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Pentoxifylline improves cutaneous wound healing in streptozotocin-induced diabetic rats

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ABSTRACT

Worldwide, 15% of the 200 million diabetics suffer from diabetic wounds. In 1997, the cost for amputation of toes and limbs that resulted from infected diabetic foot ulcers ranged from $25,000–$40,000 per incident. Increasing numbers of research have shown the positive influence of pentoxifylline (PTX) on healing skin wounds. In this study, we evaluate the effect of systemic PTX (25 mg/kg bid) on wound healing in 80 diabetic rats (DB) by secondary intention. Wounds (20 mm × 5 mm) were identically inflicted on the skin area of the backs of all rats. On day 15 following surgery, a band of skin (4 mm × 60 mm) that contained wound was extracted for biomechanical testing. For histologic analysis, both experimental (DB+PTX) and control, receiving distilled water (DB+DW) groups were further subdivided into day 3 and 7 groups. Rats were sacrificed three and seven days after surgery, and a sample from each wound was taken. All specimens were sectioned stereologically and stained with H&E. Cell counts were performed by stereological methods. Semi-quantitative evaluation of matrix metalloproteinases (MMPs) and inhibitor-1 was performed by Reversed Transcription-PCR and UVI TEC software. For statistical analysis we used student's *t*-test. Collectively, the results of this study demonstrate that there was significant improvement with PTX in all biomechanical parameters. Histologically, PTX reduced inflammation by day seven. Quantitatively, by day five, PTX reduced expression of MMPs and increased TIMP-1 expression. These findings revealed that PTX significantly improved wound healing indices in streptozotocin-induced DB.

1. Introduction

The prevalence of diabetes mellitus has increased tremendously world-wide. Complications arising from diabetes have become serious public health issues, of which one such complication is impaired wound healing. Currently, 15% of the 200 million diabetes suffer from wounds that do not easily heal (De Fronzo et al.,1992; Groop et al., 1993). Complications arising from diabetes have become serious public health issues, of which one such complication is impaired wound healing (Brem and Tomic-Canic, 2007). Healing impairment in diabetes is characterized by delayed cellular infiltration and granulation tissue formation, decreased collagen organization, diminished blood flow, increased blood viscosity and reduced angiogenesis (Goodson and Hunt, 1977; Bohlen and Niggl, 1979; Sebag et al., 1994). Studies have shown that wound healing in diabetic rats (DB) following full-thickness skin excisions is significantly delayed with decreased tensile strength in comparison to normoglycemic rats (Andriessen and Oxlund 1987; Bitar, 1998). Simpson has reported that altered blood viscosity in diabetic patients produces significant changes in microvascular flow patterns resulting in ischemia in the absence of a specific anatomical lesion (Simpson, 1985). These abnormalities are presumed to be the cause of numerous problems for diabetics such as choroidal blood flow, neuropathy, and wound healing (Simpson, 1985).

Dose-dependent administration of pentoxifylline (PTX) (Falanga et al., 1999) interferes with inflammation by increasing blood flow and modulating or blocking the inflammatory actions of interleukin-1 (IL-1) and TNF-α on neutrophils, monocytes and macrophages (Poulkaris et al., 1999). PTX inhibits the amount of free intracellular calcium in polymorphonuclears, improves ocular blood flow in patients with diabetic retinopathy (Sebag et al., 1994) and possibly diminishes tissue damage caused by neutrophils under various conditions (Sullivan et al., 1988).

Matrix metalloproteinases (MMPs) are a group of proteolytic enzymes that collectively degrade most, if not all, components of the extracellular matrix (ECM). Recent studies have explained the potential role for MMPs in inflammation, tissue destruction and scar formation (Kang et al., 2005). This capability is necessary for active remodeling of connective tissue in fetal development, cancer invasion and metastasis, as well as wound healing.

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PTX may affect some cytokines such as,Tumor necrosis factor-α (TNF-α), IL-1, interleukin-6 (IL-6), interleukin-8 (IL-8), VEGF and TGF-β1 (Poulkaris et al., 1999; Zhou et al., 2009; de Campos et al., 2008; Sullivan et al., 2001; Vuković and Lapcevic, 2006). These cytokines impact the quantitative expression of MMPs and their inhibitors (Subramaniam et al., 2008; Yager et al., 1996; Blakytny and Jude, 2006; Ferrari et al., 2010). In this study we investigate the effects of PTX on wound healing in streptozotocin-induced diabetic rats.

2. Materials and methods

This study was approved by the Medical Ethical Committee at Shahid Beheshti University of Medical Sciences, Tehran, Iran (protocol no. 89-01-91-7397). Eighty male Wistar rats that weighed 250–350 g were obtained from Pasteur Institute of Iran. Rats were maintained one per cage with free access to food and water in a room with controlled humidity and temperature (22–24 °C) on a 12-h light/dark cycle.

2.1. Induction of type 1 diabetes mellitus

Eighty rats, after a 12-h fast, each were given single intraperitoneal injections of streptozotocin (Zanosar Pharmacia & Upjohn Co., Kalamazoo, Ml 49001, USA) at a dose of 55 mg/kg body weight in distilled water (DW). Seven days after the streptozotocin injection, blood glucose measurements (Biomine, Rightesttm GM300, Biomine Corporation, Switzerland) were taken from tail blood. Type one diabetes was defined for each animal if blood glucose levels were consistently above 300 mg/100 ml one week after the injection (Karasoy et al., 2002; Vuković and Lapcevic, 2006). During this period diabetic rats (DB) showed clinical signs of diabetes mellitus such as polyuria, polyphagia and weight loss. After 30 days of consistent hyperglycemia, animals were considered eligible for the remainder of the study. During the study blood glucose levels for all rats were measured every three days.

2.2. Wounding model

To provide the incisional wound (day 0) each animal was anesthetized with ketamine (50 mg/kg) and daizepam (5 mg/kg). The backs of the rats were shaved and a full-thickness incisional wound (20 mm × 5 mm; Fig. 1) was made to the level of the panniculus carnosus muscle (Figs. 1 and 2). Wounds were not sutured or covered, but were allowed to heal by secondary intention. The wounds were inflicted in the same manner for all rats.

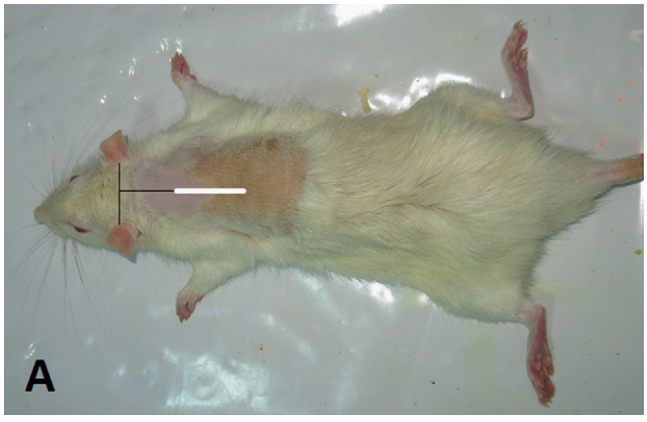

Fig. 1. White line shows the place of open wound which was consistent for all rats.

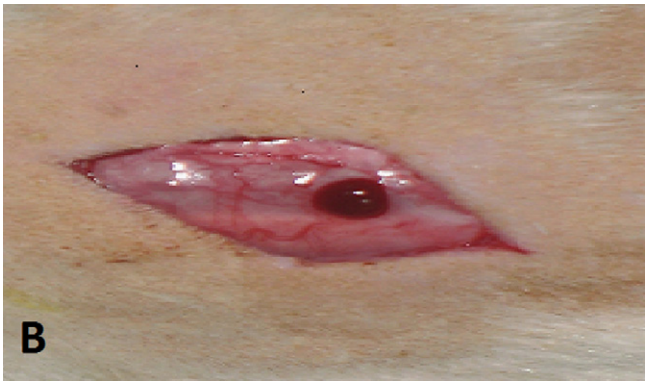

Fig. 2. Pattern of open wounds on the backs of the rats.

Fig. 3. Excised skin strip (4 mm width and 60 mm length) for biomechanical testing.

2.3. Pentoxifylline (PTX) administration

PTX (Sigma–Aldrich, St. Louis, MO, USA) was administered at a dose of 25 mg/kg (bid) until the end of each analysis (biomechanical test, histologic examination,and semi-quantitative evaluation of MMPs-1, -3 and TIMP-1 expressions) in this study. PTX was resumed seven days before the onset of the above mentioned analysis of the study.

2.4. Macroscopic assessment

Body weight and blood glucose levels were measured on the day of surgery (day 0) and every three days after surgery until the end of each analysis (biomechanical, histologic, semiquantification of MMPs and TIMP-1 gene expression).

2.5. Biomechanical test

On day 15, fourteen diabetic (experimental: $n=7$; control: $n=7$) rats were sacrificed. Strips of skin that included the healed wounds (60 mm × 4 mm) oriented perpendicular to the long axis of the body were uniformly excised (Fig. 3) All skin strips were immediately placed on a material testing machine (Zwick-Roell, Z 25-ph1F, Germany) for evaluation of the biomechanical parameters. Specimens were held in jaws and stretched at a constant speed of 6 cm/min until the skin strips were ruptured. Load–deformation curves and related parameters [work-up to maximum force (W up to F max-Nmm) and maximum stress (Rm-N/mm^2)] were analyzed by computer to compare biomechanical test parameters in the experimental (DM+PTX) and control (DM+DW) groups.