IRANIAN JOURNAL OF VETERINARY SURGERY (IJVS)
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Effect of Collagen Type I on the Hydroxyproline Content in Experimentally Induced Injury in Achilles' Tendon in Dogs

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Abstract

Objective- To determine the effect of collagen type I on hydroxyproline content in Achilles tendon in dogs.
Design- Experimental in vivo study.
Animals- A total of 12 adult dogs.
Procedure- Under general anesthesia and aseptic condition the dorsal surface of right Achilles tendon was exposed and after complete separation of connective tissue, it was splitted (striking 10 times) in full thickness in longitudinal fashion of 3cm in length in the mid-tendon area using BP blade no 15 in each one. These animals were divided into two groups of control (untreated) and experiment (treated with collagen type I) having 6 animals each. The hydroxyproline concentration was measured by modified spectrophotometer method by collecting samples from mid splitted area of injured and normal tendons of each animal from treated and untreated tendons on 30 in three dogs in each group and 90 days in remaining animals after surgery. The collected data was analyzed using student t-test at $P<0.05$% significant level.
Results- There was significant differences between hyroxyproline contents of treated tendon with that of control ones. The normal values of hydroxyproline content in control group was 97.66mg/g of dry matter (DM), whereas it was

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42.33mg/g of DM after one month and 62.06mg/g of DM after end of 3 months. These data were 97.66mg/g of DM for normal tendon and 52mg/g of DM and 83.4mg/g of DM respectively for one and three months in experimental group (P<0.05%). This study suggests using hydroxyproline content as a direct marker of the effect of collagen type I on collagen content in injured tendon.

Conclusion and Clinical Relevance- The results of this study indicated that addition of collagen type I as a local stimulator on severely injured or extensive lesion of Achilles tendon in dog will be highly useful in enhancing hydroxyproline content in the treated tendon.

Key words- Hydroxyproline, Collagen Type I, Achilles tendon, Dog**.**

Introduction

Injuries to tendons, ligaments and other soft tissues may result in complications requiring long-term rehabilitation.[1, 2] Degenerative changes and excessive mechanical forces may be considered additional uncontrolled reasons for unnoticed rupture of tendon like Achilles.[3, 4] The lesion produced in the tendon varies markedly from partial to complete disruption of the tissues and renders the affects limbs unfit for normal functioning.[5] Surgical repair of a severed Achilles tendon has been reported in dogs and cats[6], but efficient physiotherapeutic techniques are necessary for early return of normal function of affected part. However, the effect of collagen type I in severed Achilles tendon injuries has not been explored in dogs. Therefore the present study was undertaken to evaluate the hydroxyproline content after local application of collagen type I on the healing process of this tendon in dogs and its final correction between functional activity and clinical signs after surgery.

Materials and Methods

The experiment complied with the Islamic Azad University. Science and Research branch Tehran law on animal experiments and was approved by The Faculty of Specialized Veterinary Sciences.

The study was conducted on 12 adult male dogs having 25 to 30 Kg body weight with 2 to 3 years of age. The Achilles tendon of the right hind limb was exposed under deep anesthetic surgery using combinations of acepromazine maleate (0.1mg/kg), ketamine hydrochloride (5mg/kg. IM) and induction was done with thiopental sodium (10mg/kg) and anesthesia was maintained using halothane 1-2%. Splitting of Achilles tendons in all dogs was done (striking 10 times) completely in full thickness in longitudinal fashion in about 3cm in length in the mid-tendon area using Bard Parker blade no 15 in each one. These animals were divided into two groups of control (untreated) and experiment (treated with collagen type I) having 6 dogs each which were subdivided into two subgroups of one month and three months duration with 3 dogs each.. No treatment was given to control one, in experimental group, the injured area was packed with 100 mg collagen type I fibers and the area was sutured using continuous pattern with Vicryl 4-0 as routine. Skin was sutured as routine. The hydroxyproline concentration was measured by modified spectrophotometer method [7], by collecting samples (5mm in thickness) from mid splitted area of injured and normal tendons of each animal from treated and untreated tendons on 30 and 90 days after immediate euthanasia. The collected data was analyzed using student t test at P<0.05% significant level.

Results

The Achilles tendon was severed completely in longitudinal fashion and splitted using B.P.blade no15 as to have the worst shape of tendon rupture lengthwise. The degree of lameness showed by individual dog (12 dogs) was almost identical due to the similarity of the lesions. Lameness was most apparent during the first 3 days post surgery and then gradually improved in the treated limb at the 3rd weeks until the time of euthanasia on 30Th days compare to control group. Swelling at the area of operation varied between individual dogs but it was less severe in the experimental dogs. The normal values of hydroxyproline content in control group was 97.66mg/g of dry matter (DM) ,whereas it was 42.33mg/g of DM after one month and 62.06mg/g of DM after end of 3 months. These data were 97.66mg/g of DM for normal tendon and 52mg/g of DM and 83.4mg/g of DM respectively for one and three months in experimental group (P<0.05%) It was quite significantly different between untreated tendons with that of normal limb of the same animal in control group (Tab 1). This difference was quite less when treated tendon was compared with that of normal limb (Fig 1) of the same animal. There was marked increase in hydroxyproline content of treated tendon using collagen type I when compared to values of control ones (Fig 1).

Table1. The amount of Dry Matter content in the samples
Of tendons in control and treated groups

groups	Sample numbers	Normal tendon (mg/g dry matter)	Control tendon days ۳۰ (mg/g dry matter)	Control tendon in days۹۰ (mg/g dry matter)	Differences between normal and groups in 30 days (mg/g dry matter)	Differences between normal and groups in 90 days (mg/g dry matter)
Control	1	98	36.4	62.6	61.6	35.4
	2	94	43.8	59.2	2.50	34.8
	3	101	46.8	64.4	53.2	36.6
Mean±SD		97.66	42.33	62.06	55	35.6
treated	1	94	47.8	80.8	2.42	2.13
	2	89	55.8	82.6	2.33	4.6
	3	93	52.4	86.8	6.40	2.6
Mean±SD		92	52	83.4	38.66	8.6

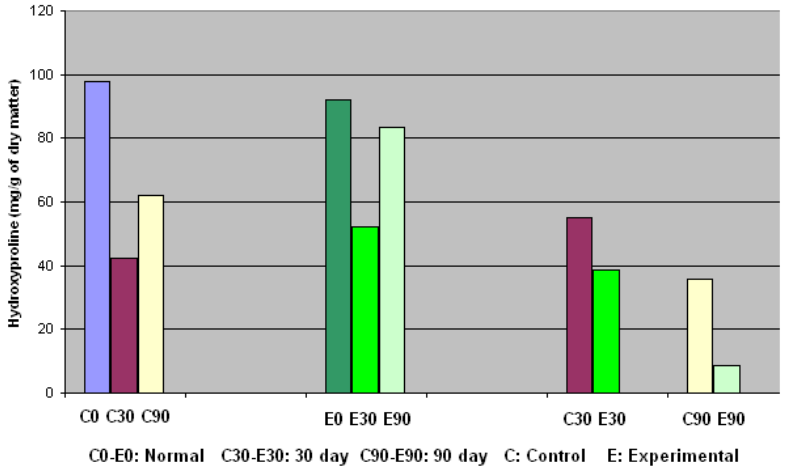

Figure 1. Showed the amount of hydroxyproline (mg/g of dry matter) normal, control and treated tendons on day 30 and 90 days

Discussion

The purpose of this study was to determine local effect of collagen type I on the severed Achilles tendon injury. The treated limb showed higher level of clinical satisfaction and functional behavior on third weeks of treatment as compared to untreated limbs. The effects of collagen type I in experimental animals induced tendopathy in the splitted area indicated the higher level of local reaction for speeding up healing as compared to that of control one. The data collected from hydroxyproline analysis positively showed significant differences between these groups. This was a very narrow difference within in group II.[8] Tendon injuries are often accompanied by injury to surrounding soft tissues or bone; consequently, healing does not take place in an isolated environment. An important factor is whether a tendon heals without formation of adhesions to adjacent tissues, resulting in decreased gliding function.[9] As to reduce the local site effects and accelerate local stimulation as far as accumulation of collagen fibers and early reorganization, local impaction of collagen type I showed, it resolves inflammation, increases tenocyte proliferation and side by side restored tendon integrity correlated to clinical signs of having full limbs weight bearing. The findings in this study provided evidence that tendonitis repair (splitted area) due to increased fibroblastic/tenoblastic activity.[10] cumulatively increased in hydroxyproline content has direct correlation in early maturation of fibroblasts and early parallel arrangement of collagen fibers and bundle formation.[11, 12] The similar findings has been reported by sharifi et al[13] concerning the effect of direct application of transcutaneous electrical stimulation on hydroxyproline content in tendon of horses and Robert et al[14] regarding the positive effect of shock wave therapy (SW) on adult tendon in rat which led to increase in mitogenic and anabolic responses of tendon tissue that brought about the clinical success of using SW treatment in resolving tendonitis. No doubt the etiology of tendonitis is multifactor including avascular changes, degenerative changes and metabolic disturbances, neural factors and neovascularization[14, 15] but the acute swelling, inflammation and matrix destruction in tendon are similar to those seen in naturally occurring tendon injuries[16]. There was a significant increase in collagen production with a model of an accelerated rate of collagen turning over and had a direct effect on biochemical properties of the tissue.[13] Early studies by Gigante et al[17]and Heidia Eriksen et al[18] showed a correlation exists among number of collagen fibrils, size, their organization, and mechanical strength. The amount of regenerating tendons considered to be good indication for relationship between mechanical strength and the absolute amount of collagen being determined as biochemical thought assays for hydroxyproline content in this study, whereas reported findings relied on the collagen profiles of mature tendons[19,20]. Application of collagen type I shown to have beneficial effects when applied to healing tendon. There was a significant increase in collagen production with a model of an accelerated rate of collagen turnover .In the treated tendon .it had a direct effect on biochemical properties of the tissue. Early studies by, Parry et al (1978)[21] and Flint et al (1984)[22] showed a correlation among collagen fibril organization, size and number of and biochemical strength. These previously published paper studies relied on the collagen the collagen profiles of mature tendons, whereas in this study, the amount of regenerating tendons as the relationship between biomechanical strength and the absolute amount of collagen as determined biochemical thought assays for hydroxyproline content. The results of this study reported here, indicated that direct application of collagen type I on severely injured or extensive lesion of tendons will be highly useful in enhancing hydroxyproline content in the treated tendon.

Conclusion

This study indicates that direct application of local impaction of collagen type I fibers into severely injured or extensive lesion of tendon will be highly useful and enhance hydroxyproline content in the treated tendon. This study suggests using hydroxyproline content as a direct marker of the effect of local use of collagen content in injured tendon.

Acknowledgements

The authors wish to acknowledge the Faculty of Specialized Veterinary Sciences. Islamic Azad University, Science and Research Center. Hesark Tehran Iran, Research Council for approval and financial support of this research work

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چکیده

بررسی اثر کلاژن تیپ ۱ برروی مقادیر هیدروکسی پرولین در ضایعات تجربی تاندون آشیل در سگ

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هدف- بررسی اثرات موضعی کلاژن تیپ ۱ در تسریع التیام ضایعات تاندون آشیل با استناد بر مقادیر هیدروکسی پرولین بافتی تاندون آشیل سگ.

طرح مطالعه- مطالعه ی تجربی.

حیوانات- در این مطالعه از۱۲ قلاده سگ بالغ در دو گروه آزمون و کنترل (شش قلاده در هر گروه) استفاده شد.

روش کار- تحت بیهوشی عمومی و شرایط کامل آسپتیک جراحی، سطح پشتی تاندون آشیل اندام راست از طریق برش جراحی در معرض قرار گرفته و پس از جداسازی کامل بافت‌های همبندی موضع برش طولی به اندازه ی سه سانتیمتر بصورت تمام ضخامت و به تعداد ۱۰ شکاف توسط تیغ بیستوری شماره‌ی ۱۵ برروی آن ایجاد گردید. این حیوانات به دو گروه شش تائی کنترل و آزمون (گروه درمان با کلاژن تیپ یک) تقسیم بندی شدند. غلظت هیدروکسی پرولین توسط روش اسپکتروفتومتری مودیفاید از طریق جمع آوری نمونه‌هائی از میان کانون‌های شکاف داده شده ی ضایعه ی تاندونی و نیز تاندون‌های سالم در روز سی‌ام در سه قلاده در هر گروه و روز نود در سه قلاده‌ی دیگر هر یک از گروه‌های آزمون و کنترل مورد ارزیابی قرار گرفت. داده‌ها تحت انالیز آماری با استفاده از student T test در $P<0/05$ ارزیابی شدند.

نتایج- اختلافات بارزی بین مقادیر هیدروکسی پرولین تاندون‌های درمان شده در مقایسه با گروه کنترل وجود داشت. مقادیر نرمال هیدروکسی پرولین در گروه کنترل ۹۷/۶۶ mg/g ماده خشک بود در حالیکه این مقدار بعد از یک ماه برابر با ۴۲/۳۳ mg/g ماده‌ی خشک و بعد از سه ماه به ۶۲/۰۶ mg/g ماده‌ی خشک رسید. مقدار این داده در گروه آزمایش ۹۷/۶۶ mg/g ماده‌ی خشک بود که بترتیب بعد از یک و سه ماه به ۵۲mg/g و ۸۳/۴mg/g ماده‌ی خشک رسید ($P< 0/05$٪). این مطالعه نشان داد که هیدروکسی پرولین بعنوان یک نمایه‌گرمستقیم از اثر کلاژن تیپ ۱ برروی محتویات کلاژن در تاندون آسیب دیده می‌باشد.

نتیجه گیری و کاربرد بالینی- نتایج این مطالعه نشان داد که افزودن کلاژن تیپ یک بعنوان یک محرک موضعی برروی آسیب‌های شدید یا ضایعات گسترده‌ی تاندنی آشیل سگ در جهت افزایش مقادیر هیدروکسی پرولین بسیار موثر واقع می‌شود.

کلید واژگان- هیدروکسی پرولین، کلاژن تیپ ۱، تاندون آشیل، سگ.