Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Effect of systemic carnitine therapy on serum fibronectin level in diabetic rats

Erkam Kömürcü, MD,[a,*] *Ömer Faruk Özkan, MD,*[b] *Ahu Sarbay Kemik, PhD,*[c] *Gürdal Nusran, MD,*[a] *Mehmet Aşık, MD,*[d] *and Emrah Arslan, MD*[e]

[a] *Department of Orthopedics and Traumatology, Faculty of Medicine, Çanakkale Onsekiz Mart University, Çanakale, Turkey*
[b] *Department of General Surgery, Faculty of Medicine, Çanakkale Onsekiz Mart University, Çanakale, Turkey*
[c] *Department of Biochemistery, Cerrahpasa Medical School, Istanbul, Turkey*
[d] *Departmant of Endocrinology and Metabolisma, Çanakkale Onsekiz Mart University, Çanakkale, Turkey*
[e] *Department of Plastic and Reconstructive Surgery, Çanakkale Onsekiz Mart University, Çanakkale, Turkey*

ARTICLE INFO

Article history:
Received 20 August 2013
Received in revised form
8 November 2013
Accepted 20 November 2013
Available online 27 November 2013

Keywords:
Carnitine
Fibronectin
Flap viability
Diabetes mellitus

ABSTRACT

Background: L-carnitine has been shown to enhance wound healing. There has, however, not been sufficient research on the effect carnitine has on diabetic wound healing. We investigated the relationship between the viability of full thickness skin grafts (FTSGs) and fibronectin (FN) serum levels in diabetic rats that were administered carnitine.

Materials and methods: A total of 40 rats were divided into four groups of 10 rats each and operated on. The FTSG model was 10 × 3 cm, with the dorsal flap extending from the tip of the scapula to the hip joint. After surgery, group 1 (nondiabetic control, $n = 10$) and group 2 (diabetic control, $n = 10$) were given a sterile saline solution at 0.9% with a dose of 100 mg/kg/d intraperitoneally for 7 d after the surgery. Group 3 (diabetic sham, $n = 10$) contained diabetic rats and did not receive any agent after the surgery. The diabetic rats in group 4 (carnitine study diabetic, $n = 10$) were given carnitine with a dose of 100 mg/kg/d intraperitoneally for 7 d after the surgery.

Results: The percentages of viable areas in groups 1–4 were 70.38 ± 6.10%, 62.66 ± 1.55%, 62.59 ± 2.94%, and 73.48 ± 4.43%, respectively. The mean levels of FN, measured in milligram per deciliter, in groups–4 were 23.57 ± 3.27 mg/dL, 21.58 ± 2.35 mg/dL, 22.04 ± 2.71 mg/dL, and 27.11 ± 2.79 mg/dL, respectively. Furthermore, we found that there was a strong positive correlation ($R = 0.509$; $P = 0.001$) between FN and the viability of the FTSG.

Conclusions: We demonstrated that administering carnitine leads to an increase in diabetic wound healing. Further increasing the levels of the FN serum might have a role in this process.

1. Introduction

Successful surgical results depend on wound healing, which in turn can depend on factors, such as the presence of infections and malnutrition, and different chronic diseases, such as diabetes mellitus (DM) [1]. DM leads to a decrease in immune system functions, including functions of polymorphonuclear leukocytes, fibroblasts, collagen synthesis,

* *Corresponding author*. Department of Orthopedics and Traumatology, Faculty of Medicine, Çanakkale Onsekiz Mart University, Çanakale, Turkey. Tel.: +90 286 263 59 50; fax: +90 286 263 59 56.
E-mail address: erkakom@yahoo.com (E. Kömürcü).

0022-4804/$ – see front matter
http://dx.doi.org/10.1016/j.jss.2013.11.1101

neovascularization, and granulation. Especially diabetic foot and wound healing surgery poses a major challenge for clinicians. However, the etiologic factors of impaired wound healing in patients with DM are not completely understood yet.

Recently, studies demonstrated that fibronectin (FN) plays a significant role in wound healing. FN is a high-molecular-weight glycoprotein that can be subdivided into two types: (1) plasma FN and (2) cellular FN. Plasma FN supports hemostasis; it helps control infections, improves epithelialization, and regulates tissue granulation [2]. However, it has been shown that plasma FN levels are reduced in diabetic wounds [3]. Several studies have found that wound healing improved with the application of topical FN in DM [3–5].

L-carnitine (Carnitene; Sigma Tau, Rome, Italy) is an endogenous ester and its intracellular functions have been well described [6]. It can enhance wound healing as well. It may be involved in repairing the energy gap that metabolic disorders and immune suppression as well as burned flaps can cause in wound healing, according to the experimental studies [7]. Previously, studies have reported imbalances in carnitine metabolism in DM [8]. Carnitine treatment has been shown to significantly improve the recovery of the ischemic myocardium in diabetic rats [9]. However, there are no reports of the use of carnitine in diabetic wounds. It could play a critical role in the treatment of disrupted diabetic wound healing.

We hypothesize that carnitine could have a positive effect on diabetic wound healing by increasing plasma FN serum levels. In the present study, we aimed to determine if there is any association between carnitine, FN, and the viability of full thickness skin grafts (FTSGs) in experimental DM in rats.

2. Materials and methods

2.1. Animals

All procedures of the present study were reviewed and sanctioned by the Animal Research Ethical Committee of the Canakkale Onsekiz Mart University Medical School (No. 212/26). All of the animal experiments were conducted in compliance with the "Guide for the Care and Use of Laboratory Animals", published by the US National Institute of Health (revised 1985). Forty male Sprague–Dawley rats, weighing 250–300 g, were used in the study. During the experimental procedure, all rats were housed under standard laboratory conditions with an artificial 12-h light–dark cycle and controlled temperatures ($22 \pm 1^{\circ}C$) as well as relative humidity. All rats were caged individually and allowed free access to food and water in polycarbonate units. The rats were observed for 7 d in the animal care laboratory to exclude any possibility of underlying diseases.

2.2. Induction of diabetes

Before starting the test procedure, the body weight of the animals was measured on an analytical balance and noted. Diabetes was induced in the rats through a single 65 mg/kg intraperitoneal injection of the ß-cell toxin, streptozotocin (STZ, freshly dissolved in sterile saline, 0.9%; Sigma Chemical Co, St. Louis, MO) in the pancreas. Using an Accu-Check Advantage glucometer (Boehringer Mannheim Corp, Indianapolis, IN), blood glucose levels were monitored. Seven days after the STZ injection, animals with blood glucose levels >300 mg/dL were defined as diabetic and used in the study [3].

2.3. Experimental groups

Animals were divided into four independent groups of 10 rats each.

Group 1 (control nondiabetic, $n = 10$):

After surgery, a sterile saline solution at 0.9% with a dose of 100 mg/kg/d (~3 cc) was given intraperitoneally for 7 d [10].

Group 2 (control diabetic, $n = 10$):

After surgery in diabetic rats, a sterile saline solution at 0.9% with a dose of 100 mg/kg/d was given intraperitoneally for 7 d.

Group 3 (sham diabetic, $n = 10$):

After surgery in diabetic rats, no agent was given.

Group 4 (carnitine study diabetic, $n = 10$):

After surgery in diabetic rats, carnitine was given intraperitoneally with a dose of 100 mg/kg/d for 7 d.

2.4. Surgical procedure

Rats were anesthetized with an intramuscular injection of ketamine HCL (50 mg/kg, Ketalar; Parke-Davis, Morris Plains, NJ) and xylazine (10 mg/kg, Rompun; Bayer, Istanbul). The dorsal skin of the rats was shaved and then prepared with Betadine (10% povidone iodine). The models of the dorsal FTSG were prepared as defined by Khouri *et al.* [11]. In this model, a 10×3 cm FTSG extending from the tip of the scapula to the hip joints was marked (Fig. 1A and B). The marked section was elevated and then sutured back to its original site with 10 separate mattress 4-0 polypropylene (Prolene; Ethicon, NJ) sutures. Then, the animals were placed in individual cages, and given food and water *ad libitum*. All rats were anesthetized on the eighth postoperative day. Blood samples were collected from the intracardiac space. The animals were then sacrificed (Fig. 2 A and B). Samples were stored at $-70^{\circ}C$ and then transported to the laboratory to measure the FN serum levels. The viability of the skin FTSGs was evaluated by measuring the surviving FTSG area using a transparent graph paper. The percentage of FTSG viability was calculated using the following formula: percentage = (surviving tissue area/total FTSG area) × 100 [10,12].

2.5. FN assay

Blood samples intended for evaluating FN levels were collected in standard sampling tubes. The samples were centrifuged at 3000*g* for 15 min. Serum samples were stored at $-70^{\circ}C$ until they could be analyzed. All samples were