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DOI: 10.1111/j.1532-950X.2007.00256.x · Source: PubMed

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Evaluation of Pentosan Polysulfate Sodium in the Postoperative Recovery from Cranial Cruciate Injury in Dogs: A Randomized, Placebo-Controlled Clinical Trial

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Objective—To evaluate the efficacy of pentosan polysulfate (PPS) for improving the recovery period and mitigate the progression of osteoarthritis (OA) of the canine stifle after extracapsular stabilization of cranial cruciate ligament (CCL) injuries.

Study Design—Randomized, blinded, placebo-controlled clinical trial.

Animals—Dogs (n = 40) with unilateral CCL instability.

Methods—Each dog had an extracapsular stabilization of the stifle with or without partial meniscectomy. Dogs were divided into 4 groups based on preoperative radiographic assessment and whether a partial meniscectomy was performed. Dogs were randomly assigned to either (3 mg/kg) PPS or placebo treatment in each group, and then injected subcutaneously weekly for 4 weeks. Lameness, radiographic changes, biological marker concentration in blood and urine, and ground reaction forces (GRFs) were collected preoperatively, and at 6, 12, 24, and 48 weeks. Data were analyzed within and between groups using repeated measures ANOVA; $P < .05$ was considered significant.

Results—No adverse reactions to PPS were reported. Thirty-nine dogs completed a minimum of 24-weeks follow-up and 33 dogs completed 48 weeks. All dogs clinically improved after surgery without differences in lameness score, vertical GRFs, or radiographic progression. Grouped and evaluated only by initial radiographic score, PPS-treated dogs improved significantly faster in braking GRFs than placebo-treated dogs. In dogs with partial meniscectomies, urine deoxypyridinoline, and serum carboxy-propeptide of type II collagen were significantly increased at 6 weeks in placebo-treated dogs compared with PPS-treated dogs.

Conclusions—PPS administered after stabilization of the cruciate deficient stifle may prove to be a useful adjunctive treatment option, although further studies are necessary to substantiate this claim.

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INTRODUCTION

OSTEoarthritis (OA) is an insidious debilitating disease that commonly affects dogs. It is characterized by focal cartilage fibrillation and erosion, synovial inflammation, and subchondral bone changes including sclerosis, osteolysis, and osteophytosis.^{1–6} Be-

cause of the progressive nature of OA, the structural cartilaginous damage is often severe before the dog is admitted for evaluation of clinical signs. Thus, investigations into compounds that specifically target the metabolic imbalances in osteoarthritic joint tissues have been ongoing for many years. Compounds that appear to alter the metabolic processes or the structure of tissues in OA

From the Department of Small Animal Medicine and Surgery, University of Georgia College of Veterinary Medicine, Athens, GA. Presented in part at the 2005 Veterinary Orthopedic Society Meeting, March 6–12, 2005 Snowmass, CO.

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Funding for this project was provided in part by Biopharm Australia Pty Ltd.

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Submitted June 2006; Accepted January 2007

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0161-3499/07

doi:10.1111/j.1532-950X.2007.00256.x

have been termed disease modifying osteoarthritis drugs (DMOAD).^{7–12}

Pentosan polysulfate (PPS; Biopharm Australia Pty Ltd, Bondi Junction, NSW, Australia) has been widely used in human medicine as an antithrombotic/lipidemic agent, and more recently has gained popularity as a potential DMOAD.^{13–19} Its antithrombotic and fibrinolytic properties may improve subchondral and synovial membrane blood flow in arthritic joints.¹⁴ In animal models of OA, it modulates cytokine action and preserves proteoglycan content, and in humans with rheumatoid arthritis (RA) it stimulates hyaluronan synthesis by synovial fibroblasts and increases the molecular weight of hyaluron in synovial fluid. PPS cofractionates with high molecular weight extracts of articular cartilage and menisci from rats after intraarticular injection. PPS may bind to the cell membrane through interaction with cell surface glycoproteins such as thrombospondin before it is internalized, possibly by pinocytosis.¹⁴ PPS has shown promise in the treatment of canine OA. In the Pond-Nuki model, PPS administered at 2 mg/kg intramuscularly once weekly for 4 weeks resulted in significantly decreased articular cartilage damage based on gross and histologic evaluation and maintenance of normal articular cartilage proteoglycan content.^{15,16} Two clinical trials using PPS have been reported; both found a favorable clinical response to treatment compared with placebo groups in short term studies^{17,18}; however in a more recent study in dogs with OA secondary to cranial cruciate ligament (CCL) deficiency, no differences were identified in either functional outcome or radiographic progression using the oral calcium PPS compared with placebo.¹⁹

We hypothesized that PPS would hasten the recovery and slow the progression of OA after CCL injury and extracapsular stabilization. Our objective was to longitudinally assess ground reaction forces (GRFs), clinical lameness, progression of radiographic OA score, and biologic markers of bone and cartilage metabolism for 1 year postoperatively.

MATERIALS AND METHODS

Animals

Forty skeletally mature dogs admitted for unilateral CCL instability were enrolled in the study, which was reviewed and approved by the teaching hospital board. Inclusion criteria included dogs between 1 and 10 years of age and weighing 15–50 kg. Each dog had a normal physical examination (except for a clinical lameness associated with a unilateral cranial cruciate rupture), normal complete blood count (CBC), and serum biochemical profile. Clinical lameness had to have been <3 months duration as determined by information provided by the owner or referring veterinarian. Each dog had a

lameness score of at least 6 (Appendix A)²⁰ and a radiographic score of <20 (Appendix B).²¹

Exclusion criteria included pregnancy or lactation in female dogs; coexisting clinically evident disease on physical examination including recent traumatic injury; severe cardiac, liver, or kidney disease, or a possibility of gastrointestinal ulceration; neoplasia; neurologic disorders; coagulopathies (including exposure to rodenticides); or infection with systemic implications. Additional exclusion criteria included inflammatory arthropathies such as RA, or infectious arthritis; intraarticular injections of any kind \leq 1 month before the start of the study; concomitant treatment with heparin or other anticoagulants; corticosteroid use (> 2 months use over the last 12 months or any use in the last 1 month); concurrent medications to treat OA or previous treatment with a washout period shorter than the following:

- 1 month for oral or parenteral dexamethasone, betamethasone, methylprednisolone, or triamcinolone.
- 1 month for oral or parenteral prednisone, prednisolone, or hydrocortisone.
- 1 month for injectable glycosaminoglycan (i.e. Adequan[®], Luitpold Pharmaceuticals Inc., Shirley, NY) therapy.
- 1 month for oral glucosamine supplementation products.
- 2 weeks for topical corticosteroid preparations.
- 1 week for phenylbutazone, flunixin, aspirin, or other NSAIDs.

Finally, the owner had to read, agree to, and sign an informed consent document.

Study Design

This was a prospective, randomized, blinded, placebo-controlled clinical trial. Based on preoperative radiographic score (low: 1–10 and high: 11–20) and whether or not a partial meniscectomy was performed intraoperatively, dogs were stratified (blocked) into 4 groups (Fig 1). Group 1 was dogs with a low initial radiographic score that required partial meniscectomy. Group 2 was dogs with a low initial radiographic score and no meniscectomy. Group 3 was dogs with a high initial radiographic score and required partial meniscectomy, and Group 4 was dogs with a high initial score and no partial meniscectomy.

In each group, dogs were randomized either for administration of PPS (at the labeled dosage of 3.0 mg/kg) or placebo (0.03 mL/kg), injected subcutaneously (dorsal midline over the spinous processes between Tables 1–4) at 7-day intervals starting 7 days after surgery for a total of 4 injections by random assignment. Both solutions had identical color, clarity, and volume injected into each dog. The investigators were blinded as to the treatment each dog received.

Preoperatively and at 6, 12, 24, and 48 weeks after surgery, blood was taken for biomarker evaluation of serum osteocalcin (OC, marker of osteoblastic activity), carboxy-propeptide of type II collagen (CPII; marker of synthesis of CPII), chondroitin sulfate 846 neoepitope (CS 846; marker of aggrecan synthesis), and Col 2–3/4 (short) neoepitope reflecting collagen cleavage type I and type II collagen (C12C). Urine samples

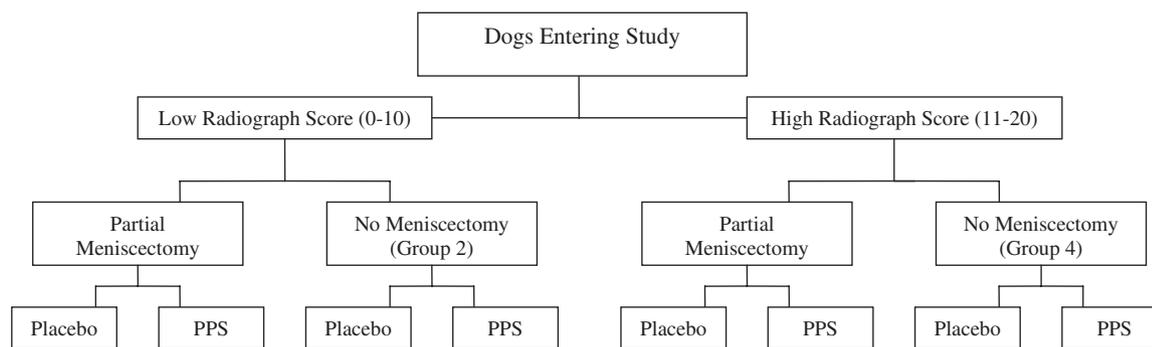


Fig 1. Diagram of the stratification scheme used in the study to block the dogs into groups before randomization.

were obtained for deoxypyridinoline (Dpd; marker of type I collagen degradation) and pyridinoline (Pyd; marker of CPII metabolism).

At each time point, each dog was evaluated subjectively for lameness and radiographic change in the operated stifle, as well as objectively by force plate analysis for GRFs; namely peak vertical force, vertical impulse, peak braking force, braking impulse, peak propulsion, or impulse propulsion (Table 1).

Radiographic Evaluation

All radiographic evaluations were performed by a radiologist who was unaware of treatment assignment. Any dog with an initial score >20 was excluded from the study (Appendix B). Each operated stifle was reradiographed and assessed at 6, 12, 24, and 48 weeks postoperatively on the same scale. For every evaluation the presence of osteophytes, subchondral sclerosis and lucencies, evidence of joint effusion, and intraarticular mineralizations including intercondylar avulsion fracture fragments were assessed and graded.

Surgical Management

Each dog's affected stifle had an exploratory arthrotomy and an extracapsular stabilization performed as described by a single surgeon (S.C.B.).²² A partial caudal pole medial meniscectomy was performed if gross damage of the medial meniscus was noted at surgery. Before surgery, each dog was administered a morphine/bupivacaine epidural, and opioid premedication. Postoperatively additional opioids were used as needed over the first 24 hours as well as transdermal fentanyl patches for 6 days in all dogs. Nonsteroidal antiinflam-

matory drugs were not used. Postoperatively, all dogs had the same rehabilitation protocol, which included limb bandaging with a modified Robert-Jones bandage for 14 days and controlled increases in leash activity and physical therapy for 12 weeks.

Gait Analysis

Ground reaction force data were collected as previously described.^{20,23,24} Briefly, the dogs were trotted over 2 identical serial biomechanical force plates (Model OR6-6-1000, Advanced Mechanical Technology Inc., Newton, MA) mounted flush with the walking surface. The plates were interfaced with a dedicated computer where data were collected, processed, and stored using dedicated software (Vetforce, Sharon Software Inc., Dewitt, MI). Video recordings were made of all trials and used to confirm gait and foot contacts. A valid trial consisted of a sole forelimb strike fully on each plate, followed by an ipsilateral hind foot strike on each force plate. Velocity and acceleration were measured by 5 photoelectric cells, placed 0.5 m apart, coupled with a triggered timer system interfaced with the computer system. The acceptable velocity range was 1.7–2.1 m/s and acceleration across the plates was restricted to -0.5 to $+0.5$ m/s².

Subjective Lameness Score

During each evaluation period, lameness while standing and walking were scored as well as signs of pain on palpation of the stifle joint and degree of weight bearing as previously described, by the same investigator [S.C.B.] (Appendix A).²⁰

Table 1. Flowchart of Treatment Scheduling and Measurement Outcomes During the 48-Week Study Period

	Week 0	Week 1	Week 2	Week 3	Week 4	Week 6	Week 12	Week 24	Week 48
Clinical examination	X	X	X	X	X	X	X	X	X
X-ray	X					X	X	X	X
Surgery	X								
Bandage removal			X						
Blood sample	X					X	X	X	X
Urine sample	X					X	X	X	X
Treatment		X	X	X	X				
Force plate	X					X	X	X	X

Table 2. Braking Peak Force Data (Mean ± SEM) for Dogs Grouped by Initial Radiographic Scores after Pentosan Polyphosphate Sodium (PPS) or Placebo Treatment

Weeks	Low Initial Score PPS Treated (n = 13)	Low Initial Score Placebo (n = 15)	High Initial Score PPS Treated (n = 6)	High Initial Score Placebo (n = 5)
	Pre	1.49 ± 0.41	2.09 ± 0.64	1.8 ± 0.41
6	2.11 ± 0.34	1.67 ± 0.5	1.46 ± 0.37	3.88 ± 1.03
12	3.00 ± 0.4	3.98 ± 0.43	3.91 ± 0.17	3.54 ± 1.08
24	4.04 ± 0.4	4.10 ± 0.33	3.83 ± 0.16	3.89 ± 0.66
48	4.19 ± 0.66	4.94 ± 0.44	5.44 ± 0.57	6.38 ± 0.88

Bold numbers are significantly different ($P < .05$) from respective baseline values.

Summation of all disuse measurement scores resulted in a cumulative limb score.

Biologic Marker Measurements

All samples were collected in the morning. Blood samples were collected from the jugular vein into serum separator tubes, allowed to clot, and then centrifuged to enable harvest of serum. Urine was obtained either by cystocentesis or free catch. A urine sample (6–8 mL) was obtained for analysis for each dog. Serum and urine samples were frozen at -80°C until assayed.

Serum OC Concentrations. Serum concentrations of OC were measured by use of a commercially available kit (Intact human osteocalcin EIA kit, Biomedical Technologies Inc., Stoughton, MA) as previously described.²⁵ The assay used monoclonal antibodies directed against the amino- and carboxy-terminal regions of intact OC. Samples were assayed in duplicate. Interassay variation was $<10.5\%$.

Serum CP II Concentrations. Synthesis of CPII was evaluated by measuring serum levels of CPII using a commercially available ELISA kit (IBEX™ Technologies, Montreal, Canada) as previously described.²⁶ Interassay coefficient of variation (CV) was $<14\%$.

Serum C12C Concentrations. Type I and II collagen degradation from collagenase-mediated cleavage was assessed by

Table 3. Radiographic Scores (Mean ± SEM) for Dogs Grouped by Initial Radiographic Scores after Pentosan Polyphosphate Sodium (PPS) or Placebo Treatment

Weeks	Low Initial Score PPS Treated (n = 15)	Low Initial Score Placebo (n = 13)	High Initial Score PPS Treated (n = 6)	High Initial Score Placebo (n = 5)
	Pre	6.93 ± 0.64	6.79 ± 0.69	15.83 ± 1.54
6	10.13 ± 1.06	8.71 ± 0.98	19.33 ± 2.64	19.0 ± 2.12
12	10.4 ± 1.19	9.54 ± 1.21	18.67 ± 2.55	21.0 ± 3.32
24	13.2 ± 1.23	10.86 ± 1.23	20.83 ± 1.78	23.0 ± 2.59
48	16.07 ± 1.52	13.38 ± 1.37	21.83 ± 1.54	27.4 ± 1.69

Bold numbers are significantly different ($P < .05$) from respective baseline values.

measuring neopeptide levels in the serum with the COL2-3/4C_{short} polyclonal antibody using an inhibition ELISA (IBEX™ Technologies, Montreal, Canada) as previously described.²⁷ Interassay CV was $<11\%$.

Serum CS 846 Concentrations. Aggrecan synthesis and repair was assessed by measuring CS 846 neopeptide levels using a commercially available ELISA (IBEX™ Technologies) as previously described.²⁸ Interassay CV was $<26\%$.

Urine Dpd and Pyd Concentrations. Urine concentrations of Dpd and Pyd were measured by use of commercially available EIA kits (Metra DPD EIA kit and Metra PYD EIA kit, Quidel Corp, San Diego, CA) as previously described.^{29,30} The competitive EIA used monoclonal anti-Pyd and monoclonal anti-Dpd antibodies. Results for Pyd and Dpd were adjusted on the basis of urinary concentrations of creatinine. Interassay variation for the Pyd assay $<11\%$ and corresponding variations for the Dpd assay was $<8\%$.

Statistical Analysis

Data (all 10 response variables) were analyzed by means of a split-plot ANOVA with the treatment factor of drug, the blocking factors of radiographic score and meniscectomy, and the repeat factor of time (SAS PROC MIXED; SAS version 8, Cary, NC). After that analysis, the data were reanalyzed again by a split-plot ANOVA without one or both blocking factors (radiographic score and meniscectomy). Post hoc analysis was performed using a Bonferroni's t-test. A P -value of $<.05$ was considered significant.

RESULTS

Forty dogs were initially enrolled in the study; however 1 dog was later excluded after a diagnosis of thyroid adenocarcinoma 7 weeks after surgery and thus 39 dogs completed a minimum of 6 months follow-up (Fig 2). Assignment to stratification groups was as follows: Group 1 had 11 dogs with 6 administered PPS and 5 placebo; Group 2 had 17 dogs with 9 PPS and 8 placebo; Group 3 had 6 dogs with 3 PPS and 3 placebo; and Group 4 had 5 dogs with 3 PPS and 2 placebo. Additionally, 4 dogs were removed from the 48-week results because of stifle instability in the contralateral limb, and another 2 dogs did not return to the hospital for evaluation at 48 weeks, thus 33 dogs completed the 48-week trial. No dog had an adverse reaction to the drug or placebo.

Mean age for all dogs was 5.4 ± 2.1 years (range, 1–10 years). Mean weight for all dogs was 36.5 ± 8.5 kg (range, 15.4–56 kg). Mean age and weight for PPS dogs was 5.7 ± 2.1 years and 36.3 ± 8.7 kg respectively whereas the mean age and weight for placebo dogs was 4.9 ± 2.1 years and 34.8 ± 8.5 kg.

Table 4. Serum Concentrations (Mean ± Standard Error) of CS 846, C12C, and Osetocalcin for Dogs Grouped by Treatment (Pentosan Polyphosphate Sodium [PPS] or Placebo) Collected During the Study

Biomarker	Treatment	Pre (0 Weeks)	6 Weeks	12 Weeks	24 Weeks	48 Weeks
Serum CS 846	Placebo	427.9 ± 68.5	399.0 ± 48.7	430.9 ± 74.1	444.4 ± 70.5	401.3 ± 61.5
	PPS	611.5 ± 90.8	316.6 ± 53.6	402.5 ± 63.9	438.8 ± 94.8	602.0 ± 185.3
Serum C12C	Placebo	0.88 ± 0.09	1.01 ± 0.09	0.9 ± 0.11	0.93 ± 0.10	0.98 ± 0.13
	PPS	0.71 ± 0.06	0.78 ± 0.06	0.77 ± 0.08	0.9 ± 0.15	0.93 ± 0.15
Serum Osteocalcin	Placebo	10.7 ± 2.6	11.2 ± 2.4	12.8 ± 2.4	13.0 ± 2.9	11.2 ± 1.6
	PPS	8.2 ± 1.2	10.3 ± 1.3	11.4 ± 2.0	16.2 ± 3.6	19.2 ± 6.5

n = 21: PPS treated group.

n = 18: Placebo treated group.

CS 846, chondroitin sulfate 846; C12C, collagen cleavage type I and type II collagen.

Lameness Score

All groups improved (had a lower lameness score) compared with preoperative baseline levels at 12, 24, and 48 weeks postoperatively regardless of treatment (Fig 3). There was no significant difference between PPS or placebo groups regardless of whether the dogs were grouped by initial radiographic score, performance of a partial meniscectomy, or by treatment alone.

GRFs

Peak vertical force and associated vertical impulse data increased at 12, 24, and 48 weeks after surgery for all dogs compared with baseline. There was no significant difference between PPS or placebo groups regardless of whether the dogs were grouped by initial radiographic score, performance of a partial meniscectomy, or by treatment alone. Peak braking force and associated braking impulse showed no significant difference between PPS or placebo groups regardless of whether the dogs were

grouped by performance of a partial meniscectomy or by treatment alone with all dogs improving from baseline at 12, 24, and 48 weeks. Interestingly, when grouped solely by radiographic score, PPS dogs had significant improvements from baseline at 12, 24, and 48 weeks, whereas placebo dogs had significant improvements only at 48 weeks (Table 2). Peak propulsion force and associated impulse data increased from the preoperative baseline at 12, 24, and 48 weeks after surgery for all dogs. There was no significant difference between PPS or placebo groups regardless of whether the dogs were grouped by initial radiographic score, performance of a partial meniscectomy, or by treatment alone.

Radiographic Score

All dogs increased radiographic scores during the 48-week period compared with baseline. There was no significant difference between PPS or placebo groups regardless of whether the dogs were grouped by performance of a partial meniscectomy or by treatment

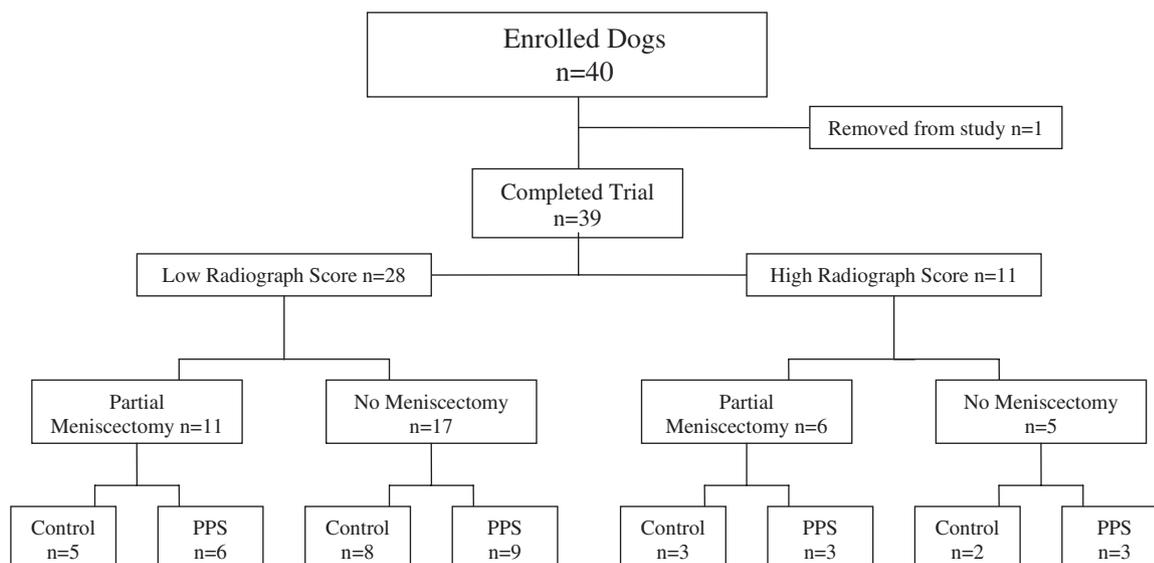


Fig 2. Final distribution of dogs into groups after stratification before randomization.

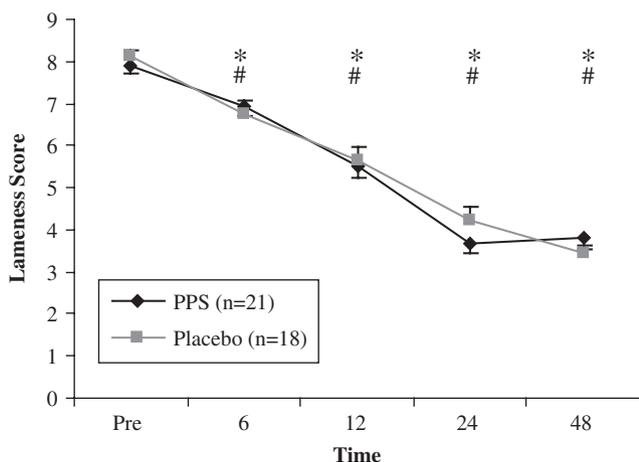


Fig 3. Cumulative lameness score over the study period. *Pentosan polysulfate (PPS) group significantly different than baseline. #Placebo group significantly different than baseline.

alone. When grouped by initial radiographic score, some differences were seen. As expected, the initial low and initial high groups were significantly different from each other at all time points. Dogs with initial low radiographic scores had significant increases from baseline at 12, 24, and 48 months in both the placebo- and PPS-treated groups. The PPS groups also had an increase from baseline at 6 weeks. In the dogs with high initial scores, placebo dogs had significant increases from baseline at all time points measured, whereas the PPS group had significant increases from baseline at only 24 and 48 weeks (Table 3).

Urine and Blood Biomarkers

At 6 and 12 weeks, all dogs had a significantly greater Dpd compared with baseline (Fig 4). More specifically,

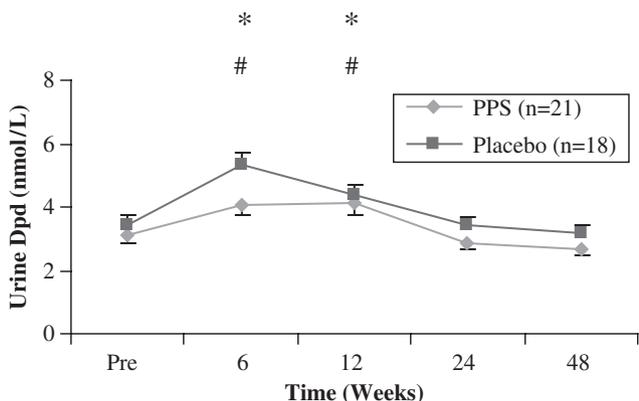


Fig 4. Concentrations of deoxypyridinoline (Dpd) in urine over the study period. *Pentosan polysulfate (PPS) group significantly different than baseline. #Placebo group significantly different than baseline.

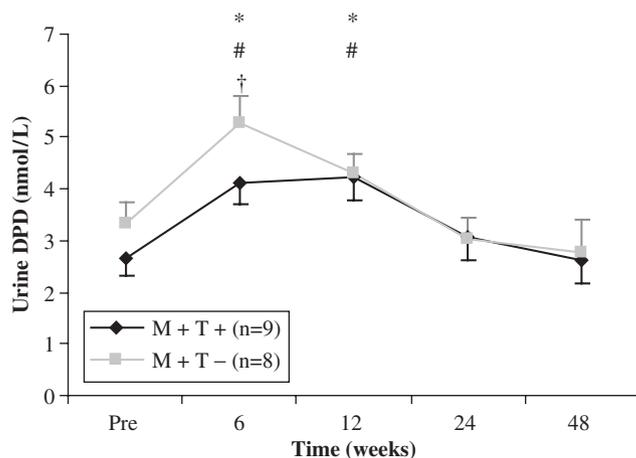


Fig 5. Concentrations of deoxypyridinoline (Dpd) in dogs where a partial medial meniscectomy was performed. *Pentosan polysulfate (PPS) group significantly different than baseline. #Placebo group significantly different than baseline. †PPS and placebo groups significantly different.

when grouped solely by whether or not a partial meniscectomy was performed, there was significantly more Dpd in the urine of placebo dogs that had a partial meniscectomy than PPS dogs that had a partial meniscectomy at 6 weeks (Fig 5). At 6, 12, and 24 weeks, all dogs had a significantly greater Pyd compared with baseline with no differences between groups (Fig 6).

Evaluation of serum concentrations of osteocalcin, C12C, and CS 846 found no significant difference between PPS or placebo groups regardless of whether the dogs were grouped by initial radiographic score, performance of a partial meniscectomy, or by treatment alone. Serum concentrations of CPII at 6 weeks after

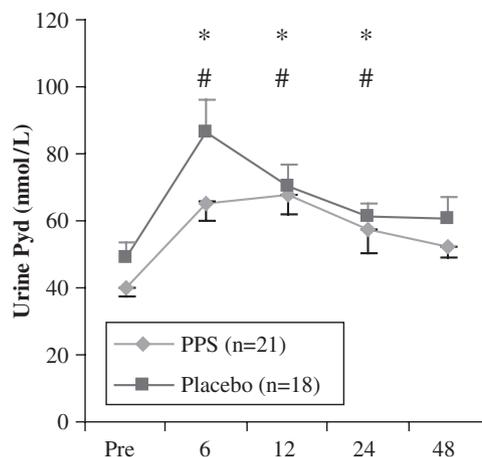


Fig 6. Concentrations of Pyd in urine over the study period. *Pentosan polysulfate (PPS) group significantly different than baseline. #Placebo group significantly different than baseline.

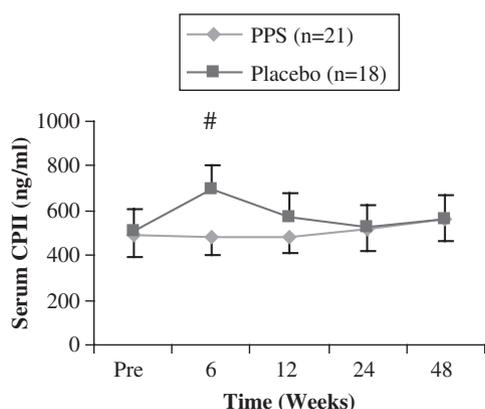


Fig 7. Serum concentrations of type II collagen (CII) over the study period. # placebo group significantly different than baseline.

surgery were significantly higher in placebo-treated dogs when compared with baseline (Fig 7). Furthermore, when grouped by partial meniscectomy there was a significant difference between placebo and PPS dogs with placebo dogs having more serum CII at 6 and 12 weeks (Fig 8).

DISCUSSION

Evaluating methodologies for medical or surgical management of cruciate injuries can be challenging as several confounding factors may play key roles in outcome. In this study, an attempt was made to limit our population to relatively acute injuries with a stipulation of lameness < 3 months, however the practicality of this attempt may have been moot as chronic partial tears may have occurred long before clinical signs were recognized. Our second attempt to lessen variation was to divide

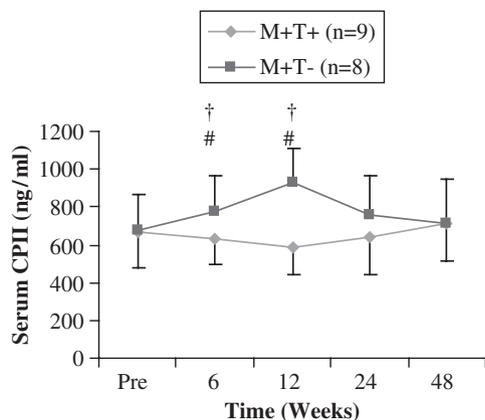


Fig 8. Serum concentrations of type II collagen (CII) in dogs where a medial meniscectomy was performed. #Placebo group significantly different than baseline. †Pentosan polysulfate (PPS) and placebo groups significantly different.

cases based upon radiographic severity and also to eliminate those with the most severe radiographic changes. Finally, there is ample evidence that meniscal injury and subsequent management directly affects the progression of OA in the knee.³¹⁻³⁶

Thus, to attempt to lessen these potential confounders, we attempted to stratify our patients before randomization to treatment group by both the radiographic score and whether a meniscectomy was performed. This stratification allowed us to perform several different statistical evaluations on the different treatment groups. For example, we found that all dogs had a significantly greater Dpd compared with baseline at 6 weeks. With the stratification used, we were able to identify more Dpd released in placebo dogs that had a partial meniscectomy than PPS dogs that had a partial meniscectomy at 6 weeks and 12 weeks; however, the stratification had a negative impact as it lowered the size of our various treatment groups and the power of the study. Thus, where significance was not found in the smaller groups, we were able to combine groups in an effort to increase the power of the study. Given that there were relatively small numbers of dogs in this study, all results that stated that there were not significant differences must be reviewed carefully.

This blinded placebo-controlled clinical study of outcomes after CCL stabilization and treatment with PPS revealed some data of interest. All dogs improved after surgery regardless of pharmacologic intervention. This finding is consistent with previous prospective data on suture extracapsular stabilizations.³⁴⁻³⁷

GRFs

Whereas overall there were no differences in the gait data between treatment groups, there was one interesting finding when the dogs were analyzed solely by initial radiographic score. When the high and low initial radiographic score groups were grouped separately and evaluated, peak braking force and associated braking impulse showed significant improvements from baseline at 12, 24, and 48 weeks in PPS dogs whereas placebo dogs had significant improvements only at 48 weeks. This more rapid increase in braking force transmission in the limb may be related to improved function, increased pain relief or both. Why grouping the dogs by initial radiographic signs detected differences between treatment and placebo is unknown. Obviously initial radiographic scores divided the braking GRFs in a fashion that exposed GRF improvements were different in each group comparing PPS with placebo. These significant braking force increases are quite remarkable because of the inherent low force magnitude measured by the force plate and the large variation usually seen in braking GRFs in the rear limbs of normal and lame dogs.^{20,23,24,38-42}

Radiographic Changes

Radiographic progression after surgery was seen in all groups with no differences between them. Increasing severity of radiographic signs is consistent with previous reports with all types of repairs in the dog.^{37,43-46} It is interesting we did not see a significant increase or a more rapid increase in radiographic scores in dogs that had partial meniscectomy and those that did not. However, this finding is most likely an example of making a type II error given the small numbers in each group of this study. It is difficult to explain the differences seen from baseline in the 2 treatment groups when dogs were grouped by initial radiographic score. Dogs that began the trial with more radiographic changes and were PPS treated had slower progression compared with their baseline than any other group including their placebo-treated high group counter parts. These data would suggest that perhaps PPS slowed the progression of radiographic changes. However, the dogs starting with lower initial radiographic scores treated with PPS appeared to have an accelerated progression as defined by radiographic scores. Alternatively these data could suggest that PPS had little to no effect on the progression of radiographic changes. Furthermore, it must be remembered that radiographic changes are not predictors of the state of the articular cartilage or the function of the limb.

Urine and Blood Biomarkers

Increases in urine concentrations of Dpd at 6 and 12 weeks in the placebo group indicate significant type I collagen degradation. Dpd is considered a sensitive marker of bone metabolism/resorption. Given previous data showing alterations in bone mineral density (BMD), femoral condylar and tibial plateau subchondral bone with cruciate deficiencies, these data are not surprising.²⁴ What is quite interesting is the apparent ability of PPS treatment to decrease the Dpd enough to show significant differences between groups at 6 weeks. Further evaluation of our data suggests that meniscectomy was a major factor elevating Dpd concentrations. Again this data is consistent with earlier works that have shown increased forces and damage in the medial compartment of the canine cruciate deficient stifle as well as significant changes in the associated subchondral bone.^{24,33,47,48} Current findings that PPS slowed bone resorption at 6 weeks are interesting, and given that the drug was only administered for 4 weeks initially and then halted begs the question of whether repeated drug administration may have continued to prevent the bone resorption. In human knee OA patients, conflicting data (elevations, loss and no change) have been found when evaluating urinary Dpd excretion and joint damage, whereas in patients with hip

OA no differences have been found.⁴⁹⁻⁵² Urine concentrations of Pyd were increased in all dogs for the first 24 weeks, suggesting significant CPII turnover. Treatment with PPS did not appear to alter this collagen turnover.

The early increases seen in serum CPII epitope concentrations of placebo-treated dogs indicates synthesis of CPII and is consistent with a significant increase in cartilage anabolic metabolism. These findings could have 2 explanations: (1) PPS suppressed the synthesis of CPII or (2) PPS slowed the catabolic state and thus less CPII synthesis was required during the recovery period. The fact that placebo-treated dogs that had partial meniscectomy also had increases in CPII at 12 weeks suggests the second explanation is more likely. Lack of significant changes in serum concentrations of osteocalcin, a marker of osteoblastic activity, C12C, a marker of collagen type I and II degradation, and CS 846, a marker of aggrecan synthesis and repair, are problematic given changes in other markers seen in the study. Whereas they may indicate the inability of PPS to alter specific metabolic activities between treatment or placebo groups, they may also be examples of committing type II errors because of low group numbers. This latter explanation may be particularly true with CS 846, given the interassay CV of nearly 25%.

In studies like this where multiple outcome measurements were used with a relatively low number of cases, caution must be taken when interpreting the results and the apparent paradox seen with conflicting data must not be overemphasized. Another important point that must be addressed is the fact that the repair methodology in this study (extracapsular suture stabilization) alone has been associated with inconsistent stifle joint stability and one may conclude many of these dogs had persistent instability.^{37,42-44,53} The instability and progression of OA changes may overwhelm the potential disease modifying effects on the joint by compounds such as PPS. Certainly it has been argued that evaluation of the cruciate deficient stifle model in the dog over a long term shows such an aggressive progression of OA that, without joint stabilization, may negate or mask significant disease modifying effects of test compounds.⁵⁴ Finally, several significant findings and trends were seen indicating improvement with PPS at the 6-week time point. These data suggest that there may be a potential to show treatment effect with PPS with greater numbers of cases or even with perhaps repeated drug dosing but confirmation of such statements is dependent on additional clinical trials.

Clinical Relevance

Certain data from this study denotes improvements with PPS over placebo at the 6-week time point. These

data suggest that there may be a potential to show treatment effect with PPS with greater numbers of cases or even with perhaps repeated drug dosing beyond the 4 weeks postoperatively used in this study.

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Appendix A. Subjective Scoring System Used to Evaluate Limb Use by Blinded Veterinarians

Variable	Score	Attribute
Lameness	1	Stands and walks normally
	2	Stands normally, slight lameness at walk
	3	Stands normally, severe lameness at walk
	4	Abnormal stance, slight lameness at walk
	5	Abnormal stance, severe lameness at walk
Weight Bearing	1	Normal weight bearing at rest and walk
	2	Normal weight bearing at rest, and favors affected limb at walk
	3	Partial weight bearing at rest, and walk
	4	Partial weight bearing at rest, and nonweight bearing at walk
	5	Nonweight bearing at rest, and walking
Response to contralateral limb lift*	1	Accepts displaced weight
	2	Mild resistance to displaced weight
	3	Moderate resistance to displaced weight†
	4	Strong resistance to displaced weight†
	5	Refuses to lift contralateral limb
Response to limb flexion and extension	1	No response
	2	Mild response (turn head toward affected limb)
	3	Moderate response (withdraws affected limb)
	4	Severe response (vocalizes or becomes aggressive)
	5	Disallows manipulation or palpation of affected limb

*Response of affected hind limb.

†Replaces contralateral limb in <10 seconds (moderate resistance) or <5 seconds (strong resistance).

Cumulative limb disuse score = sum of four scores.

Appendix B. Radiographic Evaluation

Each Determinant to Be Assigned a Value: 0 = Absent, 1 = Mild, 2 = Moderate, 3 = Severe

1. Patellar osteophytes
2. Femoral trochlear groove periarticular osteophytes
3. Lateral and/or medial condylar periarticular osteophytes
4. Femoral subchondral sclerosis
5. Distal femoral condylar remodeling
6. Subchondral cystic lucencies
7. Sesamoid periarticular osteophytes
8. Femoral intercondylar notch osteophytes
9. Proximal tibial periarticular osteophytes
10. Proximal tibial subchondral sclerosis
11. Proximal tibial subchondral cystic lesions
12. Central tibial plateau osteophytes
13. Joint effusion/capsular thickening
14. Intraarticular mineralized osseous fragments
15. Meniscal mineralization
16. Intercondylar avulsion fracture fragments

Total stifle joint score = sum of the radiographic scores at each location/determinant.