Glucosamine and chondroitin sulfate in the repair of osteochondral defects in dogs – clinical-radiographic analysis[1]

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ABSTRACT

Among the proposed treatments to repair lesions of degenerative joint disease (DJD), chondroprotective nutraceuticals composed by glucosamine and chondroitin sulfate are a non-invasive theraphy with properties that favors the health of the cartilage. Although used in human, it is also available for veterinary use with administration in the form of nutritional supplement independent of prescription, since they have registry only in the Inspection Service, which does not require safety and efficacy testing. The lack of such tests to prove efficacy and safety of veterinary medicines required by the Ministry of Agriculture and the lack of scientific studies proving its benefits raises doubts about the efficiency of the concentrations of such active substances. In this context, the objective of this study was to evaluate the efficacy of a veterinary chondroprotective nutraceutical based on chondroitin sulfate and glucosamine in the repair of osteochondral defects in lateral femoral condyle of 48 dogs, through clinical and radiographic analysis. The animals were divided into treatment group (TG) and control group (CG), so that only the TG received the nutraceutical every 24 hours at the rate recommended by the manufacturer. The results of the four treatment times (15, 30, 60 and 90 days) showed that the chondroprotective nutraceutical, in the rate, formulation and administration at the times used, did not improve clinical signs and radiologically did not influence in the repair process of the defects, since the treated and control groups showed similar radiographic findings at the end of the treatments.

Key words: degenerative joint disease, osteoarthritis, arthrosis, chondroprotective, nutraceutical.

RESUMO

Sulfato de condroitina e glucosamina na reparação de defeitos osteocondrais em cães – análise clínico-radiográfica

Dentre os tratamentos propostos para a doença articular degenerativa (DAD), os nutracêuticos condroprotetores à base de sulfato de condroitina constituem uma terapia não invasiva que favorece a manuteção da saúde da cartilagem. Além de utilizados em humanos, foram também disponibilizados para uso veterinário administrados na forma de suplemento nutricional independentemente de prescrição, uma vez que possuem somente o registro do Serviço de Inspeção Federal, que não exige testes de eficácia e segurança. A falta desses testes pelo Ministério da Agricultura

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para comprovação da eficácia e segurança de medicamentos veterinários e a carência de estudos científicos que comprovem seus benefícios geram dúvidas quanto à eficiência de tais substâncias ativas. Nesse contexto, o objetivo deste estudo foi avaliar a eficácia de um nutracêutico condroprotetor veterinário à base de sulfato de condroitina e glucosamina, na reparação de defeitos osteocondrais no côndilo femoral lateral de 48 cães, por meio de análises clínica e radiográfica. Os animais foram distribuídos em grupos tratado (GT) e controle (GC), de forma que somente o GT recebeu o nutracêutico a cada 24 horas na posologia recomendada pelo fabricante. Os resultados dos quatro tempos de tratamento utilizados (15, 30, 60 e 90 dias) mostraram que o produto na dose, formulação e no período de administração utilizados não proporcionou melhora dos sinais clínicos e não influenciou radiograficamente o processo de reparação dos defeitos, visto que os grupos tratado e controle apresentaram aspectos radiográficos idênticos ao término dos tratamentos.

Palavras-chave: doença articular degenerativa, osteoartrite, artrose, condroprotetor, nutracêutico

INTRODUCTION

Condroprotective nutraceuticals, such as glucosamine and chondroitin sulphate, are among the treatments proposed to repair the damage of the degenerative joint disease (DJD). These drugs are widely studied as they constitute a non-invasive therapy and favor the metabolism of the articular cartilage (Chard & Dieppe, 2001).

Several studies have demonstrated, through clinical and radiographic analyses, that chondroprotective nutraceuticals are effective in controlling pain and delay the progression of DJD (Reginster *et al*., 2001; Pavelka *et al*., 2002; Towheed *et al*., 2005). However, Sawitzke *et al*. (2010) questioned the effectiveness of these products and claim that the benefits of their use are controversial.

The oral administration of chondroprotectors, in the form of nutraceuticals, is widely used in human medicine and, together with the reduction of risk factors such as weight reduction and controlled exercises, constitute a therapeutic alternative in the treatment of progression of DJD in human patients. Such products intended for humans, having as the main active ingredients chondroitin sulfates and glucosamine, are controlled and certified by the National Health Surveillance Agency - ANVISA and therefore undergo testing for efficacy and safety that are required by the Ministry of Health for medicines for humans.

After being applied for controlling DJD in humans, the nutraceuticals have become available for veterinary use, mostly for older dogs and breeds predisposed to DJD as the Labrador Retriever, Rottweiler, Golden Retriever, Daschund and Sheepdogs. However, the products have in their label only the registration by the Federal Inspection Service - SIF, which inspects the manufacture of the product, but does not require testing for efficacy and safety. The lack of these tests required by the Ministry of Agriculture to confirm the efficacy and safety of veterinary medicines generates doubts about the efficiency of the various concentrations of active substances available and protects the administration of these products to animals as a nutritional supplement and trade independent of a veterinary prescription.

Thus, the aim of this study was to evaluate experimental, clinical and radiographically the effect of a veterinarian chondroprotective nutraceutical based on chondroitin sulfate and glucosamine, in the repair of osteochondral defects experimentally induced in the lateral femoral condyle in dogs.

MATERIAL AND METHODS

A total of 48 mixed breed dogs, skeletally adults with radiographic confirmation, weighing between 10 and 25 kg, from the experimental kennel of the Department of Veterinary Medicine, Federal University of Viçosa (UFV-DVT). The selected animals were housed in collective cages and fed commercial dog food once a day and water *ad libitum*.

This experimental study was approved by the Ethics Committee of the DVT-UFV, certified by Case No. 18/2008. The standards of conduct for the use of animals in teaching, research and extension of DVT / UFV were strictly followed.

The 48 animals were randomly distributed among four observation periods (15, 30, 60 and 90), according to the postoperative period and each containing 12 animals. Within each treatment, the animals were divided into two groups (TG and CG) of equal numbers. The animals of TG were the treated group, while the CG represented the control group. Table 1 shows the details of the treatments and experimental groups.

For the surgical procedure, the dogs were fasted water and solid 12 hours. Subsequently, they were sedated with acepromazine[8] (0.1 mg/kg, intravenously), induced with propofol[9] (7 mg/ kg, intravenously) and maintained with isoflurane[10] diluted in 100% oxygen.

With the animal placed in the left lateral position and surgical field properly prepared, it was performed a curvilinear skin incision in the craniolateral portion of the femoro- tibio patellar joint of the right limb, from the region of the distal femoral diaphysis to the region of the tibial proximal epiphysis. Then, divulsion of the subcutaneous tissue was performed along the same line of the skin incision and through the lateral parapatellar retinaculum and the joint capsule, as proposed by Johnson & Dunning (2005) for medial deviation of the patella and the femoral trochlear groove exposure in dogs and cats. Thus, it was possible to mark a circular area with a dermatological punch of 6 mm in diameter, on the articular surface of the lateral femoral condyle. Using a scalpel blade No. 15, the marked cartilage was removed and then the area was curetted to obtain bleeding, indicating that the subchondral bone was reached and an osteochondral lesion was created. The joint was washed with 0.9% saline solution to remove fragments of bone or cartilage, the patella was repositioned in the trochlear groove and the procedure was completed with the suture pattern "X" using the 3-0 nylon monofilament for the retinaculum and joint capsule, a simple continuous pattern for the subcutaneous tissue and the Wolff pattern for the skin, using for both planes the 4-0 nylon monofilament.

Antibiotic therapy was applied for seven days with enrofloxacin[11] at 10 mg/kg body weight, every 24 hours orally as well as analgesics in the immediate postoperative period and for a further two days with 0,3 mg/kg of morphine[12] intramuscularly every 12 hours.

All TG animals received the chondroprotective nutraceutical daily from the first day after surgery (early treatment) until to complete the treatment period. The nutraceutical was given in the form of palatable tablets, each containing 200 mg of chondroitin sulfate, glucosamine 300 mg, 24 mg of palatalizing agent and 1200 mg vehicle *q.s.p*. According to the manufacturer's recommendations, it was administered one tablet every 24 hours to dogs with 10 kg, two tablets to dogs with 11 to 20 kg, 4 tablets to dogs with 21 to 25 kg of body weight.

In the clinical assessment, the animals were observed daily for the first 15 days after surgery and then, once every seven days, until to complete the observation period of each group. The same examiner evaluated the degree of lameness, pain, swelling and the presence of wound infection (when present purulent discharge and/or suture dehiscence). The degree of lameness in the gait was evaluated as proposed by Sena (2006): grade 0 (no lameness), grade 1 (support of the limb with lameness) or grade 2 (lack of support).

To assess pain and discomfort, the operated area was manipulated and pain classified as: grade 0 (no pain manifestation), grade 1 (some pain during palpation of the joint), grade 2 (painful expressions during joint motion) or grade 3 (some pain during palpation and movement of the joint).

The circumference of the operated joint region was measured using a measuring tape to determine whether there was a change in the circumference of the joint. This parameter was also measured before surgery and at the end of the observation period in both limbs.

In the radiographic evaluation, radiographs were obtained from the femoro-tibio patellar joint, in the planes of incidence mediolateral, craniocaudal and tangential (skyline). The radiographic technique was standardized according to the distance from the apparatus to the film, kilovoltage and exposure time for each animal. The radiographic examinations were performed prior to surgery, in the immediate postoperative period and then according to the grouping of the animals: T15 - radiographic examination at 15 days after surgery; T30 - radiographic examination at 15 and 30 days after surgery; T60 - radiographic examination at 15, 30 and 60 days after surgery; T90 - radiographic examination at 15, 30, 60 and 90 days after surgery.

The lameness and pain were assessed by the chi square test. In this analysis were considered the times of treatment initiation (day 1 postoperatively), the days 5, 10 and 15 after surgery. Analysis of variance (ANOVA) with

Table 1. Detailing the experimental groups

Treatment	Number of animals	Observation period after lesion induction
G15	12 (6 TG and 6 CG)	15 days
G30	12 (6 TG and 6 CG)	30 days
G60	12 (6 TG and 6 CG)	60 days
G90	12 (6 TG and 6 CG)	90 days

G15: 15 days postoperatively; G30: 30 days postoperatively; G60: 60 days postoperatively; G90: 90 days postoperatively; TG: Groups treated with chondroitin sulfate and glucosamine; CG: Control Groups.

[8] Acepran®– Univet – São Paulo – SP - Brazil
[9] Propovan® - Cristália – Itapira – SP - Brazil
[10] Isoforine® - Cristália – Itapira – SP - Brazil
[11] Enrofloxacin® - Biovet – Vargem Grande Pay]ulista – SP - Brazil
[12] Dimorf® - Cristália – Itapira – SP - Brazil

repeated measures was used to examine changes in both the circumference of the operated limb and the non-operated limb. Additionally, it was verified that there was no interaction in the results for the circumference of the limb, and in the situations where the test found a significant interaction effect, multiple comparisons were performed using the Bonferroni test. In the case of the variable circumference of the operated region, it was also not possible to perform comparisons using ANOVA considering all times of clinical evaluation and, therefore, the same times mentioned for the statistical analysis of lameness and pain were considered.

To detect whether there has been change in the circumference of the limb between the start and end of the treatment, the analysis of variance (ANOVA) with repeated measures was also used in the same way as described above, however, the times of beginning of the treatment and the last day of evaluation were considered. A final statistical analysis was performed to determine whether there has been change in the circumference of the joint concomitantly in the two limbs. The statistical test used was the nonparametric Mann-Whitney test, taking into account the variations between the times of starting treatment and last day of evaluation, both for the operated as the contralateral limb.

In all statistical analyzes performed in this study, the value of probability greater than 0.05 was not considered significant.

RESULTS AND DISCUSSION

The lateral parapatellar surgical approach allowed for the exposure of the lateral femoral condyle, providing the means for defining the exact location of the lesion using the punch. The use of the number 15 scalpel blade allowed satisfactory excision of the cartilage fragment previously delimited. However, the curettage did not prove to be an effective method for finishing the osteochondral defect, since it should be performed until bleeding and it was found variation in the amount of bone to be curetted in order to observe the hemorrhage. Moreover, it was noted that the anesthetic directly influenced in obtaining bleeding, so that it took longer to get it when the animal was deeply anesthetized, suggesting that the method was not effective for performing standardized defects.

Except for one, all animals in the TG ingested the nutraceutical forcefully, indicating that the product was not palatable to most dogs involved in the study. The low palatability can be related to the sweet taste of the product, since the dogs belong to the order Carnivora and therefore tend to prefer food with the flavor of meat (Bradshaw, 2006).

In all operated animals there was complete healing of the surgical wound and there were no sero-purulent or bloody secretions or dehiscence.

In relation to the circumference of the joint, we observed a decrease in the values according to postoperatively period in all groups, so that, from the day 12 the value of the limb circumference tended to be stable until the day 15. Considering only the times of treatment initiation (5, 10 and 15 days after surgery), it was found significant difference between days for the parameter evaluated independent of the group in T15 ($p = 0.01$) (Table 2), T60 ($p = 0.006$) (Table 3) and T90 ($p <0.001$) (Table 4). The multiple comparison tests revealed that the difference in T15 ($p = 0.01$) occurred only between the beginning of treatment and 15 days postoperatively. At the beginning of the treatment, the value of the circumference of the operated joint was greater, independent of the study group. In T60, the multiple comparison tests revealed that differences occurred only between the day 15 after surgery and the times of treatment initiation ($p = 0.01$) and 5 days postoperatively ($p = 0.04$) , showing that on day 15 the value of the circumference of the operated limb was on average smaller than in the other two times, regardless of the group. However, in T90, the multiple comparison tests suggested significant differences between the day 15 after surgery and the times of treatment initiation ($p <0.001$), 5 ($p <0.01$) and day 10 post surgery ($p = 0.03$), so that at day 15, the value of the circumference of the operated joint was on average lower than the other three times, independently of the group. The tests also revealed a significant difference ($p = 0.02$) between the times of treatment initiation and day 10 postoperatively, with the value of the circumference of the operated joint, on average, higher at treatment initiation, regardless of group. Unlike other periods of treatment, at T30 there was no significant difference ($p> 0.05$) between days, as well as between the groups for the parameter evaluated (Table 5).

In T15, pain and lameness were observed from the first day after surgery, and the lameness was initially considered grade 2 in three animals of the TG and one animal of CG. In the other animals, the lameness was classified grade 1. At the end of treatment, only two animals still showed lameness grade 1, one from TG and the other from CG, so that the remaining animals showed no more lameness (grade 0). (Tables 2 and 3)

However, the pain was classified as grade 3 on the first postoperative day and continued this level for all animals of T15 until the end of the observation period, except for an animal of TG that had sensitivity grade 1. Considering the times of treatment initiation, 5, 10 and 15 days postoperatively, there was no significant difference between the groups of T15 for the variables lameness and pain, as well as any of the other treatment periods. Comparing the times of preoperative and final clinical evaluation, the circumference of the left limb (non-operated) of both groups of T15 showed no variation,

unlike what happened with the contralateral limb (Table 6). However, the variation CG that occurred in the operated limbs of TG and CG were statistically identical (p = 0.06). (Tables 4, 5 and 6)

In T30, except for one animal of CG that did not showed lameness (grade 0), all the other dogs showed lameness grade 1 and pain grade 3 from the first day after surgery. The development of lameness was similar for both groups, so that two animals of TG and one animal of remained in lameness grade 3 at the end of treatment, and the remaining animals were classified as lameness grade 0. Pain remained grade 3 in five animals of TG, while the other animal in this group showed no signs of pain (grade 0) at the end of treatment. In CG, the manifestation of pain after the treatment was rated grade 3 in two animals, grade 1 in two others and grade 0 in the other group members. As in T15, although there was change in the circumference of the limb operated in animals of T30 between the times of preoperative and final clinical assessment (Table 7), the Mann-Whitney test showed that the variation was not significant between TG and CG. As for the left limb (non-operated), it was found that its circumference at the height of the femoral-tibio-patellar joint had no variation during the period of treatment in both groups.

In T60, the first day after surgery, three animals of the TG showed lameness grade 2, while for the others this

Table 2. Mean values of circumference of the limbs (cm) at the height of the right femoro-tibio-patellar joint obtained from the groups of T15, as a function of the time of treatment initiation, day 5, 10 and 15 postoperative period

Group		Days			
		Beginning of treatment	**Day 5**	**Day 10**	**Day 15**
TG	Mean	22.8	22.4	21.9	21.8
	Median	23.0	22.5	22	22.0
	Standard deviation	2.4	2.6	2.4	2.5
	Minimum	19.5	18.5	18	18.0
	Maximum	26.0	25.5	24.5	24.5
CG	Mean	20.8	20.4	20.3	20.0
	Median	20.2	20.0	19.7	19.5
	Standard deviation	2.3	2.4	1.7	1.7
	Minimum	18.5	18.0	18.0	18.0
	Maximum	24.0	24.0	23.0	22.5
p value	Effect of interaction		0.85		
	Effect of day		0.01*		
	Effect of group		0.26		

* Significant difference (p<0.05), by the ANOVA test with repeated measures.

Table 3. Mean values of circumference of the limbs (cm) at the height of the right femoro-tibio-patellar joint obtained from the groups of T60, as a function of the time of treatment initiation, day 5, 10 and 15 postoperative period

Group		Days			
		Beginning of treatment	**Day 5**	**Day 10**	**Day 15**
TG	Mean	21.0	21.3	21.2	20.5
	Median	20.8	21.3	21.0	20
	Standard deviation	1.82	1.08	0.98	1.22
	Minimum	19	20	20	19.5
	Maximum	24	23	22.5	22.5
CG	Mean	22.7	21.9	21.2	20.7
	Median	22.75	21.75	21.25	21
	Standard deviation	1.42	0.97	0.82	1.4
	Minimum	21	21	20	18
	Maximum	24.4	23.5	22	22
p value	Effect of interaction		0.84		
	Effect of day		0.006*		
	Effect of group		0.51		

* Significant difference (p<0.05), by the ANOVA test with repeated measures.

parameter was rated grade 1. At the end of treatment, none of the 12 animals of the T60 showed lameness (grade 0) on the clinical examination. The pain sensitivity was rated grade 3 on the first postoperative day in all animals. At the end of treatment, two animals of TG remained with pain sensitivity grade 3, while the other four showed no more sensitivity to pain (grade 0). In the CG, however, one animal remained with pain grade 3 and two with grade 1, and the other three showed no more pain (grade 0) at the end of treatment. As for the circumference of the left limb (non-operated) of dogs of T60, there was variation in both groups, considering the times of pre-operative and final clinical assessment (Table 8), unlike what occurred in T15 and T30. The ANOVA test with repeated measures confirmed the variation of circumference in both limbs ($p = 0.001$), both in TG and in CG, so that the operated limb showed increase in its measurement from the beginning to the end of the treatment while the non-operated limb showed reduction in this measurement (Table 7).

In T90, on the first day after surgery, three animals of TG and one of CG showed lameness grade 2; two animals of TG and five of CG were classified as grade 1, whereas the remaining (one animal from each group) were rated grade 0. At the end of the treatment, none of the 12 animals of T90 showed lameness (grade 0) on the clinical examination. The pain sensitivity was classified as grade

Table 4. Mean values of circumference of the limbs (cm) at the height of the right femoro-tibio-patellar joint obtained from the groups of T90, as a function of the time of treatment initiation, day 5, 10 and 15 postoperative period

Group		Days			
		Beginning of treatment	**Day 5**	**Day 10**	**Day 15**
TG	Mean	23.33	22.83	22.67	21.75
	Median	23.25	22.5	22.5	21.75
	Standard deviation	1.08	1.47	1.86	1.67
	Minimum	22	21	21	20
	Maximum	25	25	25	24
CG	Mean	22.42	21.92	21.17	20.25
	Median	22.5	21.75	21.75	20.75
	Standard deviation	1.11	1.66	1.57	1.44
	Minimum	21	19.5	19	18.5
	Maximum	24	24	23	22
p value	Effect of interaction	0.6			
	Effect of day	<0.001*			
	Effect of group	0.34			

* Significant difference ($p<0.05$), by the ANOVA test with repeated measures.

Table 5. Mean values of circumference of the limbs (cm) at the height of the right femoro-tibio-patellar joint obtained from the groups of T30, as a function of the time of treatment initiation, day 5, 10 and 15 postoperative period

Group		Days			
		Beginning of treatment	**Day 5**	**Day 10**	**Day 15**
TG	Mean	21.6	21.4	21.4	21.8
	Median	21.8	21.5	21.3	21.0
	Standard deviation	2.1	2.1	2.6	3.0
	Minimum	18	18	17.5	17.5
	Maximum	24.4	24.5	25.5	25.5
CG	Mean	21.7	21.3	19.3	19.6
	Median	21.5	21.5	19.0	20.0
	Standard deviation	2.7	3.0	2.4	2.1
	Minimum	18.5	17.5	16	16.5
	Maximum	26	26	22	22
p value	Effect of interaction	0.6			
	Effect of day	<0.001*			
	Effect of group	0.34			

* Significant difference ($p<0.05$), by the ANOVA test with repeated measures.

3 on the first day postoperatively in all animals of the T90, except for one animal of CG that was rated grade 1. At the end of the treatment, two animals of TG remained with pain sensitivity; one was classified as grade 3 and the other as grade 2. In the CG, however, one dog remained with pain grade 2 and the other did not express pain (grade 0) at the end of treatment. Similar to T60, a reduction in the circumference of the left limb (non-operated) and an increase in the circumference of the dog operated, in both groups of T90, considering the times of preoperative and final evaluation (Table 9). But the variation was not confirmed by the ANOVA with repeated measures, with no significant difference between the measurements of operated and non-operated dogs, regardless of group.

Although Henrotin *et al.* (2005) claim that the use of chondroitin sulfate and glucosamine as chondroprotectors promotes improvement of symptoms such as lameness and pain, in this study there was no significant difference between the treated and control groups regarding the degree of lameness and pain, which was also observed by Biasi *et al.* (2005) in the treatment with 240 mg of chondroitin sulfate, subcutaneously, in dogs with unstable knees, and by Clegg *et al.* (2006), when treated osteoarthritis in humans using the same substances.

Improvement in gait was reported by Canapp *et al.* (1999) after 12 days of oral treatment with chondroitin sulphate and glucosamine of chemically induced synovitis in the radiocarpal joint of dogs. Souza (1999) performed cartilage abrasion of the femoral groove in dogs and also reported improvement in limb function after 15 days of treatment with glycosaminoglycan precursors orally administered (Tables 8 and 9).

The findings of this work, as well as those of de Biasi *et al*. (2005) and Clegg *et al*. (2006), contrast with other reports available in the literature attesting improvement in limb function associated with the use of chondroitin

Table 6. Mean values of the circumference (cm) of the right (operated) and left (non-operated) limbs, at the height of the femoro-tibio-patellar joints, obtained from the T15 groups in the preoperative period and in the final clinical evaluation

Group		Day			
		Before surgery		Last evaluation	
		Limb operated	Limb non-operated	Limb operated	Limb non-operated
TG	Mean	20	20	21.83	20
	Median	19.7	19.7	22	19.7
	Standard deviation	2.5	2.5	2.5	2.5
	Minimum	17	17	18	17
	Maximum	23.5	23.5	24.5	23.5
CG	Mean	18.1	18.4	20	18.4
	Median	17.7	18.5	19.5	18.5
	Standard deviation	1.9	1.7	1.7	1.7
	Minimum	16	16	18	16
	Maximum	20.5	20.5	22.5	20.5

Table 7. Mean values of the circumference (cm) of the right (operated) and left (non-operated) limbs, at the height of the femoro-tibio-patellar joints, obtained from the T30 groups in the preoperative period and in the final clinical evaluation

Group		Day			
		Before surgery		Last evaluation	
		Limb operated	Limb non-operated	Limb operated	Limb non-operated
TG	Mean	19.5	19.5	21.17	19.5
	Median	19.5	19.5	21	19.5
	Standard deviation	2.26	2.26	2.27	2.26
	Minimum	16	16	17.5	16
	Maximum	23	23	24	23
CG	Mean	19	19	20.33	19
	Median	18.25	18.25	20	18.25
	Standard deviation	3.05	3.05	3.46	3.05
	Minimum	16	16	16.5	16
	Maximum	24	24	26	24

sulphate and glucosamine. Thus, the assumption of Henrotin *et al.* (2005) is confirmed: that the positive results associated with the administration of substances are obtained from non-standard private pharmaceutical preparations, where there are no guarantees about their composition, pharmacokinetics and pharmacodynamics, and therefore the results cannot be extrapolated to any formulation of chondroprotector. Furthermore, the interpretation of pain is considered subjective, which generates more controversy regarding the outcomes of treatments with glycosaminoglycans (Sawitzke *et al*., 2010).

These results clearly shows that changes in the circumference of the joint of the operated limb was expressed similarly in all four periods of treatment, and when significant differences were detected, they occurred only between times, regardless of group. In all animals, the increase in measurement from the first day after surgery was associated with the swelling caused by handling of the tissue during surgery. The following observations have revealed a gradual reduction in the value of the circumference until day 15 after surgery, due to the evolution of the repair process, when, according to Woodard (2000), there is organization of the initial hematoma and formation of granulation tissue. From day 15, small reductions in the amount of the circumference of the joint were observed. However, the mean of this variable at the end of the treatment indicates that the circumference of the limb, in most cases, did not decrease by the value observed at the preoperative time. This may be associated with the healing process and regeneration of soft tissues, primarily as a result of surgical manipulation of the joint capsule, which is consistent with the reports of Johnson *et al.* (1997) and Serrato *et al.* (2007).

Dogs of T60 and T90 showed decreased circumference of the non-operated joint between the beginning and end

Table 8. Mean values of the circumference (cm) of the right (operated) and left (non-operated) limbs, at the height of the femoro-tibio-patellar joints, obtained from the T60 groups in the preoperative period and in the final clinical evaluation

Group		Day			
		Before surgery		Last evaluation	
		Limb operated	Limb non-operated	Limb operated	Limb non-operated
TG	Mean	18.58	18.58	19.17	18.42
	Median	18.25	18.25	19.5	18.75
	Standard deviation	1.77	1.77	1.66	1.88
	Minimum	17	17	17	16
	Maximum	21.5	21.5	21.5	21
CG	Mean	19.42	19.42	19.92	19
	Median	19.75	19.75	20	19
	Standard deviation	1.07	1.07	0.66	0.55
	Minimum	17.5	17.5	19	18
	Maximum	20.5	20.5	21	19.5

Table 9. Mean values of the circumference (cm) of the right (operated) and left (non-operated) limbs, at the height of the femoro-tibio-patellar joints, obtained from the T90 groups in the preoperative period and in the final clinical evaluation

Group		Day			
		Before surgery		Last evaluation	
		Limb operated	Limb non-operated	Limb operated	Limb non-operated
TG	Mean	19.83	19.83	21.25	20.75
	Median	20	20	21.75	20.5
	Standard deviation	1.47	1.47	1.75	1.6
	Minimum	17	17	18.5	18.5
	Maximum	21	21	23	23
CG	Mean	19.83	18.83	19.58	18.83
	Median	19.75	19.75	19.25	19
	Standard deviation	1.51	3.56	1.69	2.14
	Minimum	18	12	18	16
	Maximum	22	22	22	21

of treatment, however, when a significant difference occurred, it was also independent of the group. This finding may be linked to the period of confinement to which the animals of T60 and T90 were subjected for data collection, with restriction of physical activity and the consequent reduction of the muscle tone. Similar results were reported by Hoelzer et al. (2004) and Serrato *et al.* (2007).

The radiographic projection provided less overlapping of structures and the best visualization of the defect was the mediolateral, although the medial and lateral femoral condyles are not uniform and therefore do not properly overlap in this projection. It was also the projection of easier implementation, since the other ones needed hyperextension or flexion of the knee, causing discomfort to the animals and making it difficult to perform the technique. The preoperative radiographic examination revealed that all animals were adult and did not show any radiographic change in the knee joint (Figure 1A).

The radiographs of postoperative period immediately after the lesion induction, showed, in all animals, a circular area of reduced radiopacity (grade 1) in the lateral femoral condyle. This change is related to the curettage of the subchondral bone with subsequent replacement of radiopaque tissue (bone) by radiolucent tissue (clot). A circular area of decreased radiopacity in the region of the lateral femoral condyle seen on the radiographs of the imediate postoperative period continued visible at the end of the treatment (Figures 1B, 1C, 1D, 1E and 1F), regardless of group.

No radiographic changes compatible with DJD were found in the dogs, contrary to the observations of Pearson (1971), Heffron & Campbell (1979), Vasseur & Berry (1992) and Biasi *et al*. (2005), who reported the presence of osteophytes, entesiophytes and erosion of the subchondral bone in dogs with DJD, which were more pronounced in animals that were not treated with chondroitin sulfate (Biasi *et al*., 2005). The discrepancy between the data is explained by the fact that, in this study, we chose to provoke an acute injury, characterized by the removal of a fragment of articular cartilage, whereas the above mentioned authors induced instability of the knee joint, which leads to a chronic injury that, in turn, progressively degenerate the cartilage and generates the radiographic signs consistent with DJD.

The results of this study therefore support the claim by Wandel et al. (2010), in which the effect of chondroprotective nutraceuticals on pain control, improvement of limb function and retardation of radiographic signs of DJD is minimal at best.

It is noteworthy that not always radiography enables the identification of characteristic DJD lesions (Dahlberg, 1994), especially in its early stages, featuring this method as inefficient for diagnosing early disease (Listrat *et al*., 1997; Miller & Clegg, 2011). Although radiography is a low cost and broadly accessible method for veterinarians, its sensitivity in the diagnosis of DJD is low compared with techniques such as arthroscopy (Arias *et al*., 2003), computed tomography and magnetic resonance imaging (Mrosek et al. 2006; Martel-Pelletier *et al*. 2008). These techniques allow visualization of intra-articular structures without superimposition of other tissues, and in the case of magnetic resonance imaging, is also possible to evaluate the cartilage and its possible cracks quantitatively (Martel-Pelletier *et al*. 2008; Crema *et al*., 2011).

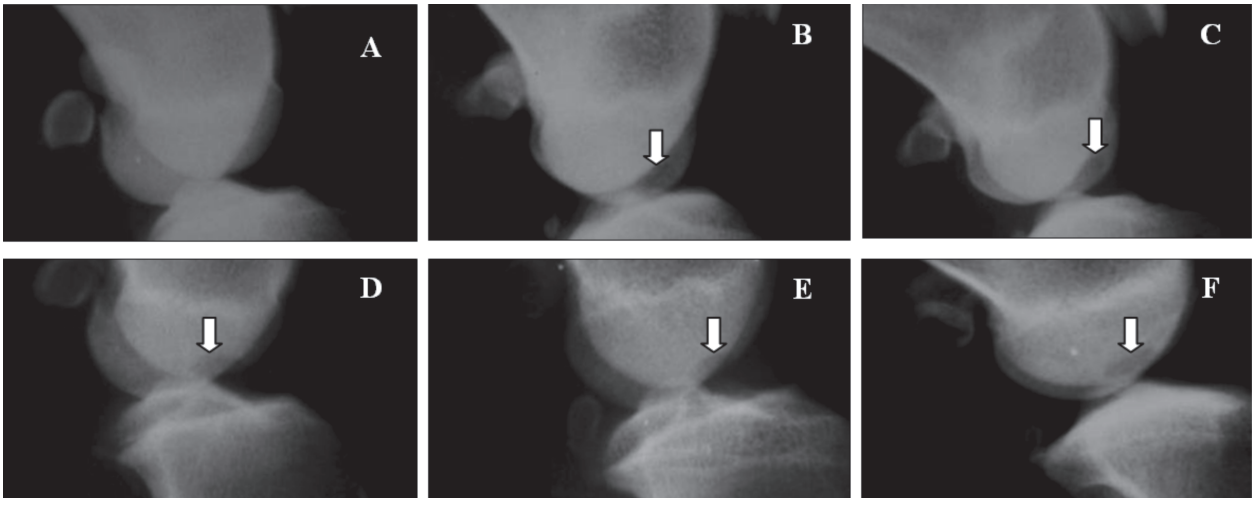

Figure 1. Mid-lateral radiographic projections of the knee of dogs subjected to surgery of induce osteochondral defect in the region of the lateral femoral condyle, to study the influence of chondroprotective nutraceutical in the repair of articular cartilage. A - Radiographic examination of the preoperative period showing radiograph normal pattern of the knee joint; B - Circular area of decreased radiopacity (arrow) immediately after the surgical procedure; C - Circular area of decreased radiopacity (arrow) at 15 days postoperatively, D - Circular area of decreased radiopacity (arrow) at 30 days postoperatively; E - Circular area of decreased radiopacity (arrow) at 60 days postoperatively, F - Circular area of decreased radiopacity (arrow) at 90 days postoperatively.

CONCLUSIONS

Based on the results of this study and in the form that it was conducted, it was concluded that the chondroprotector, at the rate, formulation and period of administration used, did not improve clinical signs and did not affect the repair process of osteochondral defects, as the treated and control groups showed similar radiographic findings at the end of the treatments.

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