# Effects of Intramuscular Polysulfated Glycosaminoglycan on Chemical and Physical Defects in Equine Articular Cartilage

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## ABSTRACT

The effect of intramuscular polysulfated glycosaminoglycan (PSG) on repair of cartilage injury was evaluated in eight horses. In each horse, one middle carpal joint had both a partial-thickness and a full-thickness articular cartilage defect created. In the contralateral middle carpal joint, chemical articular cartilage injury was created by intra-articular injection of 50 mg sodium monoiodoacetate (MIA). Horses were divided into two groups for treatment. Group 1 horses (control) received an intramuscular injection of normal saline every four days for a total of seven injections starting seven days after cartilage injury. Group 2 horses received 500 mg of PSG intramuscularly every four days for seven treatments starting seven days after cartilage injury. Horses were maintained for 12 weeks.

Horses were evaluated clinically, and their middle carpal joints were evaluated radiographically and arthroscopically at the end of the study. Joint tissues were also collected and examined microscopically. The only significant difference between groups was slightly greater matrix staining intensity for glycosaminoglycans in the radiate articular cartilage layer in MIA injected and PSG treated joints. Partial-thickness defects had not healed and the predominant repair tissue in full-thickness defects was fibrous tissue. It was concluded that using this joint injury model, 500 mg PSG administered intramuscularly had no effect on the healing of articular cartilage lesions, and minimal chondroprotective effect from chemically induced articular cartilage degeneration.

## RÉSUMÉ

Cette expérience portait sur huit chevaux et elle consistait à évaluer l'effet de l'administration intramusculaire de polysulfate de glycosaminoglycane, sur la réparation de lésions cartilagineuses. Chez chaque cheval, ou provoqua, sur une surface articulaire carpienne moyenne, une lésion qui impliquait toute l'épaisseur du cartilage et une autre qui n'en impliquait qu'une partie. Dans l'articulation correspondante de l'autre membre antérieur, on provoqua une lésion cartilagineuse par l'injection intra-articulaire de 50 mg de mono-iodoacétate de sodium. On répartit ensuite les chevaux en deux groupes. Ceux du premier, les témoins, reçurent une injection intramusculaire d'eau physiologique, à tous les quatre jours et à sept reprises, à compter du septième jour ultérieur aux lésions cartilagineuses. Ceux du deuxième reçurent une injection intramusculaire de 500 mg de polysulfate de glycosaminoglycane, à tous les quatre jours et à sept reprises, à compter du septième jour ultérieur aux lésions cartilagineuses. On garda tous ces chevaux pendant 12 semaines au cours desquelles on procéda périodiquement à leur évaluation clinique.

À la fin de l'expérience, on procéda à la radiographie et à l'arthroscopie de la partie moyenne des carpes de tous les chevaux, on les sacrifia et on préleva des échantillons des articulations précitées, pour l'histopathologie. La seule différence significative entre les deux groupes se traduisit par une coloration un peu plus intense des glycosaminoglycanes de la matrice de la couche cartilagineuse radiaire des articulations traitées par l'injection locale de monoïodoacétate de sodium ou intramusculaire de polysulfate de glycosaminoglycane. Les lésions qui n'impliquaient qu'une partie de l'épaisseur du cartilage n'étaient pas cicatrisées, alors que le tissu fibreux constituait le principal tissu de réparation de celles qui en impliquaient toute l'épaisseur. Les auteurs conclurent que dans ce genre de lésions articulaires, l'administration intramusculaire de 500 mg de polysulfate de glycosaminoglycane n'exerçait aucune influence sur leur guérison et seulement une faible protection des cartilages articulaires atteints d'une dégénérescence imputable à un agent chimique.

## INTRODUCTION

Musculoskeletal disease is a significant cause of lameness and loss of use in young performance horses (1). Of all equine musculoskeletal disease conditions, osteoarthritis or degenerative joint disease ranks highly as a cause of lameness (2,3). Numerous treatment drugs and regimens are used by veterinarians to achieve continued performance by horses affected with
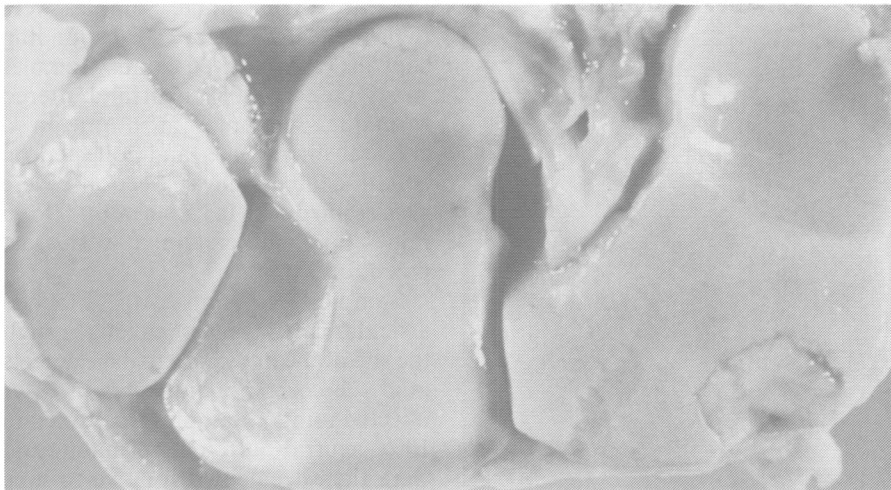
Fig. 1. Middle carpal joint, articular surface of proximal row of carpal bones, from horse 2A. This joint had both partial-thickness and full-thickness defects made on the radial carpal bone, and the horse received intramuscular PSG. Both defects are visible, with the full-thickness defect covered by smooth white tissue.

degenerative joint disease (3-6). One drug used is polysulfated glycosaminoglycan (PSG), a synthetic glycosaminoglycan that has been used to treat degenerative joint disease in people in Europe (7-10). Other reports describe the effect of PSG using different arthritis models in animals (11-18). Reported clinical benefits of PSG for arthritis in people include decreased arthralgia and improved joint mobility (10). In animal models of arthritis, further benefits cited include inhibition of destructive proteases in synovial fluid, stimulation of proteoglycan and collagen synthesis, minimization of cartilage fibrillation and erosion, and improved healing of established articular cartilage erosions (11-18). These benefits have been reported after intra-articular, intramuscular or subcutaneous use of PSG (11,12,17), and although proof of increased collagen synthesis is lacking, the other benefits are well documented.

In a clinical study in which Freund's adjuvant arthritis was induced in horses, the administration of 250 mg of PSG intra-articularly decreased carpal swelling and increased stride length (19). A chondroprotective effect was also seen when intra-articular PSG was used to treat joints in which chemical articular cartilage injury had been induced (20). The purpose of the present study was to evaluate the effects of intramuscular PSG on both physically and chemically induced injury to equine articular cartilage.

## MATERIALS AND METHODS

Eight clinically normal horses, aged two to five years, were used. All horses were free of clinical and radiographic evidence of carpal joint disease. Horses were anesthetized, positioned in dorsal recumbency, and one carpus was prepared for arthroscopic surgery. One full-thickness (8 mm x 10 mm) articular cartilage defect was made on the central aspect of the distal articular surface of the radial carpal bone in one middle carpal joint using a motorized burr. A partial-thickness articular cartilage defect (2 mm x 15 mm) was made on the axial aspect of the same bone using a curette. In the contralateral middle carpal joint, articular cartilage degeneration was induced chemically by intra-articular injection of 50 mg sodium monoiodoacetate (MIA) (Chesapeake Bay Laboratories, Hunt Valley, Maryland). Limbs selected for physical or chemical cartilage injury were alternated between horses. Phenylbutazone (2 gm orally once daily for three days) was started the day before surgery. The carpus having arthroscopic surgery was bandaged for seven to ten days.

At postsurgical week (PSW) 1, horses were randomly assigned to a control or a treatment group. Horses 1A, 1B, 1C and 1D (control group) were given 2 mL normal saline intramuscularly every four days for seven treatments. Horses 2A, 2B, 2C and 2D (treatment group) were given 500 mg PSG (Adequan, Luitpold Pharmaceuticals, Shirley, New York) intramuscularly every four days for seven treatments.

Horses were maintained in box stalls during the study. Limb circumference at the level of the middle carpal joint was measured once weekly using a tape measure. At PSW 12, horses were examined for lameness, their carpal joints were radiographed and both middle carpal joints were examined and photographed arthroscopically. Horses were then euthan-



Fig. 2. Middle carpal joint, articular surface of proximal row of carpal bones, from horse 2A. This joint was injected with MIA and the horse received intramuscular PSG. Multifocal areas of articular cartilage erosion are present.
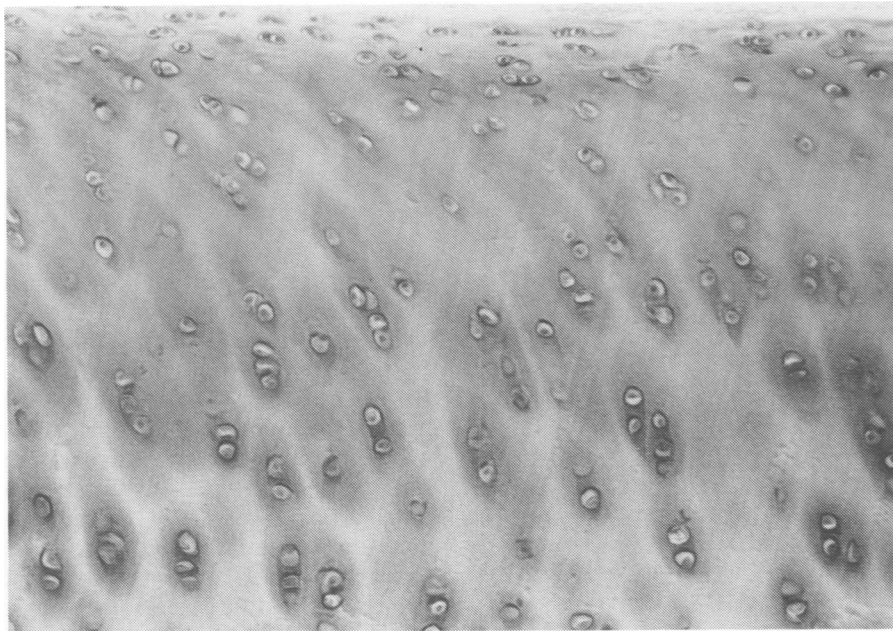
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Fig. 3. Articular cartilage from the middle carpal joint of horse 2A. This joint had physical defects made on the distal radial carpal bone and the horse received intramuscular PSG. Matrix staining is present in both the intermediate and radiate layers, but interterritorial matrix staining is slightly decreased from normal in both layers. Safranin-O fast green stain, X50.

ized, middle carpal joints were disarticulated and photographed, and joint tissues were collected for microscopic examination.

SAMPLE COLLECTION

Synovial membrane was harvested from the dorsomedial aspect of the joint, fixed in 10% neutral-buffered formalin, embedded in paraffin and sectioned and stained with hematoxylin and eosin.

Slices of articular cartilage were harvested from predetermined locations using a razor blade. Samples were collected adjacent to and from the site of the physical cartilage defects as well as from the palmar aspect of the third carpal bone. Samples were then collected from corresponding sites in MIA injected joints. Samples were fixed in 10% neutral buffered formalin, sectioned at 6 $\mu$m on a cryostat microtome, and collected on gelatin-coated slides. Sections were then stained with 0.1% safranin-0 for 5 min and counterstained with fast green for 3 min.

Samples of bone with attached articular cartilage were taken through the site of the physical cartilage defects (and the corresponding sites in MIA injected joints). Samples were fixed in 10% neutral-buffered formalin and were then decalcified for five to nine days in 10% formic acid with continuous agitation over ion exchange resin. Samples were then embedded in paraffin, sectioned at 5 $\mu$m, and stained with hematoxylin and eosin, and Alcian blue-periodic acid-Schiff (pH 2.5).

MICROSCOPIC EXAMINATION

All tissue sections were evaluated without examiner knowledge of treatment groups.

*Synovial Membrane* — Synovial membrane was evaluated qualitatively for villous hyperplasia, intimal layer hypertrophy or degeneration, and type and extent of subintimal cellular infiltrate, edema and fibrosis.

*Articular cartilage sections* — Articular cartilage samples were evaluated and graded for intensity of safranin-O staining in intermediate and radiate layers. Staining intensity was graded from 0 (no stain uptake) to 4 (maximal staining). A section of tracheal cartilage was used as a positive control for the stain. An average grade was given for each layer from the three samples from each joint.

*Bone and articular cartilage sections* — Decalcified sections of articular cartilage with subchondral bone from MIA injected joints were evaluated for articular cartilage degenerative and physical change as described below.

*Degenerative articular cartilage changes:* 0 = normal, 1 = minor loss of chondrocyte nuclear staining, 2 = eosinophilic chondrocyte nuclei and empty lacunae, 3 = empty lacunae and disorganized matrix, 4 = all chondrocytes necrotic, loss and/or collapse of matrix.
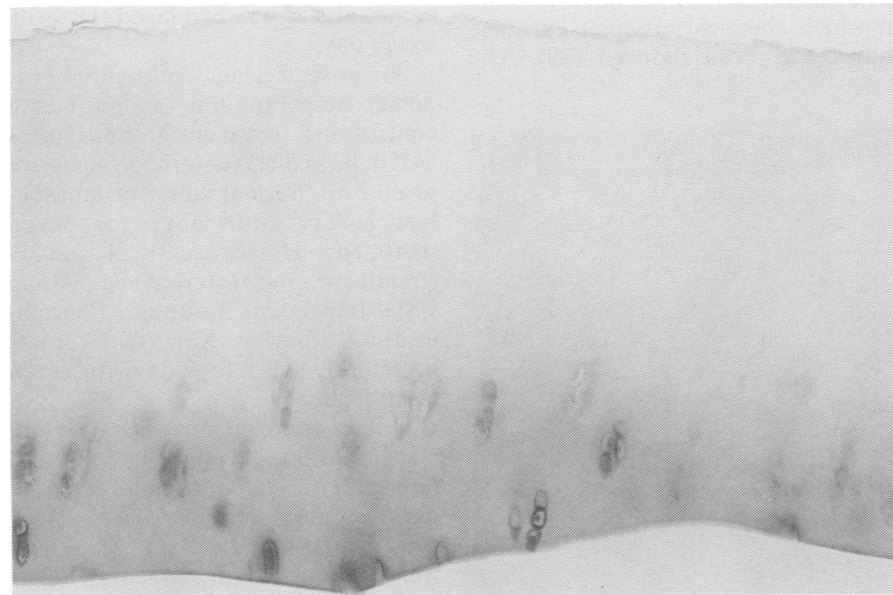


Fig. 4A. Articular cartilage from the middle carpal joint of horse 2C. This joint was injected with MIA and the horse received intramuscular PSG. Slight residual staining of articular cartilage matrix is present in the radiate layer. However, staining of the intermediate layer is absent, and loss of chondrocytes and lacunae is evident. Safranin-O, fast green stain, X50.

*Physical articular cartilage changes:*
0 = normal,   1 = surface   frayed,
2 = horizontal fibrillation, 3 = vertical
fibrillation, 4 = cartilage erosion.

Articular cartilage and bone sections from physical defect joints were evaluated for type and amount of tissue filling the defects, changes in adjacent articular cartilage, and appearance of subchondral bone below the defects.

## STATISTICAL ANALYSIS

Quantitative parameters (safranin-0 staining intensity, degenerative articular cartilage change, physical articular cartilage change) were compared between control and PSG treated horses using the Wilcoxon Rank-Sum test for two groups. A p value > 0.05 was considered insignificant.

# RESULTS

## LAMENESS

At PSW 12, mild lameness was present in four horses. Two control horses were lame in the limb with physical defects and two treatment group horses were lame in the MIA injected limb.

## LIMB CIRCUMFERENCE

Limb circumference at the middle carpal joint increased over baseline measurements in all horses, with maximal swelling occurring at PSW 1-2. Physical defect joint circumference increased to a mean of 2.8 cm over baseline in the control group and 2.0 cm in the treatment group. At the end of the study, increases over baseline were still present and were 2.5 cm and 1.8 cm respectively.

In MIA injected joints, control joints increased to a mean of 2.5 cm over baseline and treatment joints increased to a mean of 2.2 cm over baseline. At the end of the study, respective values were 2.3 cm and 2.0 cm.

## RADIOGRAPHIC FINDINGS

At PSW 12, six joints in four horses had abnormal radiographic changes. Horses 2A and 2D had a small radiolucent area at the distal radial carpal bone in MIA injected joints. Horse 2D had a similar radiographic finding in the joint that had physical



Fig. 4B. Articular cartilage from the middle carpal joint of horse 2B. This joint was injected with MIA and the horse received intramuscular saline. Loss of chondrocytes and lacunae is extensive here as well, and no safranin-O staining is present. Safranin-O, fast green stain, X50.

defects. Horses 1C and 1A had small osteophytes on the distal radial carpal bone in joints that had physical defects. Horse 1A also had a small osteophyte on the distal radial carpal bone in the MIA injected joint.

## ARTHROSCOPIC AND GROSS PATHOLOGICAL FINDINGS

In physical defect joints, partial-thickness defects had not changed in any joint over the 12 weeks of the study. Full-thickness defects in three
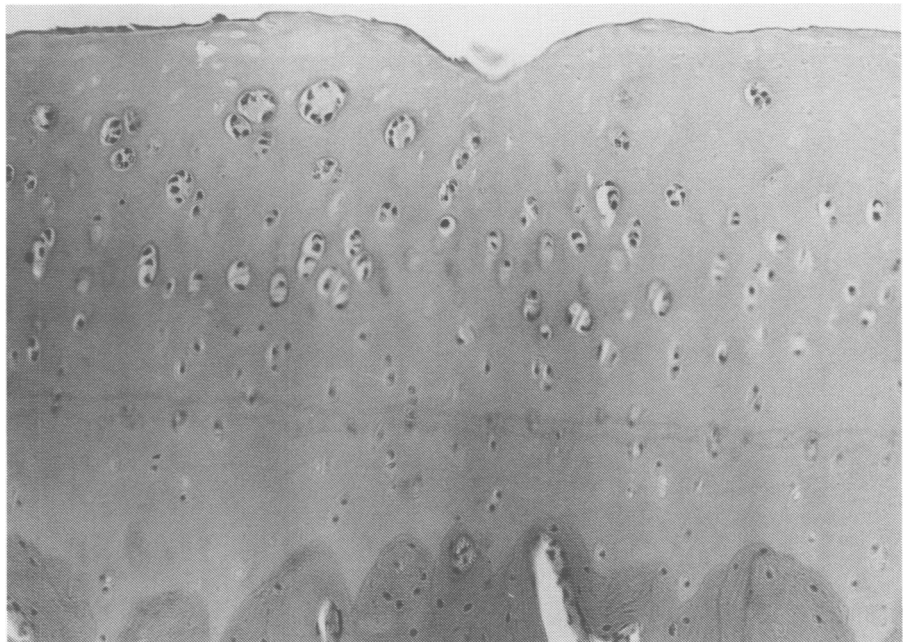


Fig. 5A. Bone and articular cartilage section from the radial carpal bone of horse 1C (horse received intramuscular saline). This section was taken through the partial-thickness articular cartilage defect. Healing of the defect has not occurred, and chondrone formation is present in the surrounding articular cartilage. H & E stain, X31.
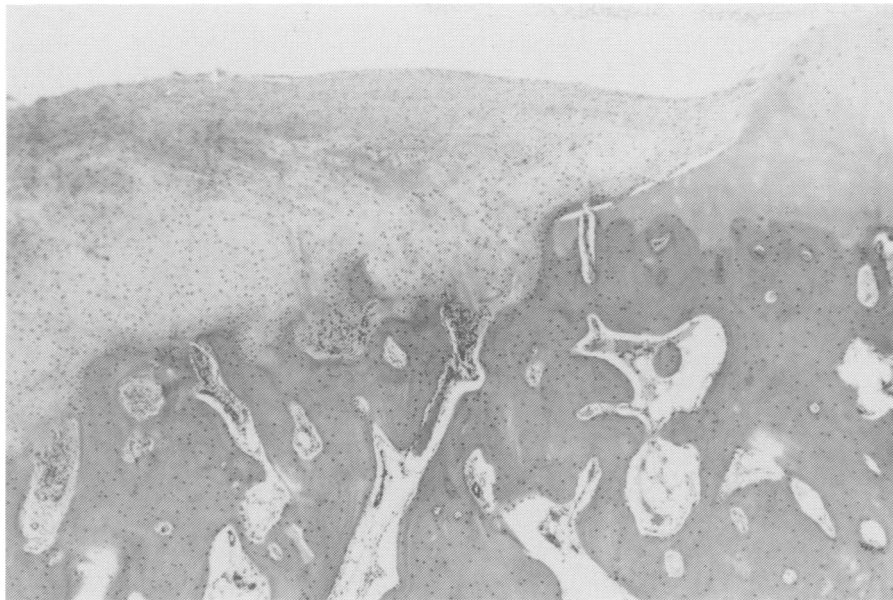
Fig. 5B. Bone and articular cartilage section from the radial carpal bone of horse 1C. Predominantly fibrous tissue is noted to be filling the defect. H & E stain, X12.5.

joints from PSG treated horses, and in all joints from control horses, were covered with smooth white tissue (Fig. 1). The full-thickness defect from horse 2C was covered with smooth red tissue. In all joints, an area of thin cartilage with raised edges was present on the third carpal bone opposite the full-thickness defect (kissing lesion). The synovial membrane appeared fibrous in the middle carpal joint of horse 2C.

In MIA injected joints, abnormal articular cartilage changes were present in all joints. In control horses, horse 1A had extensive articular cartilage loss involving much of the articular surface. Horse 1C had multifocal ($>$ 5) areas of partial-thickness cartilage erosion and horses 1B and 1D had a few ($<$ 5) areas of partial-thickness cartilage erosion. In PSG treated horses, horse 2D had extensive loss of articular cartilage, horses 2A and 2B had multifocal areas of partial thickness cartilage erosion (Fig. 2) and horse 2C had a few areas of partial-thickness cartilage erosion. Horses 1A and 2C had synovial membrane that was relatively devoid of villi.

MICROSCOPIC EVALUATION

Synovial membrane — Mild abnormal changes were consistent and were present in all joints. These changes included intimal hyperplasia, subintimal edema and subintimal fibrosis. In MIA injected joints, hemosiderosis was also a common finding. No difference between control and treatment groups was identified.

Safranin-O staining — In physical defect joints, a mild decrease in matrix staining intensity was seen in the intermediate and radiate layers of articular cartilage samples from all joints (Fig. 3). No significant difference existed between samples from control or PSG treated horses.

In MIA injected joints, a marked decrease in matrix staining intensity was seen in the intermediate and radiate layers in samples from all joints. Mild safranin-O staining was still present in the intermediate layer of articular cartilage from horse 2A but was absent in this layer in all other horses from both groups. Mild safranin-O staining was present in the radiate layer of samples from control horse 1D but was present in this layer in all PSG treated horses (Fig. 4A and B). There was significantly greater staining intensity in the radiate layer of PSG treated horses over control horses (p = 0.04).

Bone with attached articular cartilage — In physical defect joints, none of the partial-thickness lesions had healed. Empty chondrocyte lacunae were present adjacent to the lesion in all sections and a variable degree of chondrone formation was also present in all sections (Fig. 5A). No differences were observed between sections from control and PSG treated horses.

The predominant tissue type covering full-thickness defects was fibrous tissue (Fig. 5B). Fibrocartilage was
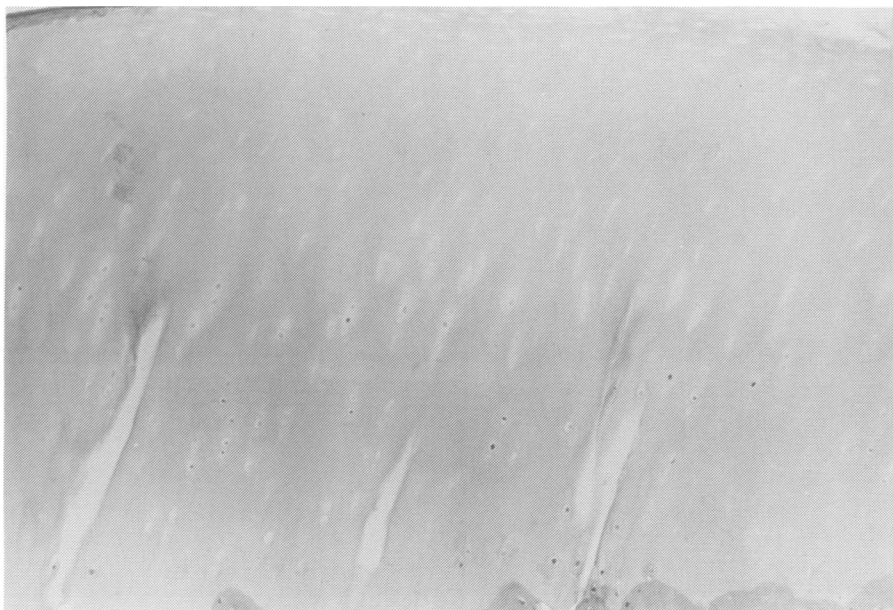


Fig. 6. Bone and articular cartilage section from horse 2C. This joint was injected with MIA and the horse received intramuscular PSG. Horizontal surface fibrillation is present as is chondrocyte loss and empty lacunae. H & E stain, X31.

variably present in covering tissue from sections from all horses, with a trend for more fibrocartilage in sections from control horses. Other than this trend, no differences were observed between sections from control and PSG treated horses.

In MIA injected joints, there was no significant difference between control and PSG treated horses for physical articular cartilage changes (p = 0.23) or for degenerative articular cartilage changes (p = 0.28). Cartilage fibrillation was present in all samples except those from control horse 1C. Degenerative cartilage changes were present in all tissue samples from control and PSG treated horses, with an average grade for severity of degeneration of 2.5 for the control horses and 2.0 for the treated horses (Fig. 6).

## DISCUSSION

Experimental models which completely reproduce the clinical signs and morphological changes of naturally occurring degenerative joint disease do not exist. However, the essential lesion of degenerative joint disease is articular cartilage degeneration and intra-articular injection of MIA has been shown to consistently induce articular cartilage degenerative changes in rats, chickens, guinea pigs, rabbits, and horses (12,13,21-25). Gross morphological and microscopic changes in articular cartilage from MIA injected joints resemble those described for naturally occurring and induced degenerative joint disease (26-29). Although this MIA model may not duplicate the sequence of events occurring in natural degenerative joint disease, it does provide a means of estimating the capacity for certain drugs to control cartilage breakdown. In our study, gross morphological and microscopic degenerative articular cartilage changes were induced in all joints injected with MIA. Changes in joints with induced physical defects were restricted to the lesion site proper and to the articular cartilage directly opposite the full-thickness defect (kissing lesion).

In our study, the only difference between control and intramuscular PSG treated horses was slightly better safranin-0 staining in PSG treated

horses in the radiate articular cartilage layer of MIA injected joints. This difference was minor in degree, with both control and treated horses having prominent articular cartilage degenerative changes. This differs from results of a previous study where PSG was given intra-articularly. In that study, physically and chemically induced articular cartilage injury was created as in the present study. Treatment group horses received five consecutive weekly intra-articular injections of 250 mg PSG. Matrix staining intensity was significantly higher in PSG treated over control joints, and PSG treated joints had less severe gross degenerative articular cartilage changes (20). In a meniscectomy arthritis model in rabbits, PSG was shown to either totally prevent or significantly ameliorate articular cartilage erosions as determined by gross and microscopic examination (17). In that study, PSG was injected intra-articularly or intramuscularly twice weekly starting one week after meniscectomy, and was continued to the end of the study at 12 weeks. Dosages used were 1 mg/kg and 10 mg/kg for intramuscular administration and 0.1 mg/kg and 1 mg/kg for intra-articular administration. All treated joints were superior to saline treated controls except in the 0.1 mg/ kg intra-articular group. A further group of rabbits were not treated for 12 weeks and then received intra-articular injections (1 mg/kg) of PSG twice weekly for eight more weeks. Articular cartilage erosions were smaller, and histological grade was superior in the treated group, suggesting a therapeutic effect on established lesions, as well as the previously described prophylactic effect. In another study, dogs underwent medial meniscectomy and were then treated with 2 mg/kg PSG subcutaneously for 54 treatments over 26 weeks (11). Treated dogs had superior scores over controls on microscopic evaluation of bone and cartilage sections, and they also had higher proteoglycan content in their articular cartilage. With a comparatively higher intramuscular dosage of PSG (10 mg/kg twice weekly for four weeks), and using a joint immobilization model of degenerative joint disease in rabbits, Golding et al further documented that

articular cartilage from PSG treated animals maintained normal proteoglycan extractability and aggregatability as opposed to saline treated controls (16). In clinical trials in people, intramuscular PSG was also reported to qualitatively ameliorate pain and dysfunction from hip and knee degenerative joint disease, to the same degree as after intra-articular administration (10,30).

Reasons for the relative lack of effect of intramuscularly administered PSG on horses in our study are unclear. Although it has been shown using labeled PSG that deposition of the drug does occur in all layers of articular cartilage after intramuscular administration (11,31), a larger dosage or longer treatment period may be required in the horse to achieve useful intra-articular concentrations. The dosage regimen used was one found to be safe and effective in a dose response study done using Freund's adjuvant model of arthritis in horses (L. Goldman, personal communication, 1986).

Pharmacokinetic and drug deposition data do not exist for PSG in horses. In rats, PSG is known to be deposited in articular cartilage within 3 h of intramuscular injection (32). In a study in rabbits, synovial fluid levels of PSG equalled blood levels within 2 h of intramuscular PSG administration (33). Maximum articular cartilage concentration of PSG in people was attained 24 h after a single 125 mg intramuscular injection and enzyme inhibitory levels of PSG were still present in articular cartilage at 96 h (34). Constant levels of PSG in rabbit articular cartilage have been documented for at least eight days after intramuscular administration (33). Although effective drug concentrations and duration of drug action for PSG are unknown, treatment periods in people are usually for eight to ten weeks, and treatment periods in some of the animal experimental studies were much longer than this (11). The drug dosage and regimen used in our study would be at the lower end of therapeutically useful dosages found in these experimental studies. Conversely, in a study done on swine and using a dosage regimen very similar to ours, aggregatability of articular cartilage proteoglycans was signifi-

cantly higher in PSG treated over control animals (35).

Marginal osteophyte formation was not a significant feature in physical defect or MIA injected joints in our study. However, in other studies using degenerative joint disease models, less osteophyte formation was seen in joints from animals receiving intra-articular or intramuscular PSG when compared to untreated control animals (15,17). We also found no effect of intramuscular PSG on healing of physical defects in articular cartilage. This was similar to observations on intra-articular PSG in horses (20) but differed from Carreno's findings in rabbits (17). Using a meniscectomy model that consistently caused cartilage erosion, he found a mild but significant reduction in erosion size in PSG treated rabbits over controls. Small animal size and rapid turnover of articular cartilage matrix in rabbits may both be factors in this finding.

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