Delay by Staged Elevation of Flaps and Importance of Inclusion of the Perforator Artery

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Abstract “Surgical delay” is aimed at increasing blood flow to adjacent angiosomes by opening choke vessels, to obtain larger and more reliable flaps. We hypothesized that staged elevation (delay) from distal to proximal, in addition to preservation perforator artery near the base will improve survival. Thirty-two Sprague–Dawley rats were divided into 4 groups and 3×8cm caudal McFarlane flaps were elevated. In “delay” groups the 3×4 cm distal part was elevated and sutured back, with complete elevation (3×8 cm) after 4 days. The deep circumflex iliac artery (DCIA) at the base was either preserved or transected. The groups were: group A (Delay/DCIA preserved), group B (Delay/DCIA transected), group C (No Delay/DCIA preserved), group D (No Delay/DCIA transected). The percentage of surviving flap area was measured after 7 days with digital planimetrics. Statistics were done with ANOVA and unpaired *T*-test. The survivals were: 98.44±3.13 % (group A), 69.33±5.51 % (group B), 76.50±8.91 % (group C) and 52.89±3.15 % (group D). Delay lead to a greater degree of survival whether the arteries were preserved ($p=0.002$) or transected ($p=0.01$). In groups where the DCIA was preserved, the delay lead to a greater area of survival ($p=0.002$). Inclusion of arteries lead to a greater degree of survival whether delay was performed ($p=0.003$) or not ($p=0.005$).

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Necropsy showed that the DCIAs were dilated bilaterally when preserved with vascular arcades reaching all viable areas. When the DCIA was transected, the gluteal perforators were dilated bilaterally with the vascular arcades reaching all viable areas. Staged elevation is an effective method of delay in both random and rando-axial flaps. When this is combined with preserving the perforator artery, survival rates are further enhanced.

Keywords Flap delay · Rat dorsal skin flap · Perforator artery · Angiosome

Introduction

The angiosome concept defined by Taylor et al. stated that when a flap is elevated without surgical delay, the anatomic territory of the next dominant perforator can be captured with safety by the artery at the base of the flap [1]. However, when the following angiosome distal to the second one is elevated there is often necrosis of the flap. “Delay phenomenon” is the ischemic preconditioning of the flap in order to open the choke vessels in between adjacent territories and capture angiosome that would be lost under non-delay circumstances. Thus, both the vascularity and the area that can be raised safely are increased.

Many methods have been proposed and investigated to improve the survival of skin flaps. These are either pharmacologic agents that aim to increase the blood flow or methods that use controlled ischemia to precondition flaps and enhance vascularity [2–6]. Dorsal rat skin flap described by McFarlane is a commonly used and accepted model in such experiments [7]. Taylor further expanded this model by identifying 3 paired angiosomes [8].

We hypothesized that staged elevation (delay) from distal to proximal will result in capturing the most distal angiosome

and result in greater length and flap survival in the rat dorsal skin flap model. In addition, preservation of the perforator artery near the base will improve survival.

Material and Methods

Thirty-two female Sprague–Dawley rats each weighing 300 to 350 g were divided into 4 groups (8 animals per group). A combination of ketamine (100 mg/ml) and xylazine (20 mg/ml) at a total dose of 0.2 ml per 100 g of body weight was administered subcutaneously followed by removal of the dorsal hair. Before and after each surgical session the incision sites were cleaned with 70 % ethanol and topical antibiotics were applied.

Surgical Technique and Groups

The rats were divided into 4 groups (8 animals each) (Table I). Under sterile conditions a caudally based midline dorsal cutaneous flap including the panniculus carnosus layer measuring 3 cm at base and 8 cm in length was marked. In groups A and B, the 3×4 cm distal part of the marked flap was elevated and sutured back. After 4 days, a full 3×8 cm flap, which included the already elevated portion, was raised and sutured back. In groups C and D, a 3×8 cm caudal dorsal skin flap was raised and sutured back. In all groups, the deep circumflex iliac arteries (DCIA) were identified bilaterally 1 cm caudolateral to the base of the flap. In groups A and C the bilateral DCIAs and vein were preserved, whereas in groups B and D these vessels were transected (Table I). The animals were given rat chow and water ad libitum and cared according to the guidelines of the animal care committee of our institution.

Data Analysis

The rats and skin flaps were observed daily for their general condition and gross appearance respectively. The survival of the skin flaps were evaluated 7 days after the completion of final operation. General appearance and percentage of surviving areas of the flaps were recorded. Photographs were obtained with animals under general anesthesia to prevent motion, with a digital camera (D70s SLR) utilizing standardized parameters (Lighting in 45° in relation to the object, column height of 40 cm and distance from the object of 40 cm). A planimetric evaluation with image analysis software (Adobe Photoshop®) was used to assess and measure the percentage of the surviving area. The area of survival of the skin flaps were expressed as a percentage of the total flap area (survival rate = surviving area/total area × 100). The measurements were performed by a researcher who was unaware of the specific groups and treatment. In addition, a necropsy was

performed to evaluate the status of the arterial system within and at the base of the flaps.

Statistical Analysis

All values are expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used in the comparison of multiple dependent means of survival areas between treatment groups. The unpaired *T*-test was used in the comparison of two independent means of flap survival. A *p*-value of <0.05 was considered statistically significant.

Results

The demarcation between the viable (soft with a normal pink color) and nonviable (rigid, dark color) areas were clearly apparent on the 7th postoperative day.

In groups A and B there was no necrosis after 4 days when the flap was raised as 3×4 cm just prior to complete elevation of the delayed flap. After the second surgery the percentage of survival was 98.44±3.13 % in group A and 69.33±5.51 % in group B (Fig. 1a, b). In group C and D the surviving areas were 76.50±8.91 % and 52.89±3.15 %, respectively (Fig. 1c, d). The gradual delay lead to a greater degree of survival whether the perforator was preserved (group A vs. group C, *p*=0.002) or transected (group B vs. group D, *p*=0.01). In groups where the DCIA was preserved (group A vs. group C), the gradual delay lead to a greater area of survival (*p*=0.002) (Fig. 2). Inclusion of the perforator lead to a greater degree of survival whether delay was performed (group A vs. group B, *p*=0.003) or not (group C vs. group D, *p*=0.005) (Table 1; Fig. 2).

Necropsy showed that the DCIAs were dilated bilaterally when preserved (groups A and C) (Fig 3). The vascular network arising from the DCIA reached to all viable areas on the flap. In rats where the DCIA was transected (groups B and D), the gluteal perforator arteries (GPA) were dilated bilaterally and the vascular network arising from these arteries reached to all viable areas on the flap.

Discussion

McFarlane in 1965 described a cranially based rat dorsal skin flap ischemia-necrosis model which was popularized and modified by many researchers [7]. However, it was not until Taylor's work when the concept of angiosomes and perforator arteries was developed [1, 8]. In many studies using the caudal or cranial rat dorsal skin flap including McFarlane's original work, a comment was not made on whether the perforator artery located at or near the base was cut or preserved. This can greatly affect the result and cause variability in the

Fig. 1 Dorsal skin flaps 7 days postop **a** Group A, gradual delay and deep circumflex iliac artery (DCIA) preserved, **b** Group B, gradual delay and DCIA transected **c** Group C, no delay and DCIA preserved **d** Group D, no Delay and DCIA transected

percentage of surviving area. Thus, this is a huge flaw in scientific methodology in many experiments using this model and should be challenged. The researchers using the McFarlane model should always standardize their models and indicate in their surgical technique whether these perforator arteries are cut or preserved unilaterally or bilaterally at or near the base of the flap. Hosnutter et al. noted on this fact and

developed the concept of rando-axial pattern flaps, where an axial artery is present at the base of a flap with a skin pedicle [9]. This was compared to a true pure random flap where the axial artery is transected and the flap was only supplied by the skin pedicle. Not surprisingly, there was 75 % survival in the rando-axial flap compared to 60 % in the pure random flap. Our results are very similar to their findings as there was

Fig. 2 Graphical chart showing percent viability vs. groups A, B, C, D

Table 1 Percent surviving area according groups

Groups	DCIA preserved	DCIA transected	
Gradual delay	Group A 98.44±3.13 %	Group B 69.33±5.51 %	(<i>p</i> =0.0003)
No delay	Group C 76.50±8.91 % (<i>p</i> =0.002)	Group D 52.89±3.15 % (<i>p</i> =0.01)	(<i>p</i> =0.005)

76.5 % survival in group C where the DCIAs were preserved and the flap was raised without delay. In our study, the DCIA near the base of the flap in groups B and D were transected converting them into “pure” random flaps and preserved in groups A and C converting them into rando-axial flaps. The surviving areas were higher in groups A and C compared to groups B and D, respectively independent of the delay procedure.

Taylor et al. described three adjacent and bilateral angiosomes in the dorsum of the rat as the lateral thoracic artery, 10th posterior intercostal perforator artery (PIA) and the dorsal circumflex iliac artery from cranial to caudal [8]. This concept was further tested and developed by other researchers [10–12]. When all three vascular territories are included in the dorsal flap model of rat, typically the distal territory goes to necrosis [7, 12]. This forms the basis of rat dorsal experimental skin models. The various surgical delay methods or the use of pharmacologic agents are thus designed to capture and improve the survival of this distal most territory. In contrast, the vascular territory close to the pedicle can feed several single adjacent vascular territories by opening choke vessels that lie in between [12]. Yang et al. have found that, the DCIA not only anastomosed with the adjacent ipsilateral PIA and contralateral DCIA, but also with the contralateral PIA [12]. In our method of gradual elevation of the flap from distal to proximal, the flap survival was improved whether the perforator artery was preserved or transected (groups A and B vs. groups C and D respectively). In addition, there

Fig. 3 Identification of the deep circumflex iliac artery (DCIA) bilaterally**Table 2** Vascular territories captured

Captured territories	DCIA preserved	DCIA transected
Gradual delay	1+2+3	1 + Part of 2
No delay	1+2	1

appears to be more angiosomes in the dorsum of the rat than described by Taylor et al., like the angiosome of the gluteal perforator artery [8]. The next distal angiosome to the DCIA is the GPA which lies 4 cm caudal to the DCIA. It emerges as a true perforator vessel via the gluteus maximus muscle of the rat. In groups where the DCIA was transected the GPA appeared to be dilated and was connected to the viable areas of the flap through a vascular network. This dilation was not seen when the DCIA was preserved. Thus it appears that whenever a perforator vessel is cut during the elevation of a flap a “burden” is placed on the artery of the next angiosome to increase its vascular supply. This raises the questions “How much of the McFarlane flap is a true random pattern skin flap?”. Since there will always be a nearby perforator artery that will be “forced” to supply the flap only the distal parts can be considered as “random”. But the more important question is “Do the researchers take into consideration to preserve or transect the base artery?”. Thus, the researchers using this flap model should always standardize the model by either careful preservation or complete transection of the arteries at the base. Unfortunately, when literature is reviewed this important fact is not even mentioned in the methodology except in a few papers [9, 12].

Controlled ischemia in a flap leads to preconditioning and enhancement of vascularity with the ultimate aim of improving survival or obtaining a larger flap. The mechanism of any kind of mechanical delay in a flap is by means of opening the choke vessels in between adjacent angiosomes as well as enhancing the collateral blood flow at the base of the flap [1, 8]. Various methods of delay have been tried in the rat model including surgically dividing part of the flap, applying clamps, suturing part of the flap, heat shock, and by using ND:Yag Laser [8, 10, 13–16]. In addition pharmacological agents have been used to improve survival on the dorsal rat skin model. Silenafil was shown to increase the flap survival rate upto 83 % (control group 71 %) when given orally and upto 97 % (control group 67 %) when delivered via a fibrin glue carrier [2, 3]. Tellioglu et al. used carnitine to obtain a flap survival rate of 85 % compared to 58 % in the control group [5]. However, in none of these models 100 % survival was achieved. In our experimental model 100 % flap survival rate was achieved in all 7 out of 8 animals in the experimental group (98.44±3.13 %). We introduce a new method of surgical delay in the rat model by gradually elevating the flap from its distal end to its base with consecutive surgeries set days apart. In our model, we used day four as the day for delay

period based on the data reported by Holzbach et al., who showed that VEGF levels increase after 3 days in the distal parts of dorsal rat flaps when surgically delayed leading to higher survival rates [17]. Our method also increases the effective length and dimensions of the skin flap raised. Taylor when describing the principles of angiosome concept that when a flap is raised based on a certain angiosome, the next angiosome can also be captured without the delay phenomenon [8]. However, in order to capture the third angiosome distally a delay procedure should be added [8]. Our data is concurrent with Taylor's principles. All there original angiosomes described by Taylor et al. on the dorsum of the rat included in the McFarlane flap can be captured with the method described (Table 2) [7, 8]. The survival was best when the gradual delay was applied to a flap, where the perforator artery was preserved close to the base of the flap (Group A). The survival was worst when no delay was performed in a flap where the perforator artery near the base of the flap is transected (Group D). In flaps where the perforator artery is transected applying the delay procedure appears to slightly improve the surviving area (Group B vs. Group D). However, when the axial artery near the base of the flap was transected as in group B, even the delay phenomenon does not lead to capturing of the 3rd territory. This is because when the artery supplying the base angiosome (DCIA) is cut, this angiosome is converted to the so called "adjacent angiosome" by shifting the base artery to GPA which is 4 cm distal to the base of the flap. When this knowledge is applied to clinical practice, a surgeon should meticulously preserve any perforator or axial vessels that are near the ultimate pedicle of a flap that is being surgically delayed.

Conclusion

Several conclusions should be drawn from this study.

1. The researchers should standardize the method of raising the McFarlane flap by either preserving or transecting the perforator arteries at the base of the flap.
2. Gradual elevation of the flap starting from its distal part can delay the flap so that a longer flap can be raised.
3. It is important to preserve the perforating or axial artery at the base of the flap whether a delay is performed or not. Preserving the artery in the more caudal angiosome distant to the flap may not be enough for optimal survival.

Further research using the same model and methodology should concentrate on capturing the 4th distal angiosome in order to achieve longer flap dimensions. In addition optimal length of delay at each surgical session and the time period in between surgical sessions in an attempt to harvest a longer flap should be investigated.

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References

1. Callegari PR, Taylor GI, Caddy CM, Minabe T (1992) An anatomic review of the delay phenomenon: I. Experimental studies. *Plast Reconstr Surg* 89:397–407
2. Sarifakioglu N, Gokrem S, Ates L, Akbuga UB, Aslan G (2004) The influence of sildenafil on random skin flap survival in rats: an experimental study. *Br J Plast Surg* 57:769–772
3. Ulusoy MG, Uysal A, Koçer U, Karaaslan O, Cuzdan SS, Ayyildiz A, Ustün H (2005) Improved flap viability with site-specific delivery of sildenafil citrate using fibrin glue. *Ann Plast Surg* 55:292–296
4. Tellioglu AT, Uras KA, Yilmaz T, Alagözlü H, Tekdemir I, Karabağ O (2001) The effect of carnitine on random-pattern flap survival in rats. *Plast Reconstr Surg* 108:959–962
5. Pang CY, Forrest CR, Morris SF (1989) Pharmacological augmentation of skin flap viability: a hypothesis to mimic the surgical delay phenomenon or a wishful thought. *Ann Plast Surg* 22:293–306
6. Fujihara Y, Koyama H, Nishiyama N, Eguchi T, Takato T (2005) Gene transfer of bFGF to recipient bed improves survival of ischemic skin flap. *Br J Plast Surg* 58:511–517
7. McFarlane RM, DeYoung G, Henry RA (1965) The design of a pedicle flap in the rat to study necrosis and its prevention. *Plast Reconstr Surg* 35:177–182
8. Taylor GI, Minabe T (1992) The angiosomes of Mammals and other vertebrates. *Plast Reconstr Surg* 89:181–215
9. Hosnuter M, Kargi E, Peksoy I, Babucçu O, Payasli C (2006) An ameliorated skin flap model in rats for experimental research. *J Plast Reconstr Aesthet Surg* 59:299–303
10. Zink JR, Syed SA, Zahir K, Thomson JG, Restifo R (1997) Transferring vascular territories from one axial pattern flap to another: a comparison of delay procedures. *Ann Plast Surg* 38:385–387
11. Ahmed SS, Pierce J, Reid M, Thomson JG, Restifo RJ (1997) A new experimental model: Vascular pedicled cutaneous flap over the middorsum of the rat. *Ann Plast Surg* 39:495–499
12. Yang D, Morris SF (1999) An extended dorsal island skin flap with multiple vascular territories in the rat: a new skin flap model. *J Surg Res* 87:164–170
13. Matsumura H, Yoshizawa N, Vedder NB, Watanabe K (2001) Preconditioning of the distal portion of a rat random-pattern skin flap. *Br J Plast Surg* 54:58–61
14. Odland RM, Kim P, Nadler D, Poole DV (1995) Nonsurgical delay of skin flaps: effect of a suture delay technique on blood flow and survival. *Laryngoscope* 105:523–528
15. Koenig WJ, Lohner RA, Perdrizet GA, Lohner ME, Schweitzer RT, Lewis VL Jr (1992) Improving acute skin-flap survival through stress conditioning using heat shock and recovery. *Plast Reconstr Surg* 90: 659–664
16. Acikel C, Kenkel JM, Ozturk S, Nojima K, Hoopman JE, Gokaslan ST, Brown SA (2005) Nonsurgical delay of dorsal rat cutaneous flap using a long-pulsed 1064-nm Nd:YAG laser with a contact cooling device. *Plast Reconstr Surg* 116:1411–1420
17. Holzbach T, Neshkova I, Vlaskou D, Konerding MA, Gansbacher B, Biemer E, Giunta RE (2009) Searching for the right timing of surgical delay: angiogenesis, vascular endothelial growth factor and perfusion changes in a skin-flap model. *J Plast Reconstr Aesthet Surg* 62:1534–1542