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Effects of intravenously administered sodium hyaluronate on equine carpal joints with osteochondral fragments under exercise

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Summary

The effects of osteochondral fragmentation and treatment with intravenous sodium hyaluronate on carpal joints of 12 horses were evaluated. All horses had an osteochondral fragment created in one middle carpal joint. Six of the horses were treated with 40 mg of sodium hyaluronate, and the other six were treated with saline once a week for three weeks. All horses underwent treadmill exercise for 56 days. Synovial fluid samples were evaluated from both middle carpal joints during the study. All horses were euthanized 72 days after surgery, and synovial membrane was obtained for histologic evaluation. Articular cartilage samples were also obtained for determining glycosaminoglycan metabolism.

Horses treated with sodium hyaluronate were less lame, had significantly better synovial membrane histologic scores, and significantly lower concentrations of prostaglandin E_2 and total protein within synovial fluid. Intravenous sodium hyaluronate treatment appears to alleviate the signs of lameness by reducing synovitis.

Keywords: osteochondral fragmentation, intravenous sodium hyaluronate, exercise, synovitis, inflammatory mediators

Auswirkungen intravenöser Injektionen von Natriumhyaluronat auf Carpalgelenke mit osteochondralen Fragmenten bei Pferden während der Arbeit

Die Autoren testeten den Einfluß der intravenösen Applikation von Hyaluronsäure auf Pferde mit osteochondralen Knochenfragmenten in den Carpalgelenken.

Die Studie wurde an 12 Pferden durchgeführt. Bei einem chirurgischen Eingriff wurde den Pferden eine Chip-Fraktur in der mittleren Carpalgelenksreihe gesetzt, sodaß sie freie Knochenfragmente aufwiesen. Sechs dieser Pferde wurden mit 40 mg/kg Na-Hyaluronat i.v. behandelt, die anderen sechs dienten als Kontrollpferde und bekamen NaCl-Lösung injiziert. Alle Testpferde wurden 56 Tage lang auf einem Laufband trainiert und bekamen drei Wochen lang jede Woche eine intravenöse Injektion.

Während der Studie wurde den Pferden Synovialflüssigkeit aus den mittleren Carpalgelenken der rechten und linken Gliedmaße entnommen. 72 Tage nach dem Versuch wurden alle Pferde euthanasiert und die Synovialmembran histologisch untersucht. Um den Glykosaminoglykan(GAG)-Stoffwechsel zu bestimmen, wurden auch Proben des Gelenkknorpels untersucht.

Das Experiment hatte folgende Ergebnisse : Die Pferde, welche intravenös Na-Hyaluronat erhielten, gingen während der Belastung weniger lahm als die Kontrollpferde. Die histologische Untersuchung ergab, daß die mit Hyaluronsäure behandelten Pferde eine bessere Struktur der Synovialmembran aufwiesen. Ihre Synovialflüssigkeit enthielt auch signifikant niedrigere Konzentrationen von Prostaglandin E_2 und einen geringeren Proteingehalt als die Gelenkflüssigkeit der Kontrollpferde.

Der Glykosaminoglykan-Stoffwechsel schien in den Gelenken der behandelten Pferde geringer als in den unbehandelten Gelenken. Die Autoren vermuteten, daß der Gelenkknorpel bei unbehandelten Pferden einen höheren GAG-Metabolismus besitzt, als bei behandelten Pferden, da die Entzündungserscheinungen durch die Hyaluronsäure-Therapie gemindert wurden.

Die Ergebnisse demonstrieren, daß Natriumhyaluronat einen positiven Effekt auf den Stoffwechsel der Synovialmembran besitzt.

Die intravenöse Therapie mit Hyaluronsäure scheint bei Pferden mit Gelenkserkrankungen die Lahmheit zu mildern und die Symptome der Synovitis zu reduzieren.

Schlüsselwörter: Osteochondrale Fragmente, Intravenöses Natriumhyaluronat, Belastung, Synovitis, Entzündungsmediatoren

Introduction

A new intravenous preparation of sodium hyaluronate (IV HA) has been developed to alleviate the need for repeated intra-articular injections in horses.[a] The chemical is extracted from the bacterial capsule of Streptococcus Equi, and is purified for intravenous use. In a clinical trial completed by the manufacturer, good to excellent results were reported in approximately 90% of the cases (Unpublished data). The purpose of the study reported here was to evaluate the use of IV HA against placebo in a controlled experiment using an osteochondral fragment model of arthritis in horses. The effects of treatment on the joints were evaluated by histologic and biochemical means.

[a]. Legend, Miles Inc. Shawnee Mission, KS.

Materials and methods

Osteochondral fragment model

Twelve horses, ages 2–7 years, and free of lameness, were placed into the study. Complete radiographic examinations were performed

on each horse. Each horse was acclimatized to a high-speed treadmill for a 2 minute trot (8 to 12 mph), 2 minute gallop (25–33 mph) and 2 minute trot.

Routine, bilateral carpal arthroscopic surgery was performed on each horse after sterile preparation and joint fluid aspiration *(McIlwraith* 1990). One milliliter of joint fluid was placed into a tube containing EDTA for determination of color, clarity, total protein (TP) and white blood cell (WBC) concentrations. The remainder of the synovial fluid was placed into a heparinized tube, centrifuged at 1,000 x g for 30 minutes and the supernatant was stored at –20 degrees centigrade for batch analysis of hyaluronate (HA), glycosaminoglycan (GAG), and prostaglandin E_2 (PGE_2) concentrations.

An 8 mm osteotome was used to create an osteochondral fragment in the distal aspect of the radiocarpal bone. The subchondral bone adjacent to the fragment was curetted with a burr to form a 15 mm wide defect for the 8 mm fragment to heal to. The reason for this was to create an incongruent area and minimize osteochondral fragment healing, as previous models without curettage resulted in healing of the fragment *(Foland* et al. 1994). Diagnostic arthroscopy was performed on the contralateral middle carpal joint to ensure absence of joint problems. After surgery, all horses were kept in stalls and runs, the bandages were changed every 3–5 days and the sutures removed 10 days post-surgery.

Experimental design

Thirteen days after surgery, both intercarpal joints were aseptically aspirated and the fluids processed as before. Six randomly-chosen horses received 40 milligrams of IV HA and the other 6 received intravenous saline. Treadmill exercise was started on day 15 and continued 5 days per week. Joint aspirations were repeated on days 20, 27, 34 and 72 after surgery. Each horse was treated again on days 20 and 27. Radiographs were repeated on day 56 and a lameness examination, including carpal flexion, was performed on each horse prior to euthanasia on day 72. Lameness was scored from 0 to 5 for severity (0=sound, 5=nonweight-bearing).

Synovial fluid analysis

Color, clarity, TP, and WBC concentrations were determined using routine clinical pathology methods (Clinical Pathology Services, Veterinary Teaching Hospital, CSU). The concentration of HA within synovial fluid was determined using a modified alcian blue technique *(Little* et al. 1990). Glycosaminoglycan concentrations in the synovial fluids were determined using a modified 1,9-Dimethyl methylene blue dye binding assay *(Carroll* 1987). The concentration of PGE_2 was also determined in each synovial fluid sample by an enzyme immunoassay technique[b] after non-polar extraction[c].

Histologic evaluation

Synovial membrane and joint capsule were obtained immediately adjacent to the chip fragment, and stained with hematoxylin and eosin (H&E). These sections were evaluated and graded blindly for cellular infiltration, synovial intimal hyperplasia, subintimal edema, subintimal fibrosis and vascularity *(Foland* et al. 1994).

Articular cartilage matrix metabolism

Articular cartilage was aseptically harvested from the weight-bearing surfaces of the ulnar, intermediate and fourth carpal bones. Four pieces, 80 to 100 milligrams (wet weight) each, were placed into Dulbecco's Modified Eagles Medium with additives, and incubated with 20 microcuries per milliliter of Sulfur-35 in media for 16 hours. All articular cartilage pieces were freeze-dried for 48 hours, dry weights were determined, and each was digested in papain. Aliquots of digested articular cartilage solution were evaluated using the cetylpyridinium chloride (CPC) precipitation technique *(MacDonald* et al. 1992).

Articular cartilage pieces, 80–100 milligram (wet weight) in size were obtained aseptically from the osteochondral fragment, the intermediate and radial facets of the third carpal bone, and from the second carpal bone. All articular cartilage pieces were freeze-dried for 48 hours and then digested in papain solution. Glycosaminoglycan content for each piece was determined by the 1,9-Dimethyl methylene blue dye binding method *(Farndale* et al. 1982).

Statistical analysis

The statistical analysis for this project utilized a split plot design. Systemic treatment with hyaluronic acid was a between horse factor and the presence or absence of an osteochondral fragment was a within horse factor. The independent variable within and between horse factors were completely randomized within the study group. A general linear model procedure (GLM)[d] was used to test both the main and interaction effects of the independent variable. When interactions existed, a least squared means procedure (LSM)[d] was used for individual treatment comparisons. The between treatment and within horse treatment effects were tested against appropriate error terms in both the GLM and LSM procedures. Additionally, randomized residual plots were made to test the assumptions that normal distribution, equal variance and mean variance of zero were present.

Data for synovial fluid parameters were evaluated over time as well as at day 72 to detect acute and chronic effects that the model and treatment may have on results.

Results

Clinical examinations

Lameness examinations on day 72 were performed on all horses at a trot, and graded 0 to 5 for severity. Lameness examinations showed 11 of the 12 horses to be lame in the limb with the osteochondral fragment. Horses treated with IV HA were less lame than control horses (Fig. 1).

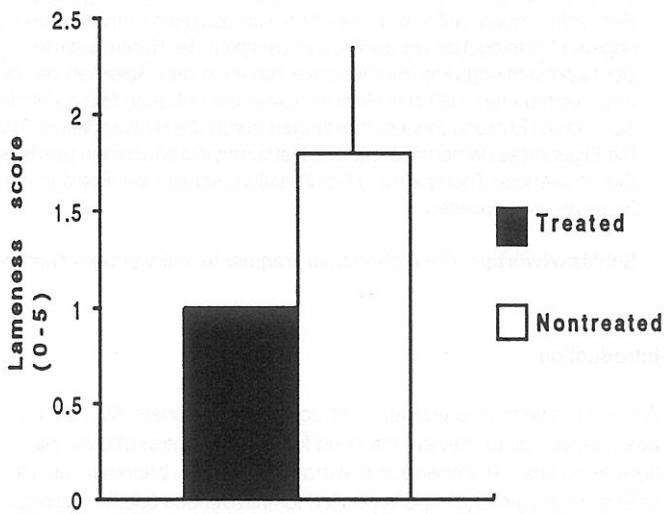

Fig. 1: Lameness scores for IV HA treated and control horses. The differences in scores are significant ($P < 0.05$).

[b] PGE_2 EIA test kit -Titer Zyme®, PerSeptive Diagnostics, Cambridge, MA.
[c] Ethyl C2 columns Amprep™, Amersham Life Sciences, Arlington Heights, Il.
[d] Statistical Analysis System, Cary, New Jersey.

Synovial fluid evaluation (Day 72)
Data from synovial fluids were evaluated and compared be-tween groups on day 72. Total protein and PGE_2 concentrations were significantly elevated in joints with an osteochondral fragment. Total protein and PGE_2 concentrations in IV HA treated horses were significantly lower than in control horses (Fig. 2). A trend was noted for the influence of osteochondral fragmentation on increasing synovial fluid GAG concentration on day 72 (p=0.0911). No effect of fragmentation or treatment were seen on other data evaluated.

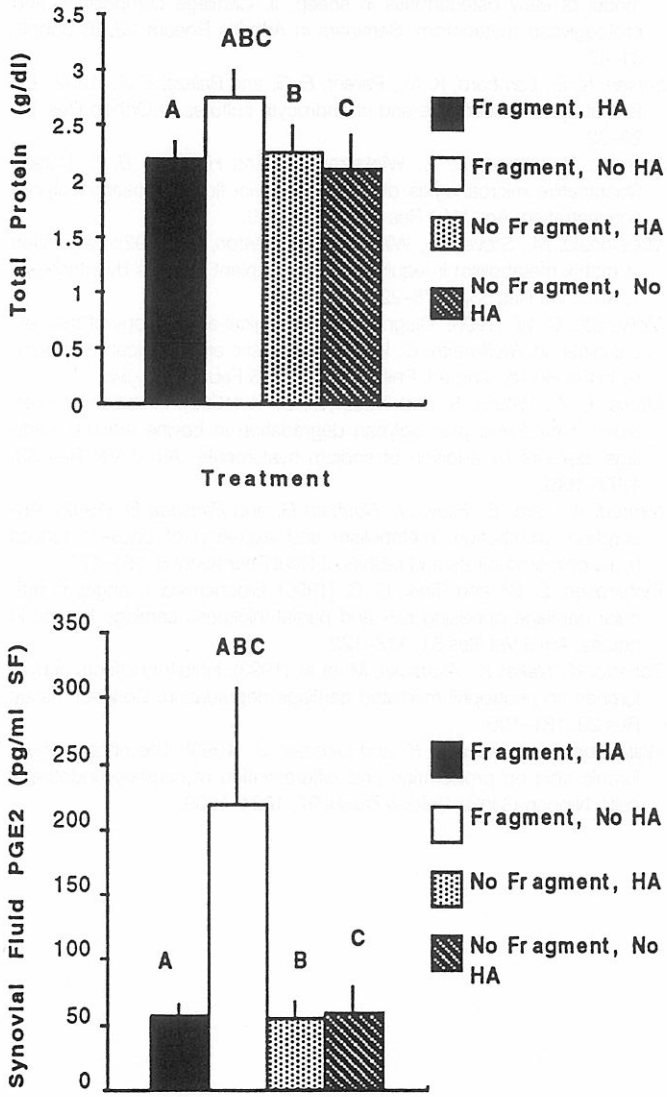

Fig. 2: Total protein and prostaglandin E2 concentrations on day 72. Same letters indicate significant differences ($P < 0.05$).

Synovial fluid evaluation (Over Time)
Osteochondral fragmentation induced significant elevation in synovial fluid total protein, PGE_2, and GAG concentrations. Treatment with IV HA however, had no significant influence on any variable evaluated. A trend ($p<0.10$) did exist however for the influence of treatment on lowering synovial fluid GAG concentrations. No effects of fragmentation or treatment were seen in other data evaluated.

Histologic evaluation
Histologic evaluation of synovial membrane revealed that joints from horses treated with IV HA had significantly less inflammatory cell infiltration into the synovial membrane and significantly less vascularity in the synovial membrane (Fig. 3).

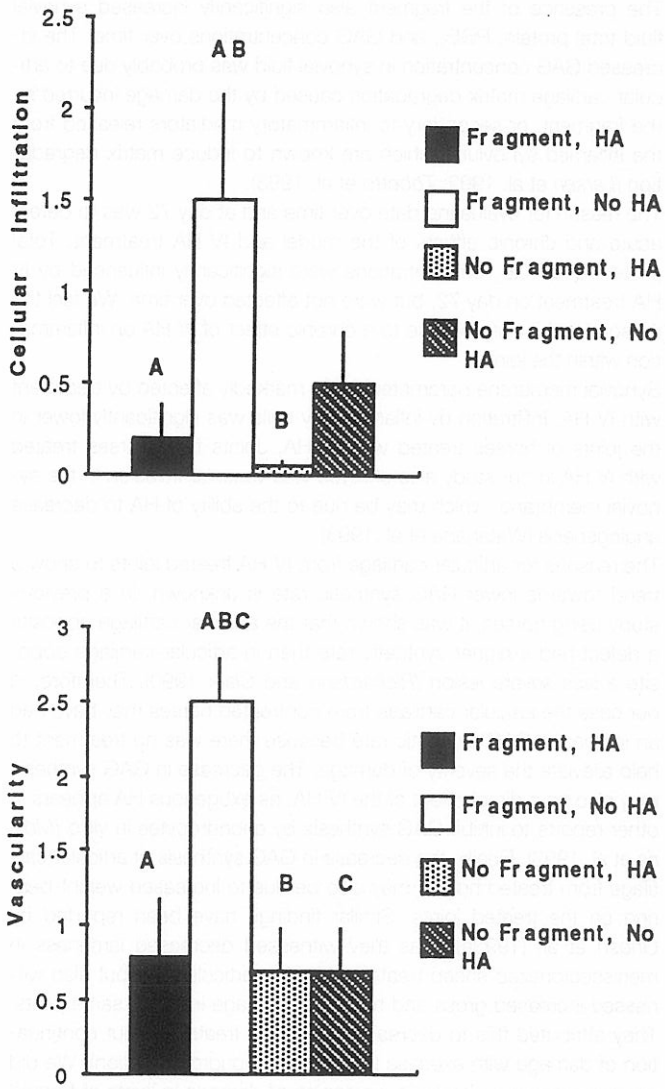

Fig. 3: Synovial membrane scores of cellular infiltration and vascularity. Same letters indicate significant differences ($P < 0.05$).

Articular cartilage matrix metabolism
Analysis of total GAG content per milligram of dry weight showed that no significant differences were evident between treatment groups. No significant differences in GAG synthetic rate were noted between groups, although a trend was noted for cartilage from IV HA treated horses to have a lower synthetic rate (p=0.093). No effect from the presence of a fragment was noted on GAG synthetic rate.

Discussion

Lameness examinations, although subjective, revealed that horses treated with IV HA were less lame at the end of the study than those not treated.
The presence of an osteochondral fragment significantly elevated synovial fluid TP and PGE_2 concentrations on day 72. The increase in TP concentration may be due to the inflammation evoked by the fragment, reflecting that this model may create a chronic, active form of inflammation. Treatment with IV HA had a significant effect on total protein and PGE_2 concentrations in synovial fluid on day 72 and ap-

peared to alleviate the effects of the fragment on these parameters in treated horses.

The presence of the fragment also significantly increased synovial fluid total protein, PGE_2, and GAG concentrations over time. The increased GAG concentration in synovial fluid was probably due to articular cartilage matrix degradation caused by the damage incurred by the fragment, or secondary to inflammatory mediators released from the inflamed synovium, which are known to induce matrix degradation *(Larsen* et al. 1992; *Tobetto* et al. 1993).

The reason for evaluating data over time and at day 72 was to detect acute and chronic effects of the model and IV HA treatment. Total protein and PGE_2 concentrations were significantly influenced by IV HA treatment on day 72, but were not affected over time. We feel the reason for this may be due to a chronic effect of IV HA on inflammation within the joint.

Synovial membrane parameters were markedly affected by treatment with IV HA. Infiltration by inflammatory cells was significantly lower in the joints of horses treated with IV HA. Joints from horses treated with IV HA in our study also showed less vascular invasion in the synovial membrane, which may be due to the ability of HA to decrease angiogenesis (Watanabe et al. 1993).

The reasons for articular cartilage from IV HA treated joints to show a trend towards lower GAG synthetic rate is unknown. In a previous study using horses, it was shown that the articular cartilage opposite a defect had a higher synthetic rate than in articular cartilage opposite a less severe lesion *(Richardson* and *Clark* 1990). Therefore, in our case the articular cartilage from nontreated horses may have had an increased GAG synthetic rate because there was no treatment to help alleviate the severity of damage. The decrease in GAG synthesis may also be a direct effect of the IV HA, as exogenous HA appears in other reports to inhibit GAG synthesis by chondrocytes in vitro *(Morris* et al. 1992). Finally, the decrease in GAG synthesis of articular cartilage from treated horses may also be due to increased weight-bearing on the treated joints. Similar findings have been reported by *Ghosh* et al. (1993a,b), as they witnessed decreased lameness in meniscectomized sheep treated with intra-articular HA, but also witnessed increased gross and histologic damage in those same joints. They attributed this to decreased pain with treatment, but continuation of damage with exercise (eg. lack of chondroprotection). We did not see gross nor histologic worsening of damage in joints of treated horses, but synthetic rate may be a more sensitive indicator of metabolism, and consequently damage. Further in vitro and in vivo studies are needed to evaluate the effects of IV HA on articular cartilage.

The exact mechanism of action of IV HA is difficult to determine. We feel that it may work at the synovial membrane level since significant effects were seen there. Further work is needed to examine the effects of IV HA on local tissues (eg. synovial membrane and endothelium) and to determine if specific cellular activities are upregulated by the drug. Other studies have shown that the HA molecule will bind to specific receptors on inflammatory cells and modulate their actions *(Nimrod* et al. 1992).

In conclusion, IV HA appeared to improve clinical signs of lameness in the group of horses studied here, and improved parameters of synovial membrane inflammation and vascularity, and inflammatory mediator release into the synovial membrane.

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