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Histological and biomechanical analysis of the effects of streptozotocin-induced type one diabetes mellitus on healing of tenotomised Achilles tendons in rats

Zhaleh Mohsenifar [a,1], Mohammad Javad Feridoni [b,1], Mohammad Bayat [b,*], Reza Masteri Farahani [b,1], Shiva Bayat [c,1], Amir Khoshvaghti [d,1]

[a] Pathology Department, Ayatallah Taleghani Hospital, Shahid Beheshti University of Medical Sciences, Tehran 1985717443, Iran
[b] Anatomy and Biology Department, Medical Faculty, Shahid Beheshti University of Medical Sciences, Tehran 1985717443, Iran
[c] Shiraz University of Medical Sciences, Shiraz, Iran
[d] AJA University of Medical Sciences, Tehran, Iran

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ABSTRACT

Background: Tendon healing is impaired in patient with diabetes mellitus. The effects of streptozotocin-induced type 1 diabetes (STZ-D) on the healing of the transected Achilles tendon in rats was studied.
Methods: In the experimental group, type one diabetes was induced via administration of STZ. The right Achilles tendon of all the rats was transected 30 days after the STZ administration. The Achilles tendons were examined for biomechanical and histological examinations.
Results: The statistical analysis showed that Young's modulus of elasticity and stress tensile load of the control group were significantly higher than those of the experimental group, and inflammation in the experimental group was significantly higher than that in the control group. At the same time, fibrosis in the experimental group was significantly lower than that of the control group.
Conclusion: Induction of type 1 diabetes by STZ significantly delayed the healing of the transected Achilles tendon in rats.

1. Introduction

Diabetes mellitus (DM) is a common and serious disease. Type 1 DM is one of the diseases with best animal models, which have been used extensively in recent years to clarify the etiology and pathogenesis of the disease as well as to investigate how to prevent its development and progression [1,2]. Several studies indicate that streptozotocin-induced diabetic rats serve as a useful model for mechanisms related to wound healing in general [3,4] and tendon inflammation in particular [5].

People with DM are more prone to develop problems with musculoskeletal system than are normal people [6,7]. Chen et al. reported that the repair of the diabetic rotator cuff could be performed with the exception of improved motion and function and thus concluded that the surgeon should remain cognizant that a higher rate of complications, infection in particular, might occur after rotator cuff repair in the diabetic population [8]. Cavanagh et al. in their review article reported that surgery to heal ulcers and prevent recurrence could include tenotomy, tendon lengthening, reconstruction, or removal of bony prominences. Be that as it may, these procedures may result in secondary ulceration and other complications [9]. Bruggeman et al. reported that diabetes mellitus is one of the risk factors of open Achilles tendon repair and is associated with an increased rate of wound complications [10].

The injured tendon is first filled by exudated fibronectin and fibrin, which form a scaffold for the migration of various cells and stimulate vascular ingrowth [5]. During tendon injury there is a requirement for cell infiltration from the blood vessel to provide the necessary reparative factors for tissue healing. However, mature tendon is poorly vascularized [11]. Recently Marolais et al. reported injured tendon exhibited a specific sequence of inflammatory cells [12]. Kawamura et al. hypothesis that cytokines produced by infiltrating macrophages in tendon healing are likely to contribute to formation a fibrous scar tissue interface rather than a normal insertion site [13].

* Corresponding author. Tel.: +98 021 22439976/2122439976; fax: +98 021 22439976/2122439976.
E-mail addresses: bayat_m@yahoo.com, mohbayat@sbmu.ac.ir, bayatenator@gmail.com (M. Bayat).
[1] Tel.: +98 2122439976; fax: +98 2122439976.

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Although the tendon healing process has been well studied in healthy animals, the impact of sustained hyperglycemia on tendon healing has not been well characterized [14,15]. Indeed, such information has critical implications for defining the appropriate non-operative and surgical management of tendon injuries in diabetic people.

The present study aimed to determine the effect of STZ-induced type 1 DM on the early phase of Achilles tendon healing in rats.

2. Materials and methods

2.1. Animals

Forty-six adult male Wistar rats (4 months old and weighing 250–350 g) were used in this study. The rats were housed in cages made of poly propylene, in a 12-h light/dark environment and provided with water and rat chaw (standard diet) *ad libitum*. All the procedures were approved by the Medical Ethics Committee of Shahid Beheshti University of Medical Sciences (protocol no 13.12347), Tehran, Iran. The rats were randomly divided into control ($n = 23$) and experimental ($n = 23$) groups.

We induced type 1 DM by the administration of a single dose of STZ. At 30 days following STZ administration [16] we transected the rats right Achilles tendons. At days 5, 10, and 15 post-surgery on the early phase of Achilles tendon healing in rats [17–19], we examined the extent of tendon repair in six rats of each control and experimental groups by light microscopy. Additionally, one tendon of each group was examined by transmission electron microscopy. At ten days post-surgery the Achilles tendons of six rats were submitted to a biomechanical test [16]. We calculated the stress tensile load (MPa/mm^2) and Young's modulus of stiffness (MPa/mm^2) [16]. Data from the diabetic rats were compared to the data from the non-diabetic control rats.

Recently Ahmed et al. investigated the effect of type 2 DM on tendon repair in 11 Goto–Kakizaki (GK) rats. In this study the rats' right Achilles tendons were transected. At two weeks post-injury the researchers assessed the intact and injured tendons by biomechanical testing, histology, and gene expression analysis. The results showed impaired tendon healing in the type 2 DM rat model. This impairment was primarily attributed to altered expression of collagen and MMPs that reflected decreased degradation of matrix proteins and impaired tissue remodeling [14]. In another study Egemen et al. studied the effect of streptozotocin- (STZ) induced type 1 DM on the biomechanical and histological properties associated with healing in rats with injured Achilles tendons [15]. At three weeks after induction of diabetes both Achilles tendons from each rat were transected, despite the number of studies that chose to transect only the Achilles tendon from one hind limb of each rat [16–19]. At weeks 2, 4, and 6, they examined the left Achilles tendons of rats by a subjective histological test. The right legs (Achilles tendons) were prepared for mechanical testing in order to measure maximum force (N). The researchers determined that the diabetic animals had significantly less proliferation of fibroblasts and lymphocytes compared to the control group. They noted a significant delay in tendon strength in the diabetic rats [15].

2.2. Induction of type 1 DM

Type 1 DM was induced in the rats of the experimental group by an intraperitoneal injection of the pancreatic β-cell toxin streptozotocin (Zanosar Pharmacia and Upjohn Co, Kalamazoo, MI, USA) freshly dissolved in sterile distilled water (pH = 7.3) at a single dose of 55 mg/kg body weight [20]. The rats of the control group received a control injection of distilled water. DM was defined as a blood glucose concentration greater than 250 mg/dl in an orbital sinus blood sample (Gm 300, Biomine, Gm H, Heerbrugg, Switzerland) seven days after the STZ injection [20]. The blood glucose level was monitored every two weeks throughout the study. All the diabetic rats were kept for 30 days after the streptozotocin injection [16] and most of them were sacrificed on 35 and 40 as well as 45 days after streptozotocin injection for examinations. The rats' weight was recorded regularly during the study period.

2.3. Tenotomy

Anesthesia was administered via an intramuscular injection of 50 mg/kg ketamine and 5 mg/kg diazepam. Antibiotic therapy with Ceftriaxone (Ceftrax, Jaber ibn Hayan, Tehran, Iran) at a dose of 50 mg/kg was administered intramuscularly immediately before surgery and at 24 and 48 hours after surgery. The tendon was approached by a medial skin incision, and the tendon was released from the surrounding soft connective tissue via this incision. After resecting the tendon of the plantaris muscle, the Achilles tendon was transected with a scalpel 5 mm above its insertion into calcaneus. Both ends of the transected Achilles tendon were approximated and immediately repaired with 4/0 nylon using the modified Kessler suture technique and the skin was subsequently closed.

In each group, 5 of the repaired Achilles tendons were prepared for biomechanical examinations, 15 of the repaired Achilles tendons for light microscopical examinations, and the remaining 3 of the repaired Achilles tendons were prepared for transitional electron microscopical examinations.

2.4. Biomechanical examination

On day 10, the rats were killed by inhalation of chloroform in a closed space. After careful dissection from the surrounding tissues, the tendon was excised from the musculotendinosus junction proximally. The tendon with the attaching calcaneal bone was removed. The transverse and anterior-posterior diameters of the specimens (repairing tissue) were measured as an index of thickness of the specimen with a digital caliper. The musculotendinosus end was fixed between two paper strips to the upper jaw, and the calcaneus was mounted onto the lower jaws of the machine. Biomechanical measurements were carried out using a material testing machine (Zwick – Roell, Germany). Tensile load was then applied at a displacement rate of 1 mm/sec. Load characteristics were directly plotted on an *X–Y* chart recorder, and a force displacement graph was obtained. All the strength characteristics of the specimens were calculated from that part of the load displacement (deformation) curve. All the specimens displayed typical load-deformation curve with an upward linear slope and a failure response at the point of failure.

Load to failure was defined as the ability of the specimen to resist a tensile load before rupture. Stress tensile load (M Pa/mm^2) was calculated by means of dividing the tensile load value by the surface area (mm^2) of the specimen. Young's modulus of stiffness was calculated by dividing the load to failure by displacement to failure (M Pa/mm^2) [18].

2.5. Light microscopical examination

The rats from the control ($n = 15$)and experimental ($n = 15$)groups were sacrificed on day 5 ($n = 5$ per group), day 10 ($n = 5$ per group), and day 15 ($n = 5$ per group). Tendons were extracted and fixed by 10% formalin saline. The samples were prepared for light microscopical examination. Five micrometer longitudinal sections, including the repairing tissue, were stained with the Hematoxylin and Eosin method and five slides from each