Effect of Low Level Laser on Hydroxyproline Content in experimentally Induced Injury in Achilles' Tendon in Dog

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ABSTRACT

Objective of study is investigating the effect of Low Level Laser on hydroxyproline content in Achilles tendon in dogs. 12 adult dogs were selected and 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 low level laser) 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. 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 91mg/g of dry matter ( DM ), whereas it was 36.33mg/g of DM after one month and 58.5mg/g of DM after end of 3 months. These data were 89 mg/g of DM for normal tendon and 50.83mg/g of DM and 82.43mg/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 Low Level Laser on collagen content in injured tendon. The results of this study indicated that additional exposure of tendon to Low Level Laser 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, Low Level Laser, Achilles tendon, Dog.

Introduction

The regeneration of tendon tissue is the main objective of tissue engineering and regenerative medicine [27]. The Achilles tendon (tendo-calcaneus communis) is the strongest tendon in the structure of the musculoskeletal system in the dog [5]. Its main function in rear-limb forward progression, and it contributes to progressive support of the hock [5]. The healing process if a tendon injury id usually difficult and uncertain because the prognosis varies widely depending on the amount of trauma and the time that elapses between trauma and treatment. The etiology of Achilles tendon injuries in dogs is usually traumatic. Depending on the trauma, the severity of the lesion may vary considerably, leading to stretching, small or partial lacerations or a complete rupture [7,21]. The main objective of surgery or local treatment for tendon injuries in the dog is to restore an adequate tensile strength to support body weight [12]. Restoring gliding function for lower mobility and use of the phalanges, which is the primary objective of tendon surgery or treatment in humans, is instead a secondary objective in the dog [12]. However, the effect of low level laser in experimentally induced severe injury in the Achilles tendon has not been explored in dogs. Therefore the present study was undertaken to evaluate the hydroxyproline content after local application of low level laser on the healing process of this tendon in dogs and its final correlations 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 30 to 35 Kgbw with 3 to 4 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 3 cm 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 Low level laser) 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 exposed daily to Low level laser 860 nm with intensity of 10mW with 63/cm energy for 10 minutes for 14 days. Skin was sutured as routine. The hydroxyproline concentration was measured by modified spectrophotometer method [23], by collecting samples (5 mm 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 were severed completely in longitudinal fashion and splitted using B.P.blade no 15 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 2nd 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 91 mg/g of dry matter (DM), whereas it was 36.33 mg/g of DM after one month and 58.5 mg/g of DM after end of 3 months. These data were 89 mg/g of DM for normal tendon and 50.83mg/g of DM and 82.43 mg/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 (table 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 low level laser when compared to values of control ones (Fig 1).

**Table 1:** The amount of Dry Matter content in the samples of tendons in control and treated groups.

<table>
<thead>
<tr>
<th>Differences between normal and groups in 90 days (mg/g dry matter)</th>
<th>Differences between normal and groups in 30 days (mg/g dry matter)</th>
<th>Control tendon in days 90 (mg/g dry matter)</th>
<th>Control tendon days 30 (mg/g dry matter)</th>
<th>Normal tendon (mg/g dry matter)</th>
<th>Sample Numbers</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.1</td>
<td>58.2</td>
<td>58.9</td>
<td>30.8</td>
<td>89</td>
<td>1</td>
<td>Control</td>
</tr>
<tr>
<td>33.8</td>
<td>51.8</td>
<td>56.2</td>
<td>38.2</td>
<td>90</td>
<td>2</td>
<td>Mean±SD</td>
</tr>
<tr>
<td>33.8</td>
<td>53.6</td>
<td>60.4</td>
<td>40.6</td>
<td>94</td>
<td>3</td>
<td>Treated</td>
</tr>
<tr>
<td>32.5</td>
<td>54.46</td>
<td>58.5</td>
<td>36.53</td>
<td>91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9.1</td>
<td>41.6</td>
<td>80.9</td>
<td>48.4</td>
<td>90</td>
<td>1</td>
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</tr>
<tr>
<td>4.4</td>
<td>33.6</td>
<td>81.6</td>
<td>52.4</td>
<td>86</td>
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</tr>
<tr>
<td>6.2</td>
<td>39.3</td>
<td>84.8</td>
<td>51.7</td>
<td>91</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>6.5</td>
<td>38.16</td>
<td>82.43</td>
<td>50.83</td>
<td>89</td>
<td></td>
<td>Mean±SD</td>
</tr>
</tbody>
</table>

**Discussion**

Tendon defects remain a major concern in orthopedic surgery because of the limited availability of tendon auto grafts [13]. The Achilles tendon is essential for normal ankle joint movement and particularly critical for activities like running or jumping and climbing [19]. There are two concepts of operative and non-operative treatment for repair of tendon injuries. Modern concepts of non-operative treatment have been shown to produce good results with acceptable complication rates [20,28]. A successful reconstruction must have the appropriate strength, durability and tension to meet the dynamic workload. As the regenerative process leads to a tissue structurally and functionally similar to the original tissue and collagenous connective is analogous to scar tissue that occurs in most parts of body [2,3,9]. The purpose of this study was to determine local effect of low level laser 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 low level laser 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 [4,6,10]. The data collected from
hydroxyproline analysis positively showed significant differences between these groups. This was a very narrow difference within in group II. 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 [1]. As to reduce the local site effects and accelerate local stimulation as far as accumulation of collagen fibers and early reorganization, local impaction of low level laser showed, it resolves inflammation, increases tenocyte proliferation and side by side restored tendon integrity correlated to clinical signs of having full limbs weight bearing [8]. The findings in this study provided evidence that tendonitis repair (splitted area) due to increased fibroblastic/tenoblastic activity [11,25]. Cumulatively increased in hydroxyproline content has direct correlation in early maturation of fibroblasts and early parallel arrangement of collagen fibers and bundle formation [18]. The similar finding has been reported by Gum Steven et al [15] and sharifi et al [26] concerning the effect of direct application of transcutaneous electrical stimulation on hydroxyproline content in tendon of horses. No doubt the etiology of tendonitis is multifactor including avascular changes, degenerative changes and metabolic disturbances, neural factors and neovascularization [17], but the acute swelling, inflammation and matrix destruction in tendon are similar to those seen in naturally occurring tendon injuries. There was a significant increase in collagen production with a model of an accelerated rate of collagen turnover [24] .In the treated tendon, it had a direct effect on biochemical properties of the tissue.

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References
