Effects of Glycosaminoglycan Polysulfate Treatment on Soundness, Hyaluronic Acid Content of Synovial Fluid and Proteoglycan Aggregate in Articular Cartilage of Lame Boars

J.J. Brennan, F.X. Aherne and T. Nakano*

ABSTRACT

Eighteen lame boars were equally assigned to two treatment groups based on initial bodyweight and leg soundness. The boars were injected intramuscularly with an aqueous solution of glycosaminoglycan polysulfate or saline on day 0, 5, 10, 15, 20 and 25 and killed on day 27. The glycosaminoglycan polysulfate treatment significantly ($P<0.05$) improved leg soundness score, and resulted in an increase ($P<0.06$) in the hyaluronic acid concentration of the cubitus joint synovial fluid, and an increase ($P<0.05$) in the proportion of aggregated proteoglycans in the articular cartilage of the medial femoral condyle. Feed intake, growth rate and articular cartilage soundness score for the cubitus and stifle joints were not significantly ($P>0.10$) affected by the treatment.

Key words: Glycosaminoglycan polysulfate, boar, lameness, hyaluronic acid, proteoglycans.

RÉSUMÉ

Cette expérience portait sur 18 verrats boiteux qu'on partagea en deux groupes égaux, en se basant sur leur poids corporel initial et l'état de santé de leurs membres. Aux jours 0, 5, 10, 20 et 25, ceux d'un groupe reçurent une injection intramusculaire d'une solution aqueuse de polysulfate de glycosaminoglycane et ceux de l'autre, seulement de la saline. On les sacrifia tous, au jour 27. Le polysulfate de glycosaminoglycane améliora de façon significative ($P<0,05$) l'état de santé des membres et provoqua une élévation ($P<0,06$) de la teneur en acide hyaluronique de la synovie des articulations huméro-radiales, ainsi qu'une augmentation ($P<0,05$) dans la proportion d'agrégats de protéoglycanes dans le cartilage articulaire du condyle fémoral médial. La consommation d'aliments, le taux de croissance et l'état de santé des cartilages des articulations huméro-radiales et fémoro-tibiales ne subirent pas une influence significative ($P>0,1$), à la suite de l'administration de polysulfate de glycosaminoglycane.

Mots clés: polysulfate de glycosaminoglycane, verrat, boiterie, acide hyaluronique, protéoglycanes.

INTRODUCTION

Osteochondrosis and osteoarthrosis are two forms of noninfectious degenerative joint disease which cause significant lameness in swine (1). Repeated intraarticular injections of hyaluronic acid have given variable results in the treatment of equine degenerative joint disease (2) and recovery rates of over 50% have been reported. The beneficial response to hyaluronic acid administration might suggest a deficiency of this glycosaminoglycan (GAG) in the synovial fluid of lame animals and a resultant failure of joint lubrication. A major disadvantage of intraarticular injection is the invasion of the joint space and the potential for subsequent joint infection. An alternative approach is the stimulation of endogenous synthesis of hyaluronic acid by the synoviocytes. Glycosaminoglycan polysulfate (GAGPS) is an artificially polysulfated glycosaminoglycan (GAG) which stimulates the synthesis of hyaluronic acid by cultured human synoviocytes (3). Intramuscular administration of GAGPS to humans suffering from rheumatoid arthritis increased the concentration of hyaluronic acid in the synovial fluid (4). It also stimulates proteoglycan (PG) synthesis (5).

Proteoglycans are the major component of the amorphous ground substance of cartilage. They contain core proteins to which polyanionic GAG's including chondroitin sulfate and keratan sulfate are covalently attached. Proteoglycans form large aggregates by interacting with small amounts of hyaluronic acid and this interaction is stabilized by a link protein (for a review see reference 6). Aggregation is thought to help immobilize PG's within the collagen network contributing to the maintenance of the biomechanical properties of articular cartilage (7). We have recently studied PG's from osteochondrotic porcine cartilage and found that PG's in severely degenerative joints were less aggregated than those from normal joints, probably due in part to proteolysis of core proteins including the hyaluronic acid binding region (8). *In vitro* studies indicate that GAGPS inhibits the activity of some lysosomal enzymes including three keratan sulfate-degrading enzymes (9) and cathepsin B (10). The inhibitory effect, if any, of GAGPS on PG degrading enzymes may cause a change in the structure of articular cartilage PG's from degenerative porcine joints.

The objective of this experiment was to investigate the effect of repeated intramuscular administration of GAGPS on articular cartilage and leg soundness scores, PG aggregation and

*Department of Animal Science, The University of Alberta, Edmonton, Alberta T6G 2P5 (Brennan, Aherne) and Department of Oral Biology, Faculty of Dentistry, The University of Alberta, Edmonton, Alberta T6G 2N8 (Nakano).

Reprint requests to Dr. F.X. Aherne.

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synovial fluid hyaluronic acid concentration in lame boars.

MATERIALS AND METHODS

Eighteen Yorkshire x Landrace boars averaging 101 kg bodyweight, which were culled because of severe locomotory problems were used. These animals were subjectively evaluated for soundness of their fore and hind legs using a score of 1 (normal) to 6 (paralysis of the hind limbs or severe lameness of the fore limbs). Based on leg soundness score and initial body weight, they were equally assigned to two treatments and penned in individual 2.4 × 1.2 m pens with woven wire flooring. A 16% crude protein diet was allowed *ad libitum*. One group of nine boars was injected intramuscularly on days 0, 5, 10, 15, 20 and 25 with GAGPS which was provided in an aqueous solution by Luitpold Werk (8000 Munich, Federal Republic of West Germany). A dose of 5.2 mg kg bodyweight$^{-0.75}$ was selected because a similar level was used successfully in the treatment of human (5) and equine (Dr. L. Goldman, Luitpold werk, personal communication) degenerative joint disease. An equivalent volume of sterile saline solution was also administered intramuscularly to each of nine control boars at 5 d intervals. After a 27 d test period the boars were slaughtered by mechanical stunning and exsanguination. Leg bones were immediately removed with cubitus and stifle joints intact and stored at 4°C during synovial fluid sampling. Synovial fluid samples were collected from the cubitus and stifle joints (11) and analysed for hyaluronic acid according to the method described by Jourdain *et al* (12). Limb bones were kept frozen at -20°C until thawed at 4°C for the evaluation of cartilage soundness and for PG analysis.

The articular cartilage of the distal femur, distal humerus and proximal radius and the distal ulnar physis were each appraised using a score ranging from 1 (apparently normal) to 6 (cartilage partially or completely separated from subchondral bone). Cartilage samples for PG analysis were then removed from the craniomedial aspect of the medial femoral condyles of each of four control and four treated boars. Each of the sites which was sampled was moderately osteochondrotic (score 3) with some evidence of tissue softening. Each sample was diced (less than 2 mm^2) using a scalpel and PG's were extracted with 5 mL of solution containing 4.0 M guanidinium chloride, 0.05 M sodium acetate (13), 0.005 M benzamidine hydrochloride, 0.1 M 6-aminohexanoic acid (14) and 0.01 M disodium EDTA adjusted to pH 5.8. Samples were extracted for 60 h on a tube rotor at 4°C. Tubes were then centrifuged and the supernatant was filtered (glass wool) into dialysis bags. The residual cartilage was resuspended in 3 mL of extracting solution, centrifuged and the supernatant collected. The combined supernatants were dialysed against ten volumes of 0.005 M sodium acetate buffer (pH 5.8) for 24 h. The dialysates were combined with washings from the dialysis bags and adjusted to a density of 1.5 g mL^{-1} using cesium chloride. The solutions were centrifuged at 95,000 × *g* (37,000 rpm) for 48 h at 10°C using a Beckman L2-65B ultracentrifuge with a 60 Ti angle rotor. The upper three-fifths (A2) and the lower two-fifths (A1) of each centrifugate were then separated. The density of fraction A1 was 1.60 g mL^{-1}. Both fractions were dialysed against five changes of distilled water for 60 h at 4°C and freeze-dried.

The cartilage residue remaining after the guanidinium chloride extract was digested with 5 mg of twice crystallized papain (Sigma Chemical Co., St. Louis, Missouri) per g dry cartilage for 48 h at 60°C (15). The digests were centrifuged and the supernatants were diluted to 50 mL with 0.15 M sodium acetate buffer (pH 6.8). Uronic acid content of 0.5 mL aliquots of the diluted digest was determined by the carbazole reaction (16) using glucuronolactone as standard.

Gel chromatography of fraction A1 PG's was performed as described by Nakano *et al* (8) using a 0.8 × 133 cm column of Sepharose 2B (Pharmacia, Upsala, Sweden). Proteoglycan aggregates from normal swine articular cartilage and glucuronolactone were used to determine void volume (V_0) and total volume (V_t) of the column respectively. The column was eluted with 0.5 M sodium acetate pH 5.8 at a flow rate of 4 mL/h^{-1}. Fractions (0.75 mL) were collected using a fraction collector and analysed for uronic acid content by the carbazole reaction (16). The percentage of aggregated and nonaggregated PG's which were excluded from or retarded in the column, respectively, were then calculated. The partition coefficient (K_{av}) of the retarded peak was calculated as $(V_t-V_e)/(V_t-V_0)$, where V_e was the retarded peak volume.

Data were analysed by a oneway analysis of covariance with initial leg soundness score included in the statistical model as a covariate (17). Means were compared using Student's t-test (17). Partial correlation coefficients between initial leg soundness scores and hyaluronic acid concentration in the cubitus and stifle joints were computed within residual sums of squares and were therefore corrected for treatment effects.

RESULTS

Leg soundness scores, performance and synovial fluid hyaluronic acid concentrations of GAGPS-treated and control animals are summarized in Table I. Overall leg soundness score was significantly ($P<0.05$) improved and there was a tendency ($P<0.06$) towards an increased hyaluronic acid concentration in the synovial fluid of the cubitus joints of GAGPS-treated boars (Table I). The hyaluronic acid concentration in the stifle joint synovial fluid was significantly ($P<0.05$) lower than that of the elbow joint and was not significantly ($P>0.10$) increased by GAGPS treatment. There was no significant effect of GAGPS administration on average daily gain, average daily feed intake or gain/feed of boars during the 27 d test period. Initial leg soundness score for the hind legs had a significant effect on final leg soundness score ($P<0.01$), hyaluronic acid concentration of the stifle joint synovial fluid ($P<0.05$) and average daily gain ($P<0.05$) of boars and was therefore included in the statistical model as a covariate. There was a significant partial correlation ($r=-0.49$, $P<0.05$) between leg soundness score for the hind limbs and hyaluronic acid concentration in the synovial fluid of the stifle joint.

Joint lesions observed were predominantly osteochondrotic and were most severe on the medial condyles of

TABLE I. Effect of GAGPS Treatment on Leg Soundness Scores[a], Performance and Synovial Fluid Hyaluronic Acid Concentration of Lame Boars

	Treatment			
	Saline	GAGPS	SEM[b]	SIG[c]
Initial leg soundness score:				
Front legs	2.22	2.56	0.48	NS
Hind legs	4.00	3.78	0.56	NS
Overall	3.11	3.17	0.41	NS
Final leg soundness score:				
Front legs	3.11	2.00	0.46	P<0.06
Hind legs	4.44	3.33	0.34	P<0.06
Overall	3.78	2.67	0.31	P<0.05
Synovial fluid hyaluronic acid (mg mL^{-1}):				
Cubitus joint	1.78	2.25	0.15	P<0.06
Stifle joint	1.51	1.71	0.18	NS
Initial weight, kg	101.4	101.5	2.1	NS
Final weight, kg	121.3	123.1	3.35	NS
Daily gain, kg d^{-1}	0.73	0.80	0.13	NS
Daily feed, kg d^{-1}	2.33	2.77	0.25	NS
Gain feed, kg	0.27	0.28	0.02	NS

[a]Scored on a scale from 1 (normal) to 6 (paralysis of hind limbs; lameness of fore limbs)
[b]SEM, standard error of mean
[c]SIG, significance of difference between means (NS, P>0.10)

TABLE II. Effect of GAGPS Treatment on Cartilage Soundness Scores[a] of Boars

	Treatment			
Site	Saline	GAGPS	SEM[b]	SIG[c]
Medial femoral condyle	2.33	2.75	0.30	NS
Lateral femoral condyle	1.67	1.19	0.24	NS
Medial humeral condyle	1.72	1.94	0.40	NS
Lateral humeral condyle	2.00	1.75	0.48	NS
Proximal radius	1.44	1.31	0.26	NS
Distal ulnar physis	1.83	2.25	0.31	NS

[a]Scored from 1 (normal) to 6 (severely osteochondrotic)
[b]SEM, standard error of mean
[c]SIG, significance of difference between means (NS, P>0.10)

TABLE III. Effect of GAGPS Therapy on the Chromatographic Properties (Sepharose 2B) of Fraction A1 Proteoglycans from Osteochondrotic Swine Articular Cartilage

	Treatment			
	Saline	GAGPS	SEM[a]	SIG[b]
Aggregated, %	10.7	19.5	1.52	*
K_{av} of retarded peak	0.34	0.32	0.03	NS

[a]SEM, standard error of mean
[b]SIG, significance of difference between means (NS, P>0.05;*, P<0.05)

the humerus and femur as was found previously (1). There was no significant (P>0.10) effect of GAGPS therapy on cartilage soundness scores for the femoral or humeral condyles, the proximal radius or the distal ulnar physis (Table II).

Aliquots of fraction A1 PG's were chromatographed on Sepharose 2B to determine the extent of PG aggregation and the relative size of the PG's retarded in the column. The results are summarized in Table III and representative chromatograms of fraction A1 PG's from control and GAGPS-treated animals are shown in Figs. 1 and 2. The GAGPS treatment caused a significant (P<0.05) increase in the percentage of fraction A1 PG's which was aggregated. The K_{av} of the retarded peak was similar (P>0.10) between the two treatments.

DISCUSSION

Reports from several countries indicate that noninfectious degenerative joint disease is a serious problem of commercial breeds of swine. Manipulations of nutritional and management factors have been unsuccessful in the prevention of joint abnormalities and no therapeutic treatment of lame pigs has been reported. Since several studies have indicated positive effects of GAGPS on human degenerative joint disease (18,19,20), we have studied the effect of this glycosaminoglycan on the joints of lame boars. The results of this study indicate that GAGPS therapy improved the leg soundness of lame boars. Although the mechanisms responsible for this improvement are not clear, the increased (P<0.06) hyaluronic acid concentration observed in the cubitus synovial fluid from GAGPS-treated animals herein may be associated with improved soundness scores in the fore-legs. Hyaluronic acid which accounts for most of the viscosity of synovial fluid, is responsible for the efficient lubrication of the joint (21). Verbruggen and Veys (4) in a study of arthritic human knee joints reported that GAGPS treatment significantly elevated hyaluronic acid concentrations in the synovial fluid. Nishikawa *et al* (22) suggested that several galactosamine-containing glycosaminoglycans (dermatan sulfate, chondroitin-6-sulfate and GAGPS) stimulate the synthesis of hyaluronic acid by cultured synovial membrane explants and that these molecules may interact with the cell membrane of the synoviocyte to stimulate hyaluronic acid synthesis. The reason for the relatively unchanged (P>0.10) low concentration of the stifle synovial fluid hyaluronic acid observed in this study is unknown. The reduced concentration of synovial fluid hyaluronic acid may be related to relatively high leg soundness scores and impaired joint lubrication by hyaluronic acid.

A recent review of the treatment of equine degenerative joint disease indicates that hyaluronic acid therapy is quite effective (2). Treatment of surgically induced fractures of the radial and carpal bones of horses by administration of hyaluronic acid to the intercarpal joint increased the weight-bearing capacity of the affected limb

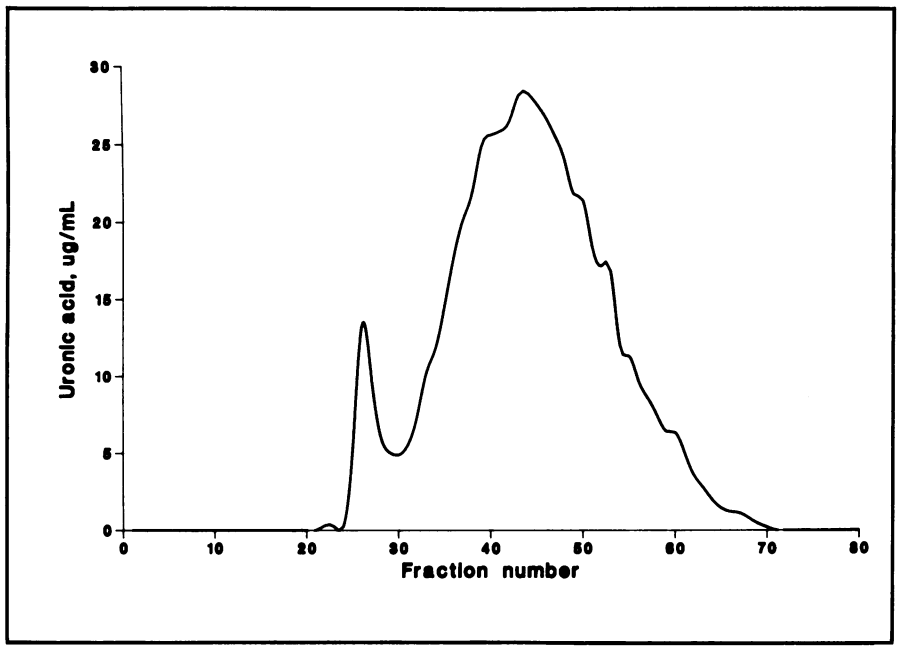

Fig. 1. Sepharose 2B column chromatogram of fraction A1 proteoglycans from ostoechondrotic articular cartilage of a lame boar which was treated with saline. Aggregated proteoglycan and glucuronolactone were used to determine void volume (V_0) and total volume (V_t) of the column, respectively.

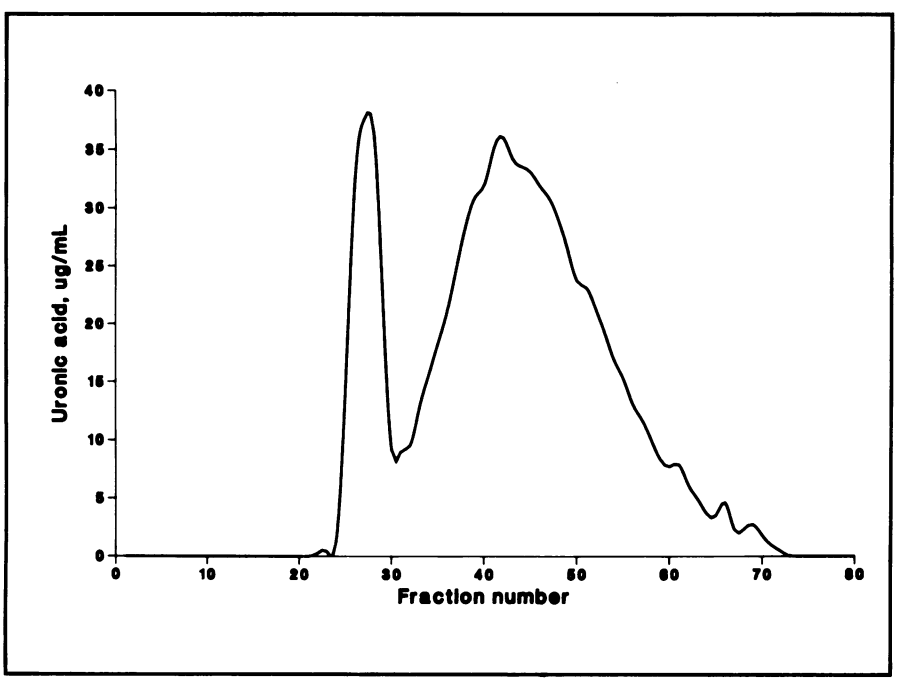

Fig. 2. Sepharose 2B column chromatogram of fraction A1 proteoglycans from ostoechondrotic articular cartilage of a lame boar which was treated with glycosaminoglycan polysulfate.

(23). It has been reported that 59 to 69% of horses recovered from osteoarthritis after intraarticular hyaluronic acid administration (24,25). The GAGPS therapy may however be a better method of increasing the hyaluronic acid concentration in the synovial fluid because the risk of joint infection is reduced. In addition, the stimulation of endogenous synthesis of hyaluronic acid may be preferable to the administration of exogenous hyaluronic acid which may differ in molecular weight or viscosity.

The compressive strength of joint cartilage is largely determined by its PG structure (7), and in general, degenerative cartilage contains a smaller proportion of aggregated PG's than does normal cartilage (7). The relatively small proportions of aggregated PG's (less than 20%, Table III) observed in this study likely reflect the pathological condition of the cartilage studied and are fairly comparable to those of PG aggregate (22.7%±S.E. 7.5) previously reported from severely degenerative porcine femoral cartilage (8).

The GAGPS therapy used in the present study demonstrated structural improvement of PG's by increasing the proportion of PG aggregates, approximately twofold (Table III). The increased proportion of PG aggregate will in turn help protect PG's from proteolytic breakdown (7). This may be due to an increase in hyaluronic acid production by chondrocytes and/or inhibition of proteinases (10) by GAGPS.

In conclusion, GAGPS administration significantly improved the leg soundness score of lame boars possibly due to an elevation of hyaluronic acid concentration in the synovial fluid. It also improved the structure of articular cartilage PG's.

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