

Ultrasound therapy during experimental tendinous injury healing in rabbits

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ABSTRACT

Efficacy of ultrasound (US) therapy in modulation of peritendinous adhesions following tendon injury in a rabbit model *in-vivo* was evaluated in adult New Zealand White rabbits (16) of either sex, which were randomly divided into 2 groups of 8 animals each (groups 1 and 2). A lateral curvilinear skin incision was given just above the left hock joint over the tendoachillis under thiopental sodium anaesthesia and after exposing the tendon a uniform area of 2 cm was crushed with toothed forceps for creation of peritendinous adhesions. The surgical wound was closed in a routine manner. In group 1 (test group) ultrasonic therapy (US) was started after 3 days of tendon surgery and was given @ 0.5 W/cm² for 5 min, daily for 10 days. In group 2 (control) no treatment was given. Clinical examination revealed no significant difference in rectal temperature, exudation, Warmth and pain scores at different time intervals. On day 14 onwards there was a significant increase ($P < 0.05$) in tendon gliding movements in US treated group and normal gliding was seen on day 30. Air tendograms revealed early organization, minimal adhesion formation and lesser thickening of tendon at the reconstructive site in the test group. Histopathology and scanning electron microscopic observations revealed that proliferative and inflammatory responses were significantly reduced in US treated group as well as early restoration of normal arrangements of collagen fibres. The ultrasonic therapy had reduced the formation of peritendinous adhesions in treated animals. This appears to be a simple and inexpensive means of refined treatment for the management of peritendinous adhesions.

Key words: Histopathology, Rabbits, Superficial digital flexor tendon, Surgery

Tendons are prone to injury due to their superficial location in the body (Kumar *et al.* 2002 b), and their repair has great limitation due to peritendinous adhesions. Formation of these adhesions may impair the function of limbs seriously (Mathews and Richards 1976) and often lead to permanent deformations. Therapeutic ultrasound recommended in the treatment of musculo-tendinous injuries in animals to minimize such postoperative complications (Porter 1998). Stimulating effects of ultrasound (US) in the healing of different tissue was also reported (Maiti *et al.* 2004, Ng *et al.* 2003, 2004).

In the present study, the effect of US therapy in the prevention of peritendinous adhesions were studied. Clinical, radiology, gross, histopathology, histochemistry and scanning electron microscopy techniques were used to evaluate the healing.

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MATERIALS AND METHODS

Adult New Zealand White rabbits (16) of either sex were randomly divided into 2 groups of 8 animals each (groups 1 and 2). The rabbits were kept in individual cages and had *ad lib.* access of water and feed. Food for 12 h and water for 6 h were withheld before the operation. All the surgical procedures were performed under 2.5% thiopental sodium anaesthesia administered intravenously to effect. Achilles tendon of left hind limb was used for creation of peritendinous adhesions. A lateral curvilinear skin incision was given just above the hock joint over the tendo-achilles. A uniform area of 2 cm was crushed with toothed forceps for creation of peritendinous adhesions. The surgical wound was closed in a routine manner. Daily antiseptic dressing of the suture line was done until complete skin healing. Streptopencillin @ 100 mg/kg and diclofenac sodium @ 2 mg/kg intramuscularly were administered for 5 and 3 days, respectively, in all the animals. In group 1, US therapy was started after 3 days of tendon surgery. The US therapy was given @ 0.5 W/cm² for 5 min daily for 10 days. In group 2, no other treatment was given after surgical intervention (control). The experimental design and treatment of these animals were approved by the Animal Care Committee of our Institute. The healing in

animals of both the groups was evaluated on the basis of the following parameters.

Clinical observations: Feeding pattern and general behaviour changes were observed daily in all the animals. The rectal temperature was recorded daily in the morning up to 10 days in all the animals. Surgical wounds were examined for their gross appearance and status of healing. Degree of exudation, warmth and pain were recorded up to day 14 post-operative and were graded as per Kumar *et al.* (2002 b). Tendon gliding movement, swelling and weight bearing was recorded on days 1,2,3,5,7,10,14, 30 and 60 post-operative and were graded (Kumar *et al.* 2002 b).

Radiological observation: Air tendonograms were taken on days 20, 40 and 60 postoperative, for the density and thickness of tendon.

Macroscopic observations: The gross findings were recorded on days 7, 14, 30 and 60 post-operative after euthanising the animals. The adhesions at the operated site were graded as per Kumar *et al.* (2002a).

Histopathological observations: The histopathological studies were made on days 7, 14, 30 and 60 post-operative, using haematoxylin and eosin staining for routine examination. Special staining techniques were used to demonstrate collagen fibres (Masson's trichrome), reticular fibres (Silver staining), elastic fibres (Verhoeff's staining) and mucopolysaccharide (Periodic Acid Schiff-Alcian blue, PAS_AB) in healing tissues.

Histochemical observations: The sections were stained by Gomori's methods for alkaline phosphatase (AP) activity. The grading was done as no AP activity (0), mild AP activity (+), moderate AP activity (++) and intense AP activity (+++).

Scanning electron microscopic observations: The tissues were fixed in 2.5% glutaraldehyde in a phosphate buffer at pH 7.2 for 24h at room temperature. Hexamethyl disilazane technique (HMDS) was used for drying of the specimens. The microstructural properties of the tendon were studied using scanning electron microscope operating at 5kV (15 WD, working distance) using different magnifications as needed for the desired observations.

Student's paired t-test was used to compare the means at different time intervals. The results of non-parametric observations were analyzed with Friedman 2-way analysis of variance by ranks.

RESULTS AND DISCUSSION

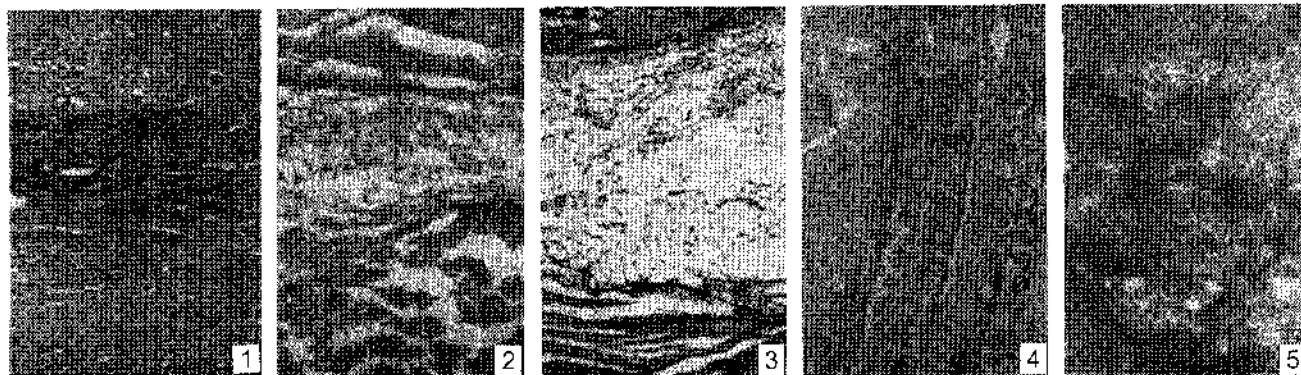
Clinical observations: Jerking of the operated limbs during their natural movement or jump was seen for 2 to 3 days in all animals. No significant difference was observed in rectal temperature, exudation, warmth and pain scores at different time intervals. Reduction in tendon gliding movement was up to day 10 in both the groups of animals. After day 14 there was significant increase ($P<0.05$) in tendon gliding movements in US treated animals and normal gliding was seen on day 30. Significant reduction ($P<0.05$) in

swelling was observed at day 7 and onwards in group 1 whereas no reduction was seen in control group. Circumference of the limb was comparable on days 30 and 60. No significant change in weight bearing scores was observed in both the groups. Surgical wounds in all the animals healed by first intention and none of the surgical wounds exhibited infection. The minimum gliding movements of tendon were seen up to day 10 postoperative in both the groups of animals. The degree of tendon gliding movements depended upon the adhesions formed. Earlier improvement in gliding movements in test group may be due to US therapy. It may be due to the fact that the ultrasound prevents/reduces the formation of adhesions with peritendinous structures (Ng *et al.* 2004).

Radiological observations: Air tendonogram in group 1 at day 20 showed that the distal third of the tendon was close adhered with the surrounding structures. Injured and adhered part appeared denser than the normal tendon. At day 40, marked reduction in the thickening of the tendon was seen. Presence of air (negative control) between the achilles tendon and tibialis posterior muscle revealed marked reduction in adhesions. At day 60, the thickness and density of the tendon further reduced. In control groups, at day 20 showed marked increase in the density and thickness of the tendon and complete adhesions of achilles tendon with skin and surrounding structures. However, the thickness of tendon and density gradually reduced as the healing progressed. Mild adhesions between achilles tendon and tibialis posterior were still present at 60 days. Similar observations were also reported by Kumar *et al.* (2002b) and Ramesh *et al.* (2003b).

Gross observations: In group 1 at day 7 easily separable adhesions were seen. Vascularity was very prominent and gliding movements were absent. Animals of control group showed severe adhesions all around the injured tendon. Presence of adhesions was characterised by foamy and reticulated appearance of soft tissue density. These adhesions indicated proliferative fibrous tissue growth from the surrounding tissues (Kumar *et al.* 2002, b). Peacock Jr. (1964) stated that the development of the fibrous tissue adhesions was a normal process of tendon healing, which indicated the cellular phase of tendon healing. Progressive organization and regression of adhesions was observed earlier in US treated group at different test intervals. The fibrous tissue growth from the surrounding tissue was organized and the presence of negative contrast around tendon was suggestive of loosening of adhesions (Kumar *et al.* 2002 b, Ramesh *et al.* 2003b).

Histopathological observations: On day 7 in group 1 fibrino-haemorrhagic exudate accumulated in the sheath containing neutrophils and fibroblasts. The injured site of tendon showed irregularly arranged fibroblasts and thin collagen fibres (Fig. 1). The neotendon formation was also visible within the mature tendon as thick strips of parallel fibroblasts in the collagen fibre matrix having number of

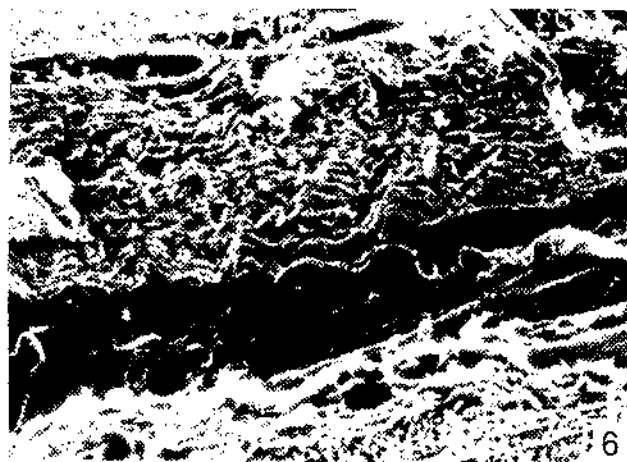


Figs 1-5. 1. Thick fibrous connective tissue adhesions all around the tendon and closely adhered tendon sheath H & E. $\times 100$ (Group 1, day 7). 2. Rarified and fragmented adhesions between tendon and tendon sheath. H & E. $\times 250$ (Group 1, day 60). 3. Loosely arranged fibroblasts with collagen and few mesenchymal cells in adhesion tissue between tendon and tendon sheath. H & E. $\times 100$ (Group 2, day 7). 4. Showing persistence of loose connective tissue adhesions. H & E. $\times 160$ (Group 2, day 30). 5. Intense (+++) alkaline phosphatase activity at the adhesion tissue. $\times 160$ (Group 2, day 7).

erythrocytes. PAS activity was noticeable in neotendon and in the healing tissue. Fine, sparse elastic fibres were also visible in the granulation tissue when the section was found stained with Verhoeff's stain. On day 14, the sheath was found loosely adhered at several places with tendon. Gridley's stained section showed fine wavy reticular fibres in neotendon and healing sheath. The consistency of tendon was less compact and the adhered sheath gets loosened due to breaking of the fibres. On day 30 tendon sheath was found completely detached from the tendon at places. The evidence of dissolution of adhesion with the cellular activity was found much better than the control of the same day. On day 60, sheath adhesions further loosened than at days 30. An empty space appeared between the tendon and the sheath. The rarified adhesion between tendon and sheath was easily visible (Fig. 2).

In control group on day 7 adhered tendon sheath showed extensive exudation and less number of fibroblasts and mesenchymal cells. Core of the tendon showed stellate shaped fibroblasts arranged parallel within collagen bundles. The sheath contains few fibroblasts, mesenchymal cells and irregularly loosely arranged collagen fibrils (Fig. 3). On day 14 tendon was tightly adhered with the peritendinous tissue and the collagen fibres and fibroblasts were more organized along the long axis of tendon. On day 30, there were no areas of necrosis, fibril lyses or signs of acute inflammation. Peritendinous adhesions showed loose and fine strands of fibrous tissue extended in the direction of the gliding (Fig. 4). On day 60, the adhesions were loose as compared to day 30 and rarified towards the tendon surface.

Histopathological examination on day 7, showed moderate to extensive exudation at the injury site in control group whereas, it was mild to moderate in US treated group. Presence of exudation and no improvement in the arrangement and orientation of collagen fibrils up to day 7 following the repair of injured tendon tissue has been reported



Figs 6-7. 6. SEM photograph showing widening of the space between tendon and tendon sheath with loosely arranged collagen fibrils. 500 $\times$ (Group 1, day 30). 7. SEM photograph showing tendon sheath adhered with tendon proper. 500 $\times$ (Group 2, day 30).

by Williams *et al.* (1984). The histological changes such as the increase in the number of fibroblasts, collagen organization, the increase in capillaries, thickening of epitenon and formation of adhesions have been observed in healing tendon (Potenza 1964). The development of adhesions between healing tendon and surrounding structures correlates with the intensity, duration of the inflammatory reaction and degree of mobilization of the tendon during the healing period. Gelberman *et al.* (1983) reported this phenomenon to be initiated from the tendinous sheath and the surrounding tissue. The proliferative changes initiated from paratenon and surrounding tissues resulted in adhesion formation (Potenza 1964, Kumar *et al.* 2002 a).

The tendon sheath was tightly adhered with tendon and subcutaneous tissue up to day 14 in animals of control group whereas, it was comparatively loose and thinner in animals of US treated group. Difference in thickness of sheath and its adhesions with the tendon was more markedly different among animals of US treated group on day 30 and 60 postoperatively. The formation of scars and granulation tissue is suppressed by US therapy, which also prevents the formation of adhesions after repair of the tendon without interfering with healing. Reduced adhesions may be the result of the decrease in the formation of new extracellular matrix due to the inhibition of mononuclear phagocytosis and lymphocytes. Therapeutic ultrasound has been used for treatment of various tendon injuries in different species of animals by various workers (Dyson *et al.* 1968, Bansal *et al.* 1992). The mechanism of ultrasound on tendon repair and function is not known completely.

Physiologic effects of nonthermal ultrasound include stable cavitation and micro streaming. They may enhance healing through increased permeability of cell membranes (Cameron 2003), increased synthesis of proteins and increased release of growth factors (Sussman and Dyson 2001). Stevenson *et al.* (1986) reported that ultrasound waves in some way may interfere or inhibit the polymerization (bond formation) of the basic molecular units for collagen formation of mature collagen from its basic molecules by its micromassage i.e. to and fro mechanical action to the tissue particles. Fragmentation of tropocollagen (basic molecule of collagen) was demonstrated by sonication (Dyson *et al.* 1969). By virtue of this property ultrasound therapy has been used in physical medicine as an aid to the clearing of fibrous adhesions (collagen) from area of surgical excision (Summer and Patrick 1964) and to soften collagenous scar (Bierman 1954). However, there are no long term studies evaluating the changes occurred with superficial digital flexor tendinitis treated with therapeutic ultrasound (Reef 2001).

The Masson's trichrome stain for selective demonstration of collagen fibres has also been used to study different aspects of tendon healing (Kumar *et al.* 2002a). Some elastin fibres at the adhesion site and injured site of tendon were visible only up to day 7. Later on elastin fibres were seen particularly

in blood vessels and the areas where blood vessels entered the tendon. These fibres may be necessary for the protection and maintenance of the vessels, which are subjected to unusual stress because of the mobility of the tendon (Greenlee *et al.* 1976). Reticular fibres (Gridley's silver staining) were observed only up to day 7 and day 14 intervals. Thereafter no reticular fibres were observed. The disappearance of reticular fibres after day 14 might be due to their maturation and transformation into collagen fibres (Dellman and Brown 1983). Periodic Acid Schiff staining for distribution and concentration revealed only mild activity up to day 7 and further no activity indicated the more concentration of mucopolysaccharides at earlier phase of injury, which later diminished out.

Histochemical observations: In test group the activity on day 7 was moderate (++) but on day 14 it became very mild (+) and almost nil on day 30 and 60 postoperatively. In control group, the intensity of alkaline phosphatase reaction was intense (+++) on day 7 (Fig.5). Later, the activity reduced to nil by day 60. The increased levels of alkaline phosphatase in traumatic area due to tissue injuries were reported to help in proliferation of fibroblasts (Soni *et al.* 1976). Further, this enzyme also appeared to be associated with the metabolic process concerning collagen formation (French and Bendit 1954). In US treated group mild to moderate activity of alkaline phosphatase as compared to control group may be attributed to the action of ultrasound therapy used.

Scanning electron microscopic observation: On day 7 the peritendinous tissue was relatively more vascular and had increased cellularity. Empty spaces between tendon and peritendinous structure ranged from 2 to 5 micron were visible. Animals of control group showed complete adherence with tendon and tendon sheath and was highly vascular. Peritendinous sheath showed irregular and loosely arranged collagen fibres. On day 14 in US group there was further widening of the space between tendon and tendon sheath and ranged from 12 to 15 micron. Tendon sheath was not found completely adhered to tendon and presence of gliding movements was indicative of loosening of adhesions. On day 30 the space between tendon and tendon sheath widened further. Tendon sheath showed loosely arranged collagen fibrils in test group (Fig. 6), whereas, in control group the tendon sheath was found adhered with tendon (Fig.7). Day 60 observations were more or less the same as on day 30. The scanning electron microscopy (SEM) was found to be of immense value in assessing the interaction between tendon and tendon sheath. Ultra structural information allowed studying the event occurring during tendon injury and healing as also reported by others (Kumar *et al.* 2000, Ramesh *et al.* 2003a).

The study showed that treatment with US therapy reduced the formation of peritendinous adhesions. This appears to be a simple and inexpensive means of refined treatment for the management of peritendinous adhesions.

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