The Effects of Passive Cigarette Smoke Exposure on the Survival of the Reverse Sural Fasciocutaneous Flap

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Abstract

Background The possibility of a person who had undergone surgery to be exposed to the ill effects of cigarette smoke is high, more so if the person lives with a smoker. With increasing popularity of reverse sural fasciocutaneous flaps, a surgeon may have to manage a person who lives with a smoker or is exposed to cigarette smoke. A clear understanding of the effects of exposure to cigarette smoke on reverse sural fasciocutaneous flaps is necessary. This study was performed to establish a clearer understanding of the effects of smoking on reverse sural fasciocutaneous flaps and evidence for preoperative patient counseling about smoking and smoke exposure.

Objective The study investigated effects of exposure to cigarette smoke on the survival of the reverse sural fasciocutaneous flap.

Methods This was an experimental observational study conducted at the Laboratory for Animal Research Unit in the Health Campus of Universiti Sains Malaysia. Twelve adult White New Zealand rabbits (*Oryctolagus cuniculus*) were divided into two groups of six. All 12 rabbits had a 2.5- × 2.5-cm reverse sural fasciocutaneous flap raised on both its hind limbs. The group exposed to cigarette smoke underwent 4 weeks of smoke exposure for 2 hours daily prior to surgery. This was then continued until analysis of the results.

The control group had no intervention. All flaps were analyzed on the seventh postoperative day, after tracing on transparent plastic sheets with the necrotic area marked followed by 2D planimetry done on a grid paper. The flaps were assessed on the total flap area and survival area percentage. It was recorded as mean ± SD. The presence or absence of infection and hematoma was also noted.

Results Twelve flaps were analyzed in each group. Total mean flap area and survival area percentage of the control group were 120.33 ± 31.03 mm² and 80.12 ± 15.75%, respectively, whereas in the cigarette smoke-exposed group, it was 121.83 ± 17.93 mm² and 62.04 ± 34.01%, respectively. The control group had two infections and one

Keywords

- ▶ passive cigarette smoke exposure
- ▶ fasciocutaneous skin flap
- ▶ rabbit experimentation

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hematoma, and the smoking-exposed group had six infections and nine hematomas. The comparison between the groups was made in terms of total flap area, survival percentage, presence of infection, and hematoma for which the p values were 0.886, 0.115, 0.083, and 0.003, respectively. The differences between right and left hind limbs were also analyzed ($p = 0.414$).

Conclusion There was no statistical difference in the results from the right and left hind limbs. There was no statistical difference in the survival of reverse sural fasciocutaneous flap between the control and smoking-exposed groups.

Introduction

A recent study in Malaysia showed a prevalence of 46.5% of smokers among adult males.¹ A relationship between ill effects on the heart, carcinogenicity, and also wound healing with smoking has been well established by many authors.¹⁻³ The negative effects of smoking in other type of flaps (free flaps and pedicle-based flaps) has been shown in the past; however, evidences are not totally conclusive.⁴⁻⁶ There are limited studies done on the effects of exposure to passive smoke on fasciocutaneous flap.^{7,8} No study focuses on the effects of cigarette smoke exposure on a reverse sural fasciocutaneous flap in a rabbit model.

Sural fasciocutaneous flap was developed according to the distribution of the sural nerve. It was first mentioned in literature in 1981 by Pontén.⁹ He concluded that it was a safe flap with an easy design for lower limb tissue defects. A reverse sural fasciocutaneous flap relies on retrograde blood supply through multiple distal perforators. This flap was introduced in 1983 and was based on the concept of reverse flow established by Botswick et al¹⁰ way back in 1976. It has gained wider usage in the reconstruction of tissue defects around the ankle, especially over the malleolus and heel. The versatility of the flap in this region with sparse blood supply is remarkable. These days some authors have even extended the reach of the flap until the forefoot.¹¹

This study was performed to establish a clearer understanding of the effects of smoking on reverse sural fasciocutaneous flaps. The purpose was to achieve a better patient selection and establish evidence for preoperative patient counseling about cigarette smoke exposure. It will be able to provide evidence for the betterment of patient care and in keeping with the principle of evidence-based medical management.

Methodology

This study was conducted between October 2012 and February 2013 in the Laboratory for Animal Research of the Health Campus of Universiti Sains Malaysia with the approval of the Animal Ethics Committee. The sample size required for this study was 15 using the calculation of two means. The reference study used was based on another animal study by Chang et al.⁸ The animal ethics body of the university, however, only approved the use of 12 rabbits to be sacrificed for the study. Hence, 12 adult New Zealand white rabbits were used for the study.

The rabbits were randomly assigned into two groups of six (control group and cigarette smoke-exposed group). The control group represented the group of rabbits that had reverse sural fasciocutaneous flap surgery done without any exposure to cigarette smoke, whereas the smoking-exposed group was exposed to cigarette smoke via placement into the smoking chamber as illustrated in ►Fig. 1.

Fig. 1 The rabbit smoke chamber illustrating the partition between the compartment and direction of smoke flow.

The smoking-exposed group of rabbits had daily 2-hour sessions of cigarette smoke exposure for 4 weeks prior to flap surgery. The rabbits were placed in a custom-built smoke chamber with two separate compartments for one rabbit and cigarette. The compartments were separated by a fenestrated partition that allowed uninhibited smoke passage. The smoke flow was maintained at low flow and unidirectional by an exhaust system at the other end. The smoke was generated from a commercially available lit cigarette. The cigarettes were lit one at a time to maintain 2 hours of smoke flow in the rabbit chamber and allowed to fully burn out. Each rabbit underwent 2 hours of smoke exposure individually. The smoke exposure was continued during the postoperative assessment period at a similar exposure time.

After 4 weeks, both the groups of rabbits had reverse-flow islanded fasciocutaneous sural flap done on both their lower limbs. The surgery was performed with loupe magnification by the surgeon under strict operative sterility and general anesthesia utilizing micro instruments. A combined intramuscular injection and inhalational anesthesia was administered. The rabbit was first injected with ketamine-xylazine (ketamine 25 mg/kg; xylazine 3 mg/kg), followed with inhalational isoflurane (flow rate was adjusted to maintain oxygenation > 90% using pulse oximeter; anesthesia was administered by a veterinarian). The shaving of the surgical area was done before cleaning and draping the surgical field. The flap area was marked and islanded to isolate the flap to be supplied solely by the sural vein; its arterial supply was done only by the fascial capillary arborization. Prophylactic antibiotic was given as routine using intramuscular enrofloxacin (Baytril 10 mg/kg dose).

The surgery was performed by a surgeon trained in plastic surgery under supervision of a microsurgeon with over 10 years of experience in microsurgery. The reverse sural fasciocutaneous flap for the study was modeled based on the flap developed by Chang et al.¹² A 2.5- × 2.5-cm fasciocutaneous flap over midportion of the posterior calf and centered at intersection of the two heads of gastrocnemius was developed. The flap borders were delineated accurately with a marker. Sterility was maintained throughout the operation. Lesser saphenous vein was identified between the two heads of gastrocnemius proximally and ligated. Sural nerve also ligated proximally. The flap was elevated completely and circumferentially islanded down to the deep fascia subfascially, but the lesser saphenous vein inflow was left intact. Flap elevations were performed under loupe magnification. The skin flap was later reapplied to the defect and sutured in place. Nonconstrictive circumferential simple dressing with paraffin net and gauze was applied. The dressing was held in place with minimal plaster, just enough to prevent slippage.

Postoperatively, daily analgesia were given in the form of intramuscular meloxicam (0.2 mg/kg/d) until completion of study (day 7 postoperatively). Flap assessment and dressing were done after intramuscular ketamine (10 mg/kg) injection, to provide comfort for the rabbit and hasten the process of assessment. The rabbits were fed and rationed water as usual. The operated hind limbs were not immobilized during the postoperative period. Dressings were held with minimal

nonconstrictive taping, just enough to prevent slippage and unwanted exposure.

The flaps were assessed at four junctures—immediately post operation and at days 1, 3, and 7 post flap surgery—in terms of color, temperature, and capillary refill time. The presence and absence of infection and hematoma were also noted. All observation was document in each rabbit's individualized proforma sheet. On day 7 post surgery, the margins of the sural fasciocutaneous flaps were traced on a transparent plastic sheet. The survival area was clearly bordered. The survival area is defined as any area without any evidence of necrosis. The presence and absence of infection and hematoma foci were also documented. Digital photographic documentation was used for this purpose (►Fig. 2A–D). The flaps were assessed for total take-up, total necrosis, infection, peripheral necrosis with infection, and extent of hematoma. The flap survival percentage was calculated by manual two-dimensional (2D) planimetry by placing the flap tracing on a grid paper.

Upon completion of the experiment, the rabbits were sacrificed with overdose of phenobarbitone sodium. This was in keeping with the protocol and requirement of the animal ethics committee. The data obtained were analyzed using the SPSS software (IBM SPSS Statistics for Windows, Version 20.0., Armonk, New York, United States) licensed to the school of Medical Sciences, Universiti Sains Malaysia.

Results

All the 12 rabbits survived the operations. Postoperatively all 24 sural fasciocutaneous flaps were assessed until completion of study and were available for final analysis. There were no omissions in this study.

The control group consisted of six rabbits representing 12 reverse sural fasciocutaneous flaps ($n = 12$ flaps). The mean total flap area for the reverse sural fasciocutaneous flap was $120.33 \pm 31.03 \text{ mm}^2$. Two flaps showed complete survival and 10 flaps showed variable levels of peripheral necrosis. The mean survival area percentage was $80.12 \pm 15.75\%$. Two flaps showed evidence of infection. Only one flap showed hematoma on the final assessment and had signs of infection as well. Eight flaps had no signs of infection or hematoma.

The group exposed to passive cigarette smoking (smoking group) consisted of six rabbits representing 12 reverse sural fasciocutaneous flaps ($n = 12$ flaps). The mean total flap area for the reverse sural fasciocutaneous flap was $121.83 \pm 17.93 \text{ mm}^2$. One flap showed complete survival and one showed complete necrosis. The remaining 10 flaps exhibited a wider range of necrosis, with 9 flaps demonstrating peripheral necrosis and 1, central necrosis. The mean survival area was $62.04 \pm 34.01\%$. Six flaps had signs of infection. Nine flaps showed hematoma on final assessment. All flaps with infection had hematoma also. Only three flaps showed no signs of infection or hematoma.

Based on calculation after cross-tabulation, likelihood of hematoma forming in the cigarette smoke-exposed group is 12 times. Chi-square test indicated significant difference between control and cigarette smoke-exposed groups in terms of presence of hematoma ($p = 0.001$). The result of

Fig. 2 Examples of flap assessment at 7 days: (A) Total take-up, (B) total necrosis, (C) infection, (D) peripheral necrosis with infection.

Fisher's exact test also confirmed this finding of chi-square test giving a p value of 0.003 (–Table 1).

Based on calculation after cross-tabulation, likelihood of infection in the cigarette smoke-exposed group is three

times. However, chi-square test indicated no significant difference between control and cigarette smoke-exposed groups in terms of presence of infection ($p = 0.083$). The result of Fisher's exact test also confirmed this finding of chi-square test with a p value of 0.193 (–Table 2).

An independent samples t -test indicated that there were no statistical differences between control and smoking-exposed groups with p value of 0.886 in terms of total flap survival area (–Table 3).

Discussion

This study was designed to assess the survival relationship of reverse sural fasciocutaneous flap and exposure to passive cigarette smoke based on a rabbit model. Even though it was calculated that the sample size would be 15 flaps per each group of the study, it was only possible to allocate six rabbits for each group (control and smoking) due to the requirements by the animal ethics committee. To maximize the number of flaps for analysis, both the hind limbs of rabbits were used for the reverse sural fasciocutaneous flap surgery and analysis (i.e., six rabbits with 12 flaps per group).

An indirect method of intermittent inhalational cigarette smoking in a purpose-designed chamber was used. The International Agency for Research on Cancer concluded that the chamber smoke exposure method is the most suitable for small animals.¹³ As no other study is available for assessing effects of smoking on survival of reverse sural fasciocutaneous flaps, intermittent inhalation of cigarette smoke is the method widely used by many other smoking-related studies in rabbits.¹⁴ No standard protocol is available on the volume of smoking, smoke exposure time, or duration of smoking outlined in the current available literature. The smoke exposure varies widely from 15 minutes per 6 hours¹⁵ to 3 hours per day.¹⁶ This is also correct on the duration of smoking, which was done up to 8 weeks in some studies. Forrest et al showed that a minimum of 2-week exposure is needed for deleterious effect to manifest in a nicotine study in rats.¹⁷ Due to presence of such a wide variation, the method of choosing 2 hourly daily smoking for 4 weeks of smoking was found most suitable for the study setting and it was approved by the University Animal Ethics Committee.

The mean flap survival percentage in the control group was $80.1 \pm 15.8\%$ that was comparable to $88.8 \pm 6.5\%$, as demonstrated by Chang et al.⁸ The differences of mean flap survival percentages between left and right rabbit hind limbs, which were $75.8 \pm 28.1\%$ and $66.38 \pm 27.27\%$, respectively, were found not to be statistically significant ($p = 0.414$). These findings support that rabbit limb vasculature is symmetrical,¹⁸ thereby validating the use of bilateral hind limb for future studies on reverse sural fasciocutaneous flaps. It is possible that this can be extended to other studies on rabbit hind limb flaps. Using both limbs can minimize the number of rabbits used in experiments. This reduces and prevents wastage, and thereby reduces number of rabbits sacrificed for future studies on reverse sural fasciocutaneous flaps.

The reverse sural fasciocutaneous flaps were further compared in terms of total flap survival area and percentage.

Table 1 Chi-square and Fisher's exact test for hematoma samples of control and smoking rabbit groups

	Value	df	Asymptomatic sig. (two-sided)	Exact sig. (two-sided)	Exact sig. (one-sided)
Pearson Chi-square	10.971 ^a	1	0.001		
Continuity correction ^b	8.400	1	0.004		
Likelihood ratio	12.221	1	0.000		
Fisher's exact test				0.003	0.001
Linear-by-linear association	10.514	1	0.001		
No. of valid cases	24				

Abbreviations: df, degree of freedom; sig., significant.

^aZero (0%) cell has expected count less than 5. The minimum expected count is 5.^bComputed only for a 2 × 2 table.**Table 2** Chi-square and Fisher's exact test for infection samples of control and smoking rabbit groups

	Value	df	Asymptomatic sig. (two-sided)	Exact sig. (two-sided)	Exact sig. (one-sided)
Pearson Chi-square	3.000 ^a	1	0.083		
Continuity correction ^b	1.688	1	0.194		
Likelihood ratio	3.104	1	0.078		
Fisher's exact test				0.193	0.097
Linear-by-linear association	2.875	1	0.090		
No. of valid cases	24				

Abbreviations: df, degree of freedom; sig., significant.

^aTwo (50%) cells have expected count less than 5. The minimum expected count is 4.^bComputed only for a 2 × 2 table.**Table 3** Independent *t*-test for total flap area samples of control and smoking-exposed groups

		Levene's test for equality of variances		t-test for equality of means						
		F	Sig.	t	df	Sig. (two-tailed)	Mean difference	Std. error difference	95% CI of the difference	
									Lower	Upper
Flap area	Equal variances assumed	2.825	0.107	0.145	22	0.886	−1.500	10.345	−22.954	19.954
	Equal variances not assumed			0.145	17.612	0.886	−1.500	10.345	−23.268	20.268

Abbreviations: df, degree of freedom; sig., significant.

Statistically, however, they were not significant. The result indicates that the likelihood of hematoma and infection is higher in the cigarette smoke-exposed group compared with that in nonexposed rabbits; however, the statistical test indicates otherwise.

Unfortunately, no other study is available in literature for direct comparison on the effects of cigarette smoke exposure on reverse sural fasciocutaneous flaps. This is in stark contrast to smoking studies of other types of flap, which show increased flap necrosis in smokers.^{4–6} However, this was in

keeping with the fact that certain type of flaps may yield better results than others.¹⁹ Consistent with these findings, no statistically significant differences were noted in the presence of infection ($p = 0.193$).

Although no statistical difference was observed with all parameters analyzed, there were some notable clinical findings between the two groups. The only total flap failure was seen in the smoke-exposed group. The range of necrosis was also wider in the smoke-exposed group (up to 100%) compared with control group (up to 42.27%). Notably, only three

flaps were with any signs of infection or hematoma in the control group compared with eight flaps in the cigarette smoke-exposed group. The results might be due to combination of effects. Increasing the sample size may improve the power of this study. This represents the most important limitation of this study. This would have improved statistical significance of hematoma formation and infection. However, the other analyzed parameters most probably will not be affected as noted by significantly higher *p* values.

Many areas can be improved in this study. The 4 weeks of total smoke exposure of the rabbits before flap surgery with additional exposure of 1 week before final assessment might be too short a period to induce microvascular changes. Short-term effects of smoking exposure has no negative effect on blood flow to the limbs and may actually cause vasodilation in large vessels.^{20,21} This, however, may be due to other ingredients obtained by direct inhalation into the blood stream

The rabbit skin is much more loose and mobile than that of humans. This may provide better resistance to effects of smoking. Moreover, the rabbit hind limb has thick fur coverage that needs to be shaved prior to surgery. A limitation of this study is that shaving potentially results in micro trauma to the skin that can induce perfusion and local vasodilation in skin that may indirectly affect the result obtained.

Future studies on reverse sural fasciocutaneous flaps should look into whether chronicity of cigarette smoke exposure actually affects the outcome (e.g., 4, 8, and 16 weeks). Evidences are inconclusive on whether abstinence from smoking is actually beneficial and the minimum period to improve survival of flaps could be studied.

The study was done not to highlight the known fact of smoking that is detrimental to tissue healing; however, it was done to add evidence to the effects of passive smoke inhalation and its detriment to flaps exposed as opposed to direct inhalation of cigarette smoke. In interpreting results of this study in clinical use, patients requiring a reverse sural fasciocutaneous flap must not be denied the use of this versatile flap as it was clearly shown that there were no significant statistical differences between the control and smoking exposure groups. However, they must be informed that the flap survival percentage is unpredictable and that there is a wider range of flap necrosis if they live in vicinity where cigarette smoke exposure is not avoidable.

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