

Evaluation of oral mucosal wound healing in diabetic rats receiving zinc supplements and Vitamin E

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ABSTRACT

Introduction: Diabetes mellitus is defined by the hyperglycemia and can be worsen by the different pathological changes including impaired wound healing. Defect in the wound healing causes delay in repairment of it and development of chronic ulcers. Zinc deficiency related to the delay of wound healing. Reduction of antioxidants such as vitamin E associated with increased blood glucose which is a risk factor of increasing complications of diabetes. The purpose of this study was to evaluate the effect of zinc and vitamin E supplementation on the process of wound healing in diabetic rats in the Pasteur Institute of Tehran. **Material and method:** 42 male Wistar rats were randomly divided into three groups of controls, diabetic and diabetic with Zinc and vitamin E supplementation. Rats became diabetic by intraperitoneal injection of streptozotocin. In each groups, an 8 mm wound was created in buccal mucosa. Then, seven rats were randomly selected in each third and seventh day, to assess the wound diameter after anesthesia. **Results:** In the follow-up of third day, the highest diameter was in the diabetic group and the lowest was in the diabetic group who received supplements. In the follow-up of seventh day, the largest diameters were for the diabetic and the lowest were for the healthy group. In the healthy and diabetic groups, the reduction in wound diameter was significant from day three to seventh. **Conclusion:** The results of this study showed that zinc and vitamin E supplementation is effective in improving the process of oral mucosal ulcer in diabetic rats.

Keywords: diabetic rats, vitamin E, wound healing, zinc.

Introduction

Diabetes mellitus is defined by hyperglycemia and can be worsen by different pathologic changes including neuropathy, retinopathy, nephropathy, cardiovascular diseases, and impaired wound healing^[1]. The number of people with diabetes is increasing due to population growth, aging, urbanization, unhealthy eating habits, increased prevalence of obesity, and sedentary lifestyle^[2]. Currently, 80% of population is living with diabetes in low and middle income countries^[3, 4]. Also, diabetes is life-threatening because of its potential micro and

macro vascular complications^[5].

Presence of defect in wound healing phase (inflammatory, proliferative, and remodeling phases) causes delay in repairing or appearance of chronic ulcers^[6, 7]. In normal patients during restoration inflammatory phase (24-48 hours after ulceration), neutrophils are the first cells that migrate to the site of injury. These cells make up about 50% of the wound area cells. After 5 days of ulceration, the number of neutrophils decreased and epithelialization and monocyte / macrophage and lymphocyte become the main cells of wound site. These cells play an important role in the recovery process by cytokine products and growth factor^[8].

In the wound remodeling phase of diabetic patients, there is an increase in apoptosis of keratinocyte, fibroblasts and endothelial cells that decrease the level of various growth factors^[9]. Understanding the role of supplements in the wound healing process can provide useful therapies for the healing of diabetic wounds.

Zinc is important in insulin activity and carbohydrate metabolism^[10]. Oxidative stress plays an important role in the pathogenesis of diabetes and its complications. Zinc is an

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important part of antioxidant enzymes such as superoxide dismutase, and zinc deficiencies impair their synthesis and subsequently increase oxidative stress [11]. Studies have shown that diabetes is associated with a reduction in zinc (hypozincemia) and an increase in the level of zinc in uric acid (hyperzincurea) [12]. In addition, zinc deficiency is very common in developing countries [13].

According to animal studies, zinc supplements modify fasting insulin and fasting glucose levels in mice [14]. Human studies have also confirmed the beneficial effects of zinc supplementation in both type one [15, 16] and type two [17, 18] diabetes. Experiments conducted on animals with low levels of serum zinc and normal animals showed that zinc deficiencies caused delayed wound healing [19].

Certain antioxidants are obtained through diet [20, 21]. One of the best one is vitamin E, a fat-soluble vitamin that helps prevent lipid damaging by free oxygen radicals [22]. When a large amount of active free radicals' attack lipids in the cell membrane or lipoprotein, the lipid peroxidase chain will broke down [20]. Vitamin E disables this reaction chain and acts as a inhibitor in lipid peroxidase chain breakdown [21].

There is a strong relationship between increased blood glucose levels and antioxidant reductions. This reduction is a major risk factor for increased complications of diabetes which can be reduced by antioxidant supplementation [23].

Most studies focus on skin restoration instead of mucosal repair, so that there is not a ratified protocol for the treatment of oral ulcers in diabetic patients [20, 24]. According to the studies, vitamin E and zinc have been reported to be effective in the process of repairing diabetic ulcers, alone [16- 19], but there is no evidence for the beneficial effect of using them simultaneously, with regard to the prevalence and consequences of non-healing wounds in diabetic patients and the inadequate success of conventional treatments and supplements, the aim of this study was to investigate the effect of zinc and vitamin E supplementation at the same time on the process of wound healing in buccal areas of diabetic rats during a month in Pasteur institute of Iran.

Methods:

This study was approved by the Ethics committee for Animal Research of the Shahed University of medical science.

In this experimental study, 42 male Wister rats, 2-3 weeks old and weighing 200-300 g, were randomly divided into three groups of 14 including controls, diabetic and diabetic recipients of Zinc and vitamin E supplement groups. they were kept in the same conditions in plastic cages with free use of water and 12 hours of darkness at 24 ° C (Rats were prepared from the center of the Pasteur Institute of Iran and stored in the same place) [1].

28 rats became diabetic by intraperitoneal injection of streptozotocin. 48 hours after they became diabetics, 2 ml of venous blood were measured to assess the blood glucose levels,

and rats with blood glucose levels more than 200 mg / dl identified as diabetic.

In the supplemented rats group, vitamin E (0.4 mg / kg manufactured by Behsa) [25] and Zinc (8.25 mg/day manufactured by Razak Company) [2] given by daily gavage for two weeks. All three groups after anesthesia (by ether gas in the desiccator) were injured by a puncture biopsy (manufactured by KAI) at a diameter of 8 mm in the buccal mucosa.

Then seven rats in the third day and seven rats in the seventh day were randomly selected, and after anesthesia the diameter of the ulcer were measuring by caliper (manufactured by Inside).

The wound diameter in each section is assessed statistically by ANOVA and post hoc tests, and repetitive ANOVA for taking time into account.

Results

Research was done on 42 rats including 14 healthy, 14 diabetic with zinc and vitamin E supplementation, and 14 diabetic ones. The diameter of the wounds based on the time tracking among three groups has been shown in table 1:

Table 1. Mean and standard deviation of the diameter of the wounds in the three groups at 3 and 7 days after

Group	Mean±SD		P value
	3 days after	7 days after	
control (N=14)	5.92±0.62	3.21±1.10	P<0.000
Diabetic (N=15)	6.78±0.71	5.93±0.53	P<0.8
Diabetic with supplement (N=12)	4.97±0.56	3.68±1.13	P<0.01
P value (ANOVA result)	P<0.01	P<0.001	

In the third day of follow-up, the highest diameter was related to the diabetic group as 6.78 mm and the lowest was related to diabetic group with supplementation as 4.97. The ANOVA test showed that these differences were statistically significant between three groups ($p < 0.01$). Post hoc test also showed that there was a significant differences within groups ($p < 0.01$).

In the seventh day of follow-up, the highest wound diameter was related to diabetic group as 5.93 and the lowest ones was related to the healthy group as 3.21. The ANOVA test showed that this diameter was statistically significant ($p < 0.01$). Post hoc test illustrated that there was a significant difference of diabetic group with healthy and supplementation groups ($p < 0.01$). However, there was no significant deference between supplementation group and the healthy ones ($p < 0.7$).

The diameter changes in the healthy group on the third day were 5.9 mm and on the seventh day it was 3.21 mm. Paired test showed that this decrease in wound diameter from days 3 to 7 was statistically significant ($p < 0.05$).

In the diabetic group, the diameter of the wound was 6.78 mm in the third day reached to 5.93 mm in the seventh day, which this decrement was not statistically significant ($p < 0.8$).

In the supplementation group, the wound diameter decreased from 4.97 mm in the third day to 3.68 on the seventh day, which was statistically significant ($p < 0.01$).

Discussion

Despite the research done in the process of wound healing in patients with diabetes, there is no proper method to improve this complication. The present has been conducted to investigate the process of oral mucosal ulcers healing in diabetic rats which were intervened by zinc and vitamin E supplementation. The current study showed that healing mucosal lesions prolonged among rats with diabetes. This delay in healing is more noticeable on the seventh day which indicated the chronicity of diabetic wounds, which supplementation with zinc and vitamin E accelerated wound healing in diabetic rats. On the third day, the smallest diameter possessed to the supplementary group indicating an increased wound healing in this group. On the seventh day, the lowest diameter possessed to in the healthy group and the next was supplement group, which there was no significant difference between the supplement and the healthy group.

Several factors such as increasing inflammatory response and expression of inflammatory cytokines, inflammatory cells (macrophages and neutrophils) [26], angiogenic factors and growth factors [27] play a role in delayed diabetic wound healing. Several studies have shown changes in the metabolic and functional profile of inflammatory cells, as well as the reduction in chemotaxis [28] and phagocytosis [29], which prolong the process of inflammation and increase the risk of infection [8].

Brizeno et al. in a study investigated the delayed oral wound healing in rats with diabetes and found that body weight and wound healing were significantly decreased in the diabetic group [1].

In the study which was conducted by Momen-Heravi et al [30], the use of zinc for 12 weeks among patients with diabetic foot ulcer had a desirable effect on the parameters of wound size, glucose metabolism, cholesterol and malondialdehyde; however, did not affect other lipid profiles and inflammatory markers. Another study by Kheiralla et al [31]. Found that 25 mg/day of zinc supplementation improved wound healing through improving immune response among patients with diabetic foot ulcer. Hyperglycemia and impaired metabolism of insulin in patients with diabetes disrupt the normal process of cytokine production, which it makes the wound become chronic [32]. Zinc level is reduced by many non-specific factors, such as infection and trauma [33]. Zinc intake increases the rate of wound healing process through improving immune response [31], the regulation of metalloproteinases [34], and zinc metalloenzymes such as alkaline phosphatase, RNA and DNA polymerase, and matrix metalloproteinases (MMPs) [35].

In skin lesions of animals with diabetes, collagen levels I and IV are significantly reduced in comparison with normal animals. These deficiencies directly relate to delayed wound healing and reduce the percentage of collagen-occupied area in the wound

because collagen is an essential component of the extracellular matrix. Therefore, the use of zinc can play a role in improving the speed of wound healing [36].

Musalma et al [37] investigated the effect of Alpha-Tocopherol as an antioxidant on the wound healing of streptocytocin-induced diabetic rats. Reduced malondialdehyde among the treatment group accompanied by increased glutathione peroxidase activity was shown that it would help enhance the wound in rats ($p < 0.05$).

Jain et al. [25] investigated on the effect of vitamin E on preventing complications and progression of diabetes. This study showed that postprandial blood sugar (PPBS), total cholesterol (TC), and diastolic blood pressure (DBP) significantly decreased. These findings revealed that vitamin E can inhibit the progression of the disease, and also decrease the onset of complications including foot ulcer, cardiovascular disease, and retinopathy.

Galeano et al. [38] showed that Raxofelast, a hydrophilic vitamin E-like antioxidant, acts as a reactive oxygen species (ROS) scavenger, and prevent lipid peroxidation in unsaturated fatty acids of cell membrane through angiogenic activity. In addition, it has been reported that the production of fibroblasts and keratinocytes also would be increased.

Generally, it is concluded that vitamin E acts as a fat-soluble antioxidant which could reduce the level of plasma malondialdehyde (MDA) through increasing glutathione peroxidase (GPx) activity, and it could increase the rate of wound healing. Zinc is required for cross-linking of collagen fibers, DNA, RNA and protein synthesis, immune function, cell proliferation [39].

Although these above-mentioned studies have been conducted on diabetic skin wound, there is no study on the effect of supplementation on oral ulcers in patients with diabetes.

Conclusion:

The results of the present study illustrated that, the simultaneous use of zinc and vitamin E in streptozotocin-induced diabetic rats might affect an increase in the amelioration of oral ulcer in the buccal mucosa. It is suggested that zinc and vitamin E supplements are prescribed for patients with diabetes if the findings of the current study confirm by large human studies.

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