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EXPERIMENTAL STUDY

Effect of sildenafil citrate on secondary healing in full thickness skin defects in experiment

Cakmak E[1], Karasoy Yesilada A[2], Sevim KZ[2], Sumer O[2], Tatlidede HS[2], Sakiz D[3]

Medicana Bahcelievler Hospital, Plastic and Reconstructive Surgery Clinic, Istanbul, Turkey.
kzeynep.sevim@gmail.com

Abstract: *Objectives:* An acceleration of the wound healing process expedites chronic wound patient's return to normal social environments significantly. Sildenafil, a cyclic guanosine monophosphate (cGMP)-dependent phosphodiesterase- 5 inhibitor has been shown to be a potent stimulator of angiogenesis through upregulation of cGMP. In our study, sildenafil was administered orally as a cost-effective supplement in the treatment of full thickness defects and chronic wounds in that manner with low incidence of side effects and morbidity.
Materials and methods: Randomly selected 72 Wistar-Albino rats were divided into the two groups, 36 rats in each group. Control group (n =36) was divided further into a secondary healing group consisting of 9 rats and a pathology group consisting of 27 rats (pathology group 1: 9 rats, 4th and 7th day of wound healing, pathology group 2: 9 rats, 10th and 14th day of wound healing, pathology group 3: 9 rats, 21st and 28th day of wound healing. Experimental group consisted of 36 rats which received sildenafil citrate (Viagra® Pfizer, Germany) for secondary wound healing to proceed.
Results: The average wound healing period in the control group was 17.89 days and in the sildenafil citrate administered group 14.56 days. The difference of the epithelialisation on full thickness defects were more prominent on days 5 and 11 postoperatively. In the sildenafil citrate applied group, on the 7th day, the defect was 25 % smaller and on the 13th day, the defect contracted by 38 %.
Conclusion: In conclusion, we believe that sildenafil citrate administered orally is a cost- effective supplement in the treatment of full thickness defects and chronic wounds in that manner with low incidence of side effects and morbidity *(Tab. 4, Fig. 7, Ref. 34)*. Text in PDF *www.elis.sk.*
Key words: sildenafil citrate, secondary wound healing.

Chronic wounds such as pressure ulcers, diabetic ulcers and venous ulcers make up approximately 70 % of all skin wounds (1). Wound healing is a complex process that involves interaction between a number of cell types, extracellular matrix proteins and growth factors. Typical properties of non-healing wounds include a deficiency in epithelisation and granulation tissue formation, arresting of wound healing process in the inflammatory phase, wound infections and wounds being unable to gain adequate strength (2, 3). Recent evidence suggested that nitric oxide might play a role in wound healing and several studies have suggested an association between low NO production and delayed wound healing (4, 5).

Sildenafil is a selective PDE-5 inhibitor. It was first defined as an antianginal drug that enhances both the cellular and endovascular vasodilatory effects of nitric oxide (6–9). However, it proved to be ineffective as an antianginal drug and was then used for erectile dysfunction patients. Lately its' effects on random skin flap models were studied by Sarifakioglu et al with the oral administration of sildenafil citrate (10).

Sildenafil citrate enhances NO by decreasing the down regulation of cGMP levels in the tissue and also by increasing the expression of NOS enzymes in the mRNA and protein levels (11–14). It has been recently reported by Derici et al, the useful effects of sildenafil on abdominal wall healing (15). Hypothesis driven studies in both animals and human subjects indicated that NO influences wound healing at a number of levels including angiogenesis, inflammatory cell proliferation, matrix deposition and remodelling. In addition, PDE-5 antagonists are shown to reduce inflammation and fight infection. NO regulates the proliferation of keratinocytes at wound edges leading to accelerated wound closure. Full thickness skin defects closes by secondary intention, requires wound contraction by myofibroblasts. The objective of this study was to investigate the effect of sildenafil citrate on the wound closure of full thickness skin defects via the evaluation of contraction, rate of epithelialisation and breaking strength in the full thickness skin defects.

Materials and methods

The study was performed on adult female and male Wistar- Albino rats weighing between 250–300 grams, at Sisli Etfal Research and Training Hospital Animal Research Laboratory.

[1]Medicana Bahcelievler Hospital, Plastic and Reconstructive Surgery Clinic, Istanbul, Turkey, [2]Sisli Etfal Research and Training Hospital, Department of Plastic and Reconstructive Surgery, Istanbul, Turkey, and [3]Sisli Etfal Research and Training Hospital, Department of Pathology, Istanbul, Turkey

Address for correspondence: K.Z. Sevim, MD, Sisli Etfal Research and Training Hospital Department of Plastic Surgery, Acibadem Koftuncuoglu Street Is Bonkasi Bloklori A6 Blok d:33, Acibadem, Istanbul 34718, Turkey. Phone: +09.053.25203679

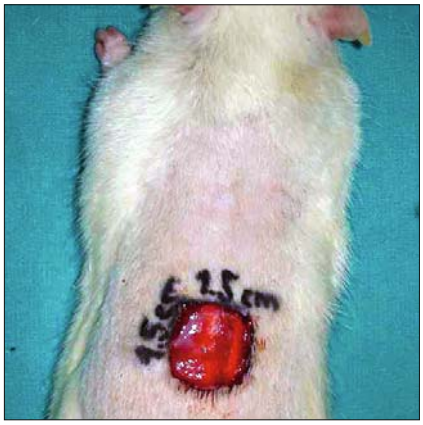

Fig. 1. 1.5 x1 .5 cm Full thickness defect created on rat dorsum.

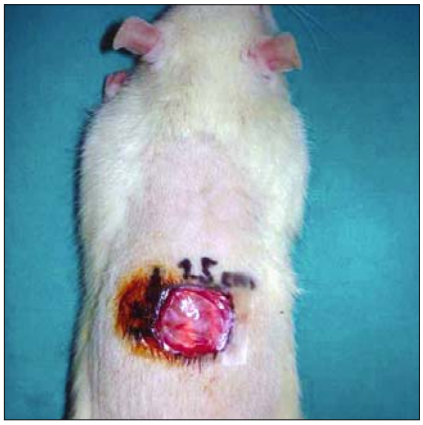

Fig. 2. Utilisation of Omiderm for wound care.

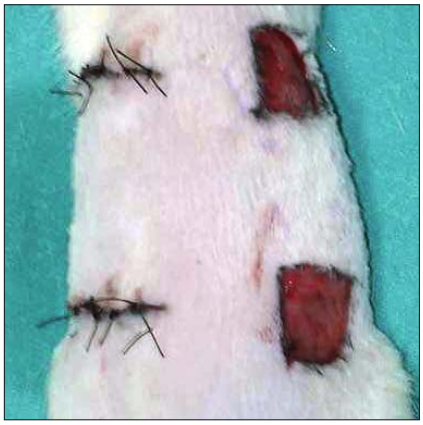

Fig. 3. Pathology group after surgery.

Housing and caging for the animals was in accordance with the National Research Council guidelines. Study was approved by the Sisli Etfal Research and Training Hospital's local ethics committee. Randomly selected 72 Wistar-Albino rats were divided into two groups, 36 rats in each group. They were kept at a 12-h light and dark cycle and were fed a standard diet with water ad libitum. All animal procedures were performed according to the National Institute of Health guidelines for the use of experimental animals. Control group (n=36) was divided further into the secondary healing group consisting of 9 rats and the pathology group consisting of 27 rats (pathology group 1: 9 rats, 4th and 7th day of wound healing, pathology group 2: 9 rats, 10th and 14th day of wound healing, pathology group 3: 9 rats, 21st and 28th day of wound healing.

Experimental group consisted of 36 rats with the same groups, which received sildenafil citrate (Viagra® Pfizer, Germany) dissolved in saline as adjunctive therapy for secondary wound healing to proceed. In all groups, the animals were anesthetized with intramuscular Ketamine HCl IM (Ketalar® 50 mg/ml, Pfizer, Germany). After shaving the dorsum of the rats and prepping the skin with chlorhexidine, in the control secondary healing group, a square shaped 1.5 x 1.5 cm full thickness skin defect including the panniculosus carnosus was created (Figs 1–3) and the wound was followed up with Omiderm dressing (16–18). After the procedure, starting from postoperative 3 days, the ungranulated areas were measured and transferred to an acetate paper which was analyzed by a computer software for healed surface area measurements. Wound healing periods were noted and no sildenafil citrate was administered in this group. In order to assess the full thickness wound in all stages (inflammation, proliferation and remodelling), 3 separate pathology groups were formed each consisting of 9 rats. On the dorsum of the rats, two 1.5 x 1.5 square defects each 2 cm apart from each other was created and biopsies marking the different stages of wound healing were harvested on 4th and 7th for the group 1, 10th and 14th days for the group 2 and 21h and 28th day for the group 3. In the sildenafil citrate applied experimental groups, SC was applied daily perorally as gavage with 10 mg/kg body weight of dosage and was first administered 2 hours after the formation of the full thickness skin defects and for the secondary healing group continued until the wound healed. In the pathology harvesting groups, SC continued until the day of sacrifice for assessing the effects in different stages of wound healing. Dressing changes were performed with Omiderm on consecutive days throughout the experiment. The animals were not sacrificed since the histopathological specimen acquired, didn't result in major morbidity in animals. The chosen dosage of sildenafil citrate was fivefold higher than the standard dose used in humans (0.7–1.5 mg/kg) due to the higher metabolism in rodents.

Histological studies were performed on formalin-fixed, alcohol dehydrated, parafin-embedded, 5 micrometer sections stained by hematoxylin-eosine and evaluated with aconventional light microscope (Olympus BX60 Microscope, Olympus, Japan). Digital images were captured using Canon EOS 500D.

The results were expressed as the mean ±SD. Wound healing intervals, surface area measurements, mechanical resistance test and comparison of breaking strength (mmHg) were assessed with SPSS v 10.01 software and comparisons between the groups were performed using the paired-sample t test and for the nonparametrical data, the Mann–Whitney U-test was performed. Differences were considered statistically significant when $p < 0.005$.

In the control and experimental groups, on the postoperative 30 days, scar tissue resistance was tested with Instron 4411 mechanical resistance testing device. For this purpose, full thickness skin on the dorsum of rats in dimensions 5 x 2.5 cm were prepared and attached to the machine and force was applied to extend the skin 10 mm/minute on the proximal and distal ends. As a result, a separation of the wound edges at maximum weight was determined.

Results

Among the control and the sildenafil administered groups, no significant difference was noted in terms of general nutrition and wound infection rates. The average wound healing period in the control group was 17.89 days and in the subcutaneously administered group 14.56 days.

The wound healing periods of the secondary healing groups are shown on Table 1. The surface area measurement of the control

Tab. 1. Comparison of wound healing period in secondary healing group versus sildenafil citrate administered group (days).

Rat #	Control	Sildenafil
1	17	15
2	19	13
3	19	15
4	15	15
5	19	17
6	17	15
7	19	13
8	19	13
9	17	15
(Mean)	17.89	14.56
Standard Deviation (SD)	1.45	1.33

Tab. 4. Mechanical test results in sildenafil administered full thickness defect groups (maximum weight=kg).

Rat#	Control	Sildenafil
1	3.658	7.544
2	4.295	3.933
3	4.134	5.497
4	5.013	8.268
5	5.852	4.423
6	3.570	6.152
7	4.718	7.624
8	3.825	6.467
9	3.893	5.989
Mean	4.11	6.21
Standard Deviation	1.10	1.46

group are in the Table 2 and Sildenafil citrate administered group's surface area measurements are shown in the Table 3.

The difference of the epithelialisation of full-thickness skin defects are shown of postoperative 5 day and postoperative 11 day (Figs 4 and 5). Wound healing periods, surface area measurements and mechanical resistance test analysis were interpreted statistically using the Mann–Whitney U-test (SPSS v 10.1).

The results were classified as significant if $p < 0.005$ was reached. In the secondary healing group, the use of sildenafil citrate significantly reduced the wound healing time ($p = 0.001$).

In the full thickness defects, the surface area measurement of the sildenafil administered group showed a significantly less time on 5th, 7th, 9th, 11th, 13th day. However, no significant difference was noted on the third day. Among the pathology gathering groups, skin biopsies were taken on 4., 7th, 10th, 14th, 21st, and 28th as planned and degree of re-epithelisation, proliferation of polymorphonuclear leucocytes, macrophages, lymphocytes, vessels and fibroblasts were evaluated. To summarize the results, the fourth day pathology groups revealed a notable amount of inflammation in the dermal layer. Re-epithelisation was not seen in neither the secondary healing control group nor the experimental group. The tenth day pathology group results revealed a capillary rich granulation tissue in both groups and the 14th day results showed that the inflammatory cell density in the dermal layer was notable elevated in the sildenafil administered group . On the 21st day, the pathology specimens proliferated fibroblasts and capillaries were lined under a perfectly formed epidermis (Figs 6 and 7). A wound's stretching force is essential in reflecting the collagen bundles and subdermal healing of the tissue. In our study, the control group showed a stretching force of 4.11 kg and in the sildenafil group showed a stretching force of 6.21 kg. Sildenafil citrate increased the mechanical resistance by 51 %.

Tab. 2. Surface area measurements in secondary healing groups (cm^2).

Rat #	3.day	5.day	7.day	9.day	11.day	13.day	15.day	17.day	19.day
1	2.23	1.58	1.08	0.70	0.41	0.22	0.16	–	–
2	1.81	1.46	0.91	0.58	0.43	0.32	0.28	0.07	–
3	2.05	1.62	1.12	0.77	0.45	0.35	0.26	0.09	–
4	2.10	1.58	0.99	0.56	0.20	0.14	–	–	–
5	2.63	2.12	1.43	0.82	0.39	0.22	0.16	0.05	–
6	2.54	1.70	0.92	0.52	0.32	0.24	0.07	–	–
7	1.96	1.38	0.82	0.61	0.52	0.28	0.18	0.07	–
8	2.68	1.67	0.90	0.62	0.41	0.29	0.22	0.16	–
9	1.99	1.28	0.76	0.47	0.32	0.13	0.09	–	–
Mean	2.22	1.60	0.99	0.62	0.38	0.24	0.18	0.07	–
Standard Deviation(SD)	0.32	0.31	0.19	0.11	0.25	0.12	0.07	0.01	

Tab. 3. Surface area measurements in sildenafil administered full thickness defect groups (cm^2).

Rat #	3. day	5. day	7. day	9. day	11. day	13. day	15. day	17. day
1	2.11	1.23	0.55	0.32	0.25	0.12	–	–
2	2.05	1.28	0.64	0.29	0.15	–	–	–
3	1.87	0.71	0.42	0.16	0.14	0.08	–	–
4	2.22	1.21	0.73	0.51	0.38	0.18	–	–
5	2.59	1.76	0.93	0.69	0.43	0.22	0.13	–
6	2.25	1.23	0.72	0.54	0.31	0.14	–	–
7	2.18	1.56	0.74	0.34	0.10	–	–	–
8	1.97	1.44	0.86	0.42	0.15	–	–	–
9	2.13	1.62	1.06	0.70	0.42	0.18	–	–
Mean	2.15	1.34	0.74	0.44	0.26	0.15	0.13	–
Standard Deviation (SD)	0.20	0.30	0.19	0.18	0.13	0.08	–	–

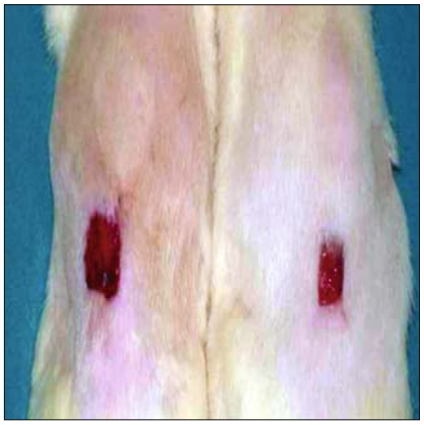

Fig. 4. Postoperative 5th day comparison between the control group and sildenafil citrate administered group.

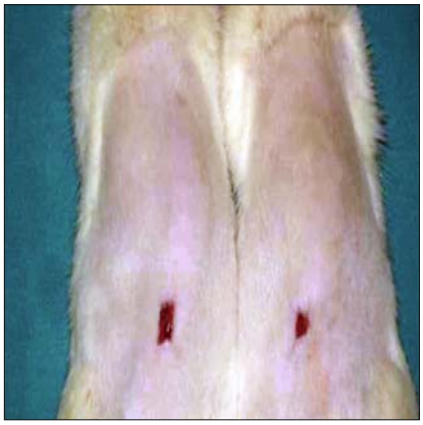

Fig. 5. Postoperative 11th day comparison between the control group and sildenafil citrate administered group.

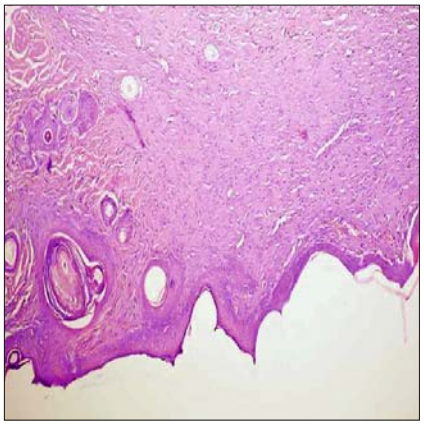

Fig. 6. Sildenafil administered secondary healing group postoperative 14th day. Superficial epithelialisation, dense fibroblast and capillary proliferation observed. H&E x100.

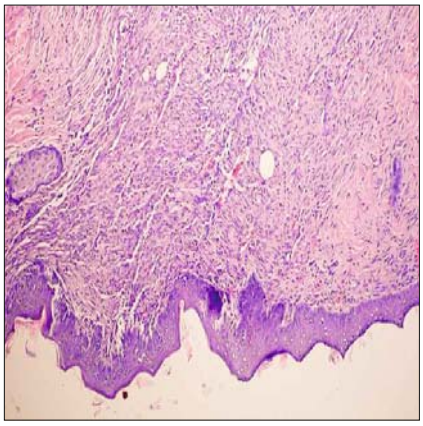

Fig. 7. Control secondary healing group postoperative 14th day. Superficial epithelialisation, decreased polymorphonuclear leukocyte count. H&E x100.

Discussion

Achieving an ideal, permanent, replacement of skin in full and deep partial thickness defects of the skin (i.e. burn injuries, chronic wounds) remains to be a significant problem. There have been many studies over the past years investigating the efficacy of various skin substitutes such as cultured epithelium, keratinocytes and integra® (19). However, treatment with skin substitutes are quite costly throughout the whole healing process. For that reason, we believe that sildenafil citrate supplemented wound therapy is a more beneficial one, especially in wounds healing through secondary intention and chronic wounds.

In clinical practice, there are reports of sildenafil citrate being utilized successfully on antiphospholipid syndrome induced refractory skin ulcerations with little adverse reactions (20, 21).

There is a report on wound healing of diabetic rats by sildenafil citrate and not recurring after 18 months of treatment due to the vasodilatory effect of sildenafil (22). There are number of studies of electrostimulation and ultrasound therapy being beneficial on secondary wound healing (22–28). Hydrotherapy, hyperbaric oxygen therapy, lasers, negative pressure therapy and a number of growth factors are reported to be useful in multiple studies.

In our study, we utilized Omiderm (a polyurethane structured layer) to aid in wound healing. Omiderm accelerates the keratinocyte proliferation process by accumulating under the exsudate and thereby upregulating the epithelisation process as well as protecting the wound from being infected by the environment (16). Nitric oxide (NO) has been discovered in 1980's and it's effects on wound healing has been extensively studied over the past decades (29–31). NO is synthetized by many types of cells and acts in neurotransmission, regulating the immune system, vessel smooth muscle relaxation and decreasing platelet aggregation to name a few of the properties. Shekhter et al studied the effect of NO on the purulent wound model that they created and reported that NO decreased the amount of inflammatory and microbial activity in the wound, increased fibroblast proliferation, contraction and epithelisation rate (32).

Sildenafil citrate decreases cGMP break-down which prevents platelet aggregation and dilates the vessels and indirectly increase the effects of NO, which in the end we believe especially aids in secondary wound healing process in full thickness defects and chronic wounds. Sildenafil citrate in literature has been administered orally, via intravenous route or locally. The oral dose was described as 1 mg/kg/ day – 20 mg/kg/ day depending on each specific case. We chose to apply it 5 mg/kg/ day by orogastric lavage, which has been proven to be effective in studies (15).

Sildenafil citrate, being a powerful and specific inhibitor of cGMP specific PDE type 5 has been utilized in erectile dysfunction and even pulmonary hypertension without any major side effects. Sarifakioglu et al has used sildenafil citrate in improving skin flap survival studies in rats and shown the effect to be dose related (10). Ulusoy et al used sildenafil citrate with fibrin glue to improve skin flap survival (30). We want to emphasize in our study the effect of sildenafil citrate in secondary wound healing specifically where wounds heal by contraction. In such wounds, the amount of growth factors transported to the wound by neovascularisation may change the whole healing process. Sildenafil citrate decreases platelet aggregation therefore we applied the sildenafil citrate 2 hours after surgery in order not to influence platelet aggregation mechanisms.

A study has been shown where sildenafil citrate increased the migration of neutrophils and monocytes in the early phase of wound healing in diabetic rats and relieved the pressure on cellular activities.

In terms of the surface area reduction in full thickness defects, a significant difference was seen after 3 days. In the sildenafil citrate applied group, on the 7th day the defect was 25 % smaller and on the 13th day the defect contracted by 38 %.

In conclusion, we believe that sildenafil citrate administered orally is a cost- effective supplement in the treatment of full thickness defects and chronic wounds in that manner with low incidence of side effects and morbidity.

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