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Effects of Sildenafil Citrate on the Hematological Parameters in the Early Phase of Wound Healing in Diabetic Rats

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ABSTRACT

In this study Investigation of effects of sildenafil citrate on some of the hematological parameters in the early phase of wound healing in diabetic rats was aimed in this study. Total 30 rats were used in the study. Rats were divided into three groups equal in number (control-non diabetic, diabetic and diabetic+Sc). While no treatment was given to the control-non diabetic and diabetic groups, sildenafil citrate was given to the diabetic+Sc group intraperitoneally with a dosage of 0.7 mg/kg/day dosage for 3 days after creating wounds: under ketamine anesthesia, a circular, full thickness, standard wound was created on the back of each rat by using a 0.8 cm sterile punch. Cardiac blood was drawn in tubes containing EDTA from each group before the creation of wounds and on days 1, 2 and 3 following the creation of wounds. Differential leukocyte counts were determined in blood samples containing anticoagulant. For this purpose, blood smears were prepared from blood samples and stained with Giemsa method. It was found that, while neutrophil counts increased in all the groups during the wound-healing periods with the increasing number of days, lymphocyte counts reduced and in control and Sc+diabetic groups, monocyte counts increased. As a result, it was concluded that Sc plays an important role in eliminating the pressure on the cellular activities in the early period of wound healing and continuance of the inflammatory process and wound healing within normal limits and also that this could be demonstrated with hematological analyses.

Key words: Rat, diabetes, wound healing, sildenafil citrate, hematological parameters

INTRODUCTION

Diabetes mellitus is a chronic hyperglycemic disorder, considered as a syndrome rather than a simple disease (Goodson and Hunt, 1979). Wound healing is a dynamic and complex process that involves a well-coordinated, highly regulated series of events, including inflammation, tissue formation revascularization and tissue remodeling (Hamed *et al*., 2010; Toker *et al*., 2009). Forever, this orderly process is impaired certain pathologic condition, such as diabetes mellitus, making the diabetic wounds a great problem for healed (Valls *et al*., 2009). On the other hand, the severity of diabetic complications can also be related to the magnitude of sub-products called advanced

glycosylation and products (AGEs), a heterogeneous group of structures found in significantly increased amounts in sera and tissues of aged and diabetic patients (Brownlee, 1992; Ptak *et al.*, 1998; Ruderman *et al.*, 1992; Vlassara, 1992). Tas *et al.* (2003) found in their study that sildenafil enhanced proliferation of new capillaries by its vasorelaxant effects in wound healing. Derici *et al.* (2009) reported that sildenafil can be used as a supporting factor in wound healing.

In wound healing, the acute inflammatory phase is characterized by a neutrophil infiltrate, replaced by mononuclear cells later. The proliferative phase is characterized by the presence of the mononuclear inflammatory cells, proliferation of fibroblasts and keratinocytes and granulation tissue formation, angiogenesis (Singer and Clark, 1999), including proliferation of endothelial cells and deposition of extracellular matrix molecules. The final phase represents maturation of the neo-formed tissue (Loots *et al.*, 1998).

Diabetes-induced impairment of wound healing is characterized by inhibition of the inflammatory response, angiogenesis and fibroplasia and defects in collagen deposition and differentiation of the extracellular matrix (Fahey *et al.*, 1991; Prakash *et al.*, 1974). It is well known that the most important repair failures are those that occur in the initial stages and they may lead to edema, reduced vascular proliferation and decreased numbers of leukocytes, macrophages and fibroblasts (Meireles *et al.*, 2008).

In this study, diabetic rats, the first phase of wound healing to changes in the cellular effects of sildenafil citrate (Sc) and hematological parameters of this process aimed to evaluate by looking.

MATERIALS AND METHODS

Animals: A total of 30 Swiss albino rats of either sex weighing 250 to 300 g obtained from the central animal house of Atatürk University were used for the study in 2009. Rats were housed individually in cages, maintained under standard conditions (12 h light-dark cycle; 25±2°C) and fed with standard pellets and water *ad libidum*. Thirty rats were randomly divided into three (control-non diabetic, diabetic and diabetic+Sc) groups with 10 animals in each group and were treated as fallows: while no treatment was given to the control-non diabetic and diabetic groups, sildenafil citrate was given to the diabetic+Sc group intraperitoneally with a dosage of 0.7 mg/kg/day dosage for 3 days after creating wounds. The study protocol was approved by the Faculty of Medicine, University of Yuzuncu Yıl's Ethical Committee and the experiments were conducted in accordance with laws concerning protection of animals.

Alloxan-induced diabetes: Diabetes was induced by the intraperitoneal injection of alloxan monohydrate (120 mg kg^{-1}) dissolved in distilled water (5%) for 3 consecutive days. Diabetes was confirmed 3 days after the last alloxan dose administration by determining the blood glucose concentration (day 6). Only animals with blood glucose levels over 250 mg dL^{-1} were used (Jaouhari *et al.*, 2000).

Surgical procedure: Under ketamine anesthesia (80 mg kg^{-1}), asepsis was provided by 10% povidine iodine (Batticon®) and the backs of rats were shaved. A circular, full thickness, standard wound was created on the back of each rat by using a 0.8 cm sterile punch.

Hematological analysis: Cardiac blood was drawn in tubes containing EDTA from each group before the creation of wounds and on days 1, 2 and 3 following the creation of wounds. Blood collected was evaluated manually in Physiology Department, YYÜ Veterinary Faculty. Differential leukocyte counts were determined in blood samples containing anticoagulant. For this purpose, blood smears were prepared from blood samples and stained with Giemsa method.

Statistical analysis: Differences between group means were assessed by a one-way Analysis of Variance (ANOVA) and post-hoc Duncan test used by SPSS/PC computer program (SPSS Inc., 1999). Results with $p<0.05$ were considered statistically significant.

RESULTS

It was seen in all the groups during the wound healing period that, while neutrophil counts increased day-by-day, lymphocyte numbers decreased and monocyte counts increased in the control group and the diabetic group that Sc was administered ($p<0.01$) (Table 1).

Varying from other groups, in the leukocyte formula of the diabetic group, increase in eosinophil count was noted during the creation and healing of the wounds ($p<0.01$). In day 1 of wound healing in the diabetic group, neutrophil percentage was found to be smaller and lymphocyte values were relatively higher ($p<0.05$). In the group that Sc was administered, although neutrophil percentage showed increase as compared to the diabetic group, it could not reach the level in the control group (Table 2).

Table 1: Distribution of leukocytes between the groups during the wound healing period (%) (n = 10)

Days	Lymphocyte	Monocyte	Eosinophil	Basophil	Neutrophil
Control					
0	63.56^{a}	6.65^{c}	3.69	0.71	25.30^{c}
1	58.50^{b}	9.50^{c}	2.67	0.67	33.67^{b}
2	48.00^{b}	14.33^{b}	4.00	0.50	33.17^{b}
3	34.17^{c}	19.67^{a}	2.33	0.67	42.83^{a}
SEM	2.51	1.25	0.41	0.09	1.57
p-value	**	**	NS	NS	**
Diabetes					
0	58.76^{b}	7.22	6.96	0.65	26.67^{b}
1	59.50^{a}	6.33	6.00	0.33	27.83^{b}
2	58.33^{a}	6.17	6.50	0.50	28.50^{b}
3	52.83^{b}	6.50	6.33	0.67	33.67^{a}
SEM	0.64	0.26	0.18	0.10	0.65
p-value	**	NS	NS	NS	**
Diabetes+Sc					
Day 0	60.60^{a}	5.27^{c}	4.93	0.60	29.12^{b}
Day 1	58.17^{a}	5.33^{c}	4.33^{b}	0.50	31.67^{ab}
Day 2	51.50b^{c}	9.17^{b}	3.17	0.50	35.67^{ab}
Day 3	44.83^{c}	14.50^{a}	3.50	0.67	36.50^{a}
SEM	1.60	0.95	0.41	0.09	1.19
p-value	*	**	NS	NS	*

*: $p<0.05$; **: $p<0.01$; NS: Non significant. Mean values within a column with no common superscript differ significantly

Table 2: Compared distribution of leukocytes between the groups during the wound healing period (%) (n = 10)

Days	Lymphocyte	Monocyte	Eosinophil	Basophil	Neutrophil
Day 0					
Control	63.56	6.65	3.69^{b}	0.71	25.30
Diabetes	58.67	7.22	6.96^{a}	0.65	26.67
Diabetes+Sc	60.60	5.27	4.93^{ab}	0.59	29.12
SEM	1.49	0.90	0.51	0.06	1.28
p-value	NS	NS	*	NS	NS
Day 2					
Control	53.50^{b}	9.50^{a}	2.67^{bc}	0.67	33.67^{a}
Diabetes	59.50^{a}	6.33^{b}	6.00^{a}	0.33	27.83^{b}
Diabetes+Sc	58.17^{ab}	5.33^{b}	4.33^{b}	0.50	31.67^{ab}
SEM	1.14	0.67	0.39	0.12	1.12
p-value	*	*	**	NS	*
Day 2					
Control	48.00^{b}	14.33^{a}	4.00^{b}	0.50	33.17^{b}
Diabetes	58.330^{a}	6.17^{c}	6.5^{a}	0.50	28.50^{ab}
Diabetes+Sc	51.50^{b}	9.17^{b}	3.17^{b}	0.50	35.67^{a}
SEM	1.42	0.93	0.53	0.12	1.14
p-value	**	**	*	NS	*
Day 3					
Control	34.17c	19.67^{a}	2.33^{b}	0.67	42.83a
Diabetes	52.83^{a}	6.50^{c}	6.33^{a}	0.67	33.67b
Diabetes+Sc	44.83^{b}	14.50^{b}	3.50^{b}	0.67	36.50b
SEM	2.00	1.42	0.51	0.11	1.06
p-value	**	**	**	NS	**

*: $p<0.05$; **: $p<0.01$; NS: Non significant. Mean values within a column with no common superscript differ significantly

In day 2 of the healing process, the highest neutrophil ratio was found in the Sc-administered group ($p<0.05$) and it was seen that lymphocyte values changed according to these values. It was seen that the Sc administered caused an increase in the monocyte percentage values starting from day 2 of wound healing as compared to the diabetic group ($p<0.01$), but it could not reach the level in the control group. It was found that this increase occurred in the lowest level in the diabetic group ($p<0.01$) (Table 2). All findings obtained in the present study were shown in Table 1 and 2.

DISCUSSION

Wound healing constitutes a big problem in diabetic patients. This problem is seen on both cellular and hümoral grounds.

Inflammatory phase of wound healing is similar to the advancement in other acute inflammatory conditions. Neutrophils migrate to the wound region within the few hours following the creation of the wound. Within the 1-2 h following the injury, monocytes migrate to the region and start to transform into tissue macrophages (Szpaderska and DiPietro, 2005). Neutrophils are the first cells that play a role in the defense of the body. Neutrophilia and leukocytosis accompanying the inflammation are seen in the blood picture in conditions like surgical operations, trauma, stress or pain (Coles, 1986). A rapid inflammatory response is required for the realization of the later stages of healing in the wound healing period (Szpaderska and DiPietro, 2005). In this

study, the percentage distribution of leukocytes found during the inflammation phase of healing in the control group and the diabetic group that Sc was administered was consistent with the classical wound healing process. In this context, it can be said that Sc corrects the impairment of wound healing seen in diabetics.

Neutrophil increase in the control group started on day 1 of wound healing, continued on day 2 and reached the peak on day 3. It was found that neutrophil increase started on day 3 of healing in the diabetic group and in the diabetic group that Sc was administered, although there was an increase in the number of neutrophils in days 1 and 2 of healing, the marked increase was in day 3 ($p<0.05$) (Table 1). This finding can be interpreted as Sc stimulated the migration of neutrophils in diabetic animals and contributed to the healing process. Similar course of the increase of the number of monocytes in the control group and the diabetic group that Sc was administered was interpreted as that Sc eliminated the delaying effect of diabetes on wound healing. It was not found any study opposite of the results of the present study.

Inflammatory cells like neutrophils and macrophages are required fro the phagocytosis of the necrotic tissue and microorganisms (Li *et al*., 2007). Following the creation of the wound, while platelets adhere on the damaged tissue, growth factors in their granules are released (LaVan and Hunt, 1990). Platelet derived growth factor (PDGF) stimulated chemotaxis for monocytes and neutrophils (Boudreaux, 1996; Gentry, 1992). Neutrophils migrating to the region with diapedes and macrophages passing to the tissue release many cytokines and growth factors (like interleukin 1-β, IL -1β and TGF β) and initiate the development of the granulation tissue (LaVan and Hunt, 1990).

It has been reported that the capillary basal membrane thickens in diabetics and this in turn impairs migration of leukocytes and elimination of metabolic wastes to cause delays in wound healing (Loder, 1988; Li *et al*., 2007; Munoz-Torres *et al*., 1996). In diabetic wounds, stimulating effects on the release of pro-inflammatory cytokines like macrophage inflammatory protein MIP-2, monocyte chemo-attractant protein-1 (MCP-1), tumor necrosis factor α (TNF α) and IL -1 α occur during the tissue healing period. This in turn elongates the time passing for reaching of neutrophils and macrophages and cause delays in tissue healing (Galkowska *et al*., 2006; Hubner *et al*., 1996; Wetzler *et al*., 2000). Decreases in anti-inflammatory cytokines and growth factors also impair the activation of leukocytes and macrophages (Maruyama *et al*., 2007).

When hyperglycemic rats were compared with the control group, marked reducing of the neutrophil count was found in the wound area (Romana-Souza *et al*., 2009; Maruyama *et al*., 2007). Wetzler *et al*. (2000) reported that migration of neutrophils and macrophages takes longer time in diabetic mice. In this study, it was observed that the period for neutrophils and macrophages to reach the wound are was elongated in the diabetic group, as consistent with the information in the literature. Neutrophil increase in the diabetic group of this study became prominent in day 3 of wound healing. This finding also parallels the literature mentioned earlier.

In conclusion, it was decided that Sc played an important role in the early periods of wound healing in diabetic rats to relieve the pressure on cellular activities and to maintain the wound healing within the normal limits and this could be demonstrated with hematological analyses. However, further studies are required to show this more clearly.

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