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Patterns of sural nerve innervation of the sural artery with implication for reconstructive surgery

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ABSTRACT

Background: Most of the literature concerning the neurocutaneous flap is related to its anatomic investigation and clinical application, and the more in-depth physiological problem such as whether the cutaneous nerve contains sympathetic fibers that innervate its accompanying vessels has never been explored.

Materials and methods: Dissection was first performed on three rabbits. In another 22 rabbits, two rabbits undergoing no surgery were used as the normal control group. In the remaining 20 rabbits, the 40 sides of hind limbs were divided into a nerve severance group, where the sural nerve was transected at its origin after creation of the proximally based sural neurocutaneous flap, and a nerve preservation group, in which the continuation of the sural nerve was preserved. The sural neurovascular bundles at four time points were harvested for immunohistochemical and Western blotting analyses of the expression of tyrosine hydroxylase (TH). An infrared thermal imager was used for measurement of the average flap temperature within the first 24 h.

Results: The sural neurovascular bundle entered the skin at 4.5 ± 1.2 cm above the lateral malleolus. The TH in the sural nerve and tunica adventitia of the sural artery showed a synchronized abated expression in the nerve severance group. The TH expression showed no decline in the nerve preservation group. The average flap temperature in the nerve severance group was higher than that in the nerve preservation group starting from 2 h after flap harvest ($P = 0.05$).

Conclusions: The cutaneous nerve has meted out sympathetic fibers to the accompanying artery, regulating its vascular tone.

In the early 1990s, Bertelli and Khoury and Masquelet *et al.* found through dissection that there was a longitudinal chain-like vascular plexus around every cutaneous nerve in the extremities which not only supplied the cutaneous nerve but also gave off branches to the surrounding skin.[1,2] Therefore, they proposed that the vascular supply to the skin partially depended on the vascular supply of the cutaneous nerves, and by including a cutaneous nerve and its vascular plexus, the

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vascularity of the flap can be strengthened. Due to the reliability of the vascular supply of the neurocutaneous flap and the potential for sensate reconstruction of the flap by coapting the cutaneous nerve into a nerve in the recipient site, the neurocutaneous flap has received wide popularity in the reconstructive field, becoming a workhorse for resurfacing soft-tissue defects in the extremities in either anterograde or retrograde way[3-7] or even in a free approach.[8] Though numerous anatomic and clinical studies have been carried out on the neurocutaneous flap, in-depth physiological studies concerning the following two issues have never been performed:(1) whether the cutaneous nerve contains sympathetic fibers that control the vascular tone of its accompanying vessels and (2) if the cutaneous nerve does contain sympathetic fibers that innervate its accompanying vessels, what kind of hemodynamic change would be brought about if the cutaneous nerve is severed in a proximally based neurocutaneous flap. Since the sural neurocutaneous flap is one of the most commonly used surgical approaches in clinical practice, we examined the aforementioned two questions in a proximally based neurocutaneous flap established in the hind limbs of New Zealand White rabbits.

The histologic demonstration of the sympathetic innervation in the early period was performed using formaldehyde staining.[9,10] More recently, a tyrosine hydroxylase (TH) immunohistochemical staining technique has been introduced to evaluate the sympathetic innervation in the human tissue and has demonstrated high specific staining.[11,12] Therefore, in this study, we employed the immunohistochemical and Western blotting methods for analyzing the location and quantity of sympathetic components, respectively, within the sural neurovascular bundle over a 7-d course after surgical intervention.

Materials and methods

Dissection of the sural neurovascular bundle

A mixture composed of 25% ammonia and white latex in a 1:1 ratio was first prepared, and a strong red dye was added to the mixture to make it red. The red mixture then underwent filtering in a funnel lined with double layers of gauze to remove large particles. A 50 mL syringe connected with a fine plastic tube was used to aspirate the mixture. Three New Zealand White rabbits were anesthetized with 20% urethan (6 mL/kg), after which the abdomen was opened and the fine plastic tube was inserted into the abdominal aorta and fastened with threads. The red mixture was then injected into the aorta until the red dye could be spotted on the hind limbs of the rabbits. The rabbits were placed in a refrigerator under a temperature of −4°C overnight to enhance the cure of the mixture. Dissection for observation of the sural neurovascular bundle was performed with the knee and ankle joints flexed in 90° on the second day after perfusion. The animal experiments were approved by the Animal Ethics Committee of Fujian Medical University and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978).

Harvest of the proximally based sural neurocutaneous flap

After anesthesia, the hair on the hind limbs of the rabbit weighing 2-2.5 kg was removed using an electric clipper, and templates with a size of 2×7 cm^2 were marked on the hind limbs of the rabbits weighing 2-2.5 kg. An incision was first made at the posteroinferior angle of the flap located at the tip of the lateral malleolus and was then extended forward and upward along the posterior, inferior, and anterior margins of the flap. Afterward, the flap was elevated in a distal-to-proximal direction at the subfascial layer, carrying a layer of connective tissue on the surface of tendo calcaneus to avoid separation of the sural neurovascular bundle from the flap. The elevation stopped when the sural neurovascular bundle was observed entering the skin, a point about 5 cm above the lateral malleolus. Finally, the proximal margin of the flap was incised and the sural neurovascular bundle was dissected free from the most proximal part of the flap and carefully protected (Fig. 1). A total of 22 rabbits were involved in this part of study, among which two rabbits received no flap harvest and served as the control group. In the remaining 20 rabbits, the proximally based sural neurocutaneous flaps were harvested on both hind limbs, but managements of the sural nerve differed, i.e., the sural nerve was severed at its origin on one hind limb (the nerve severance group), whereas on the other limb the sural nerve was left untouched (the nerve preservation group). The 20 flaps in either the nerve severance group or the nerve preservation group were each further divided into four subgroups, according to the time points after surgery, i.e., day 1, 3, 5, and 7 subgroups, with five flaps in each subgroup. At each time point, the sural neurovascular bundles were harvested with a cuff of surrounding tissues and divided into two parts: one part immersed in 4% formaldehyde for immunohistochemical analysis and the other part frozen for subsequent Western blotting analysis (Fig. 1).

Immunohistochemical analysis of TH expression

The samples were fixed in 4% paraformaldehyde for no more than 48 h, washed in phosphate buffered saline, stored in 70% ethanol, and finally embedded in paraffin blocks. Five micrometer sections were cut, transferred to microslides, and air-dried at 60°C overnight. The samples were immunohistochemically stained for TH. For the staining, slides were deparaffinized and underwent high-pressure antigen retrieval in citrate buffer (pH = 6.8). Endogenous peroxidase blocking was performed with 3% H_2O_2. The slides were then incubated with a primary mouse anti-TH antibody (Abcam, Britain). The PV-8000 (China) Universal Immuno-peroxidase Polymer Anti-Mouse/Rabbit Immunohistochemical Staining reagent Max-Vision TM system (ZSGB-BIO) was used for color development. Micrographs were taken from an optical microscope.

Western blot analysis of TH expression

The tissue for Western blot was snap frozen in liquid nitrogen before homogenization in lysis buffer. Equal amounts of protein (20 or 30 μg) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. A mouse anti-rabbit TH