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SUMMARY

We tested the hypothesis that treatment of growing, susceptible dogs with glycosaminoglycan polysulfates would mitigate the signs of incipient hip dysplasia. In one experiment, seven pups, randomly selected from two litters, were injected intramuscularly twice weekly with 2.5 mg per Kg body weight glycosaminoglycan polysulfates (Adequan®) and seven control pups received sterile buffered 0.9% saline from six weeks to eight months of age. The hip joints of the dogs were examined by radiography with dogs in the standard, legs-extended position. At eight months of age all dogs in this experiment had no femoral head subluxation on radiographic examination. The Norberg angles, a measure of coxofemoral congruity, improved from a mean of 102 ± 1 degrees in controls to 106 ± 1 degrees in the drug treated group ($p = 0.008$). These dogs were not subjected to necropsy.

In a second experiment, eight pups were randomly selected from two litters and were injected intramuscularly twice weekly with 5.0 mg per Kg glycosaminoglycan polysulfates (Adequan®) from six weeks to eight months of age. Similarly, eight control pups were injected with saline. At eight months of age hip joints were examined by radiography with dogs in the standard position, and at necropsy intra-articular tissues were evaluated macroscopically and biochemically. Eight of eight dogs in the drug treated group had no subluxation on radiographic examination, whereas four of eight control dogs had femoral head subluxation. The mean Norberg angles on the radiographs were 109.7 ± 1.6 degrees for the treated group and 101.5 ± 1.6 degrees for controls, representing a mean improvement in coxofemoral congruity

of 8.2 degrees in the drug treated dogs. The radiographic diagnoses (normal vs. dysplastic) and the Norberg angle measurements were significantly different ($p = 0.04$ and 0.002, respectively) for the treated and control groups.

At necropsy one of eight dogs in the drug treated group had cartilage degeneration, whereas four of eight dogs in the control group had cartilage degeneration. The mean pathologic score determined for the hip joints of dogs in the treated group was 1.6 ± 0.8 whereas for controls the score was 3.3 ± 1.2 ($p = 0.09$). Normal (disease-free) dogs had hip pathologic scores of zero. The mean fibronectin content of femoral head articular cartilage was reduced from 2.19 ± 0.61 μg/mg in untreated dogs to 0.59 ± 0.56 μg/mg for drug treated dogs ($p = 0.04$). Fibronectin content was used as a measure of the extent of cartilage degeneration, and the cartilage of disease-free hip joints contained 0.32 ± 0.03 μg per mg. The mean proteoglycan content of the cartilage was unaffected by the drug treatment. There was a trend for lower synovial fluid volume and lower ligament volume (more normal volumes) in the treated dogs, but the differences were not statistically significant.

Hip joint laxity was assessed with a distraction method during radiographic examinations of the dogs in both Experiments 1 and 2. The differences in laxity determinations between the drug treated and control groups were not statistically significant.

Taken together, the data suggested that intramuscular treatment with glycosaminoglycan polysulfates from six weeks to eight months of age of growing pups which were susceptible to hip dysplasia resulted in less subluxation as determined from the

standard radiographic projection. The dogs in the treated groups had closer coxofemoral congruity when they were eight months old; at necropsy the joint pathologic scores of treated dogs had a trend to improvement, but the differences were not statistically significant. The mechanism of action for this drug effect is unknown.

FOOTNOTES

a. Agway Big Red High Energy, Pro-Vet, Newark, Delaware.

b. P/D Canine, Hill's Pet Products, Topeka, Kansas.

c. Corid, MSD - Agvet - Merck Co., Rahway, New Jersey.

d. Norden Laboratories, Lincoln, Nebraska.

e. Purchased from a distributer; Butler Company, Rochester, New York, who obtained the drug, Adequan®, from Luitpold Pharmaceuticals, Inc., Shirley, New York.

f. Procedure of General Diagnostics, Organon Teknika Corp., Durham, North Carolina.

g. Picker (G5255), 500 milliampere, 90 kilovolt radiography unit, Rochester, New York.

h. Prom-Ace (10 mg/ml), Aveco, Fort Dodge, Iowa.

i. Biotol (5% solution in distilled water), Boehringer Ingelheim, St. Joseph, Missouri.

j. Dr. Gail K. Smith, personal communication

k. Beuthanasia, Schering Co., Kenilworth, New Jersey

l. Luitpold Pharmaceuticals, Inc., Shirley, New York.

m. R.J. Todhunter and G. Lust, unpublished data.

INTRODUCTION

Hip dysplasia continues to be of concern for dog owners and veterinarians because joint pain, restricted motion, osteoarthritis, and lameness are associated with the condition. It is widely accepted that hip dysplasia has a hereditary basis, but the cause of the hip abnormality is not known (for a review see Ref. #1). Some years ago preliminary evidence from several investigators suggested the usefulness of limiting food intake in growing dogs in order to delay the onset, lessen the severity of pathological signs, and even prevent hip dysplasia in some dogs[2,3,4]. This concept recently received strong support in a study which substantiated that limited food and the resulting slower weight gain during the growth period resulted in better hip conformation and less hip dysplasia[5].

Short of surgical interventions and also antiarthritic-analgesic, non-steroidal or steroidal therapy to increase joint function, there is no medical treatment available to ameliorate or reverse the progression of the disease. Several recent reports claimed that intra-articular or intramuscular injections of glycosaminoglycan polysulfates lessened the pathological changes of surgically induced osteoarthritis in stifle joints of rabbits and dogs[6,7,8,9]. Since dogs with hip dysplasia invariably have evidence of osteoarthritis in the hip joints, it was of interest to examine if prophylactic treatment of susceptible, growing dogs with glycosaminoglycan polysulfates would diminish the occurrence of the hip joint tissue abnormalities that precede or are a part of hip dysplasia. The studies described here were designed to test the hypothesis that treatment of susceptible

pups during most of their growth period with glycosaminoglycan polysulfates would mitigate the signs of incipient hip dysplasia.

MATERIALS AND METHODS

Dogs. Labrador retrievers bred and raised at this laboratory were used. Four litters of pups were studied. Experiment 1: Litter "a" was six pups from a normal male with good hip joint conformation mated to a normal female with fair hip conformation. Litter "d" was eight pups from a male with dysplastic parents, but with good hip conformation at 12 months of age at the time of mating to a female with bilateral severe hip dysplasia with evidence of degenerative joint disease. Experiment 2: Litter "f" was eight pups from a male with severe hip dysplasia with degenerative joint disease mated to a female with moderate hip dysplasia with degenerative joint disease. Litter "g" was eight pups from a male with severe hip dysplasia mated to a female with severe hip dysplasia with degenerative joint disease.

Nursing pups were supplemented with evaporated milk and cereal at three weeks of age. Dog food, dry[a] and canned meat,[b] were gradually added until weaning at five-six weeks. Dry dog food[a] supplemented with canned meat[b] was fed to six months; followed by dry food[a] only to eight months of age. All dogs were allowed dry food and water ad libitum. Pups were treated for coccidia[c] at three and six weeks of age; wormed at eight and eleven weeks, and at 24 and 27 weeks. First standard vaccinations[d] were at four and six weeks with measles, distemper and parvovirus vaccine as prescribed by the manufacturer. At 8, 12 16, and 24 weeks the dogs were vaccinated with five-way vaccine

(DA_2 PL-CPV). Individual dogs were weighed at weekly intervals and the body weights were recorded.

Drug Administration. A preparation of glycosaminoglycan polysulfates (molecular weights 4-16 kD) was purchased from a commercial company.[e] Experiment 1: An equal number of pups from each litter (4 from "a" and 3 from "d") were assigned at random to receive the drug treatment or the control treatment. Drug treated pups were injected intramuscularly with 2.5 mg glycosaminoglycan polysulfates per Kg body weight twice weekly (Monday and Thursday) alternating left and right hind legs starting at six weeks and ending at eight months of age. Untreated controls were injected intramuscularly with a matched volume of sterile phosphate buffered (pH 7.2) 0.9% saline, on the same schedule. Experiment 2: Four pups from each litter ("f" and "g") were selected at random to receive the drug treatment or the control treatment. Treated pups were injected intramuscularly with 5.0 mg/Kg glycosaminoglycan polysulfates twice weekly from six weeks to eight months of age. Untreated controls were injected with a matched volume of buffered saline in the same way as the controls for Experiment 1.

Activated partial thromboplastin time[f] was assessed on plasma of six dogs from litter "a" of Experiment 1 and from eight dogs of litter "f" in Experiment 2. The blood was collected and the analyses were done when dogs in each litter were six months old. This test utilized the reported mild heparin-like activity[9] of

glycosaminoglycan polysulfates to ascertain if the drug reached the blood.

Radiographic Examination

Dogs were examined[g] under anesthesia at four, six and eight months of age for hip joint conformation with respect to hip dysplasia. The standard legs extended, dorsal recumbency, ventro dorsal radiographic examination was done.[10,11] Dogs initially were calmed with injection of 0.5 ml tranquilizer,[h] followed by anesthesia,[i] using up to 8 ml of a 5% solution[i] administered slowly until the toe-pinch, withdrawal reflex was negative. Anesthetized dogs also were examined for the extent of hip joint distraction, an estimate of laxity, as described by Smith et al.[12]. For this procedure the anesthetized dogs were positioned on their backs in a standard plastic cradle and a foam-rubber cushion, and were tied down to the x-ray table with velcro straps. An examiner (GL) stood behind a lead-shielded, mobile board with a lead-lined window and two openings for the radiographic safety gloves to pass through. The examiner squeezed the stifles medially (femurs nearly perpendicular to the table top) while a device made by Smith et al.[12,j] was positioned between the thighs. The distraction device also was held in place by the velcro straps.

Evaluation of Radiographs

The standard legs-extended radiographs initially were evaluated as normal or dysplastic (GL, KAB), and graded further (KAB) as excellent, good or fair normal joints, or mild, moderate

or severe hip dysplasia as described in a booklet by the Orthopaedic Foundation for Animals.[13] A radiographic score was used by assigning corresponding numbers of 1, 2, 3, 4, 5, 6 to the diagnoses from excellent to severe. On the radiographs a Norberg angle measurement was made[5,10,11] and a center-edge angle measurement was made.[14] Data were recorded as degrees.

The method of Smith et al.[12] was used to determine the joint distraction distance (mm) between the geometric center of the femoral heads and acetabulae and a distraction index was calculated by dividing the distance by the radius of the femoral head.[12] This represented an estimate of joint laxity. The method of Belkoff et al.[15] also was used on the radiographs of the distracted joints to assess the percent of coverage of femoral heads by the cranial side of acetabulae. This measurement also was associated with joint laxity[15] and served as another estimate of laxity after joint distraction. The original procedure of Belkoff et al. positioned femurs at a 45° angle for distraction, whereas for the method of Smith et al. the femurs are in a neutral (more perpendicular) position.

Necropsy Examination

Not all dogs in Experiment 1 were examined at necropsy at eight months of age; the joints of some dogs in this group were examined subsequently, but observations were not available for all dogs at eight months of age and data were not included here. All dogs in Experiment 2 were killed with an overdose of barbiturate[k] at eight months of age immediately after radiographic examinations. Hip joints and shoulder joints were

dissected out and placed in crushed ice. Within two hours after death, joints were examined for cartilage degeneration, macroscopic presence of synovitis, synovial fluid and round ligament volume.[10,11,16,17] Samples of articular cartilage from the known site of lesion predilection on femoral heads[16,17] were collected and stored frozen (-20°C). Cartilage from the weight bearing region of the humeral head also was collected and frozen. Fibronectin and proteoglycan contents of the articular cartilage samples were determined as described by Burton-Wurster and Lust.[18,19] Experiments were done to establish that glycosaminoglycan polysulfates did not interfere in the ELISA test for fibronectin. This was determined by adding known quantities of glycosaminoglycan polysulfates to the extraction solutions of fibronectins and then using the ELISA.

A pathologic score was devised by assigning numbers to joint tissue abnormalities as follows: macroscopic appearance of normal synovium = 0, normal articular cartilage = 0, synovial fluid volume less than 0.3 ml = 0, ligament volume less than 0.7 ml = 0. Synovitis = 1, cartilage degeneration = 1, synovial fluid volume 0.3 - 0.8 ml = 1, greater than 0.8 ml = 2; ligament volume 0.7 - 1.1 ml = 1, ligament volume greater than 1.1 ml = 2; proteoglycan content of cartilage greater than 30 µg/mg = 0, 20 - 29 µg/mg = 1, 5 - 19 µg/mg = 2; fibronectin content of cartilage less than 0.4 µg/mg = 0, 0.4 - 1.0 µg/ml = 1, greater than 1.0 µg/mg =2. The additive joint pathologic score was averaged per dog. Normal, disease free, dogs had scores of 0.

Experimental Design and Statistical Analysis

Two experiments were done, testing the effect of two doses of glycosaminoglycan polysulfates on the manifestation of signs of hip dysplasia in Labrador retriever dogs. Experiment 1 used 2.5 mg/kg drug and Experiment 2 used 5.0 mg/kg drug. Evidence was sought for the efficacy of the drug in reducing the frequency or mitigating the signs of hip dysplasia. Both Experiments 1 and 2 had a randomized complete block design with the block being the litter. Two litters were used in each experiment. Different dams and sires were mated to produce four litters; litters were independent. An equal number of pups from each litter were assigned at random to receive either drug treatment or control treatment (saline solution). There was replication of treatments within each block (randomized complete block design with replication in each block). This allowed for a check of difference in response to the treatments between litters rather than assuming that no variation existed.

The Cochran-Mantel-Haenszel test[20] was used to assess the difference between the treated and control groups in the proportion of dogs having hip dysplasia, after controlling for litter effects. Quantitative data were analyzed as a randomized block design, where litter was the blocking factor, to test for effects of drug. Since the "a priori" research hypothesis was that the drug treatment should reduce the signs of hip dysplasia, one-sided tests of treatment effects were appropriate. Partial correlations among the quantitative measures of hip dysplasia also were calculated to check the conformity of the different

measures, after controlling for variation in the data due to litter and treatment effects.

RESULTS

General Observations

The growing dogs used in these studies tolerated the drug and placebo treatment without apparent untoward effects. The repeated injections were not painful. Pups grew at a conventional rate, and at necropsy (Experiment 2) no obvious abnormalities were noted at the sites of injections in the hind limbs.

Activated Partial Thromboplastin Time

An activated partial thromboplastin time (APTT) was determined using one litter (a) of Experiment 1 and one litter (f) of Experiment 2 when pups were six months old to assess whether the injected glycosaminoglycan polysulfate actually was utilized by the treated dogs. The data were obtained (measured in seconds) at 0, 1, 2, 3, 4, 6, 27 hours after intramuscular injection of the drug. Data are listed in Table 1. Dogs in litters (d) and (g) were not studied for APTT.

There was no difference in APTT at hour 0 for the two groups of Experiment 1 ($p = 0.6779$) or of Experiment 2 ($p = 0.7663$). The mean initial APTT were 14.83 ± 0.26 and 14.67 ± 0.26 seconds, respectively, for the treated and control groups of Experiment 1 and 14.50 ± 0.57 and 14.75 ± 0.57 seconds, respectively, for Experiment 2. For each dog in the control group, APTT did not change with time from the time of drug administration, measured as hours or as the natural logarithm of hours (i.e., the slope

was zero). However, for each drug treated dog, the APTT (after time 0) decreased linearly with the natural logarithm of time. The rate of change in APTT differed significantly between the two groups (Experiment 1: $p = 0.005$ and Experiment 2: $p = 0.0016$), with the slope for the treated group being smaller than that of the control group, as expected. The mean APTT after drug was significantly greater for the drug group than the control group (Experiment 1: $p = 0.0011$ and Experiment 2: $p = 0.0000$). The mean APTT after the start of the assay were 19.33 ± 0.46 and 14.80 ± 0.46 seconds, respectively, for the treated and control groups of Experiment 1 and 19.33 ± 0.29 and 14.00 ± 0.29 seconds, respectively, for Experiment 2.

Because data for some assay times were missing for some of the dogs in each group of Experiment 1, an analysis, which compared the two groups for APTT at four to six hours after drug, was performed. The mean APTT at four to six hours was significantly higher for the drug group than the control group ($p = 0.0031$). The mean APTT at four to six hours were 19.00 ± 0.51 and 15.17 ± 0.51 seconds, respectively, for the drug and control groups.

The mean APTT at one to two hours after drug was compared for the two experiments. There was evidence of an interaction between experiment and treatment ($p = 0.0800$), indicating that the magnitude of the difference in APTT at one to two hours for drug and control groups differed for the two experiments. For each experiment, the mean APTT at one to two hours after drug injection was significantly greater for the treated than the control group: Experiment 1: $p = 0.00005$; drug treated: $21.33 \pm$

0.33 seconds, and controls: 14.67 ± 0.33 seconds; Experiment 2: $p = 0.0001$; drug treated: 23.75 ± 0.88 seconds, and controls: 14.13 ± 0.88 seconds.

Growth of Pups

The weight (kg) of each pup was taken each week for the duration of each experiment. In Experiment 1, weights were measured starting at six weeks (1.5 months) and continued to be taken through 38 weeks (9.5 months). In Experiment 2, weights were measured during weeks six through 35 (1.5 through 8.75 months) for litter (f) and during weeks seven through 35 (1.75 through 8.75 months) for litter (g). Individual growth curves were fit to the data for each dog. Growth exhibited a curvilinear pattern in which weight increased with age but the rate of weight gain tapered off toward the end of the experiment. No lag in weight gain was found for any dog during the first weeks of the experiments nor was there a strong leveling off of weight for any dog by the termination of the experiment, although the growth rate did decline during the last few weeks of each experiment.

Figure 1 shows the average growth curves for the treated and control groups of litters (a) and (d), respectively, of Experiment 1. Figure 1 also gives the corresponding plots for litters (f) and (g) of Experiment 2. For visual clarity, the 95% confidence bands for the average curves were omitted.

The individual growth curve for each dog was summarized by three statistics: the mean weight for the duration of the experiment (mean), the rate of weight gain for the experiment (slope = linear component), and the tapering of the growth rate

(curvature = quadratic component). The values of the summary statistics of growth for each dog were used as the data in the randomized complete block model to test for an effect of drug treatment on the growth of dogs.

Table 2 gives the summary measures of growth for the two experiments. There was no statistically significant effect of drug treatment on mean weight, growth rate or tapering off of growth rate for either experiment: Experiment 1: $p = 0.4068$, 0.2702 and 0.2317, respectively; Experiment 2: $p = 0.1057$, 0.7612 and 0.8594, respectively. In addition, an analysis using the data from the two experiments was performed to test whether the effect of drug on growth differed between the two experiments. There was no significant interaction between experiment and treatment for any of the three summary measures of growth ($p = 0.3451$, 0.1048 and 0.1019, for mean, slope and curvature, respectively), indicating that the difference in response between the drug and control groups was similar for the two experiments. There was no main effect of drug treatment on any of the summary measures of growth as well ($p = 0.8437$, 0.4719 and 0.4561 for mean, slope and curvature, respectively), indicating that there was not a significant difference in growth between the treatment and control groups. Finally, there was no statistically significant difference between the two experiments in mean weight, rate of weight gain or tapering of growth rate ($p = 0.5809$, 0.4887 and 0.4965, respectively). It was concluded that the growth of pups was similar in the two experiments and no statistically significant differences in growth due to drug treatment were found.

Changes in Norberg Angle with Time

The Norberg angle of each pup was measured at four and eight months of age in Experiment 1 and at four, six and eight months of age in Experiment 2. In general, the Norberg angle increased over the period from four to eight months of age for the pups in each experiment.

For Experiment 2, one could assess whether a linear trend in Norberg angle with age existed for each pup. The mean Norberg angle over the period from four to eight months of age and the rate of change in Norberg angle over that period (the slope) were used as summary statistics in comparing time trends in Norberg angle between the treated and control groups.

Table 3 gives the mean Norberg angles at each age for the treated and control groups of each experiment as well as the mean difference in Norberg angle from ages four to eight months for the two treatment groups of each experiment. At four months of age, there was no significant difference in Norberg angle between the two groups for either experiment (Experiment 1: $p = 0.0873$; Experiment 2: $p = 0.2396$), although the mean Norberg angle of the drug group was greater than that of the control group. At eight months of age, the Norberg angle was significantly greater for the drug group than the control group for both experiments (Experiment 1: $p = 0.0081$; Experiment 2: $p = 0.0016$). For Experiment 2, the Norberg angle at six months was significantly greater for the treated group than the control group ($p = 0.0065$). The difference in Norberg angle from ages four to eight months was significantly greater for the treated group than the control group for Experiment 2 ($p = 0.0004$); whereas, for Experiment 1,

there was no statistically significant difference between groups in mean difference in angle ($p = 0.3489$).

In addition, an analysis using the data from the two experiments was performed to test whether the two experiments differed in the effect of drug on Norberg angle at four months, Norberg angle at eight months, or the difference in angle over the period of four to eight months of age. There was no significant interaction between experiment and treatment for Norberg angle at four months or Norberg angle at eight months ($p = 0.5701$ and 0.1600, respectively), suggesting that the difference in response between the drug and control groups was similar for the two experiments. However, there was a significant interaction of treatment and experiment for difference in Norberg angle ($p = 0.0226$). Hence, the separate analyses must be used for the difference in angle. There was no main effect of drug treatment on Norberg angle at four months ($p = 0.0782$), indicating that the Norberg angle at four months was not significantly greater for the drug group than the control group; however, the Norberg angle at 8 months was significantly greater for the drug group than the control group ($p = 0.0001$). Finally, there was no statistically significant difference between the two experiments for Norberg angle at either four months or eight months of age ($p = 0.4057$; $p = 0.5901$, respectively).

The improvement in coxofemoral congruity was expressed as the change in degrees ($\Delta°$) due to drug treatment and was calculated for each age of each litter as the mean angle of the treated group minus mean angle of the control. Table 3 gives the mean $\Delta°$ for each age of each experiment. Analysis of the

improvement data suggested that there was no difference between experiments for improvement at four months of age or at eight months of age ($p = 0.8520$ and 0.1358, respectively).

In summary, the effect of the drug on Norberg angle at four or at eight months of age was similar for the two experiments. There was no statistically significant treatment effect on Norberg angle at four months of age, but the Norberg angle at eight months of age was significantly greater for the drug treated dogs than the control dogs. The change in Norberg angle over the period of four to eight months of age differed for the two experiments: the difference in Norberg angle was significantly greater for the treated group than the control group for Experiment 2; whereas there was no statistically significant difference between treatment groups for Experiment 1.

Summary of Experiment 1

Experiments 1 and 2 both ended when dogs reached eight months of age. The data obtained at this time period are presented in Tables 4-8; Table 4 lists data for dogs in Experiment 1 and Tables 5-8 pertain to Experiment 2.

Data on the effects of biweekly injections of 2.5 mg/kg glycosaminoglycan polysulfate on hip joint radiographic parameters at eight months of age are listed in Table 4. Only the Norberg angle and the center-edge measurements were significantly different between controls and drug treated dogs. The center-edge angles, although determined independently, estimated joint congruity as did Norberg angles. Thus the center-edge angle determinations substantiated the Norberg angle determinations.

Summary of Experiment 2

In addition to radiographic examination, hip joints of the dogs in Experiment 2 also were observed at necropsy at eight months of age. Data for individual pups for Norberg angles, radiographic score, and pathologic score are presented in Table 5. The population mean values for the observed radiographic and intra-articular parameters are listed in Table 6. It can be noted that the radiographic diagnoses, Norberg angles, and center-edge angles were significantly different between controls and treated dogs. The mean pathologic scores were 3.3 ± 0.9 and 1.6 ± 0.9 for controls and treated respectively, but the difference was not statistically significant ($p = .09$). Biochemical analysis of articular cartilage disclosed that the mean fibronectin content was significantly different between controls and treated dogs. The fibronectin content of articular cartilage from disease-free joints was 0.32 ± 0.03 μg/mg wet cartilage (also see Tables 7 and 8).

In Table 7 values for the experimental observations (i.e., data from Table 6) are compared with values calculated for the disease-free joints and for the dysplastic joints respectively from Experiment 2. These values can be compared to previously published numbers from the literature, called "expected" values in Table 7.

Data are presented suggesting that glycosaminoglycan polysulfates treatment had no effect on the fibronectin and proteoglycan content of humeral head articular cartilage and on synovial fluid volume in disease-free shoulder joints from the dogs in Experiment 2 (Table 8).

Correlation of Measures

Partial correlations among quantitative measures of hip joint conformation were calculated to check for the conformity of the different measures, after controlling for the variation in the data due to litter and treatment effects. In Experiment 1 center-edge angle was negatively correlated with radiographic score ($r = -0.62$, $p = 0.054$) and as expected highly positively correlated with Norberg angle ($r = 0.90$, $p = 0.0004$). Femoral head coverage percent was negatively correlated with distraction length ($r = -0.94$, $p = 0.0001$) and index ($r = -0.95$, $p = 0.0001$). Distraction length was positively correlated with distraction index ($r = 0.99$, $p = 0.0001$).

For Experiment 2, radiographic score was correlated positively with synovial fluid volume ($r = 0.95$, $p = 0.0001$), and negatively with Norberg angle ($r = -0.82$, $p = 0.0006$) and center-edge angle ($r = -0.86$, $p = 0.0002$). Norberg angle was highly positively correlated with center-edge angle ($r = 0.97$, $p = 0.0001$) and with femoral head coverage percent ($r = 0.95$, $p = 0.0001$) and negatively with distraction length and index ($r = -0.90$, $p = 0.0001$) with synovial fluid volume ($r = -0.84$, $p = 0.0003$) and with pathologic score ($r = -0.85$, $p = 0.0002$). Distraction index was negatively correlated with femoral head coverage percent ($r = -0.90$; $p = 0.0001$). Pathologic score was positively correlated with fibronectin content ($r = 0.85$, $p = 0.0002$) and with synovial fluid volume ($r = 0.84$, $p = 0.0003$). Ligament volume exhibited no strong relationship with any of the other measures.

DISCUSSION

The data presented suggested that early treatment of susceptible pups with glycosaminoglycan polysulfates reduced the signs of incipient hip dysplasia. The drug treatment was continued from six weeks to eight months of age and it is not possible to predict if the beneficial effects observed at eight months of age would persist in the future. Additional studies are needed to answer that question.

The goal of this study was to ascertain a drug effect on early signs of hip dysplasia during the developmental phase of the disease, which coincides in general terms with the growth period of dogs. The initial radiographic evidence for hip dysplasia can be observed between three and twelve months of age in Labrador retrievers.[17] Six months was reported to be the most frequent age for the initial appearance of hip dysplasia and about 50% of susceptible pups had signs of abnormality on radiographs at this time; at eight months 60-70% were dysplastic. At six and eight months some dogs that appeared to be disease-free on radiographic examination had hip joint abnormalities.[17] It is not known to what extent the severity of disease in parents influences the time of disease onset in their progeny. It was reasoned that from weaning to eight months was an appropriate period to evaluate a prophylactic therapy with a promising drug.

The drug, glycosaminoglycan polysulfates (molecular weights 4-16 kD), is a product isolated from bovine tracheal cartilage and is polysulfated synthetically.[7,8,9] Previously several reports appeared with data suggesting that this drug had been effective in reducing cartilage degeneration and in inhibiting proteases

and in promoting proteoglycan formation in stifle joints of dogs and also rabbits when cruciate ligaments had been resected at surgery in order to create an unstable stifle joint and subsequently osteoarthritis.[6,7,8] Similar results also have been claimed by authors for the treatment of osteoarthritis in horses[21] and in humans.[9]

The data of the present study suggested that drug treatment resulted in improved coxo-femoral congruity as assessed by Norberg angle[5,10,11] and center-edge measurements.[14] These two measurements were made independently on radiographs in this study and provide an effective means to quantify the position of the center of the femoral head in relation to its acetabulum in the hip joint. Corresponding intra-articular effects of the drug were a trend for lower synovial fluid and round ligament volume and a significant reduction of cartilage fibronectin content. The proteoglycan content of the lesion area of femoral heads was not different in treated and control dogs. The observed joint pathologic score had a trend toward normal in the treated dogs, but the means were not statistically different between controls and treated dogs. The observations on the hip joint tissues help support the conclusion from the radiographic evaluation that drug treatment on average was associated with a better hip joint.

Of interest for the pathogenesis of hip dysplasia was that a measure of hip joint capsular laxity, i.e., femoral head distraction,[12,15] apparently was unaffected by the treatment. The mean values for femoral head coverage percent, distraction length and distraction index were not significantly different although in Experiment 2 the mean values appeared to be reduced in the

treated dogs (see Table 4 and 6). Smith *et al.*[12] proposed that a distraction index of 0.3 or less is associated with less degenerative joint disease. If our observations were substantiated after further study, it suggests the possibility that the mean laxity identified in the dogs of this study was inherently abnormal and that the drug only delayed the onset or reduced the signs of disease in the treatment groups. It would require follow-up studies to subtantiate this. An alternative explanation for the data might be that a certain amount of laxity is a phenomenon associated with hip joints, but is not critical or causal for disease progression to hip dysplasia.

The data do not readily suggest a mechanism of action for glycosaminoglycan polysulfates in this study. Possibilities include an as yet unknown effect on muscle tissues and/or on the development of bone, as well as the previously postulated[6,7,8] protective effects on cartilage and inhibitory effects on cartilage proteinases. The possibility that bone metabolism might be involved was suggested by data from a recent study[m] wherein intra-articular administration of glycosaminoglycan polysulfate caused a striking increase in technetium-99-methylene diphosphonate uptake into subchondral bone of equine carpus joints.[m,22] Other investigators recently reported that signs of hip dysplasia in dogs also were reduced and/or abated at six months of age when Labrador retriever pups were fed 25% less food than a control group. In that report[5] it was observed that the improvement of hip joint congruity persisted in the test dogs until two years of age. Those authors did not propose a mechanism of action for the limited food effect. Thus, there are now

two methods, i.e., glycosaminoglycan polysulfates therapy and limited food consumption, that promote the reduction of the signs of incipient hip dysplasia in dogs. It is reasonable to propose that future studies aimed at elucidating the mechanism of action of these two phenomena will help us in understanding the cause of hip dysplasia in dogs.

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Table 1. Effects of one intramuscular injection of glycosaminoglycan polysulfates on plasma activated partial thromboplastin time.

Hours post Injection	Experiment 1		Experiment 2	
	Untreated Controls	Drug Treated	Untreated Controls	Drug Treated
	. . . . . seconds.			
0	14.5 ± 0.6	14.8 ± 0.5	14.8 ± 0.5	14.5 ± 0.6
1	15.0 ± 0.0	20.5 ± 0.7	13.5 ± 0.5	23.0 ± 2.1
2	14.8 ± 0.5	21.7 ± 0.5	14.7 ± 0.3	24.5 ± 0.5
3	N/A*	N/A	14.0 ± 0.4	20.8 ± 0.5
4	15.5 ± 0.7	19.5 ± 0.7	13.8 ± 0.3	17.0 ± 0.0
6	14.5 ± 0.7	19.0 ± 1.4	13.5 ± 0.3	16.0 ± 0.7
27	14.5 ± 0.7	15.0 ± 0.0	14.5 ± 0.3	14.7 ± 0.5

For Experiment 1: controls and treated groups n = 3 each.

For Experiment 2: controls and treated groups n = 4 each.

Data as means and standard error.

*N/A: Data not available.

Table 2. Means of the summary data of growth for the two drug experiments.

	Experiment 1		Experiment 2	
	Untreated Controls	Drug Treated	Untreated Controls	Drug Treated
Mean Weight (kg)	15.27 (0.76)	16.16 (0.76)	17.43 (0.76)	16.88 (0.71)
Growth Rate (kg/month)	5.28 (0.29)	5.98 (0.29)	6.98 (0.29)	6.71 (0.27)
Decline in Growth Rate (kg/month^2)	-0.22 (0.02)	-0.27 (0.02)	-0.34 (0.02)	-0.32 (0.02)

Individual growth curves were fit to the data for each dog and each curve was summarized by three statistics: mean weight (the pivot point of the curve), growth rate (the linear component of the curve) and decline in growth rate (the curvature). The mean weight is the average weight of a pup over the period from 1.5 to 8.0 months. The growth rate is the rate of weight gain of a pup over the experimental period, as measured by the slope of the data. The decline in growth rate is the tapering off of increase in weight for a pup, as measured by the curvature in weight as a function of age. The standard errors of the summary statistics are given in parentheses. The drug doses were 2.5 and 5.0 mg/kg body weight for Experiments 1 and 2, respectively.

Table 3. Changes in Norberg angle with time for two experiments.

	Experiment 1			Experiment 2		
	Untreated Controls	Drug Treated	Δ°	Untreated Controls	Drug Treated	Δ°
Norberg angle at 4 months (°)	96.7 (1.5)	99.76 (1.5)	2.3 (3.3)	99.4 (1.4)	100.8 (1.4)	1.3 (3.3)
Norberg angle at 6 months (°)	N/A*	N/A		103.1 (1.6)	109.7 (1.6)	6.5 (2.4)
Norberg angle at 8 months (°)	102.2 (1.0)	106.3 (1.0)	4.0 (1.2)	101.5 (1.6)	109.7 (1.6)	8.2 (1.2)
Difference in angle	5.5 (1.4)	6.3 (1.3)		2.1 (1.1)	8.9 (1.1)	

*NA: Data not available.

Mean Norberg angle at four, six, and eight months of age and mean difference in angle from four to eight months of age for the two drug experiments. The difference in Norberg angle over the period of four to eight months of age was calculated for each dog as angle at eight months minus angle at four months. The improvement in coxofemoral congruity was expressed as the change in degrees (Δ°), calculated as mean of treated minus mean of control for each litter. The standard errors are given in parentheses. The drug doses were 2.5 and 5.0 mg/kg body weight for Experiments 1 and 2, respectively.

Table 4. Effects of intramuscular injections of 2.5 mg/Kg glycosaminoglycan polysulfates on hip joint radiographic parameters at eight months of age.

	Untreated Controls	Drug Treated	Probability p
Diagnosis	7 N; 0 D	6 N*; 0 D	0.39
Radiographic Score	2.2 ± 0.4	2.0 ± 0.4	0.52
Norberg Angle (degrees)	102.2 ± 1.0	106.3 ± 1.0	0.008
Center-edge Angle (degrees)	13.0 ± 0.8	17.7 ± 0.9	0.002
Femoral head coverage (%)	35.6 ± 3.1	40.9 ± 3.4	0.27
Distraction (mm)	6.4 ± 0.75	6.1 ± 0.07	0.80
Distraction index	0.55 ± 0.06	0.53 ± 0.07	0.85

Data as means ± standard error of the mean from Experiment 1.

*One radiograph was lost.

Table 5. Norberg angles, radiographic scores and pathologic scores of hip joints at eight months of age in control and treated dogs for Experiment 2.

Untreated Controls					Drug Treated				
Dog No.	Norberg Angle Average*	Radiographic Diag.*		Pathologic Score*	Dog No.	Norberg Angle Average*	Radiographic Diag.*		Pathologic Score*
F59	110°	N	1	0	F19	111°	N	1	0
F69	99°	D	5	5.5	F29	113°	N	1	0.5
F79	104°	N	2	0	F39	111°	N	2	1.0
F89	104°	N	1	0	F49	110°	N	2	0
G59	108°	N	2	0.5	G19	108°	N	2	1
G69	96°	D	(R2)† (L4) 3	5.5	G29	110°	N	3	2
G79	101°	D	(R4) (L2) 3	6.5	G39	104°	N	3	6.5
G89	91°	D	6	8	G49	110°	N	2	1.5

*Degree, diagnosis and score numbers are "per dog."

†R = right joint; L = left joint.

Table 6. Effects of intramuscular injections of 5.0 ug/Kg glycosaminoglycan polysulfates on hip joint radiographic and intra-articular parameters at eight months of age.

	Untreated Controls	Drug Treated	Probability p
Radiographic Diagnosis	4 N; 4 D	8 N; 0 D	0.04
Radiographic Score	2.9 ± 0.5	2.0 ± 0.3	0.10
Norberg Angle (degrees)	101.5 ± 1.6	109.7 ± 1.6	0.002
Center-edge Angle (degrees)	12.9 ± 1.5	19.3 ± 1.5	0.006
Femoral head coverage (%)	38.7 ± 2.3	41.9 ± 2.3	0.17
Distraction (mm)	7.9 ± 0.6	7.1 ± 0.6	0.20
Distraction index	0.65 ± 0.05	0.59 ± 0.05	0.23
Pathologic Score	3.3 ± 0.9	1.6 ± 0.9	0.09
Synovial fluid vol.(ml)	0.39 ± .12	0.23 ± .12	0.17
Ligament vol.(ml)	0.76 ± .06	0.68 ± .06	0.18
Cartilage			
Proteoglycan (ug/mg)	36.4 ± 2.9	36.4 ± 2.4	---
Fibronectin (ug/mg)	2.19 ± 0.61	0.59 ± .56	0.04

Data from Experiment 2; expressed as means and standard error of mean.

Table 7. Comparison of overall experimental measurements with those calculated for normal and dysplastic hip joints of the Labrador retrievers used in Experiment 2.

Parameter	Units	Normal Joints Expected	Normal Joints Observed*	Dysplastic Joints Expected	Dysplastic Joints Observed**	Untreated‡ Controls	Drug‡ Treated
Radiograph	-	N	N	D	D	N-D	N
Norberg angle	degrees	>102	109.4±1.0	<101	96.9±1.7	101.5±1.7	109.7±0.9
Center-edge	degrees	> 12	19.2±0.7	< 11	8.3±1.7	12.9±1.7	19.3±0.7
Joint laxity	mm	1-4	6.9±0.7	> 4	10.3± .7	7.8±0.7	7.1±0.5
Distraction index	-	<0.3	0.5±.02	>.3	0.83±.03	0.7±.05	0.6±0.4
Femoral coverage	%	> 40	44.7±1.0	<39	32.5±2.8	38.7±2.3	41.9±1.5
Synovial fluid	ml	< 0.3	0.2±.03	>0.4	0.7±0.1	0.4±.1	0.23±.04
Ligament volume	ml	< 0.7	0.60±.06	>0.7	1.0±0.1	0.78±.06	0.68±.06
Cartilage	-	smooth	smooth	rough	rough	variable	variable
Fibronectin	ug/mg	< 0.4	0.32±.03	>0.5	3.52±0.7	2.30±.66	0.62±0.2
Proteoglycan	ug/mg	30-45	38.2±1.7	5-29	28.6±2.8	36.4±2.9	36.4±2.4

"Expected" values represent literature values taken from references 10, 11, 12, 14, 16, 17, 18, and 19.

*Mean and SEM from joints of dogs with normal radiograph and disease-free cartilage at necropsy.

**Mean and SEM from joints of dogs with a diagnosis of dysplastic and with cartilage degeneration.

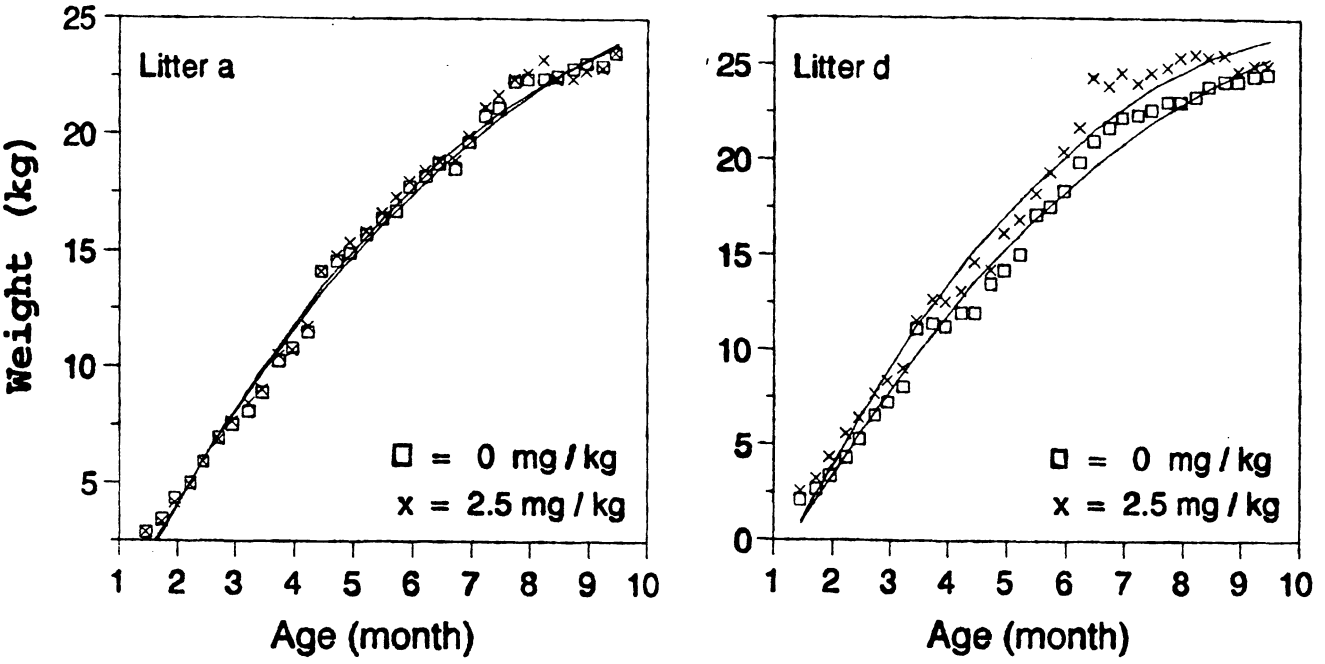
‡Experimental data from Table 6; mean values from untreated controls and from treated dogs.

Table 8. Lack of effect of glycosaminoglycan polysulfates treatment in growing dogs on cartilage proteoglycan and fibronectin content of normal shoulder joints.

	Untreated Controls	Drug Treated
Proteoglycan (ug/mg)	36.4 ± 2.9	36.4 ± 2.4
Fibronectin (ug/mg)	0.21 ± .03	0.31 ± .07
Synovial fluid (ml)	0.41 ± .06	0.48 ± .04

Data from Experiment 2; expressed as means and standard error of mean.

Experiment 1



Experiment 2

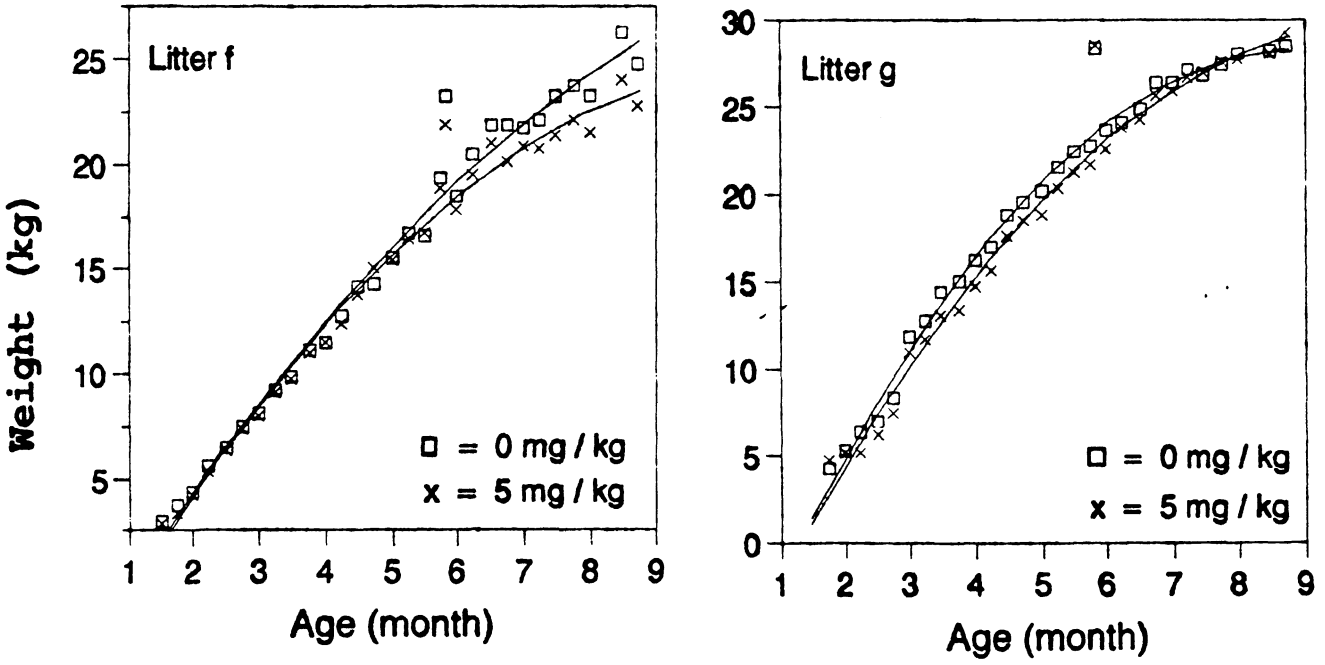


Figure 1. Growth of pups in experiments 1 and 2.