HEALING EFFECT OF TOPICAL NIFEDIPINE ON SKIN WOUNDS OF DIABETIC RATS

ABBAS EBADI[**], A. MAJID CHERAGHALI[*], HASSAN QOSHONI[*] and HOSSAIN EIMANI[*]

[*]Faculty of Medicine and [**]Faculty of Nursing, Baqyyatollah University of Medical Science, Tehran, Iran

ABSTRACT

Non-healing foot ulcers in patients with diabetes are the leading causes of complications such as infection and amputation. Ulceration is the most common single precursor to amputation and has been identified as a causative factor in 85% of lower extremity amputations. It seems that poor outcomes are generally associated with infection, peripheral vascular disease and wounds of increasing depth. Nifedipine, a calcium channel blocker that is mainly used for the treatment of cardiovascular disorders has recently been used to treat wounds caused by peripheral vascular disorders. In present study topical Nifedipine 3% has been used to treat skin wounds in normal and diabetic rats. Effects of Nifedipine were evaluated in three different phases of wound healing process. In both experiments (normal and diabetic rats) topical Nifedipine significantly improved inflammatory phase. However, maturation phase was only significantly improved in diabetic rats. Nifedipine did not affect proliferation phase in either group significantly. Overall results of this study showed topical Nifedipine improved skin wound healing process in normal and diabetic rats.

Keywords: Diabetes, Skin wounds, Topical Nifedipine

INTRODUCTION

The prevalence of diabetes and its related lower extremity ulcers mandates the need for a consistent coordinated multidisciplinary approach to wound care. Glycemic control, nutritional support, off loading the extremity, treatment of possible infection, ensuring of the appropriate blood flow, adequate and proper debridments and appropriate topical wound care are among the most used medical interventions (1). Diabetes continues to be one of the most common underlying factors associated with lower extremity amputation.

Amputation is perhaps the most important complication of diabetes that is recognized by the patients and is sometimes associated with an increased risk of reamputation of the same extremity and an elevated mortality rate in the first 3-5 years after amputation and placement of the patients in nursing homes or extended care facilities. Peripheral neuropathy, ulceration, infection and peripheral vascular disease are among the most common causes that lead to limb loss. Ulceration is the most common single precursor and has been identified as a causative factor in 85% of lower extremity amputations. It seems that poor outcomes are generally associated with infection, peripheral vascular disease and wounds of increasing depth, and the cumulative effects of these comorbidities would progressively contribute to great likelihood of amputation (2)

The prevention and treatment of chronic wound includes many stages such as the use of various wound dressing, bandages, antimicrobial agents, footwear, physical therapies and educational strategies (3).

Nifedipine, a calcium channel blocker that is mainly used for the treatment of cardiovascular disorders has been used to treat wounds caused by peripheral vascular disorders (4-7).

In this study the healing effect of the 3% Nifedipine ointment on skin wounds of diabetic rats has been investigated.

Methods and Materials

Male Wister rats, 180-200 g, were purchased from Iran Pasture Institute and housed for one week prior to any experiment in animal house, at room temperature with 12 hours dark/light cycle and free access to food and water. During the experiment each animal was housed in a

Correspondence: Dr A. Majid Cheraghali, Department of Pharmacology, Baqyyatollah Medical Science University, Tehran, Iran, Email: m.cheraghali@bmsu.ac.ir

separate cage. All solvent and chemicals were either analytical or pharmaceutical grade.

Rats were divided randomly to four experimental groups. Each group consisted of 8-10 rats. Diabetes was induced in rats by I.P. injection of 75 mg/kg of Streptozosin (8). Rats' average blood glucose was 70-80 mg/dl prior to the experiment and increased to 250-300 mg/dl. Water intake and urination were also drastically increased. Blood sugar was measured in blood sample which were obtained from the tail of the rats using Glucometer (Bohringer-Mannheim). Rats were anesthetized and part of their back skin, 1.5 cm in diameter, was removed using sterile methods. Wounded animals were housed in a separate cage and divided in four groups. Two groups, normal and diabetic, both received dressing with ointment base (placebo) for their wounds as control groups. Two other groups, normal and diabetic, both received dressing with Nifedipine ointment 3% as treatment. In all groups dressing were changed once a day. In each group samples from wound of two anesthetized rats collected on days 3 and 7-post experiment. Rest of the animals were sampled two weeks after experiments. Skin samples were immediately fixed using formalin 10% and further processed for histological studies. Three different methods of staining including routine hematoxilin and eosin staining, trichrom and mason staining and reticholin staining were used in this study. Based on published methods a three stage healing process was used for evaluation of final process of the wounds and its scoring (9-10). Data were analyzed using SPSS software and paired t-test was used for comparison of the results.

RESULTS

Wound healing process between different experimental groups was evaluated using scoring system in three healing phases including inflammation, proliferation and maturation. Results are summarized in tables 1 and 2.

Normal rats, which were treated with Nifedipine ointment, showed significant improvement in inflammatory phase. However, there was no significant difference in proliferation and maturation phase between two groups. Both inflammatory and maturation phases significantly improved in diabetic rats which received Nifedipine ointment in comparison to control group (table 2). However, in these groups proliferation phase was not significantly different.

Table 1. Comparative effect of Nifedipine ointment on wound healing process in normal rats. Data are mean of 8 separate experiments

	Healing phases scores		
	Inflammation	Proliferation	Maturation
Placebo	5.8	8.0	10.3
Nifedipine	11.3*	9.0	6.8

*P< 0.05

Table 2. Comparative effect of Nifedipine ointment on wound healing process in diabetic rats. Data are mean of 10 separate experiments

	Healing phases scores		
	Inflammation	Proliferation	Maturation
Placebo	6.5	9.3	13.6
Nifedipine	14.5**	11.7	7.4*

*P< 0.05; ** P< 0.005

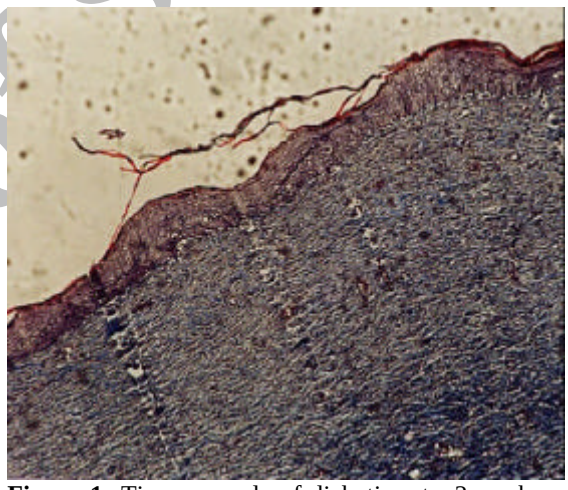

Figure 1. Tissue sample of diabetic rats, 2 weeks post treatment with Nifedipine ointment. Trichrom and Mason staining. Low number of inflammatory cells and completed granulated tissue.

DISCUSSION

Ulcers are among the most important complication of diabetes and they usually cause amputation of lower extremity. At the present there are several medical interventions to prevent and/or treat chronic wounds including different dressings, use of antimicrobial agents and wound healing drugs (11). Nifedipine, a calcium channel blocker, is being used for treatment of hypertensive venous leg ulcers and significantly improved skin wound healing process in patients received 10 mg Nifedipine three times a day (7). Ward et al used Nifedipine to treat finger ulcers in scleroderma

(12). Topical Nifedipine and Diltiazem have been successfully used for treatment of anal fission (13).

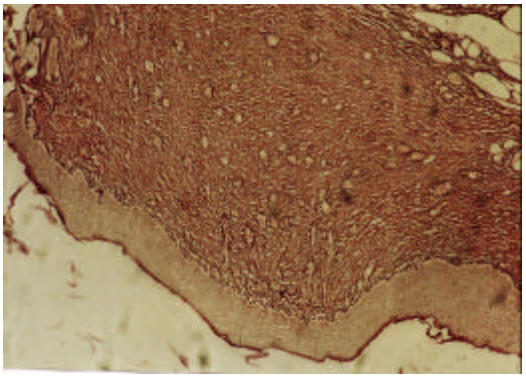

Figure 2. Tissue sample of diabetic rats, 2 weeks post treatment with Nifedipine ointment. Reticholin staining. Granulated cells.

In the present study topical Nifedipine 3% has been used to treat skin wounds in normal and diabetic rats. Effects of Nifedipine were evaluated in three different phases of wound healing process. In both experiments (normal and diabetics rats) topical Nifedipine significantly improved inflammatory phase (Tables 1-2 and Fig. 1). However, maturation phase was only significantly improved in diabetic rats (Fig. 2).
Nifedipine did not affect proliferation phase in either groups significantly.

This finding is in line with previous reports. It was previously reported that although Nifedipine significantly reduced size of vascular lesions, no anti proliferation effects was observed (14).

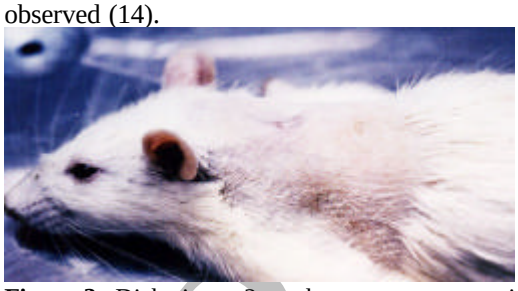

Figure 3. Diabetic rat 2 weeks post treatment with Nifedipine ointment.

Overall results of this study showed topical Nifedipine improved skin wound healing process in diabetic rats (Fig. 3).
Therefore topical Nifedipine could be beneficial in treatment of skin wounds especially in diabetic models. Although this might be due to improvement of blood circulation in lesions, more data is required to explain the exact mechanism of action of Nifedipine in improving wound-healing process.

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