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Effect of Sildenafil Citrate on MDA, GSH, Retinol, Vitamin D_3 and α-Tocoferole Levels in Wound Healing: Diabetic Rat Model

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Abstract
In this study, the effect of sildenafil citrate (SC) on Malondialdehyde (MDA), Glutathione (GSH), retinol, vitamin D3 and α-tocoferole levels were researched in the rats with diabetes and wound formation. 3 groups were formed in the study and 10 male Swiss albino rats with the weight of 250-300 g were used in each group. Wound implementation was conducted on the rats of the 1st group, diabetes and wound implementations on the rats of the 2nd group and diabetes, wound and SC implementations on the rats of the 3rd group. Normal wound care was applied to the first two groups; besides the normal wound care, SC was also applied to the third group intraperitoneally (i.p.) at 0.7 mg/kg once a day and during 3 days. Blood samples were taken before (day 0) and after (9th day) the applications. MDA and GSH were checked in the whole blood, and retinol, vitamin D3 and α-tocoferole levels were examined in the plasma. At the end of the study, MDA values of the third group were found lower ($P< 0.05$), GSH plasma retinol and α-tocoferole levels higher ($P< 0.05$) than the other groups. Consequently, it was determined that application of SC in appropriate doses and time might have a positive effect on enabling the oxidant and antioxidant balance during the wound healing in diabetes patients.

Key words: Diabetes, Wound, Sildenafil Citrate, MDA, GSH, retinol, α-tocoferole

INTRODUCTION

Diabetes mellitus (DM) is a chronic hyperglycaemic disorder and it is termed as a syndrome rather than a simple disease [1]. Diabetes, which leads to the delay of wound healing, is characterized by the inhibition of the inflammatory response, angiogenesis and fibroplasia; defects in collagen deposition; and the discrepancy of the extracellular matrix [2, 3].

Wound healing consists of four phases which are haemostasis, inflammation, proliferation and remodelling [4, 5].

While free radicals and antioxidant mechanisms are balanced under physiological conditions, the change of this balance towards the oxidants leads to severe tissue damage, known as oxidative stress [6] Free radicals affect important components of the cells, lipids, proteins, carbohydrates and DNA [7]. However, the protective role of enzymatic antioxidants such as GPx and non-enzymatic antioxidants such as vitamin E, A and GSH against free radical attack is balanced under normal conditions [8]. Sildenafil may inhibit the production of lipid peroxidation via the activity and expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [9]. One of the end products of lipid peroxidation is malondialdehyde (MDA). As a result of all these effects, free radicals lead to the oxidation of the lipids and membrane damage with autocatalytic effect [10].

There are disulfide (GSSG) forms oxidized with the reduced thiol (GSH) form, which makes up 95% of glutathione, and the ratio between GSH and GSSG is high in cells without oxidative stress [11]. Sildenafil citrate has no direct relaxing effect, but it shows this effect by inhibiting phosphodiesterase type 5 (PDE5), which is responsible for the degradation of cyclic guanosine monophosphate (cGMP), and increasing the effect of nitric oxide [12].

With this study, it was aimed to investigate the effect of sildenafil citrate on wound healing in rats, and the levels of MDA, GSH, retinol, vitamin D3 and α-tocopherole.

MATERIALS AND METHODS

Male Swiss albino rats weighing 250-300 g were used in this study. The rats were supplied from Atatürk University Experimental Animal Centre and the principles of the local ethics committee were complied with in the study. The rats were kept under standard conditions.

Formation of the groups

This study was planned in 3 groups. The rats were randomly divided as 10 rats for each group. The first group was established as wound+no diabetes, the second diabetes and wound, and the third one was established as diabetes+wound+SC.

After randomly forming 3 groups, blood samples were taken from all rats (day 0). Diabetes was created in the second and third groups using alloxan. Those with a fasting blood glucose higher than 250 mg/dl 72 hours after the last alloxan administration were diagnosed with diabetes. Skin wound was created in the rats in all groups, and their blood samples were taken after 72 hours (day 9).

Alloxan and diabetes

The diabetes was created in the animals in the second and third groups with i.p. injection of alloxan monohydrate dissolved in 5% distilled water at the dose of 120 mg/kg once a day, for 3 days [13].

Chirurgical procedure

The rats in 3 groups were anaesthetized with single intramuscular injection of 1 mg/kg xylazine hydrochloride (Bayer, Rompun, 23.32 mg/ml) and 50 mg/kg ketamine hydrochloride (Parke-Davis, Ketalar, 50 mg/ml). The wound was created as a full-thickness skin wound 1 cm in diameter on the back. The first and the second groups were only provided normal wound care, while the third group was i. p. administered 0.7 mg/kg SC (Pfizer, Viagra) once a day, or three days.

The blood (1-2 ml) taken from the hearts of the animals in all of the groups using a syringe under light anaesthesia on the day before the study (day 0) and the third day after the wound was created (day 9) was transferred to tubes containing EDTA. After MDA and GSH values were examined in the whole blood, the blood was centrifuged at 3000 rpm and the plasma were removed. Retinol, vitamin D3, α-tocopherole measurements were made in the plasma.

Analyses

The MDA level in the blood was measured with the method of taking a colourful form of the fatty acids with MDA, tiobarbutiric acid, among the peroxidation products formed by the reaction of the fatty acids with free radicals [14, 15].

The amount of GSH in blood was established with the formation of the yellow colour occurring from the reaction of sulfhydryl groups with DTBN (5',5'-(2- dithiobis-nitrobenzoic acid). The level of reduced GSH in the blood with EDTA' was measured at 412 nm in Spectrophotometer within 24 hours [16,17].

The analyses of retinol, vitamin D3 and α-tocopherole were performed by injecting into HPLC (high performance liquid chromatography) column and using diode-array detector at the wave lengths of 325, 265 and 290 nm [18, 19]. Metenol-water (98:2) was used as a mobile phase at the flow rate of 1.5 ml/min [19, 20]. C18 column (4.6 mm x 25 cm) was used to separate the vitamins. The analyses were performed using Agilent 1100 series HPLC device.

Statistical analysis

Oxidative stress parameters and vitamin levels were analysed using one-way ANOVA in SPSS 16.0 package program, and evaluated with Duncan's Multiple Range Test. Intra-group average and standard error (SEM) of the data were calculated. Intergroup statistical importance was calculated according to $p \leq 0,05$.

RESULTS

When the levels of MDA, GSH, Retinol, vitamin D3 and α- tocopherole in the blood samples taken before the beginning of the study (day 0) were compared, no intergroup statistical importance was detected for all of the groups (Table 1).

At the end of the study (day 9), the blood MDA values in Group 3 were found low (*P< 0.05) when compared to those in the other groups, while plasma retinol, α-tocopherole and blood GSH values were found high (*P< 0.05). Table 2.

DISCUSSION

It is asserted that the nonenzymatic glycation of proteins and free radical production increase in diabetes, and the tissue damage in diabetes patients results from nonenzymatic glycation of proteins [21, 22]. While Matteucci and Giampietro [23] point to an increase in plasma MDA levels in Type 1 diabetes, it was noted in other studies [24-26] that MDA levels increase in Type-2 diabetes patients and a significant decrease occurs in MDA in parallel with the decrease in blood glucose levels during the period after the treatment, in analogy to the application Sildenafil Citrate. When the findings from the current study were examined, an increase in the level of MDA was detected in all groups. It was identified that this decrease in the third group is lower than the increase in the other two groups. It is considered that this may result from the positive effect of Sildanefil Citrate on wound healing.

Table 1. Levels of MDA, GSH, retinol, vitamin D3 and α- tocopherole in the blood taken in day 0 in all groups

	Group 1	Group 2	Group 3
MDA (nmol/ml)	1.44±0.50	1.32±0.65	1.37±0.62
GSH (mg/dl)	129.08±10.44	127.53±9.06	134.33±11.33
Retinol (µg/ml)	0.49±0.04	0.51±0.05	0.47±0.07
Vitamin D3 (µg/ml)	0.015±0.001	0.018±0.002	0.020±0.002
α-tocopherole (µg/ml)	1.19±0.12	1.23±0.16	1.20±0.03

Table 2. Levels of MDA, GSH, retinol, vitamin D3 and α- tocopherole in the blood taken in day 9 in all groups

	Group 1	Group 2	Group 3
MDA (nmol/ml)	3.56 ± 0.26^{b}	5.41 ± 0.45^{a}	1.29 ± 0.15^{c}
GSH (mg/dl)	113.67 ± 8.66^{b}	111.50 ± 10.42^{b}	156.50 ± 9.72^{a}
Retinol (µg/ml)	0.63 ± 0.021^{b}	0.52 ± 0.052^{b}	1.54 ± 0.033^{a}
Vitamin D3 (µg/ml)	0.018±0.003	0.019±0.002	0.021±0.003
α-tocopherole (µg/ml)	1.10 ± 0.064^{c}	1.67 ± 0.016^{b}	2.18 ± 0.019^{a}

a, b, c; the difference between the averages indicated with different letters in the same row is important. (*P< 0.05).

Cengiz and Cengiz [21] emphasised that GSH, a natural antioxidant, may prevent the formation of free radicals, one of the complications of diabetes. In his study, Soliman [25] noted that hyperglycaemia increases oxidative stress, the antioxidant depletion formed should be considered as a risk factor in the formation of diabetes complications. A decrease in the level of glutathione was detected in 80 diabetes patients.

GSH amount was found significantly high in the early period (0-2 months) and low in the late period in Type-1 diabetes cases [27]. In Type-2 diabetes [22, 26, 28, 29], an increase in the levels of MDA and a decrease in the level of GSH were detected, and they noted that oxidative stress is important in Diabetes Mellitus as well. When the results of the study were examined, on the contrary to an increase in the levels of MDA levels in all groups, a decrease was observed in GSH in group 2, while an increase was observed in group 3. This may result from the positive effect of sildenafil citrate on wound healing.

Antioxidant vitamins have an important role in the regulation of insulin secretion [21]. The plasma vitamin E values were found high in the patients suffering from insulin-dependent diabetes mellitus, and vitamin A values were found low in the studies carried out [22, 30]. Guzel et al. [27] reported that vitamin E levels are high in late period diabetes patients and this mainly results from the transmission of a-tocopherole via lipoproteins. Furthermore, while superoxide radicals are eliminated with enzymatic dismutation, vitamins A, E and C, known as antioxidants, ensures the elimination of oxygen radicals in the organism [31]. At the beginning of the study, difference between the levels of vitamin A and E could not be detected in all of the groups. While no significant change occurred in groups 1 and 2 at the end of the study, a significant increase was observed in Group 3. It was considered that this may have resulted from the activation of the stored vitamins A and E by sildenafil citrate and increasing the amount in blood circulation.

Soliman [25] noted that hyperglycaemia increases oxidative stress, and the antioxidant depletion formed should be considered as a risk factor in the formation of diabetes complications. The findings obtained show that MDA increase in diabetes, and it is considered that early diagnosis of this increase may help clinicians in taking the measures that will prevent further intensifying of the complications of diabetes.

In their study in Yildiz et al. [32] detected a decrease in erythrocyte MDA level when compared to control group in low dose (0.7 mg/kg) and high dose (1.4 mg/kg) sildenafil citrate in rats, of which testicles they caused tortion; and they detected an increase in the levels of GSH, plasma Vitamin A and E levels, in analogy to the findings in the current study.

There are very few studies investigating the effects of SC on vitamins, on healing the wounds of diabetes patients. In the study carried out, it is considered that SC increases the amount of antioxidant vitamins, Vitamin A and E in the circulation, in healing the wounds of diabetes patients, and takes part in wound healing. It has been considered that SC may be showing this effect by inhibiting phosphodiesterase type 5 (PDE5).

As a conclusion, the formation of free radicals increases in wound healing in diabetes, while a decrease occurs in radical scavenging systems. Thus, it may be argued that diabetes patients need more antioxidants as a result of the increase in free radicals and the decrease in radical scavenging systems. The increase in lipid peroxidation is a beneficial criterion in the evaluation of the development of diabetes as it may be the preparatory factor of long-term complications. A decrease in the level of MDA, which is the determinant of skin damage, and an increase in the amount of antioxidant was detected when Sildenafil citrate was used for healing the wounds of diabetes patients. Thus, it was concluded that the use of Sildenafil Citrate would be beneficial for healing the wounds of diabetes patients.

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