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Effect of methylprednisolone use on the rotator cuff in rats: biomechanical and histological study☆

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ABSTRACT

Objective: To evaluate the influence of treatment with different doses of methylprednisolone on the mechanical resistance and possible histological alterations of the rotator cuff tendon in rats.
Methods: Male Wistar rats were divided randomly into four treatment groups: sham, vehicle or 0.6 mg/kg or 6.0 mg/kg of methylprednisolone. Changes to mechanical resistance (in N) and histological parameters (fibrillar appearance, presence of collagen, edema and vascular proliferation) of the rotator cuff tendon were evaluated. The analyses were conducted after administration of one treatment (24 h afterwards), two treatments (7 days afterward) or three treatments (14 days afterwards), into the subacromial space.
Results: Seven and fourteen days after the treatments were started, it was found that in a dose-dependent manner, methylprednisolone reduced the mechanical resistance of the rotator cuff tendon ($p < 0.05$ in relation to the vehicle group). Modifications to the histological parameters were observed on the 7th and 14th days after the first infiltration, especially regarding the presence of collagen and vascular proliferation, for the dose of 0.6 mg/kg of methylprednisolone, and also regarding the presence of collagen, edema and vascular proliferation for the dose of 6.0 mg/kg of corticoid.
Conclusion: The results obtained demonstrated a relationship between methylprednisolone use through infiltration into the subacromial space and reduction of the mechanical resistance of and histological modifications to the rotator cuff tendon in rats.

☆ Work developed at the University of the South of Santa Catarina (UNISUL), Tubarão, SC, Brazil.

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Efeito do uso da metilprednisolona no manguito rotador em ratos. Estudo biomecânico e histológico

Palavras-chave:
Corticoides
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RESUMO

Objetivo: Avaliar a influência do tratamento com diferentes doses de metilprednisolona sobre a resistência mecânica, bem como possíveis alterações histológicas do tendão do manguito rotador (MR) em ratos.

Métodos: Ratos Wistar machos foram divididos aleatoriamente em quatro grupos de tratamento como *sham*, veículo, 0,6 mg/kg ou 6 mg/kg de metilprednisolona. Alterações na resistência mecânica (em N) e em parâmetros histológicos (aparência fibrilar, presença de colágeno, edema e proliferação vascular) do tendão do manguito rotador (MR) foram avaliadas. As análises foram feitas após o tratamento com uma (24 horas após), duas (sete dias após) ou três (14 dias após) administrações no espaço subacromial.

Resultados: Após sete e 14 dias do início do tratamento a metilprednisolona reduziu, de maneira dependente de dose, a resistência mecânica do tendão do MR ($p < 0,05$ em relação ao grupo veículo). Também foram observadas modificação em parâmetros histológicos nos dias sete e 14 após a primeira infiltração, principalmente quanto à presença de colágeno e proliferação vascular para a dose de 0,6 MG/kg de metilprednisolona e presença de colágeno, edema e proliferação vascular para a dose de 6 mg/kg do corticoide.

Conclusão: Os resultados obtidos demonstram uma relação entre o uso de metilprednisolona por infiltração no espaço subacromial e a redução da resistência mecânica e modificações histológicas no tendão do MR de ratos.

Introduction

One of the most frequent causes of shoulder pain is degenerative traumatic injuries to the rotator cuff, particularly in the avascular area of the tendon,[1] which may affect individuals in any age group and may become worse with aging and through work or recreational activities.[2,3]

Approximately 54% of adults over the age of 60 years present partial tearing or complete rupture of the rotator cuff, in comparison with only 4% of those aged between 40 and 60 years.[4] Furthermore, around half of these patients do not present histories of trauma: this suggests that in these cases, the degeneration of the rotator cuff occurs gradually and results in incomplete tearing and possibly complete rupture. This event may lead to loss of shoulder function to varying degrees.[5,6] It can also occur at different levels: shoulder pain, weakness in making arm abduction movements and loss of mobility.

Under these conditions, the treatment options include conservative measures (rest and avoidance of the causal factor), pharmacological measures (non-steroidal anti-inflammatory drugs) and rehabilitation (physiotherapy), along with infiltration using corticoids in the subacromial space (ICSS).

ICSS is used in the initial treatment of pathological conditions of the rotator cuff and may produce good results with regard to pain relief and gains in range of motion, probably because of the anti-inflammatory and analgesic effects of these medications,[7,8] resulting from the anti-inflammatory effect of the corticoids. Among the corticoids used, the one seen most often is methylprednisolone, which is indicated as short-term adjuvant therapy for this condition, particularly for relieving acute crises or avoiding exacerbation, because of its solubility and short-term action, which causes less tissue damage.

However, the current clinical recommendations for local use of these medications for treating pathological conditions of the rotator cuff are that three infiltrations over the course of a year should be the maximum number, with a spacing of at least three months between them.[9] This precaution is taken because of the side effects reported in the literature, such as tendon atrophy, alterations to the healing process, structural alterations to the collagen fibers and metabolic alterations to collagen synthesis. These could lead to diminution of the biomechanical properties of the tendon and even cause complete tendon rupture.[10,11]

Few studies have examined the specific clinical effects of corticoids on the rotator cuff. The literature is basically composed of case reports and experimental studies on animals using the tendon of the sural triceps and the patellar tendon. The lack of consensus and the lack of specific studies evaluating the effect of ICSS on the tendon of the rotator cuff strengthen the idea that there is a need to comprehend and/or explain the mechanisms that are implicated in the effects of corticoids. Currently, corticoids are only used empirically to treat this condition.[12] The rotator cuff of rates is considered to be a very useful in vivo model for studying the diseases of the rotator cuff,[13] although these studies have not yet evaluated the degree of tendon resistance after clinical use of ICSS.

In this light, the present study had the primary aim of evaluating the effect of methylprednisolone on the mechanical resistance of the rotator cuff in rats, along with the secondary objective of evaluating the histological alterations relating to cellularity, vascularization and changes to collagen fiber patterns in the tendon of the rotator cuff in rats.

Materials and methods

Seventy-five adult male Wistar rats (*Rattus norvegicus*) of average weight 300 g were used. They were kept under standardized lighting conditions (dark–light cycles of 12 h each) and temperature conditions ($22 \pm 2\,^{\circ}C$), with free access to water and feed. The experiments were conducted in the laboratory of the surgical center of our university, between the hours of 08:00 and 17:00, after the animals had become acclimatized. The protocols were approved by our institution's Ethics Committee for Animal Use (no. 12.016.4.01.111).

The total sample size was subdivided as follows. A minimum group size of $n = 6$ was respected, which was considered to be the smallest number sufficient for the statistical analyses proposed, with regard to the species selected, as described in the literature on this field. The calculation for the number per group was made in accordance with the formula: $[(Z\alpha\colon 2 + Z\beta)2p^*(1 - p^*)2]\colon 2\delta$; where $p^* = (p0 + p1)/2$ and δ (delta) $= (p0 + p1)$. Four treatment groups were evaluated, which began with 18 animals each. From these groups, six animals were removed at the different observation times, i.e. respectively at the times of the first, second and third administrations of this treatment. In addition to these animals, three rats were used initially, in a pilot study to standardize the technique for substance administration and to recognize the animal's anatomy.

The animals were randomly divided between the groups, which received the following treatments: group 1 (sham), which did not undergo any intervention or receive any treatment over the course of the experimental period and served as a control group for the resistance analyses; group 2 (vehicle), which received treatment using the diluent of the corticoid tested, in accordance with the manufacturer's specification; and groups 3 and 4 (experimental), which received subacromial injections of two different doses of the corticoid tested, respectively.

The corticoid investigated was methylprednisolone at the doses of 0.6 mg/kg (group 3) or 6 mg/kg (group 4). These doses were selected based on a reference study[14] that used 0.6 mg/kg to observe deleterious effects from similar treatment on the peritenon of the sural triceps of rats. In addition, pharmacokinetic issues were taken into consideration and the dose of 6 mg/kg was chosen with the aim of investigating possible dose-effect correlations for the parameters observed in the present study, as well as possible adverse effects from its administration. The drug was administered in the subacromial space of the animals' right shoulder, at a volume of 0.1 ml/100 g of the animal's weight. Three administrations were performed. The time of application of the first dose was considered to be time zero, followed by injections at the times of 24 h and 7 days after the first administration.

For the procedure of subacromial injection, the animals received anesthesia induced by means of an intraperitoneal injection of ketamine hydrochloride plus xylazine hydrochloride (40–75 mg/kg of ketamine + 5–10 mg/kg of xylazine). The intra-articular infiltrations were made via an anterior route, with the animal in dorsal decubitus on a table that was tilted at 45° and the upper right limb rotated externally. Following this, topographically, the apex of the acromion and the humeral head were located. The needle was introduced between these two, along an oblique path going down from the horizontal, until it reached contact with the humeral head. It was then withdrawn slightly to enable administration of the volume.

Twenty-four hours after the first application (day 1), 7 days later (second application) or 14 days later (third application), counting from the start of the treatment with methylprednisolone, different groups of animals ($n = 6$) were sacrificed and dissected. The tendon of the rotator cuff was carefully removed in order to separate the tendon from the humerus. This was done by raising it from its humeral insertion and amputating it at its muscle–tendon junction, so as to form a unit that was then evaluated in accordance with the methodology described below.

Mechanical resistance test

This test was conducted in accordance with what was recorded by other authors,[15] using a bench with a manual clamp. Its upper extremity was connected to a linear dynamometer and its lower extremity was connected to a receptacle. The entire apparatus was suspended at approximately one meter from the ground. For this biomechanical test, the force ($P = m \times g$) exerted on the tendon was measured in newtons (N). The tendon unit was dissected by means of an incision above the protuberance of the scapular spine, with access superiorly to the supraspinatus muscle, together with its tendon. This was used for the resistance tests and histological evaluation. The distal portion of the tendon was deinserted from the greater tubercle of the humerus and tenotomy was performed close to the junction with the muscle. One extremity of each unit was gripped by the bench clamp and the other end was connected to the dynamometer. The receptacle at the lower extremity was filled with distilled water at a constant flow rate. In this model, as the water flowed in, the force exerted on the tendon increased, such that the corresponding force on the dynamometer produced by mass of the receptacle filled with water together with gravity could be read directly from the scale, in newtons. The magnitude of the force at the moment when each animal's tendon snapped was recorded individually.

Histological analysis

For the histological analysis, the separated tendon unit was embedded in paraffin and sections of thickness five microns (5 μ) were cut longitudinally and transversally. The slides were viewed at magnifications of 100×, 200× and 400× after they had been stained using a preparation of hematoxylin-eosin (HE), in order to observe the alterations to the collagen fiber bundles and to the nuclei and cytoplasm of the fibrocytes and fibroblasts. The parameters of cellularity, collagen thickness, occurrences of edema and vascular proliferation were evaluated and categorized as scores of 0, 1, 2 or 3, as described in a previous study.[15] These parameters or histological indicators were examined in order to demonstrate the behavior of the tendon after the interventions made in this study. All

Table 1 – Influence of treating the animals with methylprednisolone on the resistance of their rotator cuffs, as assessed using a mechanical resistance test.

Treatment	Force in N at the different observation times (number of days after the start of the treatment)		
	1	7	14
Vehicle	9.0 ± 1.0	9.3 ± 0.9	9.8 ± 0.7
Sham	12.4 ± 0.9	12.8 ± 1.9	16.2 ± 2.2
Methylprednisolone 0.6 mg/kg	7.9 ± 1.0	8.5 ± 1.6	9.6 ± 1.4[a]
Methylprednisolone 6 mg/kg	10.6 ± 3.7	6.4 ± 0.7[a]	11.6 ± 2.0[a]

Two-way ANOVA followed by Bonferroni for comparison between all the groups. $p < 0.05$.
There were no statistical differences at the different observation times between the groups treated with the different doses of methylprednisolone.
[a] In relation to the vehicle group.

the analyses were performed by a single pathologist, who was unaware of the respective treatment groups of the animals from which the samples were obtained.

The data are presented as the mean ± SEM (standard error of the mean), for values obtained from ordinal variables (tension in N, to evaluate the resistance of the tendon) or the median for categorical variables (degree of inflammation). The analysis was performed using GraphPad Instat 4.0®, using one or two-way ANOVA followed by the Kruskal–Wallis or Bonferroni test, respectively. p-Values < 0.05 were taken to be significant.

Results

The analysis on the resistance tests on the tendons obtained from the animals in the different groups revealed that the treatments on the animals using methylprednisolone at the two doses evaluated in this study did not alter the tension (in N) that was needed to break them when they were evaluated within 24 h after the first administration (Table 1).

However, as can be seen in Table 1, at the times of seven and 14 days after the first subacromial infiltration of corticoid, significant differences ($p < 0.05$) were observed in relation to the control group. On the seventh day, the mean values for the force applied to the tendons in the group treated with 6 mg/kg of the substance were lower than those obtained from animals in the control group (difference: −6.4 N; 95% CI: −13.3 to 0.4). A similar effect was observed on day 14 for the two doses of methylprednisolone evaluated (0.6 mg/kg: difference: −6.5 N; 95% CI: −14.2 to 0.4; and 6 mg/kg: difference: −6.0 N; 95% CI: −12.3 to 0.3).

Regarding the histological analysis comparing different parameters (as scores) in the treated groups in relation to the control group, the evaluation under the optical microscope revealed significant alterations ($p > 0.05$) for the parameters evaluated, at the different observation times after the treatment with methylprednisolone started, as presented in Table 2.

Discussion

This study demonstrated that there is a relationship between subacromial use of corticoids and reduction in mechanical resistance and in the histological parameters of the tendon of the rotator cuff. This strengthens the preliminary findings from similar studies.[15]

In relation to the mechanical resistance of the rotator cuff, alterations were observed starting from the third administration (after a period of 14 days), for the dose of 0.6 mg/kg of methylprednisolone. For the higher corticoid dose (6 mg/kg), significant alterations were already observed from the second administration, after a period of 7 days.

Conservative treatment using subacromial injections of corticoids for managing rotator cuff injuries is a common practice within the field of medicine, but its side effects mean that greater care is required when using this. Although several theories about the anti-inflammatory effect of this drug exist, the way in which the degeneration of the tendon tissue takes place has not been clearly expressed.

In the present study, it could be seen that 24 h after subacromial application of corticoid, there was no significant alteration ($p > 0.05$) to the resistance between the groups studied, independent of the dose used. Contrary results were observed in another study[15] that also evaluated the influence of methylprednisolone on the tendon of the sural triceps of rats. IN the latter study, it was noted that the force needed to break the tendon decreased within the first 24 h after first administration of the drug. This effect was maintained for at least the next two weeks. This divergence between the two studies was probably because in the present study, unlike the previous one, healthy tendons without any injury prior to the treatment were evaluated, given that fragmentation of the tissue might facilitate its rupture.

On the other hand, in the present study, from the time of the second administration, i.e. 7 days after the start of the treatment, a reduction in the resistance of the tendon of the rotator cuff in the animals treated with the higher dose of methylprednisolone (6 mg/kg) was noted in relation to the control group. A similar effect was recorded by other authors[14] in relation to the observation time of 7 days, although with a dose 10 times smaller (i.e. 0.6 mg/kg). Although the corticoid doses applied in the present study were different to those evaluated in the previous study, this dose–effect relationship presented greater deleterious effects on the tendon, as shown in the histological analysis performed in the present study (Table 2) and also in a previous study that used similar methodology for analyzing the Achilles tendon in rats.[14]

Table 2 – Influence of treating the animals with methylprednisolone, with regard to the resistance of the rotator cuff, evaluated using different histological parameters.

Parameter evaluated	Sham			Vehicle			Methylprednisolone 0.6 mg/kg			Methylprednisolone 6 mg/kg		
	1	7	14	1	7	14	1	7	14	1	7	14
FA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.2	0.0 ± 0.0	0.6 ± 0.2[a]	0.3 ± 0.3
PC	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.6 ± 0.2[a,b]	0.0 ± 0.0	0.8 ± 0.4[a,c]	0.5 ± 0.2
ED	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.2
VP	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.2[a]	0.2 ± 0.2	0.6 ± 0.2	0.8 ± 0.4[a]

FA, fibrillar appearance; PC, presence of collagen; ED, edema; VP, vascular proliferation. Two-way ANOVA followed by Bonferroni for comparison between all the groups. $p < 0.05$.

[a] In relation to the sham group.

[b] In relation to the vehicle group.

[c] In relation to the methylprednisolone group with 0.6 mg/kg.

According to the present findings, the adverse effects of corticoids seem to follow a dose–dependent relationship, given that although treating the animals with a dose of 0.6 mg/kg promoted significant alterations with regard to the parameters of collagen presence and vascular proliferation after the third administration (day 14) in relation to the sham group, histological alterations with the dose of 6 mg/kg had already started to be observed from the time of the second administration (day 7), regarding the fibrillar appearance and presence of collagen.

In relation to the possible mechanisms through which these corticoid effects might be occurring, the histological alterations seen in the tendons of the animals treated with methylprednisolone demonstrated possible degeneration of the tendon tissue caused by this substance. It has been suggested that after tissue damage, vascular proliferation may subsequently occur, with the aim of supplying oxygen and cellular nutrition, in an attempt to repair the damaged tissue.[16] This notion is in line with our histological findings, in which we observed increased vascular proliferation on the 14th day after the start of the treatment, with the two corticoid doses evaluated.

On the other hand, production of collagen by the extracellular matrix of the tendon is a source of resistance to tendon traction. Simply decreasing the quantity of these fibers may imply diminished tendon strength.[17] Likewise, methylprednisolone was shown to produce a lower quantity of collagen and lower resistance of the tendon of the rot, subsequent to partial injury induced by means of an incision.[18] The present study reinforces these findings, given that the presence of collagen was affected by both doses, on days 7 and 14, respectively through treatment with the doses of 6 mg/kg and 0.6 mg/kg.

Finally, in relation to cellularity, it is known that the tendon is a structure composed mainly of collagen fibers, with relatively low presence of cells, which are mainly fibroblasts.[17] Although these cells were not investigated in the present study, the findings relating to the presence of collagen indirectly suggest to us that their presence at the sites of corticoid administration increased, starting from the time of the second administration of the higher dose tested, given that these cells are responsible for production of these fibers. This idea is corroborated by a study on the tendons of the semitendinosus muscle of humans, from which it was observed that increased cellularity might suggest that the tendon was more susceptible to injury.[19]

Conclusion

The analysis on the results obtained from the present study made it possible to demonstrate a relationship between subacromial use of methylprednisolone and both diminished mechanical resistance and histological alterations in the tendon of the rotator cuff, at the doses and times evaluated in the present study.

Future studies may provide greater support regarding the mechanisms implicated in this corticoid effect, which may influence the treatment of musculoskeletal conditions during degenerative processes.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Moore KL. Anatomia orientada para a prática clínica. 5th ed. Rio de Janeiro: Guanabara Koogan; 2007.
2. Nové-Josserand L, Walch G, Adeleine P, Courpron P. Effect of age on the natural history of the shoulder: a clinical and radiological study in the elderly. Rev Chir Orthop Reparatrice Appar Mot. 2005;91(6):508–14.
3. White RH. Shoulder pain. West J Med. 1982;137(4):340–5.
4. Bartolozzi A, Andreychik D, Ahmad S. Determinants of outcome in the treatment of rotator cuff disease. Clin Orthop Relat Res. 1994;(308):90–7.
5. McLaughlin HL. Rupture of the rotator cuff. J Bone Joint Surg Am. 1962;44:979–83.
6. McMahon PJ, Debski RE, Thompson WO, Warner JJ, Fu FH, Woo SL. Shoulder muscle forces and tendon excursions during glenohumeral abduction in the scapular plane. J Shoulder Elbow Surg. 1995;4(3):199–208.
7. Adebajo AO, Nash P, Hazleman BL. A prospective double blind dummy placebo controlled study comparing triamcinolone hexacetonide injection with oral diclofenac 50 mg TDS in patients with rotator cuff tendinitis. J Rheumatol. 1990;17(9):1207–10.
8. Petri M, Dobrow R, Neiman R, Whiting-O'Keefe Q, Seaman WE. Randomized, double-blind, placebo-controlled study of the treatment of the painful shoulder. Arthritis Rheum. 1987;30(9):1040–5.
9. Lashgari CJ, Yamaguchi K. Natural history and nonsurgical treatment of rotator cuff disorders. In: Norris TR, editor. Orthopaedic knowledge update. Shoulder and elbow. 2nd ed. Rosemont: American Academy of Orthopaedic Surgeons; 2002. p. 155–62.
10. Hugate R, Pennypacker J, Saunders M, Juliano P. The effects of intratendinous and retrocalcaneal intrabursal injections of corticosteroid on the biomechanical properties of rabbit Achilles tendons. J Bone Joint Surg Am. 2004;86(4):794–801.
11. Wiggins ME, Fadale PD, Ehrlich MG, Walsh WR. Effects of local injection of corticosteroids on the healing of ligaments. A follow-up report. J Bone Joint Surg Am. 1995;77(11):1682–91.
12. Wei AS, Callaci JJ, Juknelis D, Marra G, Tonino P, Freedman KB, et al. The effect of corticosteroid on collagen expression in injured rotator cuff tendon. J Bone Joint Surg Am. 2006;88(6):1331–8.
13. Thomopoulos S, Soslowsky LJ, Flanagan CL, Tun S, Keefer CC, Mastaw J, et al. The effect of fibrin clot on healing rat supraspinatus tendon defects. J Shoulder Elbow Surg. 2002;11(3):239–47.
14. Tatari H, Kosay C, Baran O, Ozcan O, Ozer E. Deleterious effects of local corticosteroid injections on the Achilles tendon of rats. Arch Orthop Trauma Surg. 2001;121(6): 333–7.
15. Lech O, Severo AL, Silva LHP, Marcolan AM, Lütkemeyer E, Kim JH. Efeito do uso de corticoide em tendões previamente traumatizados: estudo experimental. Rev Bras Ortop. 1996;31(3):187–92.
16. Chan BP, Chan KM, Maffulli N, Webb S, Lee KK. Effect of basic fibroblast growth factor. An *in vitro* study of tendon healing. Clin Orthop Relat Res. 1997;(342):239–47.
17. Junqueira LCU, Carneiro J. Histologia básica. 11th ed. Rio de Janeiro: Guanabara Koogan; 2008. p. 103–23.
18. Mikolyzk DK, Wei AS, Tonino P, Marra G, Williams DA, Himes RD. Effect of corticosteroids on the biomechanical strength of rat rotator cuff tendon. J Bone Joint Surg. 2009;91(5):1172–80.
19. Kartus J, Movin T, Papadogiannakis N, Christensen LR, Lindahl S, Karlsson J. A radiographic and histologic evaluation of the patellar tendon after harvesting its central third. Am J Sports Med. 2000;28(2):218–26.