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Some Serum Oxidative Parameters in Normoglycemic Rats: Vascular Endothelial Growth Factor (VEGF) Application

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ABSTRACT

VEGF has the positive effect on wound healing. In this study, the effect of VEGF application on some serum parameters during wound healing process were investigated. 36 female normoglycemic Wistar rats were used (200–250 g). Dorsolateral incisional wounds (length: 4 cm) were made on the two sides of the medulla spinalis in rats. They were divided into 3 groups: untreated group (n=12), chitosan group (n=12) and chitosan+VEGF group (n=12). The rats were sacrificed on the 3rd and 7th days of post wounding. NOx, TBARs and RSH levels were determined spectrophotometrically in serum. Results were compared by one-way ANOVA ($p<0.05$). Serum TBARs levels both the chitosan treated group and the VEGF application group was found decreased when compared with untreated groups (3rd and 7th days) ($p<0.05$). Chitosan and VEGF application were effective increasing antioxidant capacity of serum on the 7th day. Serum NOx levels decreased in the VEGF treated groups on 3rd and on 7th days ($p<0.05$). It can be considered that VEGF administration has a more positive systemic effect to eliminate increased oxidative damage in the serum of normoglycemic rats.

Keywords:
VEGF application; Chitosan; Wound healing; Oxidative stress; Antioxidant.

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INTRODUCTION

Wound healing is essentially a science in which physiological and biochemical events occur at the highest level. The phases of normal wound healing are inflammation, proliferation and remodeling which cannot be completely separated from each other [1-5].

VEGF have different biological properties and bioavailability which consists of 6 isoforms in humans [6-8]. VEGF shows its effects via binding to tyrosine kinase receptors, VEGF receptor 1 and VEGF receptor 2. It has been known that hypoxia and also numerous cytokines and growth factors such as interleukin-6 (IL-6), IL-1β, epidermal growth factor (EGF) and transforming growth factor-β (TGF-β) increase VEGF expression [9].

VEGF, which contains cysteine-cysteine bonds, and chitosan, which contains active -OH/-NH_2 groups [9,10], may influence the redox balance besides their contributions to wound healing process.

VEGF is produced by various cell types such as thrombocytes, fibroblasts, macrophages, smooth muscle cells and neutrophils during wound healing process and it has receptors on endothelial cells and monocytes [9]. VEGF is a powerful mitogen factor for the endothelial cells of the lymph, arteries and veins, but lacks any mitogenic activity on other cells [9]. It has been known that VEGF synthesized in the wound bed peaked at 3-7 days and then gradually decreased at 7-14 days [9].

At very low concentrations (μM), H_2O_2 induces VEGF-A expression. It has also been proven that oxidants such as H_2O_2 and NO promote VEGF synthesis, and N-Acetyl-L-cysteine, a GSH precursor, also inhibits angiogenesis by suppressing VEGF gene expression [11, 12].

It has been reported that the disruption of synthesis of VEGF and its mediator (NO) due to excessive oxidative stress in diabetes leads to the deterioration of wound healing phases [1-4, 13,14].

Chitosan is a polysaccharide consists of β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine units. It is obtained commercially from chitin by dea-

cetylation, which is in the cell walls of some bacteria and fungi. It has been shown that chitosan attracts inflammatory cells and growth factors released from them into the wound site in the early period of wound healing process [15]. Moreover, it has been reported that it has antioxidant effect thanks to its hydroxyl and amino groups [10].

On this basis, in our study, VEGF in chitosan gel were topically administered to the wound in normoglycemic rats. It was investigated how they affect the values of serum oxidative parameters such as TBARs and NOx levels, and RSH levels.

MATERIAL AND METHODS

Ethical approval for this study was obtained from Gazi University Local Ethics Committee for Animal Experiments (G.ET-10.117). Chitosan (C3646) and VEGF (Sigma V3638) were obtained from Sigma-Aldrich. 1% Lactic acid was added to 50 mL deionized water. 3 g chitosan was added to solution and stirred. After, 50 mL deionized water was added and mixed. Obtained gel was kept at room temperature overnight before the application. Finally, 417 ng VEGF was added to obtained chitosan gel (last concentration 7 ng/mL).

Animals

All of the rats were kept on standard rodent cages with appropriate amounts of rat food and water. Rats were kept with normal light-dark cycle (12:12 h) at room temperature (25±2 °C).

Wound Model

The animals were anaesthetized with a combination of xylazine and ketamine intramuscularly. The dorsal section of rats was shaved an electric razor, and cleaned with 1% iodine tincture. 4 cm lengthy - incisional wound models were made on the two sides of medulla spinalis on the rats. 36 healthy normoglycemic rats were divided into 3 groups. There were 6 rats in each group: untreated group (n = 12), chitosan treated group (n = 12) and chitosan + VEGF group (n = 12) (7 ng/ml VEGF). Each group was divided into two in itself to be sacrificed on the 3rd and the 7th days.

Biochemical Analyses

Determination of TBARs levels

The TBARs levels of serum samples were analyzed according to Kurtel et al. [16]. The absorbance was read at 532 nm.

Determination of RSH levels

Plasma RSH was determined by spectrophotometric method [17]. The absorbance was read at 412 nm. RSH amount was determined assuming a molar absorption coefficient of 13.000 at 412 nm for 5-thio-2-nitrobenzoic acid (TNB).

Determination of NOx levels

The plasma NOx levels were measured using Griess reaction. Sodium nitrite and sodium nitrate solutions were used as standards [18].

Statistical Analysis

The data were given as the mean ± standard deviation (SD). Mean values were compared by one-way ANOVA. The level of statistically significance was set at $P<0.05$.

RESULTS

The obtained results are shown in Table 1.

Serum TBARs Level

Serum TBARs levels both the chitosan treated group and the VEGF application group was found decreased when compared with untreated groups (day 3 and day 7) ($p<0.05$). The most significant reduction occurred in the chitosan group. It can be said that use of chitosan decreased lipid peroxidation in the rat serum. There weren't any significant alterations between the chitosan and VEGF treated group when compared with each other both 3 and 7 days ($p > 0.05$) (Fig 1).

Serum RSH Level

When the chitosan group were compared with the untreated group on the 7th day, the serum RSH levels of the chitosan group showed a statistically significant increase. In addition, when the VEGF treated group were compared with the untreated group, the serum RSH level

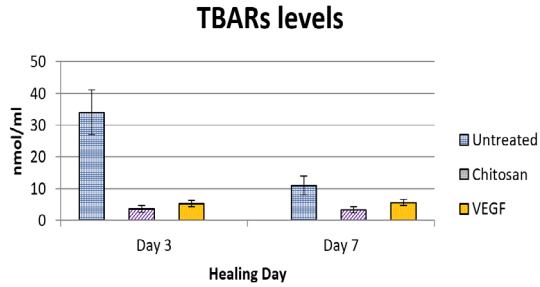

Figure 1. Serum TBARs levels.

Table 1 Serum TBARS, RSH and NOx levels in rats

	TBARS level (nmol/ml)	RSH level (nmol/ml)	NOx level (µmol/l)
Untreated groups			
Day 3	33.99 ± 7.73c,e	234.32±2.54g	9.51±1.94
Day 7	10.98 ± 3.03d,f,g	51.47±4.80	10.50±2.28f
Chitosan treated groups			
Day 3	3.57± 1.14a	223.15±2.78g	8.28±2.78
Day 7	3.32± 0.84b	393.55±9.20b	10.62±2.67f
Chitosan + VEGF groups			
Day 3	5.23 ±1.85a,c	146.18±3.12a,c,g	4.22±0.74a,c
Day 7	5.60±1.13b,d	248.78±5.40d,b	1.45±0.37b,d,g

[a] p< 0.05 when compared to untreated group (day 3), [b] p< 0.05 when compared to untreated group (day 7), [c] p< 0.05 when compared to chitosan treated group (day 3), [d] p< 0.05 when compared to chitosan treated group (day 7),
[e] p< 0.05 when compared to VEGF treated group (day 3),
[f] p< 0.05 when compared to VEGF treated group (day 7),
[g] p< 0.05 when compared to 3 and 7 day in the same group

of VEGF treated group showed a statistically significant increase on the 7th day. The serum RSH levels increased both chitosan treated group and VEGF group on the 7th day. Chitosan and VEGF application were effective increasing antioxidant capacity of serum on the 7th day (Fig 2).

Serum NOx Level

When VEGF treated groups were compared both the untreated groups and chitosan groups, NOx levels decreased in the VEGF treated groups on 3rd and on 7th days ($p < 0.05$). According to these results, it is seen that chitosan application did not change NOx levels when compared to untreated group. VEGF application decreased statistically significant in point of serum NOx levels ($p < 0.05$). VEGF administration decreased serum NO levels on both 3rd and 7th days of wound healing independent of chitosan. These results suggested that VEGF administration may have been effective by suppressing NOS enzyme levels in healthy rats (Fig 3).

DISCUSSION

There is information about that high levels of ROS are harmful by suppressing the synthesis of VEGF and its mediator (NO) in the newly developed wound tissue and that micro-levels of ROS (H2O2 and NO) are beneficial by stimulating VEGF synthesis and signaling [19-21]. Although there is no infection in the wound, it has been understood that micro-levels of oxidants formed by respiratory burst are necessary to initiate various signal processes.

Jones et al. [22] reported that cysteine-cysteine bonds present in proteins received oxidative signals from the surrounding area by the conversion of thiols to disulfides. It has been indicated that the conversion of thiols to disulfides stimulates various molecular pathways in both mitochondria and cell nuclei by leading a change in protein-DNA and protein-protein interactions [23,24]. VEGF is also a growth factor that carries cysteine-cysteine bonds in its structure. Its synthesis and signaling are stimulated by oxidants such as H2O2 and NO and are inhibited by antioxidants such as GSH and N-acetyl cysteine (NAC) [21]. It has also been reported that inadequate VEGF synthesis is effective in disrupting the redox balance of the cell. Because it has been suggested that when VEGF is released, it initiates contradictory processes that show an antioxidant effect with mitochondrial Mn-SOD and an oxidant effect with NADPH oxidase [21]. VEGF may provide a balance between oxidant

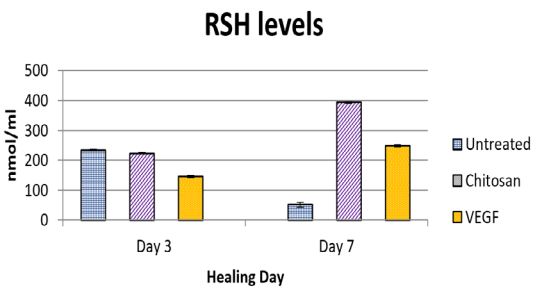

Figure 2. Serum RSH levels.

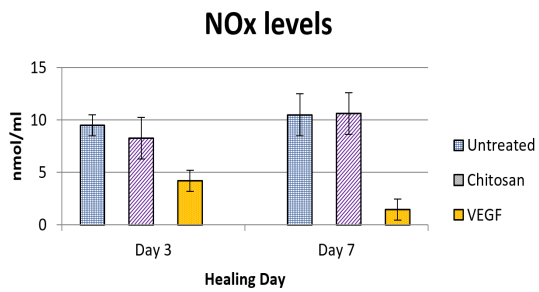

Figure 3. Serum NOx levels.

and antioxidants.

In the current study, firstly, when the chitosan and VEGF+chitosan groups were compared with the untreated group TBARs levels of the chitosan and VEGF+chitosan groups showed a decrease from the 3rd day to the 7th day. The most significant reduction occurred in the chitosan group. Both chitosan and VEGF are agents that affect the oxidation process in the media. It can be said that chitosan and VEGF showed an antioxidant effect in wound serum of healthy rats by reducing the lipid oxidation. Sönmez Çoban and Coşkun-Cevher [25] suggested that VEGF administration decreased serum TBARS levels compared to both diabetic control groups and chitosan treated diabetic groups. These results indicate that both chitosan and VEGF administration reduced lipid peroxidation in both normoglycemic and hyperglycemic rats.

When the chitosan group were compared with the untreated group on the 7th day, the serum RSH levels of the chitosan group showed a dramatically significant increase. In addition, when the VEGF treated group were compared with the untreated group, the serum RSH level of VEGF treated group showed a statistically significant increase on the 7th day. The serum RSH levels increased both chitosan treated group and VEGF group on the 7th day. Chitosan and VEGF application were effective increasing antioxidant capacity of serum on the 7th day (Table 1). On the 7th day corresponding to the proliferation phase of wound healing, serum RSH levels were increased. Both chitosan and VEGF administration increased serum antioxidant capacity. This increase was most prominent on day 7. Similarly, in the study performed by Sönmez Çoban and Coşkun-Cevher [25] in diabetic rats, increased serum RSH levels were detected on the 7th day of wound healing compared to diabetic control by VEGF application. In our study, it is thought that this effect may be due to the molecular structure of VEGF itself as an antioxidant.

According to our results, chitosan application did not change NOx levels when compared to untreated group. VEGF administration decreased serum NO levels on both 3rd and 7th days of wound healing independent of chitosan. These results suggested that VEGF administration may have been effective by suppressing NOS enzyme levels in healthy rats. Sönmez Çoban and Coşkun-Cevher [25] in their study, showed that both chitosan administration and exogenous VEGF administration increased serum NO levels in hyperglycemic rats. In contrast, we found that topical administration of VEGF reduced serum NO levels in normoglycemic rats. There is a relation between NO and VEGF in wound healing process; namely NO is one of the mediators of VEGF activity in terms of collagen deposition, nerve conduction, tissue oxygenation and restoration of endothelial function [20,26].

CONCLUSION

It may be thought that the application may have an effect on reducing oxidative stress. Further studies are needed to determine the under various conditions in relation to VEGF applications, which have an important role in wound healing.

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