Short Paper

The effect of butyric acid with autogenous omental graft on healing of experimental Achilles tendon injury in rabbits

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Summary

In this study, the role of local injection of butyric acid (BA) with autogenous omental graft was evaluated in healing of experimental Achilles tendon injury in rabbits. Nine adult male New Zealand rabbits were anesthetized and a partial thickness tenotomy was created on both hindlimbs. In treated group, omental graft was secured in place using BA soaked polygalactin 910 suture. In control group, the graft was sutured without BA. Butyric acid and normal saline were injected daily to treatment and control groups for three days, respectively. Based on the findings, on day 15 after injury, the tendon sections showed that healing rate in BA treated group was higher than that in control group. Furthermore, at days 28 and 45, comparison between BA treated and control groups demonstrated that BA increased the healing rate but with no significance. In summary, results of this study show that application of BA with autogenous omental graft can improve healing process of damaged Achilles tendon.

Key words: Butyric acid, Omental graft, Achilles tendon

Introduction

Tendons are anatomic structures placed between muscles and bones and transmit force created in muscles to bones and make joint movements possible (Ferrara, 2002). Damages to these structures affect the natural balance between stability and mobility, thus altering joint kinematics and ultimately leading to destruction of the joint (Woo et al., 2005). Blood supply to tendons is relatively poor; therefore, healing often progresses slowly. As a result, the treatment process is slow, results are variable and reinjury is common (Kuo et al., 2003; Bosch et al., 2011; Tohidnezhad et al., 2011). No treatments currently exist to restore injured tendons or ligaments to their normal condition. However, several methods have been described to improve the healing process of injured tendons. Omentum and butyric acid (BA) are two extrinsic factors which have been shown to increase tendon healing rates (Leek et al., 2010; Tracy et al., 2011).

Butyric acid is a short-chain fatty acid formed during the fermentation of complex carbohydrates by bacteria in colon (Hennigsson et al., 2001). Butyric acid has been shown to include proangiogenic properties (Tracy et al., 2011). Results of tendon and meniscal healing studies using BA-soaked sutures revealed an increased neoangiogenesis at the repair sites (Acton et al., 2004; Tracy et al., 2011). The omentum is a sheet-like tissue attached to the great curvature of stomach and contains secondary lymphoid organs called milky spots. This tissue has been used because of its healing potency for more than a century by transposing the omental pedicle on injured organs (Shah et al., 2012). Omentum has been shown to secrete many biological agents including vascular endothelial growth factor (VEGF), a variety of other growth factors and cytokines (Konturek et al., 1994). Furthermore, omentum adipocytes produce angiogenic factors such as aspletin and growth factors with demonstrated positive effects on healing processes (Zhang et al., 1997). In this study, the role of local injection of BA with autogenous omental graft in healing of experimental Achilles tendon injury was evaluated in rabbits.

Materials and Methods

Experiment design

Nine male adult New Zealand rabbits weighing 2 ± 0.2 kg were housed in Animal Housing Facility of the Faculty of Veterinary Medicine, Islamic Azad University, Garmsar Branch. Before the beginning of the experiment, rabbits were housed for two weeks at the
facility for acclimatization. Animals were supplied with
standard pellet diet and tap water *ad libitum* through the
experiment. All animals received sufficient care
according to “Guide for the Care and Use of Laboratory
Animals” published by the National Institute of Health
(NIH), USA.

**Surgical procedure**

Animals were anesthetized through intramuscular
injection of 5% ketamine hydrochloride (35 mg/kg) and
2% xylazine (5 mg/kg). Anesthesia was maintained with
inhalation isoflurane. Surgery was performed on both
hindlimbs; with one left as control. A longitudinal skin
incision was made over the Achilles tendon, and the
paratenon was identified and incised longitudinally as a
separate layer. The three bundles of Achilles tendon were
identified and the central bundle was separated bluntly
from the medial and lateral bundles (Fig. 1A). A partial-
thickness tendon defect (approximately 50% of tendon
bundle width and 1 cm length) was created, beginning at
the medial aspect of the bundle and 2 cm proximal to the
calcaneus (Fig. 1B). This partial tendon defect allowed
the rest of the tendon to act as internal splint for the non-
imobilized repair. The abdominal cavity of rabbits was
approached and a piece of the free end of greater
omentum was removed and placed into the tenotomy
gap. In treatment group, omental graft was secured in
place using No. 4/0 BA soaked polygalactin 910
(Vicryl™) suture (Fig. 1C). In control group, graft was
sutured without BA. Butyric acid and normal saline were
respectively injected locally to treatment and control
groups for three days postoperatively. After the surgery,
rabbits were recovered from the anesthesia in a heated
recovery chamber under continuous observation.
Following recovery, animals were returned to individual
cages for the rest of the experiment. Five percent
enrofloxacin (5 mg/kg, IM) was administrated to rabbits
one hour preoperatively and continued for three days.

**Histopathological studies**

On days 15, 28 and 45 post surgery, three rabbits of
each group were euthanized using sodium thiopental and
Achilles tendon specimens were collected. Specimens
were fixed in 10% buffered formalin and routinely
processed using standard procedures and then stained
with haematoxylin and eosin (H&E). Stained specimens
were microscopically studied to evaluate extent and
severity of the inflammation, angiogenesis, fibroplasia,
and complete tendon healing which were scaled from 0
to 3 by defined criteria (Sai Ram et al., 2000).

**Extent and severity of the inflammation**

- Inflammatory cells were not seen = 0
- Observation of inflammatory cells at two microscopic fields = 1
- Observation of inflammatory cells at 3-5 microscopic fields = 2
- Observation of inflammatory cells at more than 5 microscopic fields = 3

**Angiogenesis**

- Blood vessels were not seen = 0
- Existing of 0-2 blood vessels = 1
- Existing of 3-4 blood vessels = 2
- Existing of more than 4 blood vessels = 3

**Fig. 1:** A) Intact Achilles tendon, B) A partial-thickness tendon defect (approx. 50% of tendon bundle width and 1 cm length) was created, beginning at the medial aspect of the bundle and 2 cm proximal to the calcaneus, and C) Omental graft was placed into the tenotomy gap and secured with polygalactin 910 (Vicryl™) suture

**Fibroplasia**

- Recording of few thin collagen fibers with numerous fibroblasts = 0
- Recording of thin collagen fibers with very numerous fibroblasts = 1
- Recording of thick collagen fibers with numerous fibroblasts = 2
- Recording of abundant thick collagen fibers with few fibroblasts = 3

**Complete tendon healing**

- Observation of inflammatory cells; no observation of blood vessels, fibroblasts and collagen fibers = 0
- Contemporary observation of inflammatory cells, blood
vessels, fibroblasts and collagen fibers = 1
Contemporary observation of blood vessels, fibroblasts and collagen fibers; no observation of inflammatory cells 2
Observation of fibroblasts, thick and compact collagen bundle; no observation of inflammatory cells and blood vessels 3

Statistical analysis
Statistical analysis was carried out using SPSS software Ver. 16.0 (SPSS Inc., USA) and Mann-Whitney U test. Data were expressed as mean ± standard deviation (SD). Differences were considered significant when P<0.05.

Results
The average score of the histopathological changes in the two groups are shown in Table 1. Injured tendons in both treatment and control groups showed hypercellularity and formation of new blood vessels 15 days post operation. However, compared to those of control group, swelling and cellularity of the lesions decreased and fewer blood vessels were seen in treatment group (Figs. 2A, B). The average healing score in treatment group was 1, despite 0.33 in control group. The difference was not significant (P≥0.05). A mild inflammatory response was seen at day 28 post operation in control group, while this response was absent in treatment group. Results of histopathological studies at day 28 showed collagen fiber deposition in parallel alignment in treatment group. Healing seemed to be delayed in control group while the newly regenerated fibrous connective tissue was hypercellular and hyperneovascular (Figs. 3A, B).

At day 45, histopathological results showed thick collagen fibers in parallel arrangement in treatment group. However, the control group showed deposition of thin collagen fibers, high blood vessels and hypercellularity characterized with increased fibroblasts (Figs. 4A, B). Based on the findings at days 28 and 45, the mean healing rate in BA group was higher than that in control group. However, the difference was not significant statistically (P≥0.05).

Discussion
Injuries and degenerative conditions of tendons represent almost 50% of the musculoskeletal injuries treated in orthopaedic clinics (Schweitzer et al., 2010). Like other connective tissue repair processes, tendon repair process has been an attractive subject for the researchers for many years. It is well known that increased blood supply enhances the repair process in all kinds of connective tissues. In tendon healing processes,

<table>
<thead>
<tr>
<th>Day</th>
<th>BA + omental graft</th>
<th>Omental graft</th>
</tr>
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<tbody>
<tr>
<td>15</td>
<td>1 ± 0.5</td>
<td>0.33 ± 0.57</td>
</tr>
<tr>
<td>28</td>
<td>1.33 ± 0.57</td>
<td>1 ± 0.86</td>
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<tr>
<td>45</td>
<td>2 ± 0.86</td>
<td>1.83 ± 0.76</td>
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BA: Butyric acid. Difference was not significant (P≥0.05)

Fig. 2: Photomicrograph of the injured tendon in control group (A), and BA treated group (B) at day 15 post operation. Note less hypercellularity, fewer new blood vessels and better healing process in BA treated group, (H&E, ×100)

Fig. 3: Photomicrograph of the injured tendon in control group (A), and BA treated group (B) at day 28 post operation. Note less hypercellularity and more mature fibrous connective tissue and better healing process in BA treated group, (H&E, ×100)
angiogenesis is critical for the formation of vascular channels and delivery of cytokines necessary for tendon healing and remodeling (Sharma and Maffulli, 2005).

On day 15 after injury, tendon micrographs showed that healing rate in BA treated group was higher than that in control group, however the difference was not significant (P>0.05). Furthermore, at days 28 and 45 after injury, comparison between the BA treated group and control group demonstrated that treatment with BA increased the healing rate with no significant difference. Based on the previous studies, BA possesses strange vasoactive properties and is dose dependent. BA is also known to have antiangiogenic and proangiogenic affects at high and low doses, respectively (Stevenson et al., 1998; Bidder et al., 2000; Tracy et al., 2011). In 2003, Gururaj et al. showed that BA had strong antiangiogenic effects in vivo and blocked the growth of tumor cells with inhibition of vascular endothelial growth factor (VEGF). In 2010, Ciura and Jagodziński demonstrated that BA increased the formation of antiangiogenic VEGF variants in human lung microvascular endothelial cells. Leek et al. (2010) and Tracy et al. (2011) stated that tendons repaired with BA soaked sutures increased angiogenesis at the repair sites. In 2004, Acton et al. found that BA saturated sutures increased neoangiogenesis at the repair sites in meniscal healing studies. However, results of the present study showed that no significant difference existed between BA treated and control groups.

In the current study, autologous omental graft in treatment and control groups was used. The great ability of omentum in revascularization of tissues has been well documented. A number of polypeptide growth factors that possess potent angiogenic properties have recently been identified in greater omentum (Oloumi et al., 2006). This process of neovascularization allows the omentum to provide vascular support, promote function and healing in ischemic or inflamed tissues (Konturek et al., 1994; Williams et al., 1996). In 2002, Ferrara demonstrated that VEGF of omentum stimulated angiogenesis. He concluded that binding of endothelial cells to VEGF receptors possibly promoted the endothelial cell migration and proliferation, which are required for the development of new blood vessels. Furthermore, VEGF increases vascular permeability, which may contribute to angiogenesis. In 1997, Zhang et al. demonstrated that omental adipocytes were the primary source of VEGF protein.

However, no significant differences between treatment and control groups in the current study suggests that positive effects of BA and omentum in angiogenesis affect the collagen organization. The higher average healing rate in BA group compared to control group can be linked to the synergetic effect of BA and omentum on tendon healing. In conclusion, results of this study have shown that application of BA with autogenous omental graft can improve healing process of damaged Achilles tendon.

References


Konturek, SJ; Brzozowski, T; Majka, I; Pawlik, W and Stachura, J (1994). Omentum and basic fibroblast growth
Leek, B; Tasto, J; Tibor, L; Healey, RM; Freemont, A; Linn, MS; Chase, DE and Amiel, D (2012). Augmentation of tendon healing with butyric acid-impregnated sutures biomechanical evaluation in a rabbit model. Am. J. Sport Med., 40: 1762-1771.
Sai Ram, M; Anju, B; Pauline, T; Dipti, P; Kain, AK; Mongia, SS; Sharma, SK; Singh, B; Singh, R; Ilavazhagan, G; Kumar, D and Selvamurthy, W (2000). Effect of Kombucha tea on chromate (VI)-induced oxidative stress in albino rats. J. Ethnopharmacol., 71: 235-240.
Shah, S; Lowery, E; Braun, RK; Martin, A; Huang, N; Medina, M; Sethupathi, P; Seki, Y; Takami, M; Byrne, K; Wigfield, C; Love, R and Iwashima, M (2012). Cellular basis of tissue regeneration by omentum. PLoS. One. 7: 1-11.
Wright, E; Walsh, WR; Stephens, P; Evans, RO; Yu, Y; Hughes, PJ; Acton, D; Perry, A and Bruce, WJ (2004). Meniscal healing using butyric acid impregnated sutures. 50th Annual Meeting of the Orthopaedic Research Society, Poster No: 1234.