Int J Clin Exp Med 2015;8(4):5303-5309
www.ijcem.com /ISSN:1940-5901/IJCEM0005131

Original Article

Perforator pedicled sural neurocutaneous vascular flap: a modeling study in the rabbit

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Received December 22, 2014; Accepted March 17, 2015; Epub April 15, 2015; Published April 30, 2015

Abstract: Background: An animal model of a distal pedicled sural neurocutaneous flap for experimental research has not previously been established. The purpose of this study was to construct a sural neurocutaneous vascular flap model in the rabbit. Materials and methods: Thirty-five New England rabbits were divided into four groups. Five rabbits in Group A were used for an anatomical study. Red latex and gelatin-lead oxide were injected into posterior tibial arteries of five rabbits in Groups B and C, respectively. In Group D, 40 neurocutaneous flaps with a single perforator pedicle were raised bilaterally in twenty rabbits. In the right legs, 20 flaps were raised by the normal procedure. In the left legs, the perforator pedicles of 20 flaps were ligated as controls. Results: The sural nerve originated from the posterior tibial nerve. Its accompanying artery originated from the deep femoral artery and ran to the lateral malleolus following the sural nerve. A perforator of the posterior tibial artery at the superior calcaneus originated from the midpoint of the connecting line between the medial malleolus and calcaneus, and was 0.46 ± 0.03 mm in diameter at its origin. The survival rate of the flaps in the right leg 10 days after operation was 64.7 ± 8.7%. Flaps on the left side underwent total necrosis. Conclusion: The distal single perforator-based sural neurocutaneous vascular flap in the rabbit presents with anatomical stability, is easy to harvest, and has a reliable arterial supply. The developed method represents a useful animal model for the study of single perforator-based neurocutaneous vascular flaps.

Keywords: Neurocutaneous flap, perforator flap, rabbit model, sural nerve

Introduction

In 1992, Bertelli [1] and Masquelet [2] described the first neurocutaneous flap as an axial flap composed of one vein and one nerve, with arterial vascularization provided by the vascular plexus around and inside the nerve. Since then, neurovascular flaps [2, 3], neurocutaneous vascular flaps (NCVF) [4, 5], neurofasciocutaneous flaps [6, 7], and neuroadipofascial pedicled flaps [8] have been developed.

NCVF are valuable options for repairing extensive soft tissue defects in the limbs that do not involve the main artery, as the flaps are supplied by arteries around the cutaneous nerves, and long flaps along the neurosubtaneous arterial axis are available. However, the length limitation of the wide and short fascial or fasciocutaneous pedicle of the flaps would cause flap venous congestion, limited rotation of the pedicle, partial or complete flap necrosis, and bulky contour. Furthermore, post-repair swelling can result in aesthetic problems [9, 10].

In 2007, Chai et al. [11] reported a series of foot and ankle reconstructions with a distally-based sural neurofasciocutaneous flap supplied by the terminal perforating branch of the peroneal artery, and introduced the concept of the 'perforator-based flap'. Although neurocutaneous perforator flaps have gained popularity in reconstructive surgery during recent years [12-15], questions remain regarding their basic physiology, biology, and pharmacology. Cadavers have been used for anatomical studies of neurocutaneous perforator flaps [16]. However, metabolic, pharmacologic, and immunologic studies cannot be performed in cadavers or human subjects; therefore, an animal model is required for research.

Although various perforator flaps in the pedicled form in animal models have been described

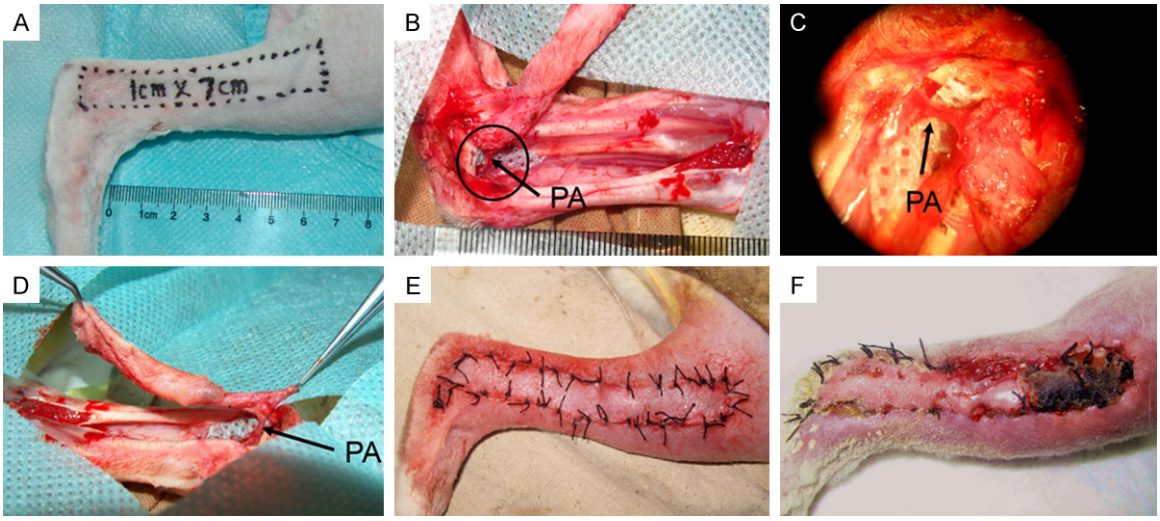

Figure 1. Flap procedure and outcome. A: Flap design. B: Exposure of the perforators. C: High power view of the circle in B. D: The flap was harvested. E: The flap was sutured *in situ*. F: Outcomes at 10 days post-operation. PA: perforator artery.

[17, 18], a neurocutaneous vascular perforator flap has not been developed. This study aimed to establish a distally-based sural NCVF supplied by a single perforator artery in the rabbit for future research.

Materials and methods

Study design

Thirty-five New Zealand white rabbits (body weight, 2.5-3.0 kg) were used in this study (Slac Laboratory Animal Corporation, Shanghai, China). All experimental animal procedures were approved by the Institute of Animal Care and Use Committee of the School of Medicine, Shanghai Jiao Tong University, and were performed in accordance with the Regulations of Laboratory Animal Care.

The animals were kept without food or fluids the night before the procedure. The rabbits were randomly divided into four groups: (A) anatomic dissection group (n = 5); (B) red latex perfusion group (n = 5); (C) gelatin-lead oxide perfusion group (n = 5); and (D) flaps modeling group (n = 20). Anesthesia was induced by napental (2 mg/kg) given in the auricular vein. Operative areas were shaved with an electric shaver. All experiments were performed on bilateral legs.

Experimental details

Anatomical dissection (Group A n = 5): After anesthesia, rabbits were fixed in the central upright position and incised along the medial thigh and the midline of the medial legs. Skin and sarcolemma were stripped aside. Subsequently, electrocautery was used to expose and observe the sural nerve, accompanying vessels, posterior tibial artery, and the origin and branches of the perforator vessels.

Vascular dyeing (Groups B n = 5 and C n = 5): After preparing the skin of the bilateral lower limbs for operation, rabbits were sacrificed by venous air embolism and dissected as described above. Subsequently, the bilateral lower limbs were broken at the level of the knee, and the posterior tibial artery, posterior tibial vein, sural artery, and sural vein were exposed. Venous indwelling needles were inserted into the sural artery and posterior tibial artery, the vessels were flushed with normal saline, and the rabbits were perfused with red latex or gelatin-lead oxide and placed in a dry place for 1-2 days.

In Group B (red latex perfusion), the location, external diameter, branch, and anastomosis of the accompanying vessel of the sural nerve, posterior tibial artery, and perforator vessel were microscopically (10-24 × magnification) measured by a vernier caliper (0.1 mm precision).

In group C (gelatin-lead oxide perfusion), in three perfused rabbits, a longitudinal incision at the anterior tibia of the bilateral legs separated the anterior from the posterior below the

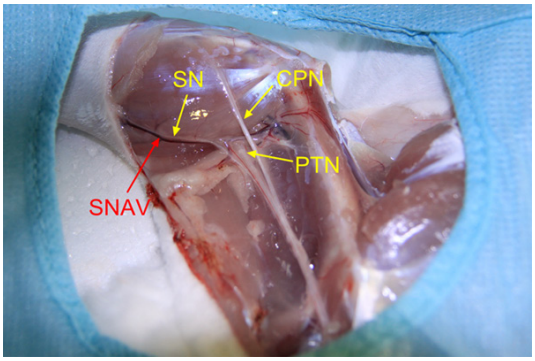

Figure 2. Anatomical dissection of the nerves in the lower extremity of the rabbit. SN: sural nerve. CPN: common peroneal nerve. PTN: posterior tibial nerve. SNAV: sural nerve accompanying vessels.

sarcolemma. Perforator vessels to the skin were marked and cut. Reverse tracking was used to find the arteries of origin. The whole integument was detached and tiled on a clipboard for X-ray graphing to observe the anastomosis of the subcutaneous vascular plexus. In the other two perfused rabbits, double incisions in the anterior and posterior sides of the bilateral legs separated the anterior from the posterior below the sarcolemma until the plantar region. The spatial relations among the cutaneous nerves, perforator vessels, main vessels, and bones were conserved, and the specimens were fixed on a clipboard for X-ray graphing.

Flap transfer (Group D n = 20): Based on the anatomy above, a distally pedicled (0.5 cm-long) sural nerve-axis flap (7 cm × 1 cm in area) (**Figure 1A**) supplied by perforator arteries of the posterior tibial artery at the superior calcaneus was designed. All procedures were performed by one experienced surgeon.

The proximal end of the flap was incised to expose the small saphenous vein, sural nerve, and accompanying vessels, which were ligated, cut, and completely separated from the deep fascia from the proximal to the distal ends of the flap until the pedicle. The pedicle skin was incised to expose the distal small saphenous vein, which was ligated and cut. The pedicle fascia was carefully stripped aside to expose the perforator arteries at the superior calcaneus and the accompanying vessels (**Figure 1B**, **1C**). A 0.5 cm perforator pedicle was created and blood flow in the middle section of the flap was observed (**Figure 1D**). When blood flow was satisfactory, the flap was sutured *in situ* (**Figure 1E**).

40 neurocutaneous flaps with a single perforator pedicle were raised in 20 rabbits. In the right legs, 20 flaps were raised by the normal procedure. In the left legs, the perforator pedicles of 20 flaps were ligated as controls.

After the operation, flaps were dressed, and penicillin (800,000 U) was immediately administered to each rabbit by a single intramuscular injection. Rabbits were able to feed conventionally in divided cages. The rabbits' posterior limbs were not immobilized, and their movement was not limited. The flaps were treated with medical alcohol every day, and a clean dressing was applied. The color and swelling of the flaps were observed. Ten days after the operation, the surviving area of each flap was measured using Image J Software (version 1.46; National Institutes of Health, Bethesda, MD, USA). The surface area was calculated in square centimeters, and the survival rate was determined (survival rate = surviving area/total area × 100%).

Statistical analysis

Quantitative data are expressed as the mean ± SD. The study results were analyzed by SPSS for Windows, Version 17.0. Chicago, IL: SPSS Inc.

Results

Anatomical observations

The sural nerve originated from the posterior tibial nerve at the posterior midsection of the femur (**Figure 2**). The nerve origins descended obliquely to the posterior until the popliteal fossa, pierced into the gastrocnemius between the lateral and medial heads, ran below the sarcolemma to the exterior posterior, passed through the sarcolemma at 5.5 cm above the lateral malleolus to run below the deep fascia in the lateral crural region, perforated out of the deep fascia, and divide into anterior and posterior branches that descended vertically. Both branches pierced into the plantar region between the lateral malleolus and Achilles' tendon.

There was one accompanying artery and vein along the axis of the sural nerve (**Figure 2**). The

Table 1. Parameters describing the posterior tibial perforator artery

Number	Position where perforator was pierced	Diameter of perforator (mm)	Length of perforator (mm)
Case 1 (L)	MOC*	0.49	11.4
Case 1 (R)	MOC	0.50	11.2
Case 2 (L)	MOC	0.46	10.9
Case 2 (R)	MOC	0.45	11.0
Case 3 (L)	MOC	0.49	11.3
Case 3 (R)	MOC	0.48	11.4
Case 4 (L)	MOC	0.46	11.1
Case 4 (R)	MOC	0.47	10.9
Case 5 (L)	MOC	0.41	10.3
Case 5 (R)	MOC	0.42	10.5

*MOC stands for midpoint of calcaneus.

accompanying artery was located anterior to the vein and sural nerve, and the vein was located between the sural nerve and the accompanying artery. The vessels accompanying the sural nerve distributed perforator vessels at the superior-middle section of the legs, and the perforators pierced into the gastrocnemius and anastomosed with intramuscular vessels. The accompanying vessels passed through the gastrocnemius sarcolemma at the superior lateral malleolus concomitant with the posterior branch of the small saphenous vein (located at the lateral sural neurovascular bundles) and divided into anterior and posterior branches while descending. The anterior branch was thicker than the posterior branch. The vascular branches anastomosed into the subcutaneous vascular plexus to nourish the integument.

Vascular observations

The diameter at the origin of the sural nerve was 0.64 ± 0.07 mm. The accompanying artery (sural artery) originated from the deep femoral artery, and descended obliquely to the posterior at the popliteal fossa. Both the sural nerve and artery pierced into the gastrocnemius between the lateral and medial heads. The sural nerve ran below the sarcolemma to the exterior posterior and divided into anterior and posterior branches approximately 5.42 ± 0.15 cm above the lateral malleolus; both branches descended into the plantar region. The sural artery divided into anterior and posterior branches and followed the sural nerve. The vascular diameter at the branching location was 0.35 ± 0.08 mm. The anterior vascular branch divided into two branch vessels at 1 and 2.5 cm above the lateral malleolus to nourish the surrounding skin, subcutaneous tissues, and the sarcolemma, and descended to the plantar region via the midpoint of the connecting line between the calcaneus and medial malleolus. The posterior vascular branch descended to the heel region at about 2 mm anterior to the Achilles' tendon.

The vein accompanying the sural nerve originated from the plantar vein, collected venous blood at the lateral crural region, and had one anastomosis with the small saphenous vein at 5.5 ± 0.25 cm above the calcaneus. The venous trunk ran concomitant with the sural nerve, ascended to the popliteal fossa, and integrated into the deep femoral vein.

The posterior tibial artery distributed one direct cutaneous perforating artery at the midpoint of the connecting line between the medial malleolus and the calcaneus; the diameter at the origin was 0.46 ± 0.03 mm, and the length of the perforator was 11.0 ± 0.37 mm (**Table 1**). The perforator vessel ran to the lateral crural region via the superior calcaneus, anastomosed with the vessel accompanying the sural nerve, and ran to the plantar region to nourish the lateral plantar region. The cutaneous perforating artery distributed two to three branch vessels to nourish the calcaneus and Achilles' tendon before the vessels anastomosed (**Figures 3**-**5**; **Table 1**).

Flap transfer

40 sural neurocutaneous flaps supplied by a single perforator of the posterior tibial artery were raised. Of these, 20 flaps in the right leg showed initial necrosis distal of the flap three days after the operation. The survival area of the right side flaps 10 days after the operation was 4.53 ± 0.61 cm^2 (1 × 4.1 cm to 1 × 5.3 cm) and the survival rate was 64.7 ± 8.7% (**Figure 1F**). Flaps on the left side underwent total necrosis.

Discussion

NCVFs are gaining popularity in plastic surgery. Since the concept of the NCVF was introduced, various animal models have been developed to study associated physiologic phenomena. Bertelli [19] reported on the earliest medial and

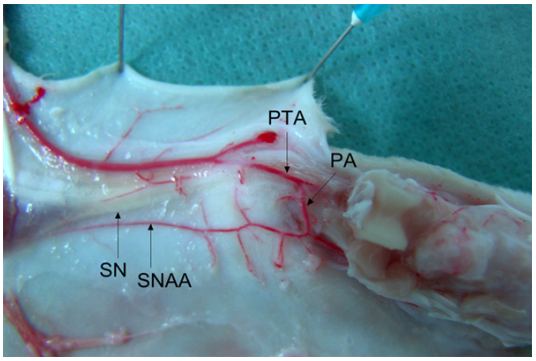

Figure 3. Vessels after the tibia calcaneus and muscle were removed. SN: sural nerve. SNAA: sural nerve accompanying artery. PTA: posterior tibial artery. PA: perforator artery.

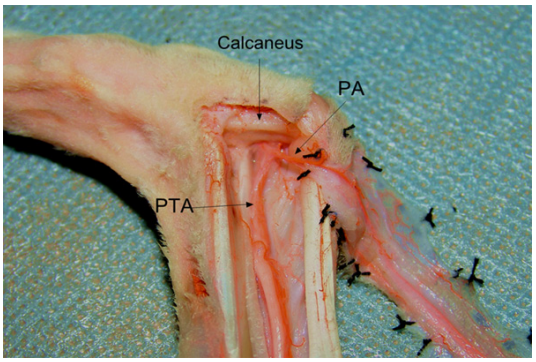

Figure 4. Vessels in a normal limb. PTA: posterior tibial artery. PA: perforator artery.

lateral NCVF model in rat hind limbs. Dogan et al. [20] constructed proximal pedicled saphenous NCVFs in a rat model. Flaps were based on pedicles of the saphenous nerve, saphenous artery, great saphenous vein, and their communicating branches and tiny fascial vessels. The authors concluded that intraneural and extraneural vascular plexus were required for flap survival. Akyurek et al. [21] modeled iliolumbar lateral femoral neurocutaneous pedicled dorsal flaps in the rat and studied the effect of pedicle structure on flap survival rate. The results showed that skin flaps survived where the artery and vein were intact, whereas mean survival rates for the neural island flap and the neurocutaneous flap were 38.2 and 44.5%, respectively. Salgarello et al. [22] developed an auricular central neurovascular bundle-based NCVF in the rabbit. They found that neurovascular plexus-conserving proximally- or distally-based NCVFs with the vessels around the ligatured flap survived entirely.

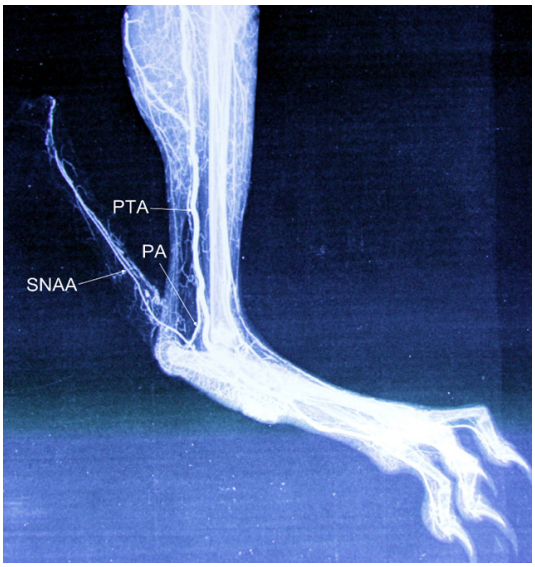

Figure 5. Angiographic analysis after the flap was raised demonstrates the course of the vascular pedicle.

Although these were well-conducted studies in validated animal models, their clinical relevance may be limited, and observations from these reports provide conflicting information about how pedicle structure affects flap survival. Most of these animal studies model proximally-based NCVFs, but distally-based NCVFs are commonly used in clinical applications. Furthermore, rat or rabbit auricular flap models cannot explain the mechanisms of venous return in NCVFs in the clinical setting, as some studies show that the rat superficial epigastric vein and rabbit auricular veins do not have valves, and the existence of valves in the vein of the rat lower limb is uncertain. To the best of our knowledge, there are no published reports of an animal model of a perforator-based NCVF. The current study investigated a clinically relevant rabbit model of a distally-based sural NCVF supplied by a single perforating artery. In comparison with the previously described animal models, this perforator pedicled flap adopted a distal pedicle. In addition, the rabbit lower limb vein is known to have valves.

The sural NCVF in the rabbit serves as a good model for research as the rabbit is a suitable size, the rabbit is cost-effective and the thin subcutaneous tissue provides an appropriate anatomical structure. The flap had a stable and reliable blood supply, the cutaneous nerve, accompanying vessels, and the perforators showed no obvious anatomical variations, the

sural nerve traverses the whole length of the lower limbs, and the perforator of the pedicle locates in the lateral malleolus region, which is similar to NCVFs used in the clinical setting.

This distal single perforator pedicled NCVF in the rabbit presented with anatomical stability. Microscopic study of the anatomy of the vessels and nerves of the lateral crural region in the rabbit model confirmed that the rabbit sural nerve arises from the posterior tibial nerve and descends obliquely to the exterior, accompanied by one artery and vein. In all animals studied, the artery originated from the deep femoral artery. The accompanying vessel follows the sural nerve, distributing nourishing branches. Meanwhile, branches anastomose with each other to form a vascular plexus around the nerve and communicate with intramural vessels and body wall vessels. Microscopy study confirmed that the posterior tibial artery distributes a perforator vessel at the superior calcaneus. This perforator traverses the calcaneus via the medial ankle joint to the exterior and distributes branches that anastomose with the vessels accompanying the sural nerve. Together, these vessels form the vascular plexus around the sural nerve to nourish the lateral crural region and the soft tissue of the ankle. This perforator is the typical direct cutaneous perforating artery.

Despite the advantages of the single perforator pedicled NCVF model in the rabbit, it is associated with some limitations. First, the anatomical structure of the rabbit is not the same as the human. Second, in our study, the flap was sutured *in situ* after being elevated and was not transferred to another region; this is different from the surgical procedure in the clinical setting. Third, histological analyses are required to substantiate our findings.

In conclusion, this distal single perforator-based sural NCVF is the first published report of a neurocutaneous vascular perforator flap model in the rabbit. It is a reliable, simple model for use in metabolic, pharmacologic, and immunologic studies. The method represents a useful animal model for future research into single perforator-based NCVFs.

Acknowledgements

The present work was supported by grants from the Shanghai Municipal Commission of Health and Family Planing Research Project (20144-Y0256).

Disclosure of conflict of interest

None.

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