ISSN- 0102-9010

EFFICACY OF POLYSULPHATED GLYCOSAMINOGLYCAN IN THE INTRATENDINOUS TREATMENT OF EXPERIMENTAL EQUINE TENDINITIS

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ABSTRACT

The aim of this study was to assess the histopathological alterations in the superficial digital flexor tendon (SDFT) of horses with experimentally-induced tendinitis and treated with polysulphated glycosaminoglycan (PSGAG). Tendinitis of the SDFT was induced in two groups (I and II) of five Arabian horses each (both sexes, 2-6 years old) by the intratendineous injection of 1 ml of collagenase (2.5 mg/ml). Seven days after injury, the horses in group I received five intralesional injections of 1 ml of PSGAG (125 mg/ml), every four days, whereas the horses in group II received saline injections of the same volume and frequency. The macroscopic changes observed 150 days after injury included thickening of the paratenon, an increase in vascularity and adhesions to the palmar surface of the SDFT. Histopathological examination revealed an extensive area of fibroplasia and neovascularization, with poorly organized collagen fibers and hypercellular thickening of the endotenon. There were no significant differences in the regeneration of the tendon between collagenase-treated and control groups, thus indicating that the intralesional administration of PSGAG had no beneficial effect in the treatment of experimentally-induced tendinitis.

Key words: Collagenase, equine, polysulphated glycosaminoglycan, tendinitis

INTRODUCTION

Repetitive forces on tendon structures during athletic activities result in a predisposition to degenerative microlesions which culminates in partial or total rupture of the tendon. Tendinitis induced experimentally with collagenase of bacterial origin results in lesions very similar to those occurring naturally, including rapid fiber dissolution, cellular necrosis, vascular damage, hemorrhage and inflammation [14,21].

Tendons are elongated cylindrical structures composed of dense connective tissue. The collagen fibers have a characteristic cross-striation produced by the superposition of molecules of tropocollagen which forms collagen fibrils arranged in bundles or fascicles parallel to the longitudinal axis of the tendon. The fascicles are surrounded by loose connective tissue, the endotenon. The epitenon surrounds the entire tendon unit. In the metacarpal region, a sheath of dense connective tissue, the paratenon, surrounds the tendon. Collagen provides tensile strength to the tendon and is predominantly (95%) type I in normal mature tendons, with a small proportion of types III, IV and V. Between the bundles of collagen fibrils, there is a small quantity of ground substance and connective cells. The most active cells in tendons are fibroblasts which have a large, clear, oval nucleus. The quiescent cells known as fibrocytes, have a smaller nucleus that is elongated and darker, and are dispersed in a parallel manner among collagen fibrils. The fibroblasts synthesize collagen fibrils as well as glycoproteins and proteoglycans of the extracellular matrix [1,7].

Within the tendon, blood vessels of the epitenon form longitudinal canals which anastomose to create a complex vascular system [18]. During tendon repair, neovascularization is intense and gradual, reaching a maximum 60 days after the lesion [5]. Extrinsic repare predominates in most cases and results from stimulation of the peritenon to proliferate and to supply the cells and capillaries necessary for scar formation. This process is also responsible for the formation of restrictive adhesions of the tendon to adjacent tissues, thereby restricting the gliding function of the healed tendon [18].

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The use of anti-inflammatory drugs to treat tendinitis has been broadened recently with the introduction of polysulphated glycosaminoglycans (PSGAG). According to Henninger [6], PSGAG inhibit lysosomes and diminish the degree of inflammation. Smith [15] reported that PSGAG reduced collagenase activity and the activation of macrophages and improved the orientation of tendon collagen fibrils during repair. In addition, PSGAG inhibit the synthesis of prostaglandin E_2 [4], the activity of the complement cascade [12], the influx of leukocytes to the site of inflammation and the production of superoxide free radicals and interleukin-1. There is also a dose-dependent effect on the metabolism of fibroblasts and tenocytes, resulting in increased production of collagen, non-collagen proteins and sulphated glycosaminoglycans [17].

The aim of this investigation was to histologically evaluate the efficacy of PSGAG given intratendineously to treat tendinitis induced by treatment with collagenase.

MATERIAL AND METHODS

The study included 10 clinically normal Arabian horses of both sexes, 2-6 years old. The animals were randomly distributed in two groups of five animals each, with group I being the treated group and group II the control group. The SDFT of the left limb was lesioned by the intratendineous administration of 1 ml of collagenase type I solution (2.5 mg/ml) (Type I collagenase: C-0130, Sigma) in the central region of the tendon. The correct location of injection was determined ultrasonographically.

One week after induction of the lesion, the horses in group I received 125 mg of polysulphated glycosaminoglycan (Adequan, Luitpold) in 1 ml injected at two sites along the lesion. A total of five injections were given at four-day intervals. The horses in group II (controls) received injections of saline under the same conditions.

The limbs were wrapped with a compressive bandage and the horses were confined to stalls for 60 days and then remained in paddocks for up to 150 days, after which they were killed with a high dose of Thiopental followed by a rapid intravenous infusion of potassium chloride. The experimental protocol was approved by the Institutional Animal Care and Use Commitee at the FCAV/UNESP. The palmar metacarpal region of the left thoracic limb was carefully dissected to isolate the lesioned area and to determine the adhesions between the SDFT and the skin and/or deep digital flexor tendon (DDFT). The paratenon was examined for its thickness, vascularization, opacity and adhesion to the SDFT. Each parameter was scored as having a normal or moderately to severely altered appearance.

With the tendon cut transversally, the classic parameters of consistency, size and staining were examined. The consistency of the tissue was characterized as firm, soft or gelatinous. The area of the section was classified as being mildly (< 50% of section area), moderately (> 50% of section area) or severely increased relative to the area of a section of normal tendon. The appearance of the cross-sections was described based on their staining.

Samples were collected from tendon scar tissue of the left limb and from normal tendon of the contralateral limb for standard histological processing and the preparation of frozen sections. The tendon segments were fixed in n-hexane (Labsynth) at -14ºC and sectioned longitudinally and transversally in slices 5-7 µm thick using a cryostat. All of the samples were stained with hematoxylin-eosin (HE) and Masson's trichrome method for collagen and muscle [9].

Each section was analyzed qualitatively using the following parameters: a) vascularization, cellularity, parallelism and retraction of the collagen fibers, b) the presence of inflammatory and fibrocartilaginous cells, and c) characterization of the interfascicular connective tissue. The vascularization and cellularity were scored as low (< 20 vessels), intense (20-50 vessels) or very intense (> 50 vessels). The extent of parallelism and retraction of the collagen fibers was classified as present, partial or absent. Four fields were examined in each of three sections per animal.

The results were analyzed statistically by the non-parametric Wilcoxon test. A value of $p < 0.05$ indicated significance [16].

RESULTS

The lesioned area was easily identified by its hyperemic appearance following removal of the skin and subcutaneous tissue. No adhesions were seen between the SDFT and the skin and the DDFT. The paratenon was of normal thickness in four animals (two in each group), moderately thickened in five animals (three in group I, two in group II), and very thick in only one animal (group II). The paratenon was moderately vascularized in seven tendons (three in group I, four in group II) and very evident in three cases (all group II). The paratenon was also translucid in seven animals and slightly to totally opaque in three others. Adhesion of the paratenon to the SDFT was seen in six animals (three in each group), but was severe in only one of them (group II).

Cross-sections of the tendons showed a variable consistency from firm (one in each group) to gelatinous (two in each group) and an increased cross-sectional area (30% greater) relative to the normal tendon. The lesions were characterized by a predominantly more pallid and uniform area, with loss of the fascicular pattern, indicating areas of repair. Areas of hyperemia were also observed in all of the tendons, indicating hemorrhagic foci.

Regardless of the treatment, histopathological examination revealed intense to very intense vascularization, with a large number of residual, newly formed vessels in all of the tendon fragments. The ratio of

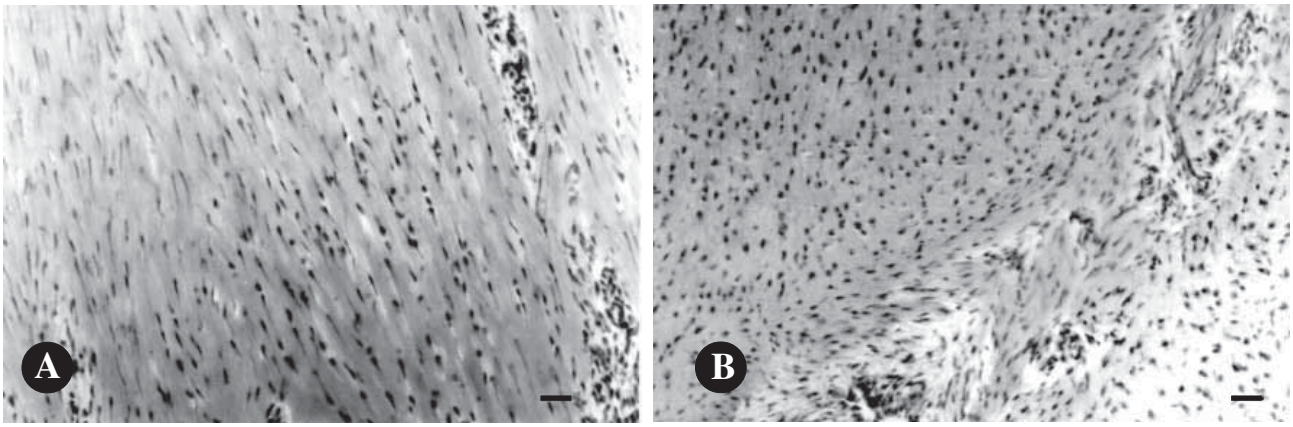

Figure 1. **A**) Longitudinal section of the TFDS from the left limb of a horse from group II showing very intense cellularity, fibroblasts with round or slightly elongated nuclei, partial parallelism of fibers and the absence of retraction. H&E staining. **B**) Cross-section of the SDFT from the left limb of a horse from group I, showing a thick, hypercellular endotenon. H&E staining. Bars = 100 μm.

cells to extracellular matrix increased, characterized by the presence of intense cellularity, and numerous fibroblasts with round to slightly elongated nuclei were organized in parallel among the collagen fibers.

The longitudinal and parallel arrangement of the collagen fibers was partial in all of the animals. Collagen fiber retraction was absent in five animals (three in group I, two in group II) and was partial in the remaining cases.

The number of inflammatory cells was unappreciable. When present, they were identified as macrophages and plasma cells in the proximity of blood vessels. Fibrocartilaginous metaplasia, characterized by the presence of isolated fibrocartilaginous cells, was occasionally observed at the lesion site. The endotenon was hypercellular, with cells proliferating in a disorderly manner among collagen bundles and appearing thicker than normal with evident and abundant vascularization. The most frequently observed microscopic changes in the lesioned tendons are shown in Figure 1.

DISCUSSION

As show here, the use of collagenase was effective in inducing tendinitis in the SDFT of horses. This finding agrees with other studies on tissue repair in this species [8,14,21] and with reports on the treatment of tendinitis [3,13].

Following removal of the skin and subcutaneous tissue, the tendon lesions in both groups were easily observed macroscopically, which corroborates the observations by Williams [21]. The lesioned tendons showed hyperemic areas with neovascularization.

The adhesions between the SDFT and surrounding structures were moderate. Treatment with PSGAG had no significant effect in restoring the gliding surface. These observations differ from those of Webbom [19] who noted occasional adhesions in this region in chronic tendinitis. This same author viewed the paratenon as a barrier limiting the inflammatory response in the central metacarpal region [20].

The thickness of the paratenon and the presence of adhesions on the palmar surface of the SDFT observed here have also been seen in subacute and chronic tendinitis [19,21].

Normal tendon appeared firm in cross-section, with a well-defined fascicular pattern. This pattern underwent changes in lesioned tendons, which were soft and gelatinous in six animals and had an increased cross-sectional area. Areas of repair were seen as pallid regions with a loss of the fascicular pattern and the presence of hemorrhagic foci characteristic of tissue with fibrovascular granulation. Similar alterations were described by others [19,21] in studies of naturally occurring tendinitis. Residual signs, mainly hemorrhage, can persist for long periods, with regions of chronicity [10]. The latter investigators observed hemorrhagic foci in tendon scars up to seven months after the lesion, indicating that damage had occurred in immature and mechanically fragile scar tissue, followed by new cycles of repair.

The freezing of tendon samples described by others [10,19] was more effective than paraffin embedding and improving the quality of the samples for histopathological examination by facilitating tissue sectioning.

Normal tendon tissue showed wavy collagen fibrils arranged in parallel bundles separated one from another by rows of elongated fibroblasts, with fine septa of connective tissue organized among the fas-

cicles. The scar tissue was characterized by hypercellularity and intense to very intense vascularization with fibroblasts containing round to slightly elongated nuclei partially organized among the collagen fibers. The endotenon was hypercellular, with abundant vascularization that was poorly organized and thicker than that of normal endotenon. The longitudinal and parallel arrangement of the fibers was partial in all of the animals, with retraction completely absent in five horses and partial in another five. These histological changes in the tendon scar 150 days after the induction of lesions were similar to findings reported by others [2,3,10,14,21] for the pathophysiology of clinical and enzymatically induced tendinitis. Silver *et al*. [14] reported hypercellular scar tissue with little subdivision into fascicles, but abnormal histological and biochemical characteristics 14 months after enzymatically-induced lesions.

The small number of inflammatory cells seen after 150 days of repair corroborated the results of Williams *et al.* [21]. Others [11,19] reported the occasional occurrence of fibrocartilaginous cells in the metacarpal region of normal tendons. According to these investigators, chondroid metaplasia was frequently observed in the tendons of older horses, in the muscle-tendon junction and in areas covered by synovial fluid. The influence of these cells on the pathogenesis of lesions of intrinsic origin is still controversial. These cells are supposedly present at sites that undergo greater traction forces [11].

In conclusion, the macroscopic and histopathological findings of this study indicate that the intralesional injection of PSGAG in this model of tendinitis had no beneficial effect on tissue repair and restoration.

ACKNOWLEDGMENTS

The authors thank Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) that supported this work (Proc. 99/02699-2).

REFERENCES

1. Banks WJ (1992) *Histologia Veterinária Aplicada*. 2nd ed. Manole: São Paulo.
2. Crevier-Denoix N, Collobert C, Pourcelot P, Denoix JM, Sanaa M, Geiger D, Bernard N, Ribot X, Bortolussi C, Bousseau B (1997) Mechanical properties of pathological equine superficial digital flexor tendons. *Equine Vet. J*., *Suppl.* **23**, 23-26.
3. Foland JW, Trotter GW, Powers BE, Wrigley RH, Smith FN (1992) Effect of sodium hyaluronate in collagenase-induced superficial digital flexor tendinitis. *Am. J. Vet. Res*. **53**, 2371-2376.
4. Frean SP, Lees P (2000) Effects of polysulfated glycosaminoglycan and hyaluronan on prostaglandin E_2 production by cultured equine synoviocytes. *Am. J. Vet. Res*. **61**, 499-505.
5. Hataka A, Moraes JRE, Paura D, Fávaro AF, Moraes FR, Perecin D (1999) Evolution of the vascular pattern in deep flexor tendon healing. An experimental study in dogs. *Braz. J. morphol. Sci*. **16**, 203-208.
6. Henninger R (1994) Treatment of superficial digital flexor tendinitis. *Vet. Clin. North Am. Equine Pract.* **10**, 409-424.
7. Junqueira LC, Carneiro J (1999). *Histologia Básica*. 9th ed. Guanabara Koogan: Rio de Janeiro.
8. Keg PR, Van den Belt AJM, Merkens HW, Barneveld A, Dik KJ (1992) The effect of regional nerve blocks on the lameness caused by collagenase induced tendinitis in the midmetacarpal region of the horse: a study using gait analysis, and ultrasonography to determine tendon healing. *J. Vet. Med.* **39**, 349-364.
9. Luna LG (1992) *Histopathologic Methods and Color Atlas of Special Stains and Tissue Artifacts*. Johnson Printers: Downers Grove.
10. Marr CM, McMillan I, Boyd JS, Wright NG, Murray M (1993) Ultrasonographic and histopathological findings in equine superficial digital flexor tendon injury. *Equine Vet. J.* **25**, 23-29.
11. Moraes JRE, Ferraro GC, Shimano AC, Camargo MHB, Moraes FR (2002) Propriedades mecânicas de três padrões de sutura no reparo de tendão do músculo flexor profundo do dedo em eqüinos. *Braz. J. Vet. Res. Anim. Sci.* **39**. (in press).
12. Rashmir-Raven AM, Coyne CP, Fenwick BW, Gaughan EM, Andrews GA, Debowes RM (1992) Inhibition of complement activity by polysulphated glycosaminoglycans. *Am J. Vet. Res*. **53**, 90-93.
13. Redding WR, Booth LC, Pool RR (1999) The effects of polysulphated glycosaminoglycan on the healing of collagenase induced tendinitis. *Vet. Comp. Orthop. Traumatol.* **12**, 48-55.
14. Silver IA, Brown PN, Goodship AE, Lanyon LE, McCullagh KG, Perry GC, Williams IF (1983) A clinical and experimental study of tendon injury, healing and treatment in the horse. *Equine Vet. J*., *Suppl*. **1**, 5-22.
15. Smith RKW (1992) A case of superficial digital flexor tendinitis: ultrasonographic examination and treatment with intralesional polysulphated glycosaminoglycans. *Equine Vet. Educ*. **4**, 280-285.
16. Stell RGD, Torrie JH (1960) *Principles and Procedures of Statistics*. McGraw-Hill: New York.
17. Trotter GW (1996) Polysulfated glycosaminoglycan (Adequan). In: *Joint Disease in the Horse* (McIlwraith CW, Trotter GW, eds). pp. 270-280. Saunders: Philadelphia.
18. Watkins JP (1992) Tendon and ligament biology In: *Equine Surgery* (Auer JA, ed). pp. 910-915. Saunders: Philadelphia.
19. Webbon PM (1977) A post mortem study of equine digital flexor tendons. *Equine Vet. J*. **9**, 61-67.
20. Webbon PM (1978) A histological study of macroscopically normal equine digital flexor tendons. *Equine Vet. J*. **10**, 253-259.
21. Williams IF, McCullagh KG, Goodship AE (1984) Studies of the pathogenesis of equine tendinitis following collagenase injury. *Res. Vet. Sci.* **36**, 326-338.

Received: June 6, 2002
Accepted: January 14, 2003