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The Effect of Diclofenac on Tendon Healing: An Experimental Study in Guinea Pigs

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Abstract

Background: Whether non-steroidal anti-inflammatory drugs have a detrimental effect on tendon regeneration is still a matter of debate. As such, the purpose of this study is to determine the effect of diclofenac on tendon healing.

Methods: Sixty four guinea pigs were randomly divided into two main groups (group A: histological study, group B: biomechanical study). Then tenotomy and repair of right Achilles tendon were done. Half of the animals in each group received diclofenac (1 mg/kg/bid) and the other half received placebo. Half of the animals in each group were sacrificed at 2 weeks and the remaining animals at 6 weeks post-operation. In group A, histological study for determining maturity of healing of tendons in diclofenac and control groups was done, and in group B, tensile force to failure of tendons at two and six weeks of post tenotomy was determined.

Results: After two weeks, of the group A1, four animals in the experimental and five in the control group were labeled as relative immature. Four of the animals in the experimental and two in the control subgroups were labeled as relative immature. These differences were not significant. In group A2, five animals in the experimental and three in the control group were in the relative mature and three of those in the experimental and four of the control group were labeled as relative immature group. In group A1, the tensile force to failure was 24.175 N and 25.371 N in the experimental and control groups, respectively. In group B2, mean force to failure was 41.019 N in the experimental and 39.743 N in the control group. There was no significant difference between both groups.

Conclusion: Diclofenac at the dosage of 1 mg/kg/bid did not appear to affect histological and biomechanical characteristics of tendon healing.

Keywords: Diclofenac; Tendon Healing; Guinea Pigs

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs) are often used to manage pain after tendon and ligament injury or surgery. NSAIDs inhibit cyclooxygenase, which converts arachidonic acid into prostaglandins, the substance integral to the inflammatory process.[1,2] Because the initial stage of the healing process of tendon is inflammation, the concern is that anti-inflammatory medications might interfere with this process. However, there are limited data to support their use for tendon and ligament injuries.[3,4] Therefore, this study assessed the effect of Diclofenac administration on the healing process of the tendon in guinea pig model.

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Materials and Methods

This prospective, experimental case control study was performed on 64 male guinea pigs. The animals were short hair English, brown and white races, with a mean weight of 500 grams. The animals were provided by the Laboratory Animal Research Center of Shiraz University of Medical Sciences. Animal

handling, maintenance and experimentations were done in accordance with the guidelines set by the Institutional Animal Ethical Committee. The animal room was well-ventilated and had a regular 12:12-h light/dark cycle throughout the experimental period. Four animals were excluded from the study due to early post-operation death probably due to complications of anesthesia. The animals were divided into 2 main groups (group A: histological study and group B: biomechanical study). Then under general anesthesia, using ketamine (45 mg/kg interaperitoneally) and Xylazine (5 mg/kg interaperitoneally), the right thigh, knee and leg of the animals were prepared and draped. Next, through 2 cm longitudinal incision on the distal posterior border of the leg, Achilles tendon was identified and tenatomy of the tendon about1 cm above the insertion to calcaneous was done. Afterwards, the tendon was repaired by 4/0 round Nylon sutures, and the skin sutured by Nylon 3/0. Dressing and long leg cast in the flexion of the knee and plantar flexion of the ankle was applied. For prevention of infection, penstep (mixture of penicillin and streptomycin) was injected intramuscularly for 3 days in all animals.

Group A (histological study) was divided into two subgroups based on the time of the histological study; subgroup A1 (two weeks) and subgroup A2 (six weeks). Group B (biomechanical study) was also divided into two subgroups based on the time of the study (B1, two and B2, six weeks). Then in each group (A1, A2, B1 and B2) eight of the animals received diclofenac 1 mg/kg intramuscularly twice daily for two weeks and for the rest of the animals placebo (equal volume of distilled water) was injected for two weeks. Groups A1 and B1 were sacrificed at two weeks post-operation with a painless method (inhalation of Co_2) and the Achilles tendon complexes were resected.

This was preformed by removing the peritendinous structures with careful dissection about the healing tissue. In group B1, the tendon complexes were kept in 0.9% saline to prevent tissue changes after drying and within 90-120 minutes of resection, each specimen was evaluated biomechanically for tensile force to failure by uniaxial tensile loading, using a Hounsfield tensiometer machine (Model H 50 KS) at a velocity of 50 mm/min. Failure load was expressed as maximum force, measured in Newton (N), which caused tendon rupture. At six weeks post-operation, groups A2 and B2 were also sacrificed and similar procedures were applied. In groups A1 and A2, resected tendons were fixed in 10% buffered formalin. They were processed and embedded in paraffin. Sections of 5 micrometer thickness were prepared and stained with hematoxylin and eosin. Histopathologic studies were performed under light microscopy. In the blind way, the pathologist assessed the resected Achilles tendon specimens for the degree of healing. Histological assessment was carried out to evaluate the cellularity, vasculrity, collagen density, collagen fiber organization and orientation, inflammation and adhesion of the healing tendons. Tendon sections were scores 1, 2 and 3, ranging from relatively immature to relatively mature tendon organization with or without inflammatory reaction.

Score 1: collagen density was less than 50%, with loose to semidense network structures, high cellularity, neovascularization and mononuclear cells.

Score 2: collagen density was over 50%, with high network structure, orientation and organization of collagen bundles along the axis of the tendon, absence or a little inflammatory reaction.

Score 3: normal tendon structure.

The data were analyzed with SPSS software (version 11.5, Chicago, IL, USA) and for comparison of results, using Mann-Whitney and Fisher Exact tests. A p value less than 0.05 was considered significant.

Results

Sixty guinea pigs entered this study; all being male with a mean weight of 500 grams and mean age of 4 months. Group A and B had the same weight and age. There was no wound infection in the animals. Weight gain was about 20 grams after 2 weeks and 55 grams after 6 weeks.

Hematoxylin and eosin stained tendon sections showed a relatively immature or relatively healing process (Figure 1). In relatively immature sections, the area between the cut ends was filled with loose or semi-dense connective tissue matrix, predominant active fibroblasts with large nuclei, neovascularization and infiltration of mononuclear inflammatory cells in comparison with normal tendon. In relatively mature sections, the collagen tissue was of high density, some degree of organization of bundles with fewer fibroblasts and blood vessels (Figure 2). After two weeks of the group in histological study.

(Group A1), four sections (50.0%) in the experimental and five (71.4%) in the control groups were labeled as relative immature and four (50.0%) in the

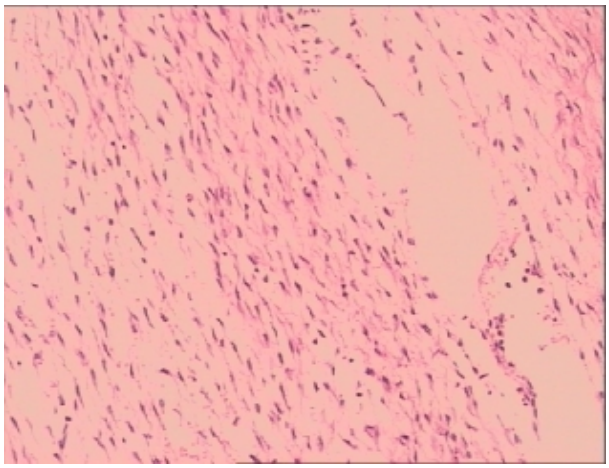

Fig 1: Relative immature healing after 6 weeks in diclofenac group (H&E x200)

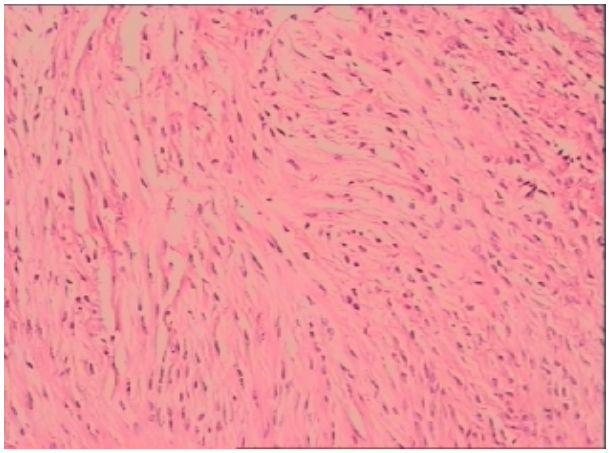

Fig 2: Relative mature healing after 6 weeks in diclofenac group (H&E x200)

experimental and two (28.6%) in the control group were labeled as relative mature. These differences were not statistically significant (*P*=0.608). In group A2 (histological study after 6 weeks), five (62.5%) in the experimental and three (42.8%) in the control group were in the relative mature and three (37.5%) in the experimental and four (57.2%) in the control group were classified as relatively immature. These findings were also not significant (*P*=0.619).

In biomechanical study after 2 weeks (Group B1), also tensile force to failure in the experimental group was 24.175 N and 25.371 N in the control group. There was no significant difference between the force to failure value of the two groups (*P*=0.700). In group B2 (biomechanical study after 6 weeks), the mean force to failure was 41.019 N in the experimental and 39.743 N in the control group. This difference was also not significant (*P*=0.770).

Discussion

Tendons heal very slowly through the formation of scar tissue following chemical, physical, or mechanical injuries and rarely regain their original strength.[5-7] In general, the sequence and time course of events that occur during tendon healing closely resemble those reported in many other tissues. Healing of the injured tendon proceeds through three phases: inflammation, regeneration and remodeling. The initial phase involves an inflammatory response. The regeneration phase is characterized by cellular proliferation and matrix formation with tendon cell proliferation and migration to the repair site, being followed by the maturation and reorganization phase.[8-11] NSAIDs inhibit tissue inflammation by repressing cyclooxygenase activity, thereby leading to a reduction in proinflammatory prostaglandin synthesis. NSAIDs can also repress inflammation by inducing apoptosis, activating peroxisome proliferators-activated receptors and inhibiting neutrophil aggregation and degranulation.[12] Diclofenac can induce shedding of adhesive molecules located on neutrophils, thereby inhibiting their locomotion and ability to invade inflamed tissues. Because prostaglandins can play an important role in the accumulation of inflammatory cells in many tissues and leukocyte, subsets may exacerbate tissue injuries. It is believed that reducing PGE_2 production and inflammatory cell accumulation through COX inhibitors will lead to a reduction in tendon injury. However, the effects of NSAIDs on connective tissue repair have produced diverging and contradictory results.[13,14]

In our study, 60 guinea pigs were selected for the evaluation of histological and biomechanical effects of Diclofenac on tendon healing. Among the laboratory animals, we selected the guinea pig, but Thomas et al. and Yuan et al. did their studies, using rabbits and rats, respectively.[3,4] We used guinea pig because the size of Achilles tendon was optimal for our procedure and their care was much easier in comparison to larger animals. In addition, guinea pigs have rapid growth and high reproductive rate. We used 64 animals and our sample size was greater than that in similar studies.[3-5]

Male pigs were selected for prevention of any probable effect of hormonal changes on tendon healing. The animals were mature with the mean weight of 500 grams, because the mechanical study was simpler and also collagen fiber maturity was completed.

We selected diclofenac among NSAIDs because this drug is routinely used by so many patients as analgesic. The route of administration was intramuscular due to its simple and high bioavailability.

The histological appearance was evaluated in a blind fashion. For two weeks, in group A1 (the group in histologic study) four animals of diclofenac and five of the control group were relatively immature. Also, four of the first and two of the second group were considered as relatively mature. In group A2, five animals in the experimental and three of the control were in the relatively mature and three of the first and four of the second group were classified as relatively immature. This was not statistically significant. The results of the histological study are comparable to those obtained by Thomas et al. and Yuan et al.[2,3]

In the biomechanical study, after 2 weeks tensile force to failure in the experimental group was 24.175 N and it was 25.371 N in the control group. In group B2, mean force to failure was 41.019N in the first and 39.745N in the second group. This was also comparable to the result obtained by Thomas et al, but in Yuan's study a significant decrease was found in failure load to failure in fluriboprofen compared to the control group.[2,3]

In our study in group A1, there were two cases of tendonitis and in group A2 there were two cases of adhesion of repaired tendon to overlying skins, all of which were among those in the control group. These results are not statistically significant but are suggesting that NSAIDs may prevent tendonitis and later adhesions in some tendon injuries.

Diclofenac in a dose of 1mg/kg did not appear to affect histological and biomechanical characteristics of healing of tendons but may reduce the possibility of tendonitis and adhesion. This study suggests that diclofenac may be used in conjunction with tendon injury without any adverse effect on tendon healing and the patients may benefit due to the low risk of adhesion.

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Conflict of interest: None declared.

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