BASIC RESEARCH

DIACERHEIN VERSUS GLUCOSAMINE IN A RAT MODEL OF OSTEOARTHRITIS

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PURPOSE: The purpose of this study was to compare the chondroprotective effect of diacerein and glucosamine regarding degenerative changes and articular stiffness in an experimental model of arthritis.
METHODS: Twenty rats underwent medial meniscectomy on the right knee. Ten animals were given diacerhein, and 10 were given glucosamine, from day 1 to the third month postoperatively, when all of them were killed. Histological and functional analysis of the knees were performed (measurement of maximum extension).
RESULTS: All operated knees showed more limited extension values and more degenerative changes as compared to nonoperated contralateral sides. A comparison of the two drugs showed that the degree of articular stiffness was significantly lower with diacerein, although degenerative changes were similar.
CONCLUSIONS: 1) Prophylactic use of diacerein leads to lower degree of articular stiffness when compared to glucosamine; 2) The prophylactic chondroprotective effects of diacerein and glucosamine are histologically similar.

KEYWORDS: Rats. Knee. Osteoarthritis. Anthraquinone. Meniscectomy. Glucosamine.

INTRODUCTION

Osteoarthritis (OA) is the most prevalent articular disease in the elderly.[1] The process is characterized by changes in the structure and function of the articulation, mainly due to a degenerative process that takes place in the articular cartilage.[2]

Destruction of articular cartilage results from a failure of chondrocytes to maintain the balance between synthesis and degradation of the extracellular cartilage matrix. Proinflammatory cytokines, such as interleukin-1 (IL-1) produced by macrophages, monocytes, synovial cells, and chondrocytes play an important role in the development of the disease.[3]

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Currently, nonhormonal anti-inflammatory agents (such as non steroidal anti-inflammatory drugs – NSAIDs), which produce adverse reactions but do not change the disease's natural history,[2,4-6] are used to relieve pain and inflammation.

Management of OA has been changed by the current knowledge of the physiology and pharmacology of the articular cartilage, as well as by the use of specific new drugs, such as glucosamine,[7] diacerein,[8] and unsaponifiables.[9]

Diacerein is an anthraquinone derivative with an inhibitory effect on production of cytokines by the synovial membrane and chondrocytes, as well as on the bioactivity level of IL-1 receptors.[5]

The aminomonosaccharide glucosamine is a major component of the glucosaminoglycans in articular cartilage.[7] Its effects include stimulation of physiologic proteoglycan synthesis and decrease in the activity of catabolic enzymes such as metalloproteases.[2]

A number of studies[1-5,7,8,10-18] have shown that the use of

diacerhein and of glucosamine is beneficial, because both drugs reduce symptoms and change the articular structure in OA.

OBJECTIVE

The purpose of this study was to functionally and histologically compare the effect of diacerein to that of glucosamine in an experimental rat model of OA.[10]

MATERIALS AND METHODS

The working material in this study consisted of 20 adult male Wistar rats. The minimum number of animals required to test the action of a potent drug is 4.[10] We used 10 animals in each group to improve the safety margin.

The minimum age for inclusion was 8 weeks, and the minimum weight was 180 grams. Rats older than 16 weeks and weighing more than 390 grams were excluded.

All rats were anesthetized with halothane and underwent total medial meniscectomy of the right knee as described by Rezende.[10]

The surgical technique consisted of the following steps: 1) creation of a medial longitudinal access pathway, 2) divulsion of the musculature of the medial knee aspect, 3) sectioning of the medial collateral ligament, 4) total medial meniscectomy, 5) suture of medial collateral ligament, and 6) suture of the musculature covering the medial collateral ligament. Surgery was performed using a microscope.

The rats were divided into 2 groups as follows: Group D, consisting of 10 rats that were given a 50-mg/kg/day oral dose of diacerein from the first day to the third month postoperative (PO), and Group G, consisting of 10 rats that were given a 240-mg/kg/day oral dose of glucosamine during the same period as Group D.

The two drugs were provided by supplying laboratories.

All rats were subjected to the same maintenance conditions and were stimulated to walk freely.

In the third postoperative month, all animals were subjected to euthanasia by intraperitoneal injection of ketamine (10 mg/kg) plus twice the anesthetic dose of xylazine (6.4 mg/kg) to ensure painless death due to respiratory arrest.

Both knees of all animals were dissected from the coxofemoral region to the ankle region, leaving the articular capsule intact. After the dissection, the maximum extension angle of each knee was measured (Figure 1), with 0 degrees corresponding to the maximum possible extension. In order to minimize any possible bias, all operations and measurements of the extension level were performed by the same surgeon.

The specimens were fixed in 10% formalin and submitted to histological study. All knees were kept in this solution for 1 day and then demineralized in 7.5% nitric acid for 2 to 3 days. After embedding the tissue in paraffin blocks, tibial sections were prepared and stained with hematoxylin-eosin and Alcian-blue. The articular cartilage injuries found in the rats' knees were evaluated and recorded using the Mankin score (Table 1) referred to by Armstrong.[4] In this system, the higher the score, the higher the level of OA. The entire histological evaluation was performed by the same pathologist. The histopathologist was blindes to group distribution when this analysis was made.

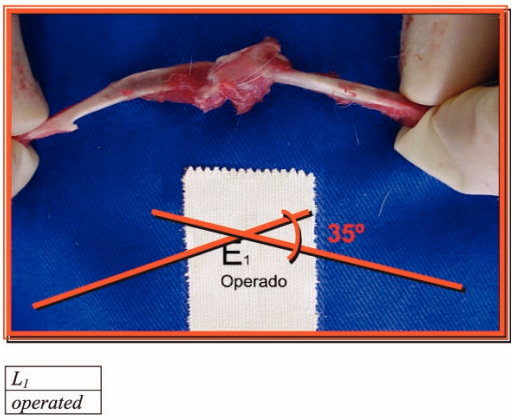


Figure 1 - Measurement of maximum extension angle

Table 1 - The Mankin score

Variable	Score
Surface	0 = normal
	1 = irregular
	2 = fibrillation / vacuoles
	3 = blisters and erosion
Hypocellularity	0 = normal
	1 = small decrease in chondrocytes
	2 = large decrease in chondrocytes
	3 = no cells
Clones	0 = normal
	1 = occasional duos
	2 = duos or trios
	3 = multiple nested cells
Alcianophilia	0 = normal
	1 = small decrease in color
	2 = large decrease in color
	3 = no color

A statistical analysis provided the mean values, standard deviation (SD), standard error of the mean (SEM), maximum values (max), minimum values (min), and number of cases (n).

We used the Wilcoxon test for paired samples and the Mann-Whitney U-test for nonpaired samples. The level of significance was set at 5% (a = 0.05).

RESULTS

The functional analysis of each knee was performed by analyzing the maximum extension angle (which reflects the degree of articular stiffness according to the experimental model, or the fixed flexion-extension of the knee), as shown in Table 2. We found that the extension angle of nonoperated knees for both groups was similar (25.6 degrees for the group receiving diacerein and 32.2 degrees for the group receiving glucosamine, $P = 0.18$). The mean extension angle of operated knees of the group receiving diacerein (38.5 degrees) was statistically different from operated knees of the group receiving glucosamine (54.1 degrees, $P = 0.009$) and different from their nonoperated contralateral sides (25.6 degrees, $P = 0.002$). Operated knees of rats receiving glucosamine were also statistically different from their nonoperated controls ($P = 0.003$).

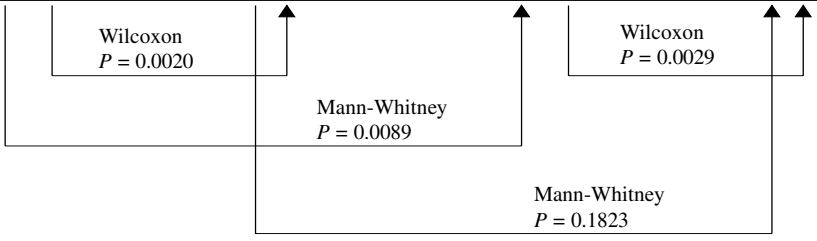
The histological analysis using the Mankin score showed mild levels of degenerative changes in both operated and nonoperated knees of both groups. The operated knees showed degenerative changes that were consistently higher than their respective contralateral controls ($P = 0.0195$ for both groups) (Figures 2 and 3). The histological scores of nonoperated sides of the groups receiving diacerein (4.3) and glucosamine (3.8) were not significantly different ($P = 0.2$). The levels of degenerative changes in the meniscectomized sides for the groups receiving diacerein (5.4) and glucosamine (6.6) were also similar ($P = 0.28$), as shown in Table 3 and Figure 4.

DISCUSSION

In clinical studies and trials, the administration of diacerein and glucosamine has produced a beneficial

Table 2 - Evaluation of knee extension level, with 0 degrees corresponding to maximum extension

	EXTENSION LEVEL DIACEREIN - GROUP D		GLUCOSAMINE - GROUP G	
	OPERATED	NON-OPERATED	OPERATED	NON-OPERATED
MEAN	38.5	25.6	54.1	32.2
MAX	30	15	20	10
MIN	50	40	70	65
SD	7.84	7.68	15.40	14.25
SEM	2.48	2.56	4.76	4.50
n	10	10	10	10



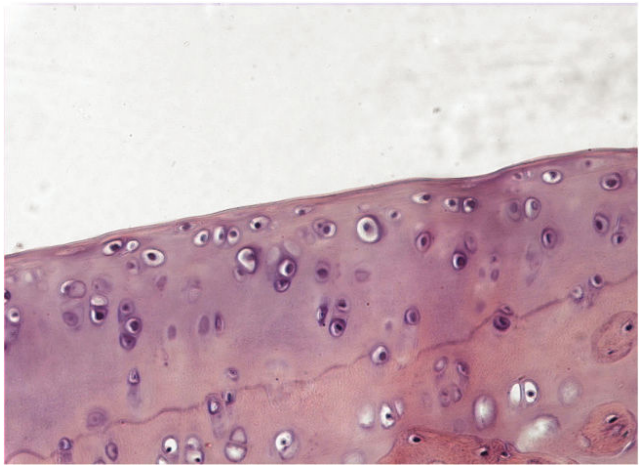


Figure 2 - Diacerin group, case 1, Nonoperated knee (left). Mankin Score = 2

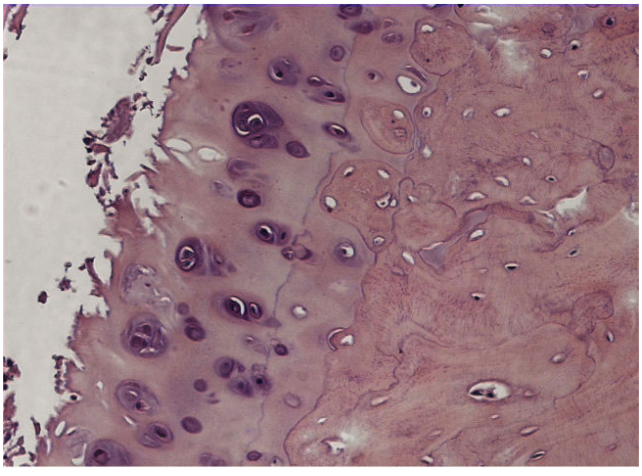


Figure 3 - Glucosamine group, case 5, operated knee (right). mankin score = 9

Table 3 - Evaluation by the Mankin score

	THE MANKIN SCORE			
	DIACEREIN – GROUP D		GLUCOSAMINE - GROUP G	
	OPERATED	NON-OPERATED	OPERATED	NON-OPERATED
MEAN	5.4	4.3	6.6	3.8
MAX	7	6	10	10
MIN	3	2	1	0
SD	1.35	1.06	2.91	3.16
SEM	0.42	0.33	0.92	0.10
n	10	10	10	10

Wilcoxon
P = 0.0195

Wilcoxon
P = 0.0195

Mann-Whitney
P =.2799

Mann-Whitney
P = 0.2176

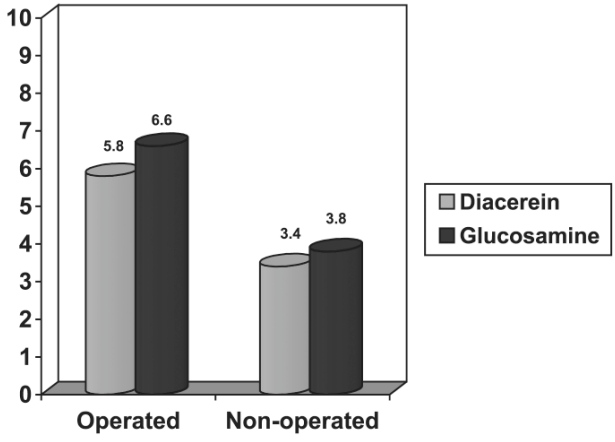


Figure 4 - Level of histological changes of operated knees and nonoperated knees receiving diacerein and glucosamine (Mankin score)

change in the structure of osteoarthritis, demonstrating a chondroprotective efficacy that delays the progression of the disease.[1-7,9-17]

Currently used NSAIDs act on symptoms only, have significant side effects,[2] mainly concerning the GI tract, and there is evidence that these drugs induce accelerated progression of knee and hip arthritis.[6] (it is not known whether this occurs due to a direct harmful effect on the cartilage or to increased mechanical overload after pain relief). Since OA is a chronic disease, the use of NSAIDs is even more limited. The drugs evaluated in the present study, on the other hand, apart from acting on the symptoms, interfere with the evolution of the disease itself, as mentioned; because they produce minimal side effects,[1,2,10] they can be administered for long periods. Both medications employed in the present study have also been used in multicenter, randomized, double-blind trials that employed each of these drugs for long periods (up to 3 years), showing the delay of arthritis progression[1,13] with minimal side effects.

The fact that we were not able to find reports comparing the use of diacerein and glucosamine led us to perform the present study.

Regarding the prophylactic administration of a drug, we were concerned with individuals (or animals, in the present case) that had not yet presented arthritis. The disease would appear due to ageing and stimulus (in this case, total medial meniscectomy), and the aim here was to prevent such degenerative changes by administering medications.

In this trial, diacerein was administered at a dose of 50 mg/kg/day, while glucosamine was administered at a dose of 240 mg/kg/day; the doses are higher than those used in humans because metabolic rates are higher in rats.

We chose the experimental model of Rezende[10] because it shows OA signs as early as 3 months postoperatively, mainly on the tibial surface, which was the structure selected for study in this trial. In addition to arthritis, this experimental model leads to limited range of motion of the knee, mainly concerning extension, because the access pathway used in meniscectomy is relatively large for these animals and because tibial collateral ligament sectioning is required, followed by suture, causing stiffness due to fibrosis. We included extension in our analysis, considering 0 degree as maximum extension, ie, the lower the value, the better the knee function.

The best functional results were observed with diacerein, where the mean extension was 38.5°, while that of glucosamine was 54.1°, which was significantly different. This may be due to the interleukin-1-inhibiting power of diacerein,[3,5,14,15] which limits the fibrocicatricial process

during the perioperative period and leads to wider ranges of motion. In vitro studies have shown that glucosamine inhibits the effects of interleukin-1, although some authors state that this inhibiting dose is greater than the maximum serum dose of glucosamine (absorption limit).[19, 20,21] Perhaps, glucosamine at physiological dosages acts rather by stimulating the production of matrix components than by inhibiting the effects of interleukin-1. It still has to be determined whether or not, in the long run, this limitation of the range of motion in the rat group receiving glucosamine would lead to major degenerative changes. Perhaps the combination of glucosamine and chondroitin, already used in humans,[22,23] would provide better functional results, due to the increased possibility of inhibiting the effects of interleukin-1. Further studies are required to answer this question.

When we compared operated and nonoperated knees, we noticed different (significant) results both histologically and functionally, which show that both diacerein and glucosamine will not fully prevent degenerative changes and articular stiffness after total medial knee meniscectomy. However, the Mankin score in operated knees (5.4 in Group D and 6.6 in Group G) shows mild levels of osteoarthritis in both groups (maximum score: 12). This arthritis score is lower than that found in previous studies where rats underwent meniscectomy, but were not given drugs, and were killed at 3 months postoperatively.[10,19] This finding is also valid for the nonoperated sides, with Mankin scores of 4.3 and 3.8, for Group D and G, respectively. Thus, the nonoperated knees of both groups showed degenerative changes that were lower than the expected value for the animal's age, which were a Mankin score of 5 in previous trials in unmedicated animals, either due to overload on the nonoperated knee or due to ageing.[10,19]

A histological comparison between the two drugs showed similar results in the operated knees, as mentioned above, with no significant differences. This trial had no control group because this had already been done in our Service.[19] Our study showed that diacerein can delay arthritis secondary to meniscectomy and to ageing or overload of the contralateral side.

The purpose of the present study was to explore whether or not glucosamine has a chondroprotective effect similar to that of diacerein. Although from the histological perspective, the effects appeared to be similar, from the functional perspective, this was not the case, based on the range of motion of the knee; treatment with diacerein was associated with a better range of motion of the operated knee than that associated with treatment with glucosamine.

CONCLUSIONS

We conclude that prophylactic use of diacerein leads to a lower degree of articular stiffness when compared to glucosamine under conditions of experimentally induced osteoarthritis; while the prophylactic chondroprotective effects of diacerein and glucosamine are histologically similar.

RESUMO

Rezende MU de, Gurgel HM de C, Vilaça Junior PR, Kuroba RK, Lopes ASS, Phillipi RZ, et al. Diacereína x glicosamina no modelo de artrose em ratos. CLINICS. 2006;61(5):461-6.

OBJETIVO: O trabalho foi realizado com o objetivo de comparar o efeito condroprotetor da diacereína em relação ao da glicosamina quanto às alterações degenerativas e à rigidez articular num modelo experimental de artrose.
MÉTODOS: Vinte ratos foram submetidos à meniscectomia medial do joelho direito. Dez animais receberam diacereína, e dez glicosamina, todos do primeiro dia ao terceiro mês pós-operatório, quando foram sacrificados. Foram realizadas análise histológica e funcional (medida da extensão máxima) dos joelhos.
RESULTADOS: Todos os joelhos operados apresentaram amplitude de extensão mais limitada e maiores alterações degenerativas, em relação ao lado contra-lateral não operado. Ao compararmos as duas drogas, a rigidez articular foi significantemente menor com a diacereína, e as alterações degenerativas foram semelhantes.
CONCLUSÕES: 1- O uso profilático da diacereína leva à menor rigidez articular em relação a glicosamina. 2- O efeito condroprotetor profilático da diacereína é semelhante, histologicamente, ao da glicosamina.

UNITERMOS: Ratos. Joelho. Osteoartrite. Antraquinona. Meniscectomia. Glicosamina.

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