Experimental Study of the Effects of Changes in Arteriovenous Pressure Difference on the Survival Area of a Reverse Island Flap

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In this article, the effect of the change of arteriovenous pressure on the survival area of reverse island flap was studied. The vein and saphenous artery in rabbits were selected to design the reverse island flap experimental model. Rabbits were divided into 4 groups: A: Control Group; B: Part anastomosis of the saphenous artery group; C: Part anastomosis of the vein group; and D: No superficial veins group. After surgery, for all four groups, flaps were assessed by general observation, radionuclide scans for the survival rate, and histology. We found that the survival rate of flaps in Group B was overtop than the other three groups ($P < 0.05$). The radioactive material (RM) in group B could be seen clearly, whereas the RM in Groups A, C, and D existed transiently. At ten days post-operation, Group B had more capillary regeneration and blood cells contrast to the other three groups. Increasing blood supply can improve the survival rate of flaps, but simply promoting venous return has no significant effect on improving the survival rate of flaps.

Keywords: Reverse-flow island flaps, Pressurization, Venous drainage, Survival rate
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INTRODUCTION

A distally pedicle flap or reverse-flow island flap are effective methods to repair soft tissue defects and have been widely used in the clinic [1,2]. However, necrosis of the skin flap has always been a problem for clinicians [3]. Disturbance of blood circulation has already become a popular research topic in skin flap necrosis. Blood circulation disorders include insufficient arterial blood supply (BS) and Venous return disorder. The skin flap necrosis caused by inadequate arterial BS or obstruction of venous reflux remains controversial. From February 2011 to August 2013, we performed saphenous artery reverse island flaps (RIF) in New Zealand rabbits. By changing the arteriovenous pressure difference, we observed changes in flap survival area and demonstrated the relationship between flap survival area and arterial BS.

MATERIALS AND METHODS

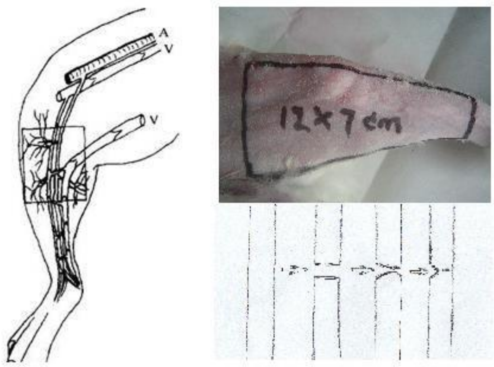
Materials

Forty adult male New Zealand White rabbits weighing 250-300 g were obtained from the Experimental Animal Centre of Soochow University, China. All the experimental rabbits were housed in separate cages with free access to fodder and water. This experiment was reviewed and approved by the Committee for the Management and Use of Laboratory Animals, and all operations were carried out in accordance with the procedures for animal care and use. All researchers followed the approved ethical procedures during protocols.

Surgical Procedures

The rabbits were randomly divided into four groups, Each group had 10 rabbits. After general anesthesia, 3% sodium pentobarbital (2 mL/kg) was injected into the ear vein of rabbits. Once anesthetized, the rabbit was fixed in a pronated position, and the bilateral hind limbs were sheared and sterilized. Then, a reverse-flow island flap above the knee 12.0 cm in length and 7.0 cm in width with a 0.5-cm-wide pedicle containing the saphenous nerve, saphenous artery and saphenous vein was successfully established. A flap 1.0 cm in width was designed. The ratio of length to width of the flap was 2:1 (Fig 1). After the flap was cut, the communicating branches of the superficial vein and venae profundae were ligatured. A semi-permeable membrane was placed between the flap and deep tissue. The flap was intermittently sutured to the surrounding soft tissue with 3-0 sutures.

Figure 1.
Anatomical diagram of the lower limb of the experimental animals; design of the saphenous artery retrograde island flap; flap vascular clip anastomosis



Group A (control): the saphenous artery and saphenous vein were adequately exposed, ligatured and cut. The great saphenous vein was then exposed, ligatured and cut at the proximal part of the flap. The communicating branches of the superficial vein and venae profundae were ligatured, and only neurovascular bundles were reserved to connect with the surrounding tissue at the pedicle of the flap (Fig 2). The specific surgical procedures defining each experimental group are as follows:

Figure 2.
The specific surgical procedures defining each experimental group

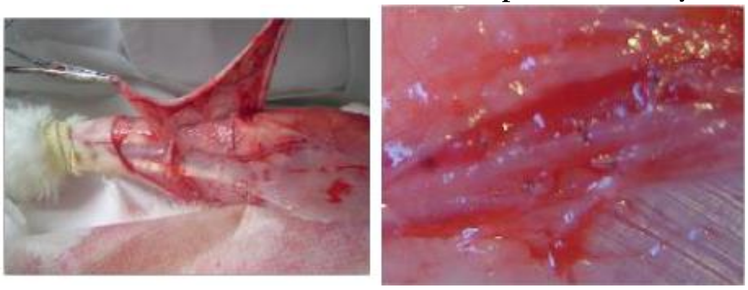
After saphenous artery and great saphenous vein ligation (not pictured), the flap is lifted to the far end. After the flap was cut, the communicating structures in the deep tissue were ligatured. Postoperative treatment: a translucent membrane is placed in between the flap and deep tissue to block deep tissue drainage in skin flap; the flap suture in situ.

Group B: the distal saphenous artery of the flap was cut and trimmed to half of its original diameter, and then an end-to-end anastomosis was performed (Fig 3).

Group C: the distal superficial veins were cut and trimmed to half of their original diameter, and then an end-to-end anastomosis was performed (Fig 4).

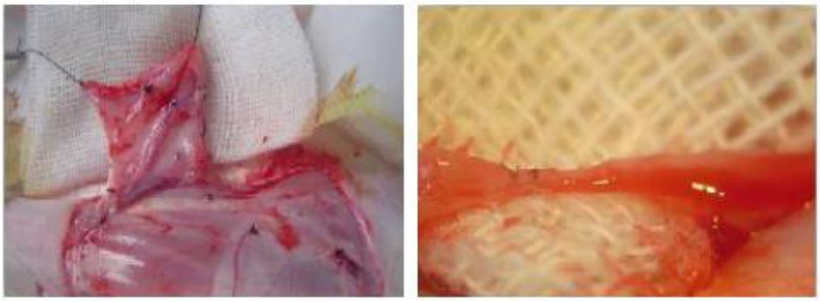
Group D: the superficial vein trunk in the pedicle of the flap was ligatured. The saphenous artery and saphenous vein were retained.

Figure 3.
End-to-end anastomosis after cut the distal saphenous artery of the flap



The distal saphenous artery was cut and trimmed to ½ of its diameter, and then end-to-end anastomosis was performed to simulate the distal peripheral artery.

Figure 4.
End-to-end anastomosis after cut distal superficial veins



After the great saphenous vein near heart is cut, the lumen is trimmed by 1/2, and then end-to-end anastomosis simulates the distal peripheral vein.

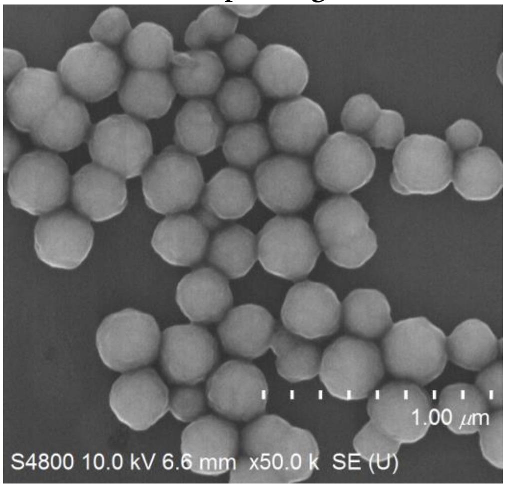
General Observation

After the operation, the color and swelling of the flaps and the growth of the fur were observed.

Nuclide Scan

In recent years, the application of nanomaterials in nuclide imaging has been widely studied [4], with advantages such as extending the duration of the strongest radioactivity. A nuclide scan was performed on the 1st and 2nd day post-operation. Radioactive nano-tracers (99mTc-Sb_2S_3, Fig 5) was slowly injected into the rabbit ear vein, and sequential scans were performed by 1000 Kev SPECT for 5 min at 0 h and 24 h after injection to observe the distribution of radioactive eikonogen in the flaps. The radioactive images were recorded. During the scanning process, a 1-mm-thick tin plate was placed under the flaps to prevent pertechnetate from getting into the muscle, which may influence the effects of the flaps.

Figure 5.
Scanning electron microscope image of nanoscale developer



Survival Area (SA) of the Flap

Once the SA of the flap was stable, the area of the surviving flap was measured. The application of model grid-tests was performed to measure the area of the flaps.

Histology

When there were no changes in the survival area of the flaps, the rabbits were sacrificed by air embolism. After the survival flaps were cut off, the tissue between the necrotic and survival regions of the flaps was prepared for specimens. The specimens were fixed with 40% formaldehyde and embedded in paraffin to prepare slices. The vessel distribution in the flap and presence of hemocytes in the vessels were observed under a 10 × 40 microscope.

Statistics Process

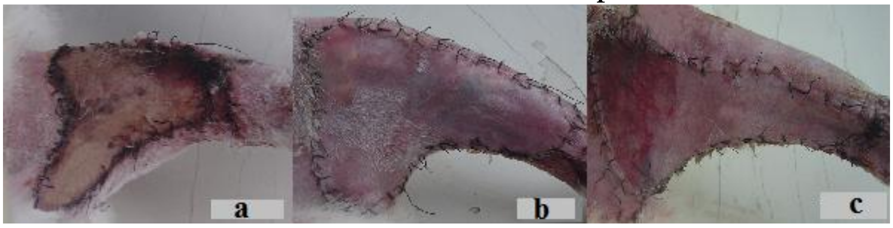
Data were analyzed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA). Quantitative data were presented as mean ± SD. T-test was used to compare the two groups. And count data were presented as percentage (%). χ^2 test was used to compare the two groups. Differences were considered statistically obvious at $P < 0.05$.

RESULTS AND DISCUSSION

General Observation of Flaps

For Group A, 7 cases were completed. The saphenous vein gradually became engorged from the proximal part of the flap to the distal part within 20 minutes post-operation. As blood flowed, the saphenous vein expanded gradually and became engorged, which presented as a dark purple, cord-like vein. Dull-red venous blood also exuded from the edge of the flap. The color of the flap gradually darkened within 3 days after operation. One week post-operation, the color of the flap was pale and completely necrotic (Fig 6-a). Nine cases were completed in Group B. One case was completely necrotic, which was excluded from statistical calculations. The saphenous vein engorged rapidly and evenly once the vascular clamps were loosened following the operation. The flap showed dull-red edema early (within 3 days after surgery), and it gradually became ruddy 3 days later (Fig 6-b, c) without evidence of tension blisters. Eight cases were completed in group C. One case acquired an infection after operation, and one case was excluded from statistical analysis because the entire flap was necrotic. The flaps in this group showed tumidness after surgery, which was much lighter than in the control group. Eight cases were completed in group D. While one case showed infection after operation, one case, which was totally necrotic, was excluded from statistical analysis. Because of poured artery post-operation, blood became plentiful inside the flap, which caused a congested state and even engorgement. From the third day, the distal part of the flap was darker in color and was accompanied by a black scab, which expanded to the pedicle of the flap.

Figure 6.
General observation of flaps.

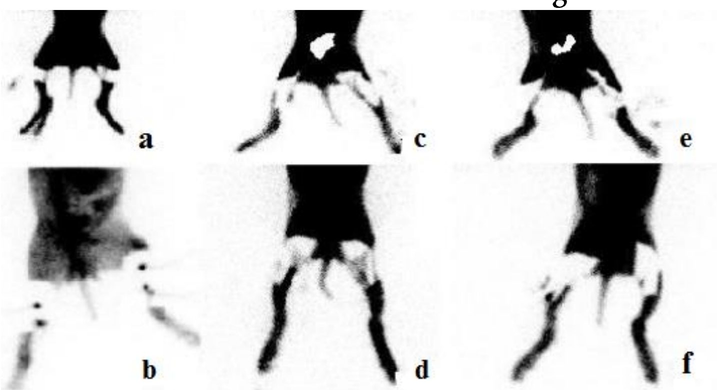


(a) Completely necrotic skin flap with a pale color. (b) 10 days post-operation; skin flap, is ruddy in color; all survived and also have hair. (c) Post-operation, skin flap is ruddy in color and only small areas of black necrosis are observed.

Nuclide Scan of Flaps

Nuclide scan showed that the radioactive material through the flap could be seen clearly on the day of operation. Nuclide scanning again on the second day post-operation did not show obvious radioactive material through the flap (Fig 7-a,b). In group B, Nuclide scans showed that the radioactive material through the flap could be seen clearly on the day of surgery, and the radioactive material increased slightly on the next day compared with the previous day (Fig 7-c,d). Nuclide scans showed that the radioactive material through the flaps of group C and group D could be seen clearly on the day of operation, but Nuclide scanning again on the second day post-operation showed that radioactive material through the groups did not change significantly (Fig 7-e,f).

Figure 7.
Results of radionuclide scanning.



(a) On the day of surgery, nuclide scans were performed for visible radionuclide distribution; (b) After the second day, of radioactive material was not obvious on the nuclide scan. (c) Both sides are based on the experimental group. On the day of surgery, nuclide scan images showed visible nuclide evenly distributed. (d) Both sides are based on the experimental group. On the day of surgery, nuclide scan images showed visible nuclide evenly distributed. (e) Left for group C. Group D is on the right, and the day after surgery, the nuclide scan indicated obvious nuclides. (f) The nuclide scan images on the second day post-operation showed no significant change.

SA of Flaps

The entire flap was removed from all the experimental animals, which completed the experiment. The area of surviving tissue within these samples was measured to determine the SA of the flap. The mean SA of the flap area observed for each group was B>D>C>A. Group B was statistically obvious contrast to Groups A, C, and D ($P < 0.05$). Among them, Group B was highly statistically obvious contrast to group A ($P < 0.01$). Group C and D had no obvious difference. The results are shown in Table 1 and Table 2.

Table 1.
Survival rate of flap area at the completion of each experiment

Group	The flap survival rate (%)								
A	76.25	35.94	64.65	56.36	60.43	34.55	65.89		
B	93.10	85.25	54.37	75.35	90.53	87.67	80.39	87.35	86.46
C	56.42	75.53	86.46	45.37	77.43	87.46	46.56	56.47	
D	39.58	69.86	52.37	76.28	65.43	74.53	75.38	85.69	

Note: the area of skin flap survival rate = area/total area of the flap

Table 2.
The descriptive data

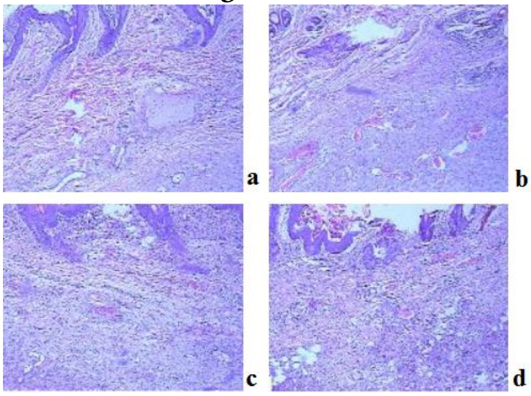
Group	Cases (n)	Mean (%)	Mean (95% confidence interval	
			lower limit (%)	upper limit (%)
A	7	56.30	41.85	70.74
B	9	82.27	73.27	91.27
C	8	66.46	52.03	80.89
D	8	67.39	54.99	79.78

Note: the flap survival area mean is greater compared to the other three groups ($P < 0.05$), 0.05 ($P < 0.001$), 0.037, 0.049

Histology of Flaps

After the boundaries of the flap survival were clear, the experimental animals were sacrificed by air embolism. The entire flap structure (composed of the both the survival part and the necrotic part) was removed, fixed with 40% formaldehyde solution, and made into paraffin sections. Sections were observed under a 10 × 40 microscope. The flaps of Group A showed more fibrous scar tissue (Fig 8 a). Capillary regeneration was more evident and a greater number of visible blood cells remained in Group B (Fig 8 b). Group C and D showed visible capillary regeneration and blood collection, but only a small amount of blood capillary regeneration (Fig 8-c,d).

Figure 8.
Histological detection.



(a) Flap with increased fibrous scar tissue. (b) Skin flap with capillary regeneration and visible blood cells. (c) Only a small amount of blood capillary regeneration in skin flap. (d) Site with a small amount of blood capillary in regeneration.

Discussion

The RIF is a special type of flap in which the pedicle contains distal vasculature. The arterial supply and venous drainage of the RIF are in the opposite direction of normal physiological blood flow. The retrograde blood flow of those flaps includes distal artery anastomosis, aortic arch or arterial rings. Distally based flaps are broad and narrow. In general, a distally based flap refers to a flap with a pedicle located distally (away from the heart), such as the RIF. The narrow concept of distally based flaps refers to the vascular pedicle of the flap from one end away from the heart, and the normal major BS flows from the distal direction into the flap. However, the blood circulation between a distally based flap and a RIF still has significant differences. The blood flow of distally based flaps exhibits normal physiology, whereas that of RIFs is non-physiological. Blood circulation normally runs according to the artery, capillaries and vein route at the level of microcirculation. Normal blood circulation of body surface tissues (skin, subcutaneous tissue and fascia) is vertically run, in which BS is from near to far, and venous return is from far to near.

The main reason for flap necrosis is disruption of the local blood circulation. Blood circulation disorders include inadequate venous stasis and arterial BS. Most scholars consider that venous stasis blocks venous blood return and venous conge stion is a major reason for skin flap necrosis [5-7]. Distal vein blood introduced into the flap through the superficial vein increases blood flow load inside and blocks the blood circulation of the flap, which is detrimental to skin flap survival. In a study by Sonmez et.al. [8] introduced a new method to reduce postoperative venous congestion of nerve island flap. They indicated that the venous congestion problem after the flap was removed could be solved by inserting the small saphenous vein into the nerve kitti after the valve was destroyed with a vein stripping device. Xi et.al [9] also believed that after surgery, blood of the distal reflux flowed through the flap via the superficial vein and increased the pressure within the veins, which caused poor venous return and edema in the flap, thereby influencing flap survival.

Experimental studies from Tanaka [10] in Japan have shown that blood flow and survival area are closely related. When the vein returned, the flap area was either unaffected or completely congestive. In other words, when venous outflow obstruction, with or without affecting other aspects, leads to the stagnation of blood flow within the flap, the blood circulation is not smooth, and complete congestive flap necrosis will occur. When the arterial pressure is greater than in the superficial, deep and accompanying veins, vein intrusion is impossible. Intrusion occurs only when the arterial venous pressure is less than venous pressure. Swelling of the entire flap could cause congestion but will not cause flap necrosis.

Most of the flap necrosis is partial necrosis in all clinical. We consider arterial BS deficiency to be the primary cause [11]. To increase the pressure inside the artery and increase the BS of the flap, the arterial pressure was made greater than the line with dry superficial venous and deep venous pressure, to prevent backward venous flow. Due to prolonged artery perfusion, the flap of the afferent vein pressure was elevated and filled with blood, which led to the closing of the venous valve, and the flap venous returned to normal [12,13].

Many scholars have performed experimental and clinical research to improve the skin flap BS in order to improve the SA of flap. Hallock et al. [14], in an SD rat abdominal experimental study, confirmed that the flap survival area with a pedicle towards no relationship was dependent on the arterial BS of the flap. Chang et al. [15] confirmed by animal experimental study that the distal artery pressure (i.e., the contralateral superficial inferior epigastric artery or arteriae circumflexa ilium profunda) can more effectively increase skin flap survival than the proximal artery pressure (i.e., the ipsilateral superficial inferior epigastric artery). By increasing the venous outflow channel to promote venous return and thereby further reducing venous pressure, flap survival area was increased [16]. Miyamoto et al. [17] reported that with a retrograde flap inferior to the normal artery, the survival area is larger. The end with end-to-end anastomosis results in blood flow and survival of the flap to make the blood, which confirmed that the artery affects a certain area of the flap survival. In view of the aforementioned facts, we designed the saphenous artery retrograde island flap, increasing the skin flap BS and increasing the flap survival area. According to the results of our experiment, the experimental groups showed varying degrees of skin flap necrosis. Numerical comparisons between Group B and Groups A, C and D were 0.002 ($P < 0.01$), 0.037 ($P < 0.05$) and 0.05 ($P < 0.05$), respectively. The differences were statistically significant, especially the difference between group A and B. In experimental group B, large amounts of blood capillary regeneration and a large number of blood cells within the lumen of free, but not the aggregated deposition, were seen under the microscope. Experimental results show that when the arterial supply is unchanged, the flap survival area is limited. However, increasing the BS through the arteries can increase the survival area of the skin flap.

This study design reported here replicates clinical practice by using New Zealand white rabbits to perform a saphenous artery retrograde island flap. In group B, increasing arterial BS in the flap by pressurization made the total arterial pressure much higher than the venous pressure inside the flap. Venous engorgement in the flap was visible post-operation, and over time, as reconstruction of the blood circulation occurred within the flap structure, the degree of venous engorgement gradually reduced. On next day, radionuclides were still present in the flap and had increased. Even though there was reduced venous pressure in the group C flap, there was no increase in arterial supply, and arterial supply was still insufficient. Arterial BS of limited scope still cannot effectively reduce the area of skin flap necrosis. The Group D flap pedicles with dry superficial vein ligation reduced the distal limb venous reflux continuously, further reducing the flap venous stasis. However, because the backflow of the superficial vein stem is blocked to a certain extent within the skin flap venous reflux, the internal arterial pressure did not increase. The deficient arterial BS phenomenon still exists, resulting in skin flap necrosis.

In the clinic, the survival area of reverse-flow island flaps has been a major concern and an important problem. When the effect of lower venous pressure and elevated arterial pressure on the survival area of flaps were compared, we can see that increasing the BS could effectively increase the survival area of flaps, which provides us with a basis to perform artery anastomosis during the flap repair process. Therefore, we regard insufficient arterial BS to be a problem, while a dry superficial vein could promote venous return to some extent. If it is conditional, we can anastomose a superficial dry vein and the vein within surrounding tissues, which will have a subsidiary effect on increasing the survival area of flaps.

CONCLUSION

We studied the effect of arteriovenous pressure on the SA of reverse island flap.The results show that increasing the BS could increase the SA of flap. Simply promoting venous return cannot effectively improve the SA of flap.

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