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Effect of nicotine treatment and withdrawal on random-pattern skin flaps in rats

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Abstract

Background: Tobacco use is associated with a high incidence of skin necrosis after surgery. The ideal timing for the cessation of tobacco use before plastic surgery has not, however, been precisely determined. The aim of this work was to define the ideal duration of nicotine withdrawal prior to random-pattern skin flap surgery in rats.

Methods: Groups of 11 animals were subcutaneously injected with saline or nicotine (2 mg/kg) twice a day and subjected to random-pattern skin flap surgery according to the following protocol: Group I: continuously injected with saline 4 weeks before and 1 week after the surgery; Group II: injected with nicotine for 4 weeks until the day of the surgery; Group III: injected with nicotine for 4 weeks until one day before the surgery; Group IV: injected with nicotine for 4 weeks until 5 days before the surgery; Group V: injected with nicotine for 4 weeks until 10 days before the surgery; Group VI: continuously injected with nicotine for 4 weeks before and 1 week after the surgery. McFARLANE skin flaps were performed on the dorsal skin, and the rats were sacrificed 1 week after the surgery.

Results: The necrotic area was smaller in group I (8.85 cm^2) than in group II (12.15 cm^2), III (12.88 cm^2) and VI (14.84 cm^2) (ANOVA $p<0.0001$). There was no difference between groups I, IV (10.13 cm^2) and V (9.27 cm^2).

Conclusions: In conclusion, 5 days before surgery was considered the ideal time for nicotine withdrawal in this experimental model.

Keywords: Nicotine; McFARLANE skin flaps; Necrosis; Tobacco; Rats

Introduction

Tobacco use is associated with a higher frequency of skin necrosis after surgery both in clinical practice (Rees et al., 1984; Reifkohl et al., 1986; Souza and Psillakis, 1989) and experimental studies (Campos et al., 2001; Van Adrichen et al., 1996). Nicotine, one of the main components of tobacco, is responsible for hemodynamic and endothelial cell changes that favor thrombosis. Evidence shows that serum levels of nicotine decrease after tobacco withdrawal, decreasing the risk of tissue necrosis. The ideal moment for cessation of tobacco use before plastic surgery has not, however, been precisely determined.

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The skin flap is a commonly used model for testing new procedures in plastic surgery. The viability of skin flaps is dependent upon their morphology: they can be axial (with an individualized vascular pedicle) or random (with a random-pattern pedicle). Hence, the uncertain vascular support common to random skin flaps may be taken into account when defining their sizes and shapes. An inadequate length to width ratio may result in ischemia and necrosis (Ferreira et al., 1987). Necrosis of a skin flap may be due to extrinsic or intrinsic factors, some of them influenced by nicotine use. Extrinsic factors may be systemic (hypotension, vascular diseases and infection), local (compression, temperature and tension) or associated with the surgical technique applied. Blood flow is the single most important intrinsic factor.

The aim of this work was to study the ideal time for nicotine withdrawal in order to prevent drug-related increase in flap necrosis in rats.

Methods

Animals

Sixty-six Wistar rats, weighting between 244 and 298 g, were obtained and housed at experimental animal facilities of the Health Sciences Institute (ICS—Federal University of Bahia). The animals were maintained with commercial balanced rat chow and water *ad libitum*.

Nicotine

Pure nicotine (SIGMA) was diluted into a sterile saline solution to a concentration of 2.5 mg/ml.

Nicotine treatment and surgical procedure

The animals were divided into six groups of 10–11 animals:

Group I: (control) animals were subcutaneously injected with saline (0.2 ml), twice a day, for 4 weeks before and 1 week after the surgical procedure.

Group II: the rats were injected with nicotine (2 mg/kg) (Forrest et al., 1987) twice a day, for 4 weeks before the surgery. In the week following the surgery nicotine was replaced by saline in the injections.

Group III: the animals were injected with nicotine for 4 weeks and nicotine administration was stopped one day before the surgery. Nicotine was replaced by saline in the injections given the day before and in the week after the surgery.

Group IV: the rats were injected with nicotine for 4 weeks and nicotine administration was stopped 5 days before the surgery. Nicotine was replaced by saline in the injections given in the 5 days preceding and in the week after the surgery.

Group V: the animals were treated with nicotine for 4 weeks and nicotine administration was stopped 10 days before the surgery. Nicotine was replaced by saline in the injections given 10 days preceding and in the week after the surgery.

Group VI: the rats received nicotine for 4 weeks before and 1 week after the surgical procedure.

In the surgical procedure, the animals were anesthetized by intraperitoneal injection of pentobarbital (40 mg/kg). A 10cm × 4 cm skin flap, cranially based at the level of the inferior scapular angle, was dissected from the rat's dorsum (McFarlane et al., 1965). After the procedure, the flap was sutured back on its bed by means of simple stitches of nylon monofilament (Campos et al., 2001; Laurence et al., 1984; Nolan et al., 1985; Gomes et al., 1994; Van Adrichen et al., 1996).

All the animals were sacrificed 1 week after the surgery. All experiments were conducted in accordance with the Oswaldo Cruz Foundation guidelines for experimentation with animals. The animals were sacrificed in a closed chamber with sulfuric ether. Then, a decal of the whole flap was performed in order to define the transition between necrosis and normal tissue. This decal, obtained with a pencil and a sheet of translucent tracing paper, was then analyzed to evaluate the necrotic area (Sigma Scan program—Jandell Scientifics, San Francisco, CA).

Expression and statistical significance of results

Necrotic areas were expressed in cm^2, as mean + standard deviation or as median and quartiles (box-plot graph) of measurements obtained from animals of the same group. The significance of the differences observed among the groups was tested using one-way analysis of variance (ANOVA). When the F-test was found to be significant, the difference between two groups was identified using the Student–Newman Kleus' test. The level of rejection of the null hypothesis was fixed at $p < 0.05$.

Results

The general goal of this study included the confirmation of the deleterious effect of nicotine in the skin flap model. In order to accomplish this, a control group (Group I) of animals that had not been exposed to nicotine, a group that received nicotine up to the day of surgery (Group II) and a group of animals that received nicotine in both pre- and postoperative periods

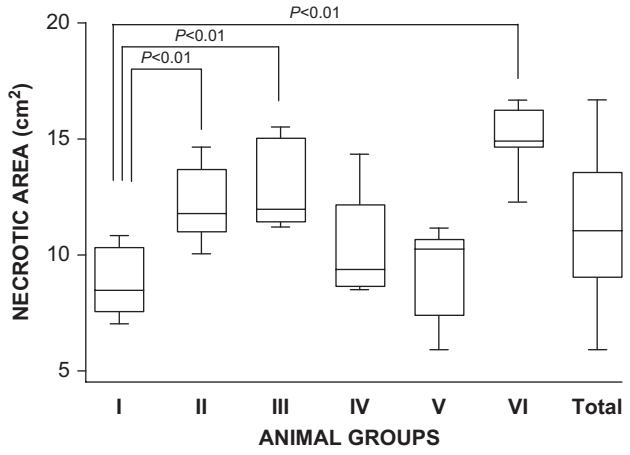

Fig. 1. Box-plot data from measurements of necrotic areas (cm^2) in skin flaps of groups of Winstar rats treated with nicotine and subjected to random-pattern skin flap surgery after different periods of nicotine withdrawal: Group I: continuously injected saline with 4 weeks before and 4 weeks after the surgery; Group II: injected with nicotine for 4 weeks until the day of the surgery; Group III: injected with nicotine for 4 weeks until one day before the surgery; Group IV: injected with nicotine for 4 weeks until 5 days before the surgery; Group V: injected with nicotine for 4 weeks until 10 days before the surgery; Group VI continuously injected with nicotine for 4 weeks before and 1 week after the surgery.

(Group VI) were compared (Fig. 1). The necrotic area observed in the animals of Group I ($8.85 \pm 1.37\,cm^2$) was significantly smaller ($p < 0.01$, ANOVA) than that observed in the animals of Group II ($12.15 \pm 1.63\,cm^2$) and in the animals of Group VI ($14.84 \pm 1.56\,cm^2$).

Next we compared the control group (Group I) with groups exposed to nicotine discontinued at different times in the preoperative period (Fig. 1). In Group III, the drug was withheld 1 day before the surgery; in Group IV, nicotine was withdrawn 5 days before the surgery; and in Group V, the drug was withheld 10 days before the surgery. The area of skin necrosis observed in the animals of Group I was significantly smaller ($p < 0.01$, ANOVA) than that observed in the animals of Group III ($12.88 + 1.76\,cm^2$). Although the necrotic areas in the animals of Group IV ($10.13 + 1.96\,cm^2$) and Group V ($9.27 + 1.87\,cm^2$) were slightly larger than those observed in the animals of group I, such difference was not statistically significant.

Discussion

There are several studies reporting deleterious effects of cigarette smoke and nicotine on the integrity of skin flaps (Rees et al., 1984; Van Adrichen et al., 1996). The purpose of this study was to answer the question asked by surgical clinicians working with cutaneous flaps, namely: "What would be the ideal time for nicotine exposure to be discontinued in order to prevent its deleterious effects on skin flaps?" Although some studies in the literature deal with this problem (Kaufman et al., 1984; Craig and Rees, 1985; Forrest et al., 1991; Gomes et al., 1994) we found no systematic comparison of different periods of nicotine withdrawal before skin surgery.

In the present study, we compared animals in which nicotine was withheld at different times in the preoperative period. Our data show that nicotine withdrawn at least 5 days before surgery significantly decreases the deleterious effect of this drug on the skin flap. It also shows that cessation of tobacco use at least 10 days before the surgical procedure virtually abolishes the risk of additional necrosis in the skin flaps. Our findings agree with others reported in the literature that show an increase in the necrotic area of skin flaps in animals exposed to nicotine until the day of the surgery (Rees et al., 1984; Van Adrichen et al., 1996). They are also in accordance with two other studies (Forrest et al., 1991; Gomes et al., 1994) that show that animals exposed to nicotine until 1 or 2 weeks prior to surgery experienced no significant increase in the necrotic areas of the skin flaps in comparison with the control (non-nicotine-treated) group.

Other histological findings of the present work included: acute (96%) and chronic inflammation (33%) with microabscess (55%), incipient fibrosis (88%), neovascularization (97%), edema (72%) and thrombosis (31%). There were, however, no significant differences in the intensity of these events between the nicotine-treated and the control groups.

Vasoconstriction, a phenomenon reported by other authors (Forrest et al., 1991; Gomes et al., 1994), was not observed in this study. One of the possible explanations for the absence of this finding in our samples is the fact that this event does not persist for longer than 24 h (Reifkohl et al., 1986; Forrest et al., 1991; Gomes et al., 1994), and our specimens were collected 1 week after surgery.

Conclusion

The authors involved in the present study conclude that the ideal timing for nicotine withdrawal in random-pattern skin flaps, in rats, is 5 days before the surgical procedure.

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