Original Article
Effects of arterial blood supply on the survival of reverse-flow island flaps: an experimental study

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Abstract: Objectives: This study aimed to investigate the effect of arterial blood supply on the survival area of retrograde island flap. Methods: The vein and saphenous artery in rabbits were selected to design the reverse-flow island flap experimental model. Forty rabbits were randomly divided into four groups: control group (group A), partial anastomosis of the saphenous artery group (group B), partial anastomosis of the vein group (group C), and no superficial vein group (group D). Flap survival was observed postoperatively, the survival area was measured, and the survival rate was compared. Blood distribution in the flap at different time points was observed by radionuclide imaging. Results: The blood vessel distribution and blood cell status were observed histologically. The survival rate of flaps in group B was higher than that of the other three groups (P < 0.05). The radioactive material (RM) could be seen clearly in group B, whereas those in groups A, C, and D existed transiently. The RM in group B was higher than that in groups A, C, and D (P < 0.05). On postoperative day 10, group B had more capillary regeneration and blood cells than the other three groups (P < 0.05). Conclusions: Increasing blood supply can improve the survival rate of flaps, but simply promoting venous return has no significant effect on the survival rate of flaps.

Keywords: Reverse-flow island flaps, pressurization, venous drainage, survival rate

Introduction
Distal pedicle flaps or reverse-flow island flaps are effective in repairing soft tissue defects and have been widely used in clinical settings [1, 2]. However, necrosis of the skin flap has always been a problem for clinicians [3]. At present, impaired blood circulation has already become a popular research topic in skin flap necrosis. Blood circulation disorders include insufficient arterial blood supply and venous return disorder. The skin flap necrosis caused by inadequate arterial blood supply or obstruction of venous reflux remains controversial. For example, Radomski et al. [4] found that only a thin skin or fascial flaps through the venous pedicle with numerous side branches located under the flap surface and along the central flap axis may survive with “to and fro” blood circulation. Wang et al. [5] showed that the flow-through blood supply nourished by the perforator lateral branch improved flap survival, with dilatation of collateral vascular anastomoses and increased neoangiogenesis. However, Park et al. [6] found that the distant venous drainage procedure may increase the survival of four-territory flaps with arterial pedicle only. Insufficient arterial blood supply can lead to flap necrosis; however, the relationship between the reverse-flow island flap survival area and arterial blood supply has not been clarified. Therefore, we created saphenous artery reverse-flow island flaps in New Zealand rabbits. The aim was to investigate the effect of arterial blood supply on the survival area of retrograde island flap. Changing the difference in arteriovenous pressure caused changes in the flap survival area and revealed the relationship between the flap survival area and arterial blood supply.

Materials and methods

Materials
Forty adult male New Zealand white rabbits, weighing 2.5-3.0 kg, were obtained from the Experimental Animal Centre of Soochow Uni-
Increasing blood supply can improve the survival rate of flaps

Figure 1. A. Anatomical diagram of the lower limb of the experimental animals, design of the saphenous artery retrograde island flap, and flap vascular clip anastomosis. B. After the ligation of the saphenous artery and great saphenous vein (not pictured), the flap is lifted to the far end. After the flap is cut, the communicating structures in the deep tissue are ligatured. Postoperative treatment: a translucent membrane is placed in between the flap and deep tissue to block deep tissue drainage in the skin flap; the flap suture in situ. C. The distal saphenous artery is cut and trimmed to half of its diameter, and end-to-end anastomosis is then performed to simulate the distal peripheral artery. D. After the great saphenous vein near the heart is cut, the lumen is trimmed by half, and end-to-end anastomosis simulates the distal peripheral vein.

University, China. All experimental rabbits were housed in separate cages with free access to food and water. Animal experiments were ratified by the Animal Ethics Committee of the Second Affiliated Hospital of Soochow University (Approval No. 2021032201) and conducted following the Guide for the Care and Use of Laboratory Animals (NIH). Efforts were made to reduce the number of animals used and minimize their suffering during the study.

Surgical procedures

Forty rabbits were randomly divided into four groups, with 10 rabbits in each group. For general anesthesia, 3% sodium pentobarbital (2 mL/kg) was injected into the ear vein of rabbits. After anesthesia induction, the rabbits were fixed in a pronated position, and both 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 with a width of 1.0 cm was designed with a length-to-width ratio of 2:1 (Figure 1A). After the flap was resected, the communicating branches of the superficial and deep veins were ligatured. A semipermeable membrane was placed between the flap and deep tissue. The flap was intermittently sutured to the surrounding soft tissue with 3-0 sutures.

Group A (control group): The saphenous artery and 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 and deep veins were ligatured, and only neurovascular bundles were reserved to connect with the surrounding tissue at the pedicle of the flap (Figure 1B). The specific surgical procedures defining each experimental group are as follows:

Group B: The distal saphenous artery of the flap was cut and trimmed to half of its original diameter, and an end-to-end anastomosis was then performed (Figure 1C).
Increasing blood supply can improve the survival rate of flaps

Group C: The distal superficial veins were cut and trimmed to half of their original diameter, and an end-to-end anastomosis was then performed (Figure 1D).

Group D: The superficial vein trunk in the pedicle of the flap was ligatured.

General observation

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

Nuclide imaging

In recent years, the application of nanomaterials in nuclide imaging has been widely studied [7], with advantages such as extending the duration of the strongest radioactivity. Nuclide imaging was performed on postoperative days 1 and 2. Radioactive nano-tracers (99mTc-Sb2S3) were slowly injected into the rabbit ear vein, and sequential scans were performed by 1000 Kev SPECT imaging for 5 min at 0 h and 24 h after injection to observe the distribution of radioactive eikonogen in the flaps. Radioactive images were obtained. During the scanning process, a 1-mm-thick tin plate was placed under the flaps to prevent the pertechnetate from getting into the muscle, which may influence the effects of the flaps.

Survival area of the flap

Model grid tests were performed to measure the flap area. Once the survival area of the flap was stable, the area of the surviving flap was measured by the grid method [8]. Flap survival rate (%) = surviving flap area/total flap area × 100%.

Histology

At 10 days after the operation, when the flap survival area was stable, that is, the survival area did not decrease, the rabbits were sacrificed by air embolism. After the surviving flaps were cut, the tissues between the necrotic and surviving regions of the flaps were prepared as specimens. The specimens were fixed with 40% formaldehyde and embedded in paraffin to prepare sections. Sections were stained with hematoxylin-eosin. The vessel distribution in the flap and the presence of blood cells in the blood vessels were observed under a microscope (10 × 40) after staining. Five fields were randomly observed under the microscope (× 400, each field of view 0.72 mm²). The number of blood vessels in each field of view was counted. For any blood vessel, as long as the structure was discontinuous, the branch structure was also counted as a blood vessel. The mean was taken as the number of capillaries in this case. The number of capillaries and blood cells in each group was compared.

Statistical analysis

Data were analyzed using IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, NY, USA). Quantitative data were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was used for multi-group comparison, and the Turkey test was used for pairwise comparison. Count data were presented as percentages (%), and the Chi-square test was used for comparison. Differences were significant at P < 0.05.

Results

General observation of flaps

For group A, seven cases were analyzed. The saphenous vein gradually became engorged from the proximal part of the flap to the distal part within 20 min after the 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 flap gradually darkened within 3 days after the operation. One week after the operation, the flap appeared pale and completely necrotic (Figure 2A). For group B, nine cases were analyzed, and one case was completely necrotic. The saphenous vein engorged rapidly and even ly once the vascular clamps were loosened after the operation. The flap showed dull-red edema 3 days after the operation, and it gradually became ruddy 3 days later without evidence of tension blisters (Figure 2B). For group C, eight cases were analyzed, with one case of postoperative infection and one case of complete necrosis (Figure 2C). For group D, eight cases were analyzed, in which one case had a postoperative infection and one was completely necrotic. Because of
Increasing blood supply can improve the survival rate of flaps

the poured artery after the operation, blood became plentiful inside the flap, which caused congestion and even engorgement. From day 3, the distal part of the flap was darker and was accompanied by a black scab, which expanded to the pedicle of the flap (Figure 2D).

**Nuclide imaging of flaps**

In group A, radionuclide imaging was performed on the day of operation, and radionuclide passage was seen (Figure 3A). Radionuclide imaging was performed again on postoperative day 2, and the radionuclide passage in the flap was not obvious (Figure 3B). For group B, radionuclides passed through the skin flap on the day of operation (Figure 3C), radionuclides can still pass through on postoperative day 2, and it was significantly increased compared with the previous day (P < 0.05, Figure 3D). For group C, radionuclides passed through the skin flap on the day of operation (Figure 3E), radionuclides still passed through on postoperative day 2, and no significant change was found when compared with the status on the day of operation (P > 0.05, Figure 3F). For group D, radionuclides passed through the skin flap on the day of operation (Figure 3G), radionuclides still passed through on postoperative day 2, and no significant change was observed when compared with the status on the previous day (P > 0.05, Figure 3H). The statistical results are shown in Figure 3I.

**Survival area of flaps**

The entire flap from all the experimental animals that completed the experiment was removed. The area of the surviving tissues within these samples was measured to determine the survival area of the flap. The mean survival area of the flap observed for each group was B > D > C > A (Table 1). Group B was significantly different from groups A, C, and D (P < 0.05).
Increasing blood supply can improve the survival rate of flaps

Table 1. Survival rate of the flap area after each experiment

<table>
<thead>
<tr>
<th>Group</th>
<th>Flap survival rate (%)</th>
<th>Mean (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>76.25</td>
<td>35.94</td>
</tr>
<tr>
<td>B</td>
<td>93.10</td>
<td>85.25</td>
</tr>
<tr>
<td>C</td>
<td>56.42</td>
<td>75.53</td>
</tr>
<tr>
<td>D</td>
<td>39.58</td>
<td>59.86</td>
</tr>
</tbody>
</table>

Note: The area of skin flap survival rate, surviving flap area/total area of the flap. A. Control group. B. Partial anastomosis of the saphenous artery group. C. Partial anastomosis of the vein group. D. No superficial veins group. *P < 0.05, compared with group B.

Among them, group B was highly different from group A (P < 0.01). Groups C and D had no obvious difference. **Figure 4** shows the comparison of the flap survival rate among each group.

**Histology of flaps**

After the boundaries of flap were clearly shown, the experimental animals were sacrificed by air
Increasing blood supply can improve the survival rate of flaps

Figure 4. Comparison of the flap survival rate between groups. A. Control group. B. Partial anastomosis of the saphenous artery group. C. Partial anastomosis of the vein group. D. No superficial vein group. *P < 0.05, **P < 0.01.

embolism. The entire flap structure (composed of both the survival part and necrotic part) was removed, fixed with 40% formaldehyde solution, and made into paraffin sections. Sections were observed under a microscope (10 × 40). The flaps of group A showed more fibrous scar tissues (Figure 5A). Capillary regeneration was more evident, and a greater number of visible blood cells remained in group B (Figure 5B). Groups C and D showed visible capillary regeneration and blood collection, but only a small amount of blood capillary regeneration (Figure 5C, 5D). Table 2 compares the capillaries and blood cell numbers in each group.

Discussion

The reverse-flow island flap is a special type of flap in which the pedicle contains distal vasculature. The arterial supply and venous drainage of the reverse-flow island flaps are in the opposite direction of the 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. Generally, a distally based flap refers to a flap with a pedicle located away from the heart, such as the reverse-flow island flaps. 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 blood supply flows from the distal direction into the flap. However, the blood circulation between a distally based flap and the reverse-flow island flaps still has significant differences. The blood flow of distally based flaps exhibits normal physiology, whereas that of the reverse-flow island flaps is non-physiological. Blood circulation normally runs according to the artery, capillary, and vein route at the level of microcirculation. Normally, blood circulation of body surface tissues (skin, subcutaneous tissue, and fascia) runs vertically, in which blood is supplied from near to far, and venous return is from far to near.

Flap necrosis is mainly caused by the disruption of the local blood circulation. Blood circulation disorders include inadequate venous stasis and arterial blood supply. Most scholars consider that venous stasis blocks venous blood return and venous congestion, which causes skin flap necrosis [9-11]. Distal vein blood introduced into the flap through the superficial vein increases the blood flow inside and blocks the blood circulation of the flap, which is detrimental to skin flap survival. Sonmez et al. [12] introduced a new method to reduce postoperative venous congestion of the nerve island flap. They indicated that the venous congestion after removing the flap could be solved by inserting the small saphenous vein into the nerve-based pedicle after the valve was destroyed with a vein stripping device. Xi et al. [13] also believed that after surgery, the 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 by Tanaka et al. [14] in Japan have shown that blood flow and survival area are closely related. When the venous blood returned, the flap area was either unaffected or completely congestive. In other words, venous outflow obstruction, with or without affecting other aspects, will lead to the stagnation of blood flow within the flap, resulting in complete congestive flap necrosis. When the pressure in the artery is greater than that in the superficial, deep, and accompanying veins, vein intrusion is impossible. Intrusion occurs
Increasing blood supply can improve the survival rate of flaps

Table 2. Capillaries and blood cell counts in each group

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood capillary (n)</th>
<th>Blood cell count (10^9/L)</th>
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<tbody>
<tr>
<td>A</td>
<td>2.3 ± 1.16*</td>
<td>3.52 ± 0.45*</td>
</tr>
<tr>
<td>B</td>
<td>7.3 ± 1.16</td>
<td>6.34 ± 0.77</td>
</tr>
<tr>
<td>C</td>
<td>2 ± 1.05*</td>
<td>2.58 ± 0.65*</td>
</tr>
<tr>
<td>D</td>
<td>1.8 ± 0.78*</td>
<td>2.71 ± 0.58*</td>
</tr>
<tr>
<td>F</td>
<td>63.390</td>
<td>78.970</td>
</tr>
<tr>
<td>P</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Note: A. Control group. B. Partial anastomosis of the saphenous artery group. C. Partial anastomosis of the vein group. D. No superficial vein group. *P < 0.05, compared with group B.

Figure 5. Histological detection (magnification =200 ×; scale bar =200 µm). A. Control group, the flap with increased fibrous scar tissue. B. Partial anastomosis of the saphenous artery group, with skin flap with capillary regeneration and visible blood cells. C. Part anastomosis of the vein group, only a small amount of blood capillary regeneration in skin flap. D. No superficial vein group, site with a small amount of blood capillary in regeneration.

only when the arterial pressure is less than the venous pressure. Flap swelling could cause congestion but will not cause flap necrosis.

Most cases of flap necrosis are partial necrosis. We consider arterial blood supply deficiency as the primary cause [5]. To increase the pressure inside the artery and the blood supply of the flap, the arterial pressure was greater than the line with superficial venous and deep venous pressure which prevented backward venous flow. With prolonged artery perfusion, the afferent vein pressure of the flap was elevated, blood filling led to the closing of the venous valve, and the flap venous state returned to normal [15, 16].

Many scholars have performed experimental and clinical studies to improve the blood supply in the skin flap and flap survival. In an SD rat abdominal experimental study, Hallock et al. [17] confirmed that the survival area of the flap is not related to the distal or proximal (or upper or lower) orientation of the pedicle, but closely related to the internal arterial blood supply of the flap. Chang et al. [18] confirmed the animal experimental study that the distal artery pre-
Increasing blood supply can improve the survival rate of flaps

Increasing blood supply can improve the survival rate of flaps (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., ipsilateral superficial inferior epigastric artery). By increasing the venous outflow channel to promote venous return and thereby further reduce venous pressure, the flap survival area was increased [19]. Miyamoto et al. [20] reported that the survival area of retrograde arteries is smaller than that of normal arteries. The end-to-side anastomosis and end-to-end anastomosis of the blood vessels result in the survival of the flap in the area with blood flows. This confirmed that the artery affects the survival area of the flap to a certain extent. Therefore, we designed the saphenous artery retrograde island flap, which increased the skin flap blood supply and flap survival area. The results of our experiment indicated significant differences, especially between group A (control group) and group B (partial anastomosis of the saphenous artery group). In group B, blood capillary regeneration and numerous blood cells were observed within the free lumen, but not aggregated deposition. Experimental results show that when the arterial supply is unchanged, the flap survival area is limited. However, increasing the blood supply through the arteries can increase the survival area of the skin flap.

This study replicated clinical practice by using New Zealand white rabbits to perform a saphenous artery retrograde island flap. In group B, the increased arterial blood supply 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 after the operation, and over time, with the reconstruction of the blood circulation in the flap, the degree of venous engorgement gradually reduced. On the next day, radionuclides were still present in the flap and their level increased. Despite the reduced venous pressure in the flap of group C, there was no increase in arterial supply, and arterial supply was still insufficient. Arterial blood supply of limited scope still cannot effectively reduce the area of skin flap necrosis. In group D, flap pedicles with ligation of the superficial vein trunk reduced the distal limb venous reflux continuously, further reducing the flap venous stasis. However, because the backflow in the branch of the superficial vein was blocked to a certain extent within the venous reflux of the skin flap, the internal arterial pressure did not increase. The deficient arterial blood supply phenomenon persisted, resulting in skin flap necrosis.

In the clinic, the survival area of reverse-flow island flaps has been a major and important problem. Comparison of the effect of lower venous pressure and elevated arterial pressure on the survival area of flaps revealed that increasing the blood supply could effectively increase the survival area of flaps, suggesting the benefits of performing arterial anastomosis during the flap repair process. Therefore, we identify insufficient arterial blood supply as a problem, whereas a superficial vein could promote venous return to some extent. If it is conditional, we can anastomose a superficial vein and the vein within surrounding tissues, which will have a subsidiary effect on increasing the survival area of flaps.

This study has some limitations. First, there were only 10 rabbits in each group, resulting in an insufficient sample size. Second, anastomosis outcome is depending on the skill of the operator, so it’s difficult to ensure the uniformity of vascular anastomosis. Third, for radionuclide imaging of flaps, data were not quantified, and the comparison results were only observed by the naked eyes, which may cause errors. Finally, in the observation of the perfusion index of flaps, regional tissue oxygenation and microanalysis were not detected; thus, there may be some deviations in the quantitative analysis.

Conclusion

We studied the effect of arteriovenous pressure on the survival area of reverse-flow island flaps. The results show that increasing the blood supply could increase the survival area of flaps. Simply promoting venous return cannot effectively improve the survival area of flaps.

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Increasing blood supply can improve the survival rate of flaps

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Disclosure of conflict of interest

None.

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