ORIGINAL ARTICLE

Effect of sildenafil citrate in nicotine-induced ischemia: An experimental study using a rat model

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H Baykan, I Ozyazgan, C Tayyar Selçuk, M Altıparmak, M Özköse, K Özyurt. Effect of sildenafil citrate in nicotine-induced ischemia: An experimental study using a rat model. Can J Plast Surg 2013;21(4):217-220.

Recent experimental and clinical studies have demonstrated the negative effects of nicotine on the viability of skin flaps. Necrotic damage to skin flaps can result in significant complications including delayed wound healing, dehiscence and wound contraction. Phosphodiesterase type 5 inhibitors, such as sildenafil citrate, have a protective effect in ischemic injuries of the brain, kidney, myocardium, spinal cord, ileum and testes. In the present study, the authors evaluated the effect of sildenafil citrate on the viability of skin exposed to nicotine-induced ischemia in Sprague Dawley rats. In the preoperative period, the rats were divided into three groups of 10 rats each. Group C was treated with subcutaneous saline and group S and group N were treated with 2 mg/kg nicotine, administered subcutaneously twice per day for 28 days. McFarlane flaps were created in all experimental animals using an incision measuring 7 cm × 3 cm. Postoperative treatment varied among the groups: group S was treated with 20 mg/kg/day sildenafil citrate, while group C and group N were treated with equivalent doses of saline for seven days. A laser Doppler flow meter was used to monitor the microvasculature. Preoperative measurements of the microvasculature revealed decreased blood flow in group N and group S, both of which were treated with subcutaneous nicotine. During the postoperative evaluation, a trend toward increased blood flow was observed in group S compared with the group with nicotine-induced ischemia treated with saline alone postoperatively (group N). A visual fluorescein dye test was used to predict skin viability and demonstrated diminished skin viability in group N and group S ($P<0.05$) during the preoperative period. Following treatment with sildenafil for seven days, a statically significant improvement in skin viability was observed in group S ($P<0.05$). Nicotine decreased blood flow within the skin and impaired skin viability, while postoperative application of sildenafil significantly ameliorated the ischemic effects of nicotine and improved skin viability. Future studies will be required to evaluate the clinical use of sildenafil for the improvement of blood flow in ischemic injury of the skin.

Key Words: *Flap; Ischemia; Nicotine; Sildenafil citrate*

L'effet du citrate de sildénafil en cas d'ischémie induite par la nicotine : une étude expérimentale sur un modèle de rat

De récentes études expérimentales et cliniques démontrent les effets négatifs de la nicotine sur la viabilité des lambeaux cutanés. Les dommages nécrotiques des lambeaux cutanés peuvent s'associer à d'importantes complications, y compris le délai de guérison de la plaie, la déhiscence et la contraction de la plaie. Les inhibiteurs de la phosphodiestérase de type 5, tels que le citrate de sildénafil, ont un effet protecteur sur les lésions ischémiques du cerveau, du rein, du myocarde, de la moelle épinière, de l'iléon et des testicules. Dans la présente étude, les auteurs ont évalué l'effet du citrate de sildénafil sur la viabilité de la peau exposée à une ischémie induite par la nicotine chez des rats de Sprague Dawley. Avant l'opération, les rats ont été divisés en trois groupes de dix rats. Le groupe C a reçu un traitement de solution physiologique par voie sous-cutanée, tandis qu'on a administré au groupe S et au groupe N un traitement de 2 mg/kg de nicotine par voie sous-cutanée deux fois par jour pendant 28 jours. Les chercheurs ont créé des lambeaux de McFarlane chez tous les animaux expérimentaux au moyen d'une incision de 7 cm × 3 cm. Le traitement postopératoire variait selon les groupes : le groupe S a reçu 20 mg/kg/jour de citrate de sildénafil, tandis que le groupe C et le groupe N ont reçu des doses équivalentes de solution physiologique pendant sept jours. Ils ont utilisé un débitmètre Doppler au laser pour surveiller les microvaisseaux. Les mesures préopératoires des microvaisseaux ont révélé une diminution du débit sanguin dans le groupe N et le groupe S, tous deux traités par de la nicotine sous-cutanée. Pendant l'évaluation postopératoire, les chercheurs ont observé une tendance vers l'augmentation du débit sanguin dans le groupe S par rapport au groupe N, qui avait subi une ischémie induite par la nicotine traitée au moyen d'une simple solution physiologique après l'opération. Un test visuel de coloration à la fluorescéine a permis de prédire la viabilité cutanée et d'en démontrer une diminution dans le groupe N et le groupe S ($P<0,05$) pendant la période préopératoire. Après le traitement au sildénafil pendant sept jours, les chercheurs ont observé une amélioration significative de la viabilité cutanée dans le groupe S ($P<0,05$). La nicotine réduisait le débit sanguin de la peau et nuisait à la viabilité cutanée, tandis que l'application postopératoire de sildénafil atténuait considérablement les effets ischémiques de la nicotine et améliorait la viabilité cutanée. De futures recherches s'imposent pour évaluer l'utilisation clinique du sildénafil pour améliorer le débit sanguin en cas de lésion ischémique de la peau.

Recent experimental and clinical studies have demonstrated the negative effects of nicotine on the viability of skin flaps (1-7). Nicotine induces vasoconstriction in peripheral vascular structures, and diminishes gas and nutrient exchange in peripheral tissues (8,9). Ischemia, venous congestion, the development of hematomas and other factors may influence skin flap viability. Necrotic skin flaps can result in significant complications, including delayed wound healing, dehiscence and wound contraction. The distal portions of skin flaps are more prone to necrotic damage. Chronic vasoconstriction can result in pathophysiological changes that impair the perfusion of oxygen into the skin flap. Tissues with absent or limited capillary blood flow will become necrotic over time (5,7,10). Smokers are at an increased risk of developing necrotic damage following skin flap surgery, which may result in additional risk to the patient and necessitate additional complex surgical procedures (11). However, pharmaceutical agents, including sympatholytics, vasodilators, calcium channel blockers, hemorheological agents, prostaglandin inhibitors, anticoagulants, glucocorticoids and free radical scavengers, may have beneficial effects. Surgical delay is preferred by many physicians and improves survival of the skin flap (9,12,13).

Sildenafil citrate, a specific inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type-5 (PDE-5), has been primarily used for the treatment of erectile dysfunction. Sildenafil citrate causes dilation of the peripheral arteries and veins as a result of enhanced nitric oxide (NO) synthesis (13). Several studies have demonstrated the inhibition of lipid peroxidation by cyclic adenosine monophosphate and cGMP analogues (14-16). PDE-5 inhibitors have a protective effect in ischemia/reperfusion injury to the brain, kidney, myocardium, spinal cord, ileum and testes (17-22).

The aim of the present study was to assess the effect of sildenafil citrate on the viability of random-pattern skin flaps exposed to nicotine-induced ischemia in Sprague Dawley rats.

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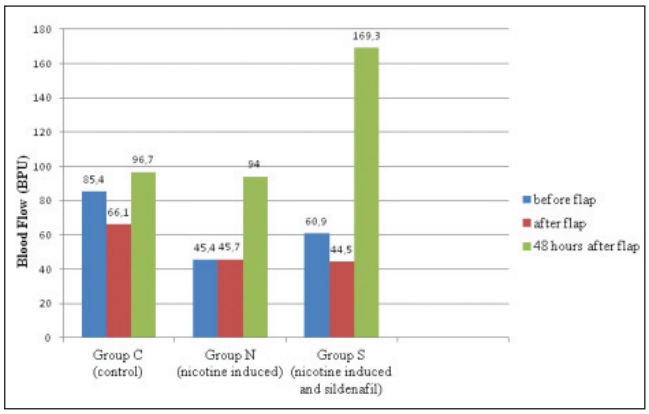

Figure 1) *Blood flow according to experimental stage. BPU Blood perfusion units*

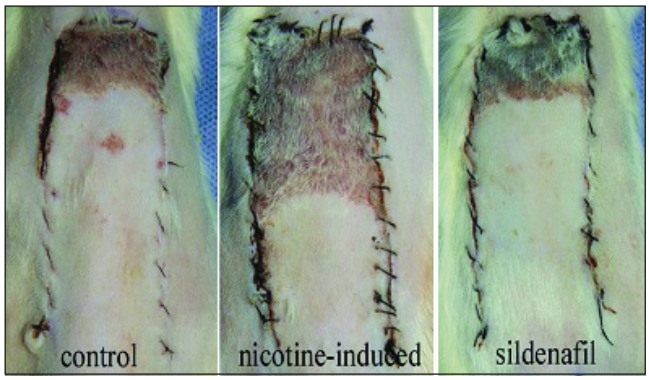

Figure 2) *Macroscopic evaulations of flap survival on postoperative day 7*

METHODS

The present study was conducted at an experimental clinical research centre and with the approval of the local ethics committee. Female Sprague Dawley rats weighing between 250 g and 300 g were used. Experimental animals were housed individually in single cages under 12 h day-night cycle and were fed a standard diet and water ad libitum. The rats were randomly divided into three groups, with 10 rats in each group in the preoperative phase: group C was treated with 0.5 mL saline, administered subcutaneously; and group S and group N were treated with 2 mg/kg nicotine, administered subcutaneously twice per day for 28 days (8). Subcutaneous ketamine-hydrochloride and xylazine were administered for anesthesia at 60 mg/kg and 10 mg/kg, respectively. Random-pattern, caudal pedicle McFarlane flaps with a 7 cm × 3 cm flap size were cut using a scalpel (23) in all experimental animals from each group. In the postoperative phase, group S was treated twice daily with sildenafil citrate at 10 mg/kg, while group C and group N were treated with equivalent doses of saline by gavage for seven days (24).

A laser Doppler flow meter (Model MP 100, Biopac Systems, USA) was used to monitor the microvasculature. Microvascular measurements were performed in the critical zone of the skin flap during the preoperative period immediately after the operation and on the second postoperative day using only the weight of the probe under similar physical and environmental conditions. The duration of the procedure was standardized to 3 min. The mean blood flow rate over a minimal oscillation period of 50 s was recorded for later analysis. The measurements recorded represent the relative perfusion of the microvasculature with red blood cells or blood perfusion units (BPU). Data were collected using an interactive program (Acknowledge, version 3.7.2, Biopac, USA) that was also used to analyze the BPU data.

The fluorescein dye tests were performed 30 min after the flap was sutured. Exactly 0.3 mL of 10% sodium-fluorescein was administered by intraperitoneal injection. The ratio of fluorescein-stained (viable portions of flap) and nonstained (nonviable portions of flap) tissue was calculated from images collected using transparent paper with millimeter grid markings in a dark room and a fluorescent lamp. Viable and nonviable portions of the flaps were evaluated macroscopically on postoperative day 7. In addition, the relative improvement in individual skin flap viability was calculated by comparing the fluorescein-stained areas at 30 min with the macroscopically viable area on postoperative day 7. At the end of the study, all rats were euthanized using high-dose ketamine.

Statistical analysis

The comparison and analysis of skin flap blood flow at different times and under different experimental conditions, and the fluorescein staining rates of skin flaps in each experimental group were performed using SPSS (IBM Corporation, USA). The Kolmogorov-Smirnov test was used to test normality for continuous variables. A one-way ANOVA test was applied to evaluate differences among groups according to normally distributed parameters. Where differences were observed, the Tukey test was performed to evaluate differences between experimental groups in a pairwise manner. For non-normally distributed parameters, differences in the dataset were evaluated using the Kruskall-Wallis test. Where significant variation existed, the Mann-Whitney U test was used to evaluate differences between groups in a pairwise manner. A paired Student's *t* test was used to evaluate differences in normally distributed values of blood flow measurements taken at two different time points in the same animal. The Wilcoxon test for related samples was used to evaluate the statistical significance of differences in changes in non-normally distributed parameters between experimental groups; $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Two rats each in group S and group C and one rat in group N died during the study. In the preoperative phase, the mean (± SD) skin blood flow in the dorsal skin was 85.4±42.4 BPU, 45.3±14.0 BPU and 50.8±18.8 BPU in group C, group N and group S, respectively. Importantly, skin blood flow was significantly decreased in the nicotine-treated groups (group N and group S) ($P<0.05$).

The blood flow values were measured immediately after the flap was sutured in all groups as follows: group C 66.1±25.6 BPU; group N 45.7±16.2 BPU; and group S 44.4±21.4 BPU. No significant difference compared with the preoperative skin BPU was observed in any of the experimental groups ($P>0.05$). The fluorescein dye test was performed 30 min after suturing the flap and resulted in the following fluorescein-staining ratios: area 66.0±6.9%, 40.2±14.8% and 38.7±10.3% in group C, group N and group S, respectively. The viable portions (fluorescein stained) of the skin flaps in the nicotine-treated groups (group N and group S) were significantly decreased relative to group C ($P<0.05$).

The mean blood flow values in group N and group S on postoperative day 2 were 94.8±54.8 BPU and 169.2±138.0 BPU, respectively. The blood flow values of group N and group S were significantly increased on postoperative day 2 relative to the initial blood flow values ($P<0.05$). However, the mean blood flow values did not dffer significantly between group N and group S on postoperative day 2 ($P>0.05$). Blood flow increased significantly in the nicotine-treated groups from between the day of the procedure and postoperative day 2 ($P<0.05$), with blood flow increasing in group S relative to group N. However, this difference was not statistically significant ($P>0.05$). There was no change in blood flow in group C (Figure 1).

The percentage of viable skin area was 81.0±7.0%, 65.8±8.9% and 76.9±10.6% in group C, group N and group S, respectively, on the seventh postoperative day (Figure 2). Nicotine decreased skin flap viability significantly ($P<0.05$), and sildenafil treatment significantly increased the skin flap viability in nicotine-damaged skin flaps ($P<0.05$). No statistically significant difference between group C and group S was observed.

TABLE 1
Blood flow, fluorescein staining and survival measurement according to treatment group

Group	Blood flow (blood perfusion units)			Ratio of fluorescein staining after flap, %	Flap survival, %	Change in flap survival relative to fluorescein staining, %
	Before flap	After flap	48 h after flap			
C (n=8)	85.4±42.4	66.1±25.6	96.6±42.2	66.0±6.9	81.0±7.0	23.9±17.2
N (n=9)	45.3±14.0*	45.7±16.2	94.0±54.8	40.2±14.8	65.8±8.9*	84.2±46.5*
S (n=8)	50.8±18.8*	44.4±21.4	169.2±138.0	38.7±10.3	76.9±10.6**	124.8±98.8

*Data presented as mean ± SD. Group C Subcutaneous saline; Group N Nicotine-induced ischemia treated with saline alone; Group S Nicotine-induced ischemia treated with 20 mg/kg/day sildenafil citrate. *P<0.05 compared with group C; **P<0.05 compared with group N*

In addition, the proportion of flap viability improvement between 30 min after the procedure and the seventh postoperative day were 23.9±17.2%, 84.2±46.5% and 124.8±98.8% in group C, group N and group S, respectively. The difference in the rate of skin flap improvement between group C and group S was statistically significant (P<0.05). Animals in group S had 40% more viable skin area compared with group N animals. However, the difference between group S and group N was not statistically significant (P>0.05). The blood flow measurements, proportional flap viability and the improvement rate of all groups are summarized in Table 1.

DISCUSSION

Random-pattern flaps are an indispensable method of treatment in plastic and reconstructive surgery. Nicotine is the primary stimulant contained in cigarettes. Nicotine induces vasoconstriction that is damaging to the peripheral vasculature (1,2,8,9). Various agents may be used to prevent or treat nicotine-induced vascular damage. In our previous study, we demonstrated that angiotensin (1-7) protects against the damaging effects of nicotine in randomized skin flaps (25).

In the present study, we observed ischemia and decreased viability in nicotine-treated skin flaps, similar to the model of Forrest et al (8). Insufficient blood flow results in ischemia that increases in severity from the proximal to the distal portion of the skin flap. Necrosis is the inevitable consequence of ischemia in the distal portions of a skin flap (10). Sawada et al (26) have divided flaps into three parts: the distal zone; the proximal zone; and the critical zone. They hypothesized that limiting ischemia in the critical zone may prevent necrosis and increase skin flap viability. Therefore, we measured blood flow in the critical zone of the skin flap.

In the present study, we hypothesized that sildenafil increases the viability of random-pattern nicotine-treated skin flaps through vasodilation of arteries and veins in the critical zone. To the best of our knowledge, the present study was the first to assess the effect of sildenafil in treating nicotine-damaged skin flaps. We observed that nicotine administration significantly decreased skin blood flow; this confirmed that our experimental model of nicotine-induced ischemia of the skin was appropriate for evaluating the potential effects of sildenafil (9). However, on postoperative day 7, skin flap viability was increased in the sildenafil-treated group compared with the group receiving nicotine alone. This result indicates the beneficial effects of sildenafil on skin flap viability in nicotine-induced ischemia (13,14,24,27,28).

Several studies have reported that oral, intraperitoneal or topical sildenafil citrate increases skin flap viability in random-pattern experimental skin flaps. Sarifakioglu et al (24) reported that oral sildenafil increased skin flap viability in a dose-dependent manner. They suggested that sildenafil may have both vasodilator and anti-aggregant activity in damaged skin. However, in another study, subdermal injection of sildenafil citrate resulted in decreased skin flap viability (27). Other researchers have suggested that sildenafil citrate improves skin flap viability by enhancing expression of vascular endothelial growth factors (28). Similar results were obtained by administering sildenafil throughout the skin bed suspended in a fibrin matrix. This mode of application increased the degree of vascularization and skin viability effecting a dose-dependent manner (29). However, Tsai et al (30) reported that sildenafil had no effect on the degree of vascularization, and that the increase in skin flap viability resulting from sildenafil treatment was primarily attributable to enhanced vasodilation. Similarly, we observed that sildenafil treatment enhanced skin flap viability following nicotine-induced ischemia but had no significant effect on blood flow. However, nicotine-derived ischemia may result in vasodilation via NO release. Therefore, blood flow is likely to increase in nicotine-treated skin flaps. Sildenafil also enhances NO release and subsequently causes vasodilation, although our results did not indicate a significant increase in blood flow following sildenafil treatment of nicotine-induced ischemic skin flaps.

According to our results and findings from previous studies, sildenafil enhances skin viability in random pattern flaps. While it is clear that sildenafil treatment limits nicotine-induced ischemic damage in skin flaps, the precise biochemical mechanism reamins unclear. Previously unknown or alternative mechanisms, rather than vasodilation or increased vascularization, are possible. Further studies evaluating these mechanisms will inform novel clinical approaches to wound healing and improve our ability to prevent ischemic injuries of the skin.

DISCLOSURES: The authors have no financial disclosures or conflicts of interest to declare.

ACKNOWLEDGEMENT: The authors are grateful to Dicle University DUBAP for their sponsorship and English editing of the manuscript.

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