 gene and according to the dose of corticosteroid [41]. The competitive interaction between the steroid–receptor complex and

cFos cJun heterodimers [56–58] and an MPA-induced increase in inhibitory factor of nuclear factor Kappa B [59, 60] will also affect the manner in which corticosteroids affect inflammation.

Combined, the presence of these multiple variables may help to explain apparent contradictions between *in vitro* and *in vivo* data and why 'corticosteroid-induced arthropathy' has been difficult to study and explain at both a clinical and experimental level. Furthermore, it appears clear that caution should be exercised when extrapolating the detrimental effects of intra-articular MPA on normal joints to their clinical effects in inflamed joints.

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