British Journal of Plastic Surgery (1987), **40**, 295–299

Dose and time effects of nicotine treatment on the capillary blood flow and viability of random pattern skin flaps in the rat

C. R. FORREST, C. Y. PANG and W. K. LINDSAY

Divisions of Plastic Surgery and Surgical Research, The Hospital for Sick Children, and Departments of Surgery and Physiology, University of Toronto, Canada

Summary—The deleterious effects of nicotine treatment on skin haemodynamics and survival of 4 × 10 cm acute random pattern skin flaps constructed on the dorsum of the rat were studied. Rats were injected subcutaneously with 0.2 ml of saline containing varying doses (0, 1, 2, 4 or 8 mg kg^{-1}; bid) of nicotine for 5 weeks, starting 4 weeks before flap surgery. It was observed that nicotine treatment at the dose of 2 mg kg^{-1} (bid), or higher, significantly ($p < 0.05$) decreased the length and area of skin flap survival compared with the control. This dose of nicotine treatment also significantly ($p < 0.05$) decreased the capillary blood flow and distal perfusion in the skin flaps compared with the control. However, the detrimental effect of nicotine treatment on the survival of acute random pattern skin flaps was not seen if the treatment was started 2 instead of 4 weeks preoperatively. It is concluded that nicotine may cause hypoperfusion and necrosis in acute random pattern skin flap surgery, and the deleterious effects are time-dependent.

Clinical and experimental evidence is accumulating to indicate that cigarette smoking may increase the risk of skin flap necrosis. Rees *et al.* (1984) suggested in a retrospective study that cigarette smokers had a significantly higher incidence of skin slough after rhytidectomy procedures compared with non-smokers. Subsequently, experimental work showed that skin flap survival in rats (Kaufman *et al.*, 1984; Lawrence *et al.*, 1984; Nolan *et al.* 1985) and hamsters (Craig and Rees, 1985) was significantly decreased by exposing these animals to cigarette smoke in smoking chambers. It was suggested that cigarette smoking contributed to tissue ischaemia in skin flap surgery (Reus *et al.*, 1984). Cigarette smoking had also been reported to impair digital blood flow and wound healing in the human hand (Sarin, Austin and Nickel, 1974; Mosely and Finseth, 1977). However, the effect and mechanism of cigarette smoking on the haemodynamics of skin flaps have not been studied in the human or laboratory animal.

Carbon dioxide, nitrous oxide, hydrogen cyanide and nicotine have been identified as the major byproducts of cigarette smoke which may have potential deleterious effects on the cardiovascular system. However, epidemiological studies (Hill and Wynder, 1974) on the detrimental effect of cigarette smoking in man indicated that the increase in the risk of developing cardiovascular disease was related to the levels of nicotine in the cigarette, but not to the number of cigarettes smoked. Furthermore, it was observed that exposure of rabbits to carbon monoxide alone or in combination with hydrogen cyanide and nitric oxide up to 12 weeks did not cause any damage to peripheral blood vessels (Hugod, 1979). Much of the recent research on the pathogenesis of cigarette smoking related cardiovascular diseases has been focused on the deleterious effect of nicotine rather than the gaseous byproducts.

The objective of this research project was to study the dose related effect of nicotine on the skin capillary blood flow and viability in acute random pattern skin flaps constructed on the dorsum of the rat.

Materials and Methods

Animals

Male Sprague Dawley rats were used for experimental animals. These animals were kept in individual cages housed in a temperature (24°C) and light (07.00–19.00 hours) controlled room. All animals were offered the same commercial rat diet

and tap water ad libitum, but food was withheld the evening before surgery.

Experiment 1: The aim of this experiment was to study the dose response effect of nicotine treatment on the length and area of skin flap viability. Eighty male rats (460 ± 13 g; $\bar{X} \pm$SEM) were randomly assigned into 5 groups (N = 16). Each group of rats was injected subcutaneously with varying doses of nicotine (0, 1, 2, 4 or 8 mg kg^{-1}; bid) in 0.2 ml of normal saline for 5 weeks. At the end of the 4th week, a 4×10 cm caudally-based acute random pattern skin flap was constructed on the dorsum of each rat under pentobarbital anaesthesia (35 mg kg^{-1}; i.p.), observing sterile technique. The animal was awakened, returned to its cage and was given tetracycline (50 mg kg^{-1}) in its drinking water for 3 days postoperatively. The length and area of skin flap survival was assessed at the end of the 5th week, using a template technique (Sasaki and Pang, 1980).

Experiment 2: The preceding experiment was repeated except that the acute random pattern skin flap in each rat was raised after 2 weeks instead of 4 weeks of nicotine treatment to study the time effect of nicotine treatment on skin flap viability.

Experiment 3: The aim of this experiment was to study the effect of nicotine on capillary blood flow and distal perfusion in acute random pattern skin flaps. Thirty-four male rats (483 ± 21 g; $\bar{X} \pm$SEM) were divided into 2 groups. The control and treatment groups of animals received subcutaneous injections of 0.2 ml normal saline or saline containing nicotine (2 mg kg^{-1}; bid) respectively for 5 weeks. At the end of this injection period, a caudally-based 4×10 cm acute random pattern skin flap was raised under pentobarbital anaesthesia. Six hours after flap surgery, the animal was re-anaesthetised with pentobarbital and the skin capillary blood flow, distal perfusion and cardiac output were studied, using the radioactive microsphere technique (Pang et al., 1984; Neligan et al., 1985). Briefly summarised, ^{57}Co-labelled microspheres (16.1 ± 0.3 μm; $\bar{X} \pm$S.D.) obtained from New England Nuclear (Boston, MA) were used. The left and right femoral arteries were catheterised (PE-50 tubing) for collection of a reference blood sample (0.786 ml/min) by a Harvard withdrawal pump, and for monitoring of mean arterial blood pressure (Hewlett Packard, model 78205) respectively. The left ventricle was catheterised via the right carotid artery for microsphere (80,000–100,000/kg) injection.

The animal was killed with an overdose of pentobarbital (100 mg kg^{-1}) at the end of the blood flow study. Each flap was marked and cut into 1 cm segments. Samples of normal skin (1×10 cm) were obtained from both flanks. All skin segments and samples were weighed, and were measured by a gamma counter (Beckman, model 8000). The capillary blood flow in each segment of the skin flaps was calculated, using the formula and method described previously (Pang *et al.*, 1984).

Statistics

Calculation of mean ± SEM, t-test and analysis of variance were performed on a microcomputer (HP-150), using the programmes provided by the manufacturer. Duncan's multiple range test was used for multiple comparisons of means (Duncan, 1955). The specific statistics used and the levels of significance observed are described in the legends of each table and figure presented.

Results

Experiment 1: The length and area of skin survival were similar in the control rats and treatment rats injected with nicotine at a dose of 1 mg kg^{-1} twice daily (Fig. 1). However, nicotine treatment at the dose of 2 mg kg^{-1} (bid) or higher, significantly ($p < 0.05$) decreased the length and area of skin flap survival compared with the control (Fig. 1).

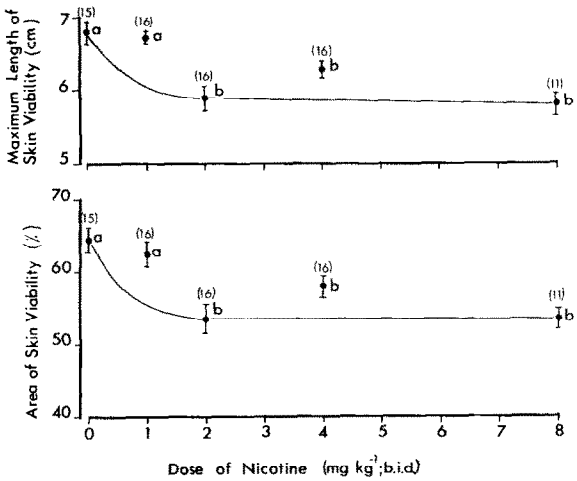

Fig. 1

Figure 1—Length and area of skin survival in 4×10 cm caudally based acute random pattern skin flaps constructed on the dorsum of the rat. Skin viability was assessed 7 days postoperatively. Values are mean ± SEM. Mean values without a common letter are significantly different ($p < 0.05$); one-way analysis of variance followed by Duncan's multiple range test. Numbers in parentheses indicate the number of flaps for each mean value.

Five out of 16 rats died with convulsions within the first week of nicotine treatment at the dose of 8 mg kg^{-1} twice daily. Perhaps this dose was too high for rats which had never been exposed to nicotine. However, the remaining rats in this treatment group tolerated the nicotine treatment well after the first week.

Experiment 2: In this experiment, the acute random pattern skin flaps in all treatment groups were raised 2 weeks instead of 4 weeks after nicotine treatment. No significant change in length or area of skin flap viability was seen between the control and treatment groups.

Experiment 3: The mean values for cardiac output, mean arterial blood pressure and wet weight of skin flaps and normal skin samples were similar in the saline (control) and nicotine treated animals at the end of the 5th week (Table 1). However, the total capillary blood flow in the acute random pattern skin flaps was significantly ($p < 0.01$) lower in the nicotine treated animals compared with the control (Fig. 2). The capillary blood flow in the normal skin samples in the nicotine treated rats (0.063 ± 0.006 ml min^{-1} g^{-1}) was significantly lower ($p < 0.05$) than that seen in the saline treated control rats (0.113 ± 0.008 ml min^{-1} g^{-1}).

Table 1 Comparison between saline and nicotine treated animals in experiment 3.

	Saline	*Nicotine*
Body Weight (g)	499 ± 3^a	460 ± 10^a
Mean Arterial Blood Pressure (mm Hg)	102 ± 2^a	102 ± 3^a
Cardiac Output (ml min^{-1} kg^{-1})	209 ± 20^a	254 ± 29^a
Wet Weight of Skin Flaps (g)	10.3 ± 0.6^a	10.0 ± 0.3^a
Wet Weight of Normal Skin Samples (g)	1.79 ± 0.8^a	1.68 ± 0.1^a

Values are mean ± SEM. Means without a common letter are significantly different; two tailed t-test ($p \leq 0.05$). There were no significant differences between the saline treated and the nicotine treated animals in the parameters measured above.

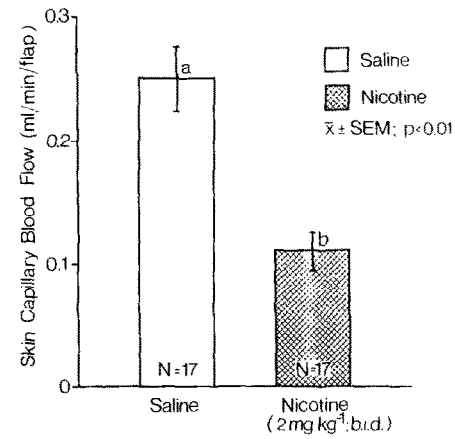

Fig. 2

Figure 2—Total capillary blood flow in acute random pattern skin flaps constructed on the dorsum of the rat. Values are mean ± SEM (ml min^{-1} $flap^{-1}$). Mean values without a common letter are significantly different ($p < 0.01$); two-tailed t-test.

The distal perfusion in the control and treatment skin flaps was studied by calculating the total blood flow of each centimetre of skin (normalised to unit tissue weight) from the pedicle to the distal portion of the flap. It was observed that the skin capillary blood flow was significantly ($p < 0.05$) lower in each 1 cm segment of the random pattern skin flap in the nicotine treated rats compared with saline treated control (Fig. 3). In addition, nicotine treatment reduced the distance of blood perfusion from the pedicle to the distal end of the flap compared with the control.

Discussion

This is the first report to demonstrate that nicotine can significantly decrease capillary blood flow, distal perfusion, and length and area of acute random pattern skin flap survival in the rat. These deleterious effects were seen at the dose of 2 mg kg^{-1}, given twice daily for 4 weeks before surgery. However, this same dose of nicotine did not produce

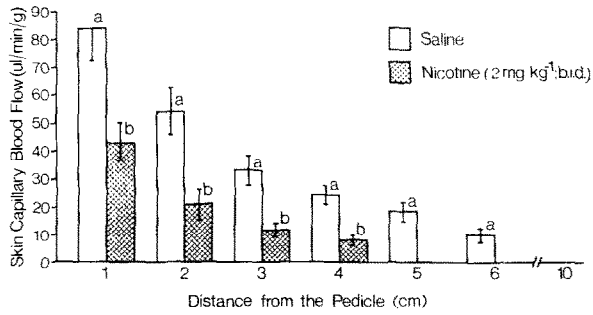

Fig. 3

Figure 3—Distal perfusion in the acute random pattern skin flaps constructed on the dorsum of the rat. Values are mean ± SEM (µl min^{-1} g^{-1}; N = 34). Mean values without a common letter are significantly different ($p < 0.05$); two-tailed t-test.

any significant deleterious effects when the rats were pre-treated with nicotine for only 2 instead of 4 weeks before surgery. This observation indicated that the deleterious effects of nicotine treatment are time dependent, and the treatment has to be given longer than 2 weeks preoperatively. These results are consistent with those reported by other investigators (Falcone and Ruberg, 1980) who failed to observe any deleterious effect of nicotine treatment (1.2 mg kg^{-1} day^{-1}) on skin flap viability in rats, given for a period of 5 days preoperatively.

The mechanism of nicotine action in the pathogenesis of hypoperfusion and skin necrosis in acute skin flaps observed in the present experiments is not known. So far, four hypotheses are available in the literature to explain the detrimental effect of nicotine on peripheral blood vessels: (i) nicotine causes direct endothelial cell damage (Hladovec, 1978), (ii) nicotine stimulates the release of norepinephrine in the sympathetic nerve terminals (Stewart *et al.*, 1973; Cryer *et al.*, 1976), (iii) nicotine stimulates the release of catecholamines from the adrenal glands (Armitage, 1965) and (iv) nicotine affects the local synthesis and/or release of prostaglandins and thromboxane (Kaijser and Wennmalm, 1978; Sonnenfeld and Wennmalm, 1980; Wennmalm, 1982). Experiments are now in progress in our laboratory to identify the specific mechanism of nicotine action which causes hypoperfusion in skin flap surgery in the rat and pig. In addition, we are also studying the long-term dose response effect of nicotine treatment (3–4 months preoperatively) on skin blood flow and viability, and the reversibility of the deleterious effect of nicotine after cessation of nicotine treatment prior to skin flap construction. This information is important for clinical management of cigarette smoking patients who are scheduled for flap surgery.

Acknowledgements

This research was supported by the Education Foundation of the American Society of Plastic Surgeons and the National Institutes of Health (HL 30212), USA. The authors wish to acknowledge the excellent technical assistance from Mr Arthur Chau and Mrs Margaret Scott.

References

Armitage, A. K. (1965). Effects of nicotine and tobacco smoke on blood pressure and release of catecholamines from the adrenal glands. *British Journal of Pharmacology*, **25**, 515.

Craig, S. and Rees, T. D. (1985). The effects of smoking on experimental skin flaps in hamsters. *Plastic and Reconstructive Surgery*, **75**, 842.

Cryer, P. E., Haymond, M. W., Santiago, J. V. and Shah, S. D. (1976). Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *New England Journal of Medicine*, **295**, 573.

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, **1**, 42.

Falcone, R. E. and Ruberg, R. L. (1980). Pharmacologic manipulation of skin flaps: Lack of effect of barbiturates or nicotine. *Plastic and Reconstructive Surgery*, **66**, 102.

Hill, P. and Wynder, E. L. (1974), Smoking and cardiovascular disease: Effect of nicotine on the serum epinephrine and corticoids. *American Heart Journal*, **87**, 491.

Hladovec, J. (1978). Endothelial injury by nicotine and its prevention. *Experientia*, **34**, 1585.

Hugod, C. (1979). Effect of exposure to 0.5 ppm hydrogen cyanide singly or combined with 200 ppm carbon monoxide and/or 5 ppm nitric oxide on coronary arteries, aorta, pulmonary artery and lungs in the rabbit. *International Archives Occupational Environment and Health*, **44**, 13.

Kaijser, L. and Wennmalm, A. (1978). Nicotinic acid stimulates prostaglandin synthesis in the rabbit heart without releasing noradrenaline. *Acta Physiologica Scandinavica*, **102**, 246.

Kaufman, T., Eichenlaub, E. H., Levin, M., Hurwitz, D. J. and Klain, M. (1984). Tobacco smoking: Impairment of experimental flap survival. *Annals of Plastic Surgery*, **13**, 468.

Lawrence, W. T., Murphy, R. C., Robson, M. C. and Heggers, J. P. (1984). The detrimental effect of cigarette smoking on flap survival: An experimental study in the rat. *British Journal of Plastic Surgery*, **37**, 216.

Mosely, L. H. and Finseth, F. (1977). Cigarette smoking: Impairment of digital blood flow and wound healing in the hand. *The Hand*, **9**, 97.

Neligan, P., Pang, C. Y., Nakatsuka, T., Lindsay, W. K. and Thomson, H. G. (1985). Pharmacologic action of isoxsuprine in cutaneous and myocutaneous flaps. *Plastic and Reconstructive Surgery*, **75**, 363.

Nolan, J., Jenkins, R. A., Kurihara, K. and Schultz, R. C. (1985). The acute effects of cigarette smoke exposure on experimental skin flaps. *Plastic and Reconstructive Surgery*, **75**, 544.

Pang, C. Y., Neligan, P. and Nakatsuka, T. (1984). Assessment of microsphere technique for measurement of capillary blood flow in random skin flaps in pigs. *Plastic and Reconstructive Surgery*, **74**, 513.

Rees, T. D., Liverett, D. M. and Guy, C. L. (1984). The effect of cigarette smoking on skin-flap survival in the face lift patient. *Plastic and Reconstructive Surgery*, **73**, 911.

Reus, W. F., Robson, M. C., Zachary, L. and Heggers, J. P. (1984). Acute effects of tobacco smoking on blood flow in the cutaneous microcirculation. *British Journal of Plastic Surgery*, **37**, 213.

Sarin, C. L., Austin, J. C. and Nickel, W. O. (1974). Effects of smoking on digital blood-flow velocity. *Journal of the American Medical Association*, **229**, 1327.

Sasaki, G. H. and Pang, C. Y. (1980). Hemodynamics and viability of acute neurovascular island skin flaps in rats. *Plastic and Reconstructive Surgery*, **65**, 152.

Sonnenfeld, T. and Wennmalm, A. (1980). Inhibition by nicotine of the formation of prostacyclin-like activity in rabbit and human vascular tissue. *British Journal of Pharmacology*, **71**, 609.

Stewart, J. E., Booker, W. M. and West, W. L. (1973). Chronic nicotine administration and norepinephrine synthesis in the isolated guinea pig heart. *Archive International Pharmacodynamics*, **201**, 362.

Wennmalm, A. (1982). Interaction of nicotine and prostaglandins in the cardiovascular system. *Prostaglandins*, **23**, 139.

The Authors

Christopher R. Forrest, BSc, MD, Research Fellow, Division of Plastic Surgery, the Hospital for Sick Children, Toronto, Ontario.

Cho Y. Pang, MSc, PhD, Senior Scientist, Research Institute, the Hospital for Sick Children, and Associate Professor, Departments of Surgery and Physiology, University of Toronto, Toronto, Ontario.

William K. Lindsay, MD, MSc, FRCS(C), FACS, Professor and Head, Division of Plastic Surgery, the Hospital for Sick Children, and University of Toronto, Toronto, Ontario.

Address all correspondence and requests for reprint to Dr Cho Y. Pang, The Hospital for Sick Children, Toronto, Ontario, Canada M5G 1X8.