Effects of transcutaneous electrical stimulation on the healing of surgically severed Achilles tendon in rabbits

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Abstract: The effect of Transcutaneous Electrical Stimulation on the healing of tendon in rabbit was evaluated using 10 young clinically normal, healthy New-Zealand white male rabbits, aged 6 months and weighing 3. 6 – 0. 8 Kg. All the rabbits were anaesthetized by using combinations of xylazine, ketamine, acepro mazine and diazepam. Splitting of 2cm full thickness of right Achilles tendon was conducted in all rabbits with scalpel (10 times), followed by their division into two groups of 5 rabbits each. In group I, the tendon was allowed to heal without the application of any treatment, but in group II, TES threapeutic regimens were begun on 3rd day after splitting at a 1W/cm^2 for 24 minutes with 70 Hz frequency and 1. 1 mA intensity daily for 15 days. The full surface of electrodes covered with pad soaked in water properly, which were placed in closed contact with skin in proximal part of splitted tendon (2 positive electrodes) and distal part(2 negative electrodes),were subsequentely fixed with adhesive tape. After 30 days, biopsy was collected from injured part for histomorphological evaluation. The staining procedure employed was Haematoxylin and Eosin stain. Results: The union was observed to be comparatively much better in group II (treated one) presenting the least inflammatory reaction, parallel and advanced stage of collagen formation with minimum cellularity when compared to the control one. Transcutaneous Electrical Stimulation(TES) for two weeks had positive and stimulatory effect on tendon healing, with the least inflammatory cells being regular and parallel bundle of collagen fiber formation in the treated group. TES as a physical method of therapy proved quite effective in tendon healing and faster remodelling of tendon fibers. *J.Vet.Res. 62,2:21-25,2007.*

Key words: transcutaneous electrical stimulation, tendon, healing, dog.

Introduction

Achilles tendinitis is one of the most common of all sports injuries and a cause of locomotion dysfunction in small and large animals. Surgical repair of a severed Achilles tendon has been recorded in dogs (Kramer et al 1993, Hann *et al.,* 1995) and cats (-Mughunnam and Reinke,1994). A part from local and systemic treatment, efficient physiotherapeutic techniques are necessary for an early recovery of the normal function of the affected part(Batch et al 1991). The phenomenon of neovascularisation of the Achilles tendon -bone junction after low-energy shock wave application has been reported (Wang et al 2002) and therapeutic ultrasound on the healing process of Achilles tendon (Tendocalcaneus) in wistar rat after tenotomy (decunha et al 2001). As TES proved quite effective in the reduction of pain and swelling especially at soft tissue levels (Reger et al 1999; Leo et al 1998), this study was undertaken to determine the effect of TES on the rate of repair of splitted Achilles tendon of rabbits.

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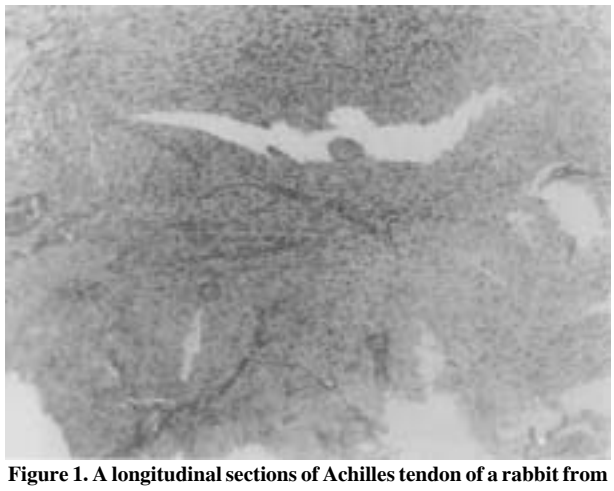

Figure 1. A longitudinal sections of Achilles tendon of a rabbit from the control group(I) at day 30 after splitting; a- Initial stage of healing; b- Neovascularisation in healing tissue. (HandE × 100).

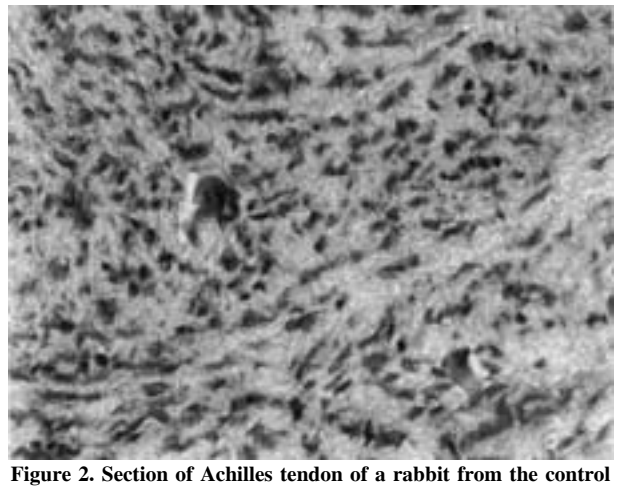

Figure 2. Section of Achilles tendon of a rabbit from the control group (I) at 30 days after splitting a- Arrow showing mixture of fibroblast and new formation of matrix connective tissue; b-Mixture of mature collegen fibers; c-collagen fibers arranged in longitudinal fashion. (HandE × 400X).

Materialsand Methods

The study was conducted on 10 clinically healthy New Zealand white male rabbits, weighing 3.6–0.8 kg and aged 6 months, maintained under similar housing management and feeding conditions. These rabbits were divided into two groups (control I and experimental, II) of 5 rabbits each.

No treatment was applied in the control group, whereas in the experimental one,TES was given for 24 minutes for two weeks after the 3rd day of surgery.

Tendon-splitting: The area around and proximal to the right hock was shaved and prepared for tendon splitting.

The effective anesthesia was induced by intramuscular injection of ketamine hydrochloride 35 mg/kg$^{-/Bw}$, xylazin 5 mg/kg^{-BW}, diazepam 1mg/kg^{-Bw} and acepromazine maleate 1mg/kg^{-Bw} combinations. A linear incision was made on the lateral aspect away from the line of the Achilles tendon, 2-3 cm proximal to the point of it's insertion on the tuber calcanei. After dissecting the fascia, the skin was reflected and the Achilles tendon was exposed and elevated with the help of two artery forces. The Achilles tendon was splitted 2 cm in length and full thickeness using a scalpel blade (10 times). The subcutaneous tissue and skin were closed as a usual practice.

Post-operatively, all the rabbits were given daily 60/000 iu penicillin G, Gentamycin 5mg/kg^{-} intramuscularly daily for 3 days. The wound was bandaged for the first 5 days and then allowed to heal without the bandage. Diluted povidone iodine(10%) was used for local application on the wound every alternate day for 10 days. The suture was removed on the 10th day.

In group I, no treatment was used. TES regimens were given to group II rabbits for 24 minutes with 70 Hz frequency for two weeks. Each rabbit was kept in a Specially designed box so as to keep them restrained during therapy. The full surface (1.5 cm^2) of electrode covered with pad-soaked in water properly was fixed in close contact in proximal part of the tendon above the severed part (2 positive electrodes) and the distal part (2 negative electrodes), which were then fixed at place using an adhesive tape.

Clinical observation was made for 30 days and tendon biopsy was collected from each case under general anesthesia and the repaired Achilles tendon was exposed for gross observation. Then 1 cm full thickeness in length of the central portion of the tendon was collected with the help of a scalpel blade for histomorphological interpretation. It was fixed in 10% buffered formalin solution.

The samples were processed for paraffin sectioning using the cedar wood oil method (Luna,1968). Longitudinal section of 5 µm thickness was used for histomorphological staining with H and E stain.

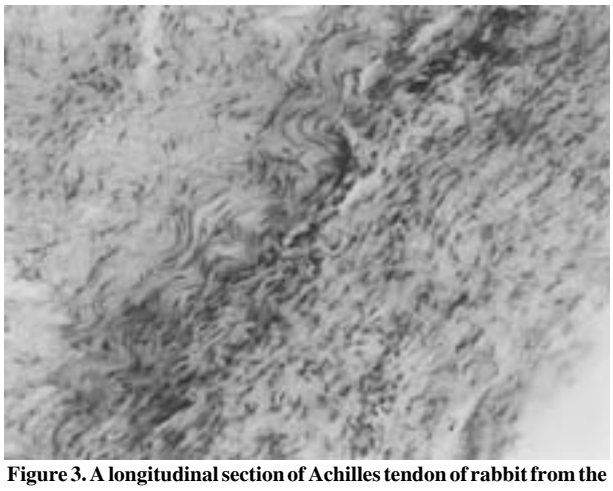

Figure 3. A longitudinal section of Achilles tendon of rabbit from the experimental group (II) at 30 days after splitting, showing; a-mature collagen fiber with initial stage of parallel arrangement; b- newly formed tenocyte cells (HandE × 200 X).

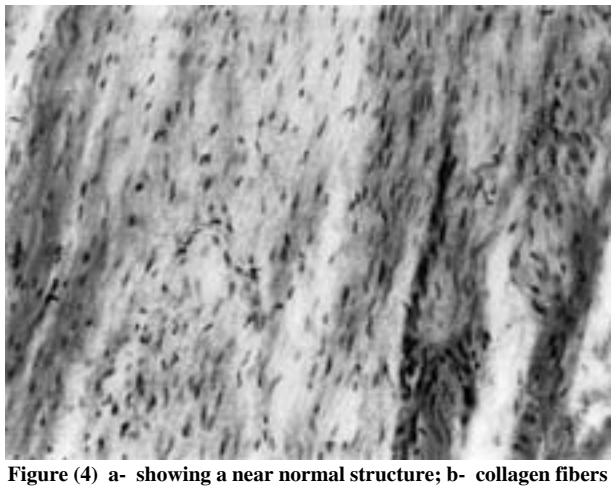

Figure (4) a- showing a near normal structure; b- collagen fibers with a wavy appearance of repaired Achilles tendon; c- newly formed tenocyte cells in the experimental group(II) at day 30 after splitting. (HandE × 200X).

Histomorphologically the sections were assessed for fibroblastic activity and maturation of the collagen. The grading of tendon healing was performed as follows:

Grade 1: was assigned when the majority of the healing area showed fibroblastic activity with the formation and synthesis of immature collagen fibers.

Grade 2: was assigned when the fibers had started arranging longitudinally and had an equal proportion of fibroblast and maturing collagen fibers.

Grade 3: was assigned when the fibroblastic activity had stopped and the collagen fibers started maturing with bundle formation at the initial stage.

Grade 4: was assigned to a structure comparable with a nearest normal tendon.

Results

The features of gross and macroscopic examination of splitted tendons showed continuity and unision in both the groups of rabbits, but the Achilles tendons in group II showed less and week peritendinous adhesions as compared with those in group I. A slight palpable thickening was observed at the site of repair at day 30 after the splitting tendon.

Histomorphology: The histological sections of the control rabbits (group I) showed union between splitted bundles of tendon at day 30. They were marked by an increase in inflammatory cells with irregular arrangement of collagen fibers with the presence of newly formed fibroblasts, fibrosis and tendonitis depicting a combination of grade I and II activity.

In group II (-treated), the union was comparatively better and the junction of old and new tendon was clearly visible. There was homogenicity without any inflammatory reaction of the union site. Bundle formation had initiated and at some locations it was more mature and organised at the healing site as compared with the control one. The newly formed tendon exhibited more compact parallel bundle formation, presenting less cellularity with highly formed fibrosis with normal epitendon and peritendon, presenting almost regularly arranged collagen fibers containing newly formed fibroblasts and tenocytes,indicating grade IIIand IV activity.

Discussion

Tough the design was same for both the groups, the disappearance of pain and swelling at the site of surgery in the group II rabbits manifested earlier than that of the control one. Actually these findings were similar to those reported following ultrasonic therapy and diathermy therapy of traumatised tendon in dogs. (Bansal, et al 1992/ Bansal, et al 1990). Generally, stimulation produces analgesic effects(Leo, et al 1986) similar to that of ultrasound therapy, which is recommended for injuries to tendons and joints in the

equine(Ferguson, 1981; Lang,1980). TES has both thermal and nonthermal effects in the tissue (Stanish and Gunniagsom 1988), as electrodes delivering 10 to 20 A current hastened recovery of injured athletes suffering from ruptured ligaments and tendons. Other studies have demonstrated the positive effect of microcurrents on tendon repair in animal models. Nessler and Mass's (1987), reported that in conducting microelectrical stimulation of tendons, 91 percent increased proline uptake than the controls tendons after 7 days while hydroxy proline activity increased by 225% versus the control one. As this study confirmed that tenoblastic repair was enhanced by microcurrent stimulation, these findings are similar to the reports of Thawer and Houthon 2001, Reger *et al.,* 1999 and Stanish 1984.

The repaired tendon usually undergoes degeneration because of an avascular and a nutritional phenomenon (Potenza 1964) but in this study however, no necrosis at the site of splitting was observed due to sufficient nutrition and increased blood supply provided to the treated part thereby increasing the rate of protein synthesis in fibroblasts, which are responsible for collagen production. An adequate blood supply is necessary for tendon healing as reported by-Koth and Sewell (1988). The effect of ultrasound therapy reported by Mummery, 1978; Webster, 1980 and Cleary *et al.,* (1988) reported that electrical stimulation does effect diffusion rate and membrane permeability of the fibroblasts These changes could alter the rate of protein synthesis in the fibroblasts and thus effect tissue repair (Owoeye *et al.,* 1987). Similar findings of this study confirmed the presence of more content and better alignment of newly formed collagen fibres in the treated rabbits(group II) at the healing site. Based on the histomorphological findings and clinical supportives evidences, TES positively enhances Achilles tendon healing in the rabbits.

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