

Short Paper

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

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(Received 5 Mar 2014; revised version 5 Jul 2014; accepted 16 Aug 2014)

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 (Henningsson *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

neangiogenesis 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 asleptin 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-immobilized 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 administered 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

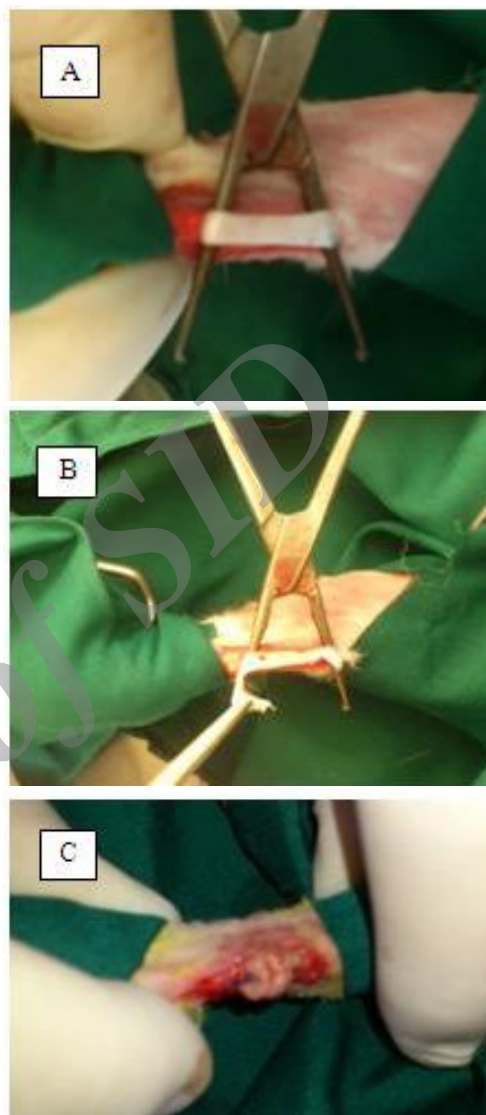


Fig. 1: A) Intact Achilles tendon, B) A partial-thickness tendon defect (approx. 50% of tendon bundle width and 1 cm length) was created, beginning at the medial aspect of the bundle and 2 cm proximal to the calcaneus, and C) Omental graft was placed into the tenotomy gap and secured with polygalactin 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 \pm 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 ($P \geq 0.05$). 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

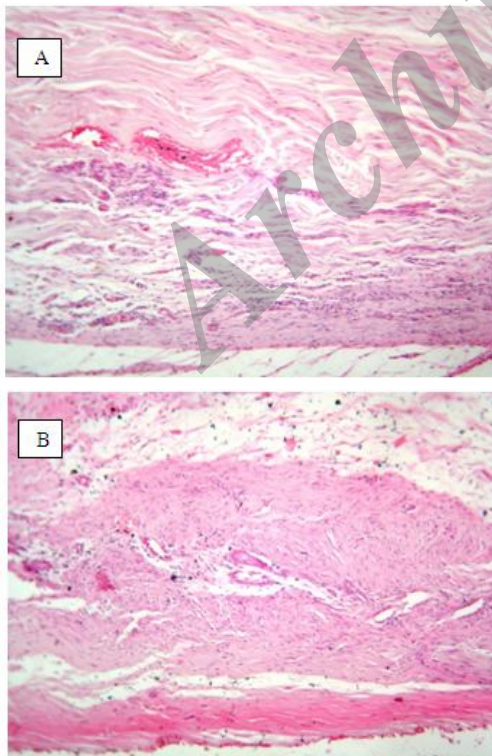


Fig. 2: Photomicrograph of the injured tendon in control group (A), and BA treated group (B) at day 15 post operation. Note less hypercellularity, fewer new blood vessels and better healing process in BA treated group, (H&E, $\times 100$)

Table 1: The average score of the histopathological changes in the two groups

Day	BA + omental graft	Omental graft
15	1 \pm 0.5	0.33 \pm 0.57
28	1.33 \pm 0.57	1 \pm 0.86
45	2 \pm 0.86	1.83 \pm 0.76

BA: Butyric acid. Difference was not significant ($P \geq 0.05$)

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 ($P \geq 0.05$).

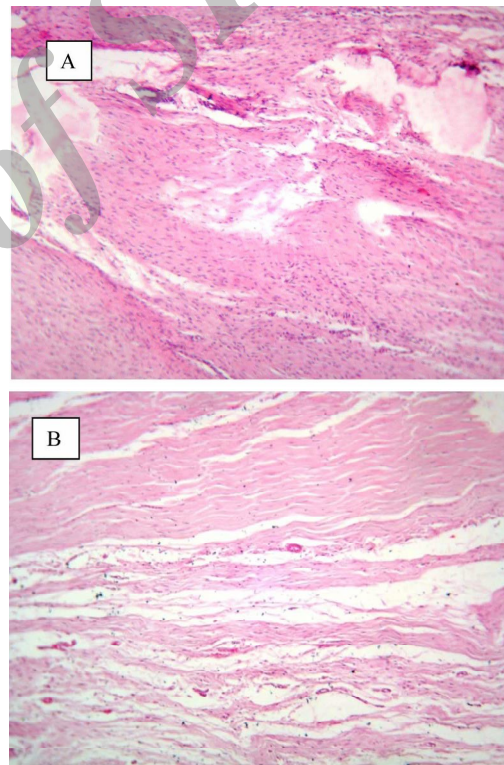


Fig. 3: Photomicrograph of the injured tendon in control group (A), and BA treated group (B) at day 28 post operation. Note less hypercellularity and more mature fibrous connective tissue and better healing process in BA treated group, (H&E, $\times 100$)

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,

angiogenesis is critical for the formation of vascular channels and delivery of cytokines necessary for tendon healing and remodeling (Sharma and Maffulli, 2005).

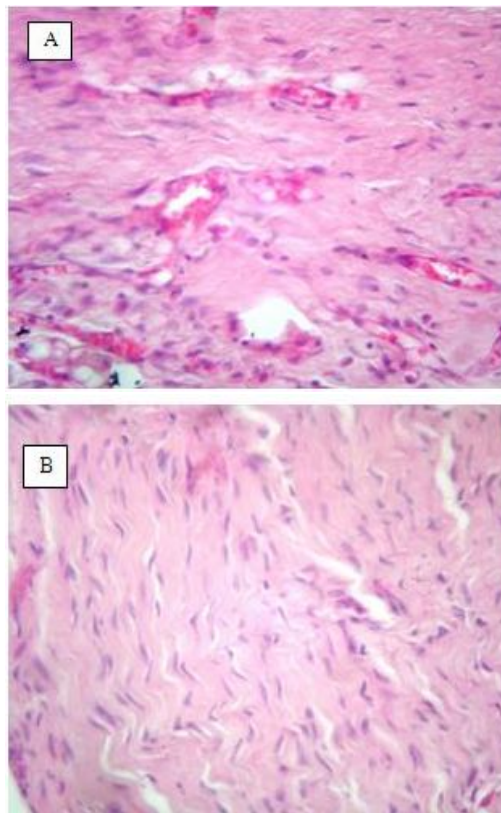


Fig. 4: Photomicrograph of the injured tendon in control group (A), and BA treated group (B) at day 45 post operation. Note less hypercellularity, fewer new blood vessels, more mature fibrous connective tissue and better healing process in BA treated group, (H&E, $\times 400$)

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 ($P \geq 0.05$). 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 effects 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 Jagodzinski 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 synergic 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.

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مطالعه اثر اسید بوتیریک همراه با پیوند امتوم خودی بر التیام تاندون آشیل در خرگوش

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(دریافت مقاله: ۱۴ اسفند ۱۳۹۲، پذیرش نهایی: ۲۵ مرداد ۱۳۹۳)

هدف از انجام این مطالعه، ارزیابی تزریق موضعی بوتیریک اسید همراه با پیوند امتوم خودی در التیام نقیصه ایجاد شده در تاندون آشیل خرگوش می‌باشد. برای انجام این تحقیق ۹ قطعه خرگوش سفید نیوزیلندی تهیه و با استفاده از ترکیب کتامین و زایلازین، بیهوش شدند. در ادامه و پس از آماده سازی آسپتیک و شان گذاری در پای راست و چپ، در بالای استخوان کالکانئوس شکافی در پوست داده شد. پس از رویت تاندون آشیل، برداشت ناقص تاندون در ناحیه میانی آن انجام گرفت. سپس این فضا با استفاده از بافت امتوم خودی پر شده و در گروه آزمایش، ابتدا و انتهای آن با استفاده از نخ پلی گالاکتین ۹۱۰ آغشته به اسید بوتیریک، به دو سر قطع شده تاندون بخیه گردید. همچنین در گروه‌های مورد مطالعه و کنترل به ترتیب تزریق اسید بوتیریک و نرمال سالین، به صورت روزانه و تا روز سوم ادامه یافت. به منظور مشاهده روند ترمیم ضایعات و

پاسخ‌های بافتی و مطالعه هیستوپاتولوژی در روزهای ۱۵، ۲۸ و ۴۵، سه قطعه خرگوش به روش انسانی معدوم شده و پس از جداسازی، تاندون مورد ارزیابی هیستوپاتولوژی قرار گرفت. نتایج فاکتورهای مورد مطالعه میانگین التیام بالاتری را در گروه درمان شده با بوتریک اسید در مقایسه با گروه کنترل نشان داد، اگر چه این اختلاف معنی‌دار نبود. بنابراین بر اساس نتایج حاصله می‌توان بیان داشت تزریق بوتریک اسید همراه با پیوند امنوم خودی می‌تواند روند التیام را در جراحی تاندون آشیل در خرگوش بهبود بخشد.

واژه‌های کلیدی: اسید بوتریک، پیوند امنوم خودی، تاندون آشیل