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A new lateral thoracic artery perforator flap design with multiple vascular territories in rats

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ABSTRACT

Background: To describe a new design for an extended lateral thoracic artery (LTA) perforator flap and investigate its anatomical, dynamic, and potential territories.
Materials and methods: To assess vascular territories, rats were randomized according to LTA perforator flap type into the surgical groups A, hemidorsal island flap; B, entire dorsal island flap; and C, reduced-size dorsal island flap.
Results: On postsurgical day 7, the surviving flap areas were 95%, 92%, and 89% in groups A, B, and C, respectively. Necrosis most commonly occurred in the contralateral LTA territories in groups B and C. The immunoreactivities of intercellular adhesion molecule 1 and vascular endothelial growth factor receptor 2 in dynamic territories, as choke vessel markers, were increased.
Conclusions: We clarified the LTA perforator flap nomenclature and defined its pedicle course and anastomosing patterns; furthermore, we demonstrated that the LTA perforator did not anastomose with its counterpart because of its unidirectional, oblique, and craniocaudal course. The LTA perforator flap was found to be a good model comprising multiple vascular territories and exhibiting continuous necrosis.
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Introduction

Over the course of years, the preference of reconstructive surgeons has undergone a change from random- and axial-pattern flaps to perforator flaps; this is because the donor sites of perforator flaps cause lower postoperative morbidity than those of conventional flaps.[1] Different perforator flap models have been described, including pedicled and free flaps, and their physiology, including hemodynamics, physiology, and wound-healing properties, studied.[2,3] In addition, the dorsal random pedicle skin flap in rats has also been the focus of several studies.[4–9] With the decrease in random-pattern flaps usage in reconstructive surgery because of a better understanding of the vascular basis of dorsal skin, various axial-pattern rat skin flaps harvested from the flank and lateral aspect of the hip[5] and thoracic region of the dorsum[6] and middorsum[7] according to the location of the iliolumbar,[5] lateral thoracic[7] and 10th posterior intercostal[7] vessels, and deep circumflex iliac artery,[8] respectively have been described.

According to the angiosome concept, all tissues are viewed as being a part of distinct vascular territories. A named source artery supplies each of these territories. Choke anastomotic vessels act as a link between adjacent vascular territories, and

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their behavior influences flap survival.[10] The main cutaneous perforators of the rat dorsum emerge from the lateral thoracic artery (LTA), posterior intercostal artery (PIA), and deep circumflex iliac artery (DCIA).[11] However, it was reported that the cutaneous territory of the DCIA supplies the axial-pattern flap.[10] Owing to the similarity of DCIA and LTA to their human body counterparts with respect to standard anatomical locations, they would be more appropriately named as iliolumbar and thoracodorsal arteries, respectively.[10]

Our literature review using electronic biomedical databases revealed that the number of perforator flaps located on the dorsum of rats is limited.[12-14] A thoracodorsal artery perforator (TAP) flap supplied by a lateral thoracic vessel, which has a skin island on the lateral thoracic region was reported by Kayano *et al*.[12] However, no surgical technique or flap dimensions were provided by these studies.[12] Another study described a 13 × 6-cm extended dorsal flap supplied by an LTA perforator (LTAP) and noted flap distal necrosis, with a flap survival rate of 67% in China.[13] An LTAP flap with smaller dimensions of 3 × 6 cm was also described.[14] Furthermore, these reports described different flap dimensions, flap survival rates, and nomenclature.

Here, we report a novel, definable, and reproducible LTAP flap model that may correlate with a human TAP flap. To determine optimal flap design, the cutaneous territory of the LTAP flap, including the anatomical territory supplied by the LTA, dynamic territory adjoining the anatomical territory, and potential territory adjoining the dynamic territory, was investigated using three different flap designs: the hemidorsal (three-territory island flap) LTAP flap nourished by the LTAP and which captures the cutaneous territories of the ipsilateral PIA and DCIA; the entire dorsal LTAP flap (six-territory island flap) nourished by a single LTAP and which captures the cutaneous territories of the bilateral PIA, DCIA, and contralateral LTA; and a four-territory island LTAP flap nourished by a single LTAP and which captures the cutaneous territories of the bilateral PIA and contralateral LTA. The aforementioned flap designs may help in defining an extended LTAP flap that can be used to evaluate a wide range of drugs or other approaches[10] which may increase flap survival rate.

Tissues of vascular territories are linked to one another via reduced-caliber choke anastomotic vessels.[10] Arteriogenesis, which is the growth of collateral vessels, is an effective mechanism for revascularization in adults that increases the lumen size of existing small collaterals. For its initiation, shear stress activates endothelial cells in the vascular wall, followed by the recruitment and adhesion of the macrophage and lymphocyte and remodeling of the vascular wall.[15] CD11b and intercellular adhesion molecule 1 (ICAM-1) used to indicate monocyte adhesion and infiltration associated with arteriogenesis were evaluated on anatomical, dynamic, and potential territories of the LTAP flap.[16] Vascular endothelial growth factor (VEGF) can induce growth in vessels, and it exerts its angiogenic functions through VEGF receptor 2 (VEGFR2), which is an endothelial cell surface tyrosine kinase receptor.[17] VEGFR2 upregulation has been detected at the tips of angiogenic sprouts located in watershed areas that supply blood from the pedicle to the potential territory.[16]

Materials and methods

The Ethics Committee of Akdeniz University, Medical School (Antalya, Turkey) approved all procedures. Twenty-four male Sprague–Dawley rats (body weight, 250-300 g) were caged individually under a 12-h light and 12-h dark cycle at 22°C and at 30% humidity. Individual cages were used to prevent cannibalization, and automutilation was not observed in any group.

Animals were housed in individual cages at 22°C and were fed the same food and tap water *ad libitum*. All animals were anesthetized intraperitoneally using a ketamine (100 mg/mL) and xylazine (20 mg/mL) combination. All dorsal hair was removed using an electric shaver; sterile conditions were maintained for all surgical procedures.

Three different flap models

To assess the vascular territories that can be supplied by a single LTAP, numbered rats were randomly divided into three groups. In group A, a hemidorsal (three-territory island flap) 4 × 11-cm LTAP flap was designed based on the most cranial perforator of the LTA on the right side. Bony landmarks were used to mark the flap on the rat dorsum. The location of the upper margin was level with the cranial upper border of the scapula, whereas the lower margin was located 1 cm below the iliac crest. The medial margin was on the midline of the dorsum and the lateral margin was on the right posterior axillary line. In group B, the entire dorsal LTAP flap (six-territory island flap) was designed based on the most cranial perforator of the LTA on the right side and measured 8 × 11 cm. The upper margin was at the same level as the cranial upper border of the scapula, whereas the lower margin was located 1 cm below the iliac crest, with the lateral margins on the right posterior axillary line. In group C, an 8 × 8-cm four-territory island LTAP flap was designed based on the most cranial perforator of the LTA on the right side. The upper margin was at the same level as the cranial upper border of the scapula, whereas the lower margin was located 1 cm below the last rib, with the lateral margins located on the right posterior axillary line (Fig. 1).

Flap elevation

With the rat in the supine position, a marked skin flap on the dorsum was raised in a cranial-to-caudal direction. All flaps were harvested by a senior surgeon (A.A.). A circumflex scapulary artery branch (an axillary artery branch), the iliac branches of the iliolumbar artery along its caudal section, and the middle part of the dorsal skin that was supplied by the dorsal branches of the 12th costal artery were located and cauterized (Fig. 2A). The most cranial perforators of the lateral thoracic vessels were defined and included, with the entrance to the skin flap located on the superolateral side. The courses of the lateral thoracic vessels were traced down to the axillary vessels (Fig. 2B). Only one single perforator of the LTA was included in the flap, and the flap was elevated beneath the panniculus carnosus, was repositioned and then sutured

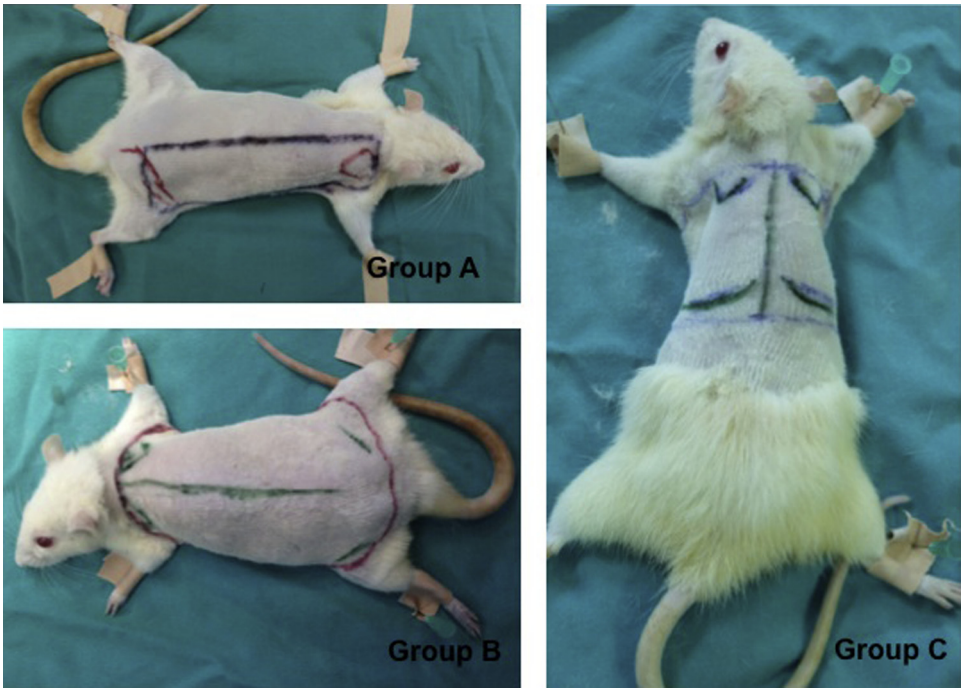


Fig. 1 – Preoperative markings of the LTA perforator skin flap. The flap was marked using bony landmarks on the rat dorsum. (Color version of figure is available online.)

using a continuous 4-0 polypropylene suture (Fig. 2C; see Video, Supplemental Digital Content 1, demonstrating flap dissection).

After the surgical procedures were completed, the flap surface areas were drawn onto transparent sheets and named according to the first surface area.[16] On postoperative day 7,

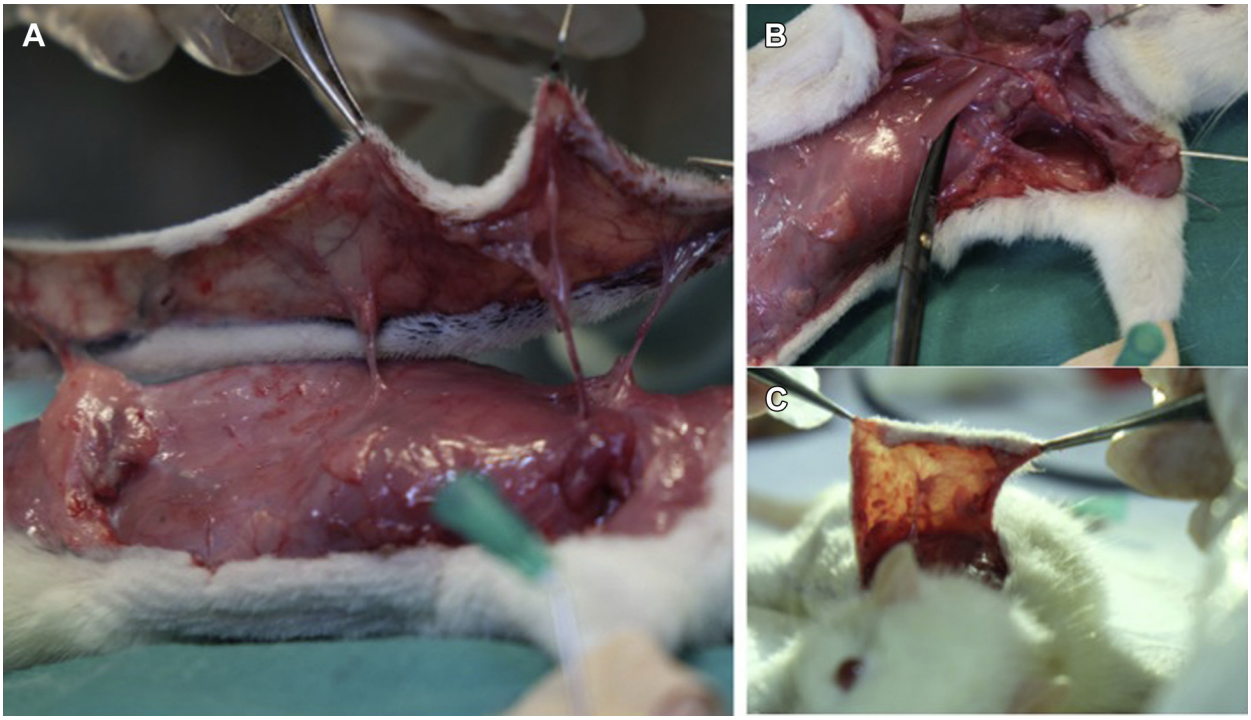


Fig. 2 – (A) The rat dorsal skin is nourished by the circumflex scapulary artery, LTA, dorsal branches of the 12th costal artery, and iliac branches of the iliolumbar artery in the craniocaudal direction. (B and C) Only a single perforation of the LTA was included in the flap, which was elevated beneath the panniculus carnosus. (Color version of figure is available online.)

the entire flap areas along with the necrotic margins were traced onto transparent sheets, and the areas of intact skin and tissue were measured. The necrotic area of the flap was measured for each rat using Adobe Photoshop 6.0 CE (Adobe Systems Incorporated, San Jose, CA) by counting the number of pixels per square centimeter. This is the basis for automatic wound-measuring devices, photoediting software, and computerized photogrammetry methods.[17]

Flap viability assessment

The animals were monitored daily. The ultimate survival of animals with all flap types was evaluated on postoperative day 7 by gross inspection of the flap color compared with that of normal skin.

Microangiography

Microangiography was performed on postoperative day 7 to demonstrate the vascular architecture of the flap, as described previously.[18] For the microangiography, intraperitoneal injections of a combination of ketamine (100 mg/mL) and xylazine (20 mg/mL) were used. In brief, we performed the following procedures. First, a neck incision was made, and a common carotid artery was exposed and cannulated using a 24-gauge catheter. Gelatin (5 g) was dissolved in 100 mL of tap water heated to 40°C; 100 mg of water-soluble red lead oxide was added to the tap water. The obtained lead oxide and gelatin mass was injected into the rat via the carotid artery and was stopped when the limbs turned red. The flaps were stored at 4°C for 12 h. Radiography was performed on all flaps using a soft X-ray machine (Mammo Diagnost UC, Philips, Hamburg, Germany) at 24 kV and 10 mAs using Microvision-C mammography film.

Histology and immunohistochemistry

All rats were euthanized on study day 7, and the full-thickness skin samples obtained from three different areas, including the anatomical territory supplied by the LTA as a source artery (A), the dynamic territory (D) immediately adjoining the anatomical territory, and the potential territory (P) immediately adjoining the dynamic territory, were excised and stored in 4.5% buffered formaldehyde solution. The fixed samples were processed in paraffin and stained with hematoxylin and eosin according to standard histology protocols. Samples were analyzed for signs of edema, inflammation, congestion, and necrosis using the following quantitative intensity scale: negative, 0; weak, 1; moderate, 2; and strong, 3.

Expressions of the vascular endothelial markers ICAM-1, VEGFR2, and CD11b were detected by staining tissues with purified rabbit antirat ICAM-1 (ab124760) polyclonal, VEGFR2 (ab2349) polyclonal, and CD11b (ab75476) monoclonal antibodies (Abcam, Cambridge, UK), respectively. Standard procedures for this staining were performed as recommended by the manufacturer.

The immunoreactivities of VEGFR2, ICAM-1, and CD11b were evaluated by quantitatively measuring the staining intensity (negative, 0; weak, 1; moderate, 2; and strong, 3). The expression was considered positive when expressed in >10% of the cells and when the staining was moderate or strong.

Statistical analyses

All statistical analyses were performed using the NCSS 2007 package program (NCSS, Kaysville, UT). Data were assessed using descriptive statistical methods (mean ± standard deviation), Friedman test for repeated measurements of multiple groups, Kruskal-Wallis test for comparisons among groups, and Dunn's multiple test for subgroup comparisons were used. A P value of <0.05 was considered to indicate statistical significance.

Results

Macroscopic and angiographic evaluation

When the skin islands were increased to 11 × 4 cm in group A (vertically oriented TAP flap), the surviving flap and necrotic area percentages proportional to the total flap surface area on day 7 were measured (Table 1). The mean surviving flap and necrotic area percentages were 95.8% and 4.8%, respectively, in group A; 92.3% and 7%, respectively, in group B (extended TAP flap, 11 × 8 cm); and 89% and 11%, respectively, in group C (horizontally oriented TAP flap, 8 × 8 cm). The minimum and maximum rates of survival and necrosis for each group are presented in Table 1. In group A, macroscopic assessment and angiography identified flap necrosis in the ipsilateral distal third territory, which can be referred to as the potential territory (P) (Fig. 3). Furthermore, there was no necrosis in three of the test subjects. Group B rats had an extended version of the TAP flap, with flap necrosis observed in the cranial contralateral upper part and the distal part of the flap, which corresponded to areas supplied by the contralateral LTAP and ipsilateral DCIA, respectively (Fig. 4). To optimize the flap, group C was then planned, and the distal third of the flaps in groups A and B were discarded because of constant necrosis that occurred in the cranial contralateral upper part of the flap (P) (Fig. 5). Angiographically, the anatomical, dynamic, and potential territories were more obvious and helped establish the TAP flap vasculature. A possible explanation of the

Table 1 – The intact flap surface area and necrotic area of the rats in all groups measured on the 7 after surgery.

Flap surface (%)	*n*	Mean	Min	Max
Flap survival area				
Group A	8	95.8 ± 4.8	88.0	100.0
Group B	8	92.3 ± 5.0	87.0	100.0
Group C	8	89.1 ± 7.8	78.0	97.0
Necrotic Flap area				
Group A	8	4.2 ± 4.8	0.0	12.0
Group B	8	7.7 ± 5.0	0.0	13.0
Group C	8	11.3 ± 8.0	3.0	22.0

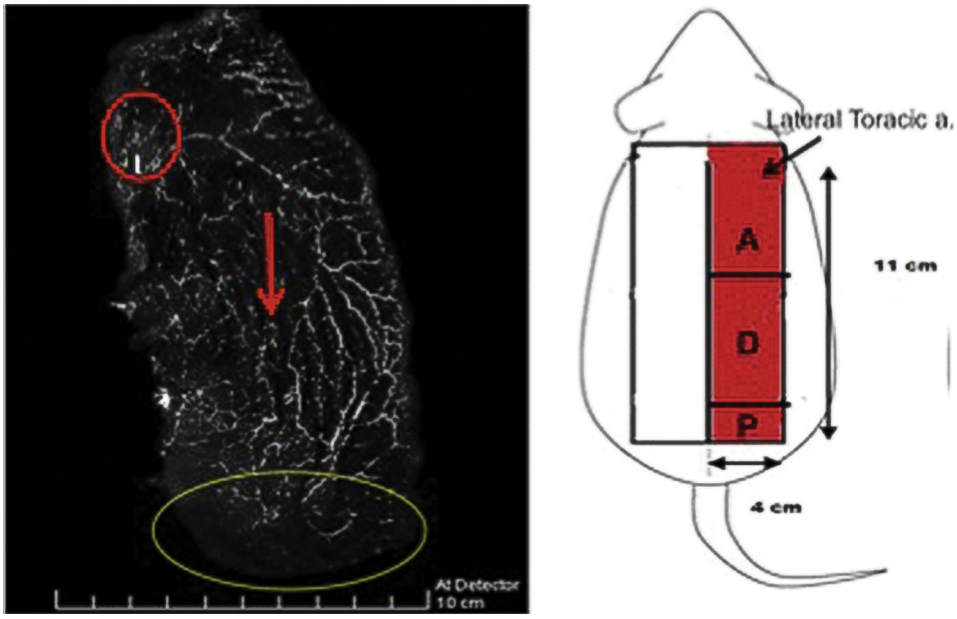


Fig. 3 – Macroscopic and angiographic examination of group A anatomical territory (A), dynamic territory (D), and potential territory (P). (Color version of figure is available online.)

reduced flap survival area in group C (89%) relative to that in group B (92%) is the association of constant necrosis that occurred in the anatomical territory of the contralateral LTA.

Histology and immunohistochemistry

Tissue samples were obtained from the anatomical, dynamic, and potential territories, as defined by macroscopic and angiographic evaluations of each group and were individually assessed. No statistically significant differences were observed between the anatomical, dynamic, and potential territories in terms of interstitial edema, congestion, necrosis, or neovascularization ($P > 0.05$).

CD11b, ICAM-1, and VEGFR2 immunohistochemical immunoreactivity were also quantified by counting the number of immunopositive cells per blood vessel after flap elevation on day 7 (Table 2). There were no statistically significant differences in the numbers of CD11b, ICAM-1, or VEGFR2 immunoreactive cells in the three different territories within group A ($P > 0.05$). The number of cells that were

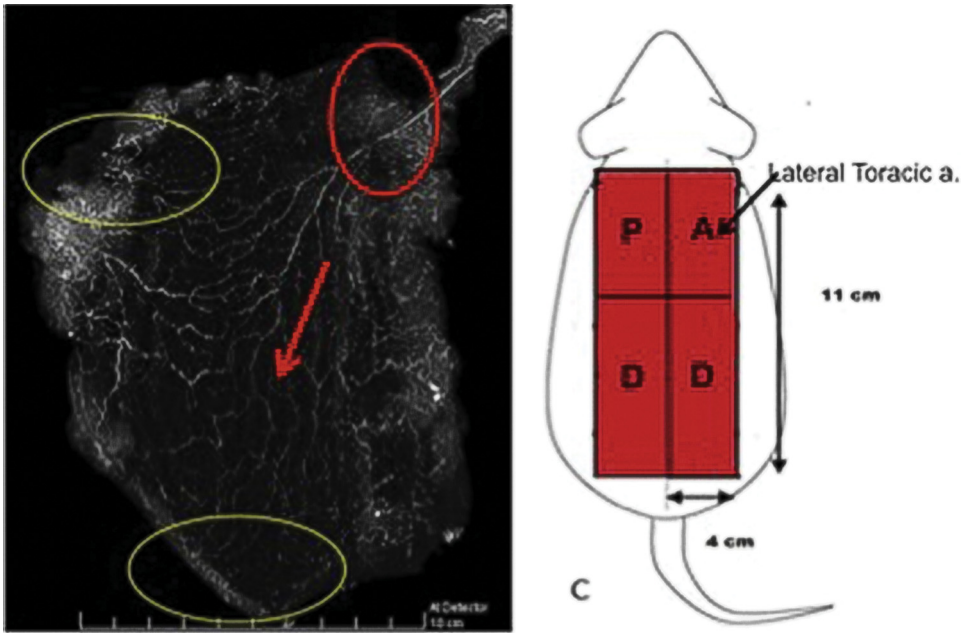


Fig. 4 – Macroscopic and angiographic examination of group B anatomical territory (A), dynamic territory (D), and potential territory (P). (Color version of figure is available online.)

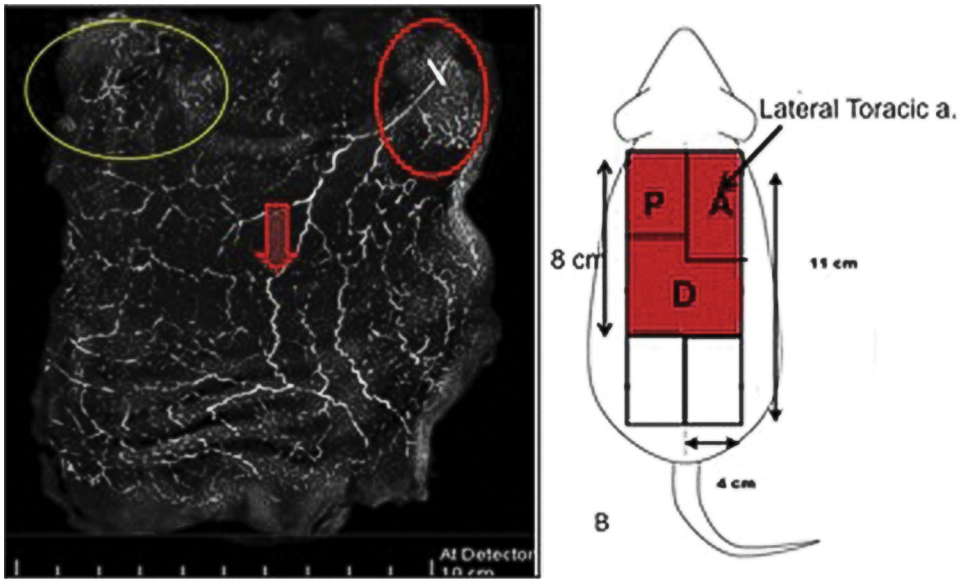


Fig. 5 – Macroscopic and angiographic examination of group C anatomical territory (A), dynamic territory (D), and potential territory (P). (Color version of figure is available online.)

immunoreactive to ICAM-1 and VEGFR2 was significantly increased in the dynamic territory relative to those in the anatomical and potential territories of group B ($P < 0.05$). There were no statistically significant differences among the territories in the expression of CD11b in group B ($P > 0.05$). Moreover, in group C, ICAM-1 immunoreactive cells were significantly increased in the dynamic territory relative to the anatomical and potential territories ($P < 0.05$; Fig. 6).

Discussion

Despite the considerable knowledge of the vasculature of the dorsal skin, investigations on multiple territory perforator flaps with predictable necrosis remain popular. In this study, we demonstrated the first single LTAP flap encompassing a defined artery and vein that was raised on the dorsum of the rat. The LTAP model was preferred for the dorsal area because the dorsal area is larger and shows less contraction than the abdominal area.[19] The dorsal flap placement may also hinder autocannibalization.

The cutaneous maximus muscle in animals is analogous to various human fascial tissues and extends across the back, trunk, and abdomen of the rat. The superior portion of the cutaneous muscle is easily dissected from the skin; however, its inferior portion tightly adheres to the skin and cannot be easily dissected.[14] Opinions on the definition of "true perforator" are divided between those of Wei *et al.*[20] and Hallock and Rice.[21] The former described "true" perforators as only including cutaneous vessels that penetrate a muscle, whereas

Table 2 – The data of immunohistochemistry.

	Anatomic	Dynamic	Potential	P value
Group A				
CD11b	1.63 ± 0.518	1.75 ± 0.707	1.75 ± 0.707	0.907
ICAM-1	1.75 ± 0.463	2.25 ± 0.463	1.88 ± 0.641	0.169
VEGFR-2	2.25 ± 0.707	2.75 ± 0.463	2.25 ± 0.707	0.217
Group B				
CD11b	1.62 ± 0.52	1.50 ± 0.76	1.50 ± 0.53	0.895
ICAM-1	1.37 ± 0.52	2.50 ± 0.53†	1.38 ± 0.52	0.001*
VEGFR-2	2.00 ± 0.00	2.75 ± 0.46†	2.13 ± 0.64	0.008*
Group C				
CD11b	1.75 ± 0.46	1.75 ± 0.46	1.63 ± 0.74	0.881
ICAM-1	1.87 ± 0.35	2.50 ± 0.53†	1.88 ± 0.64	0.039*
VEGFR-2	2.25 ± 0.46	2.50 ± 0.76	1.75 ± 0.71	0.089

* Statistically significant.
† Increased expression.

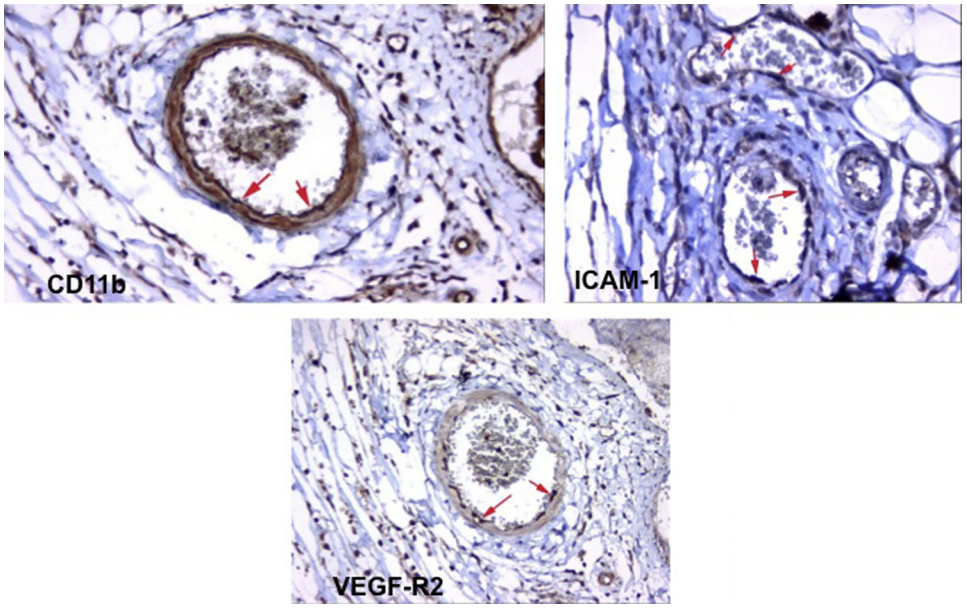


Fig. 6 – Full-thickness skin samples from the anatomical territory (A), dynamic territory (D), and potential territory (P) were evaluated by immunohistochemical analysis. On day 7, CD11b, ICAM-1, and VEGFR2 immunoreactivities were quantified by counting immunopositive cells per blood vessel. (Color version of figure is available online.)

the latter stated that a perforator follows through a muscle to the source artery. Because rat skin does not separate easily from the muscle, the LTAP flap has many characteristics similar to those of a direct cutaneous perforator flap. Zhuang *et al.* stated that the nomenclature of the dorsal vasculature should be revised and that DCIA and LTA vessels should be renamed as iliolumbar and thoracodorsal arteries, respectively, so that their names would correspond to their human body counterparts according to standard anatomical locations.[10] On the basis of our own anatomical studies, we suggest that these single LTAP flaps with a dorsal island flap be referred to as TAP flaps in rats.

An extended LTAP flap is able to cover multiple vascular territories. The included skin island was particularly designed to cover the hemidorsum, LTA anaostomoses, PIA, and DCIA. Flap survival rate was 95% because of the large diameter and unidirectional course of the perforator in this model. This model may be useful in nonischemic flap studies to confirm perfusion in the flap. In groups B and C, the LTAP flap possessed a skin island that extended from the upper border of the scapula to 1 cm below the iliac crest and from the right posterior axillary line to the left posterior axillary line. The flap was designed in a manner such that it has large and extended dimensions (11 × 8 cm and 8 × 8 cm for groups B and C, respectively) across the midline. In groups B and C, these flap procedures resulted in 92% and 89% survival rate, respectively. Flap necrosis typically occurs in the contralateral LTA territories. Our results revealed that the LTA anastomosed with the ipsilateral PIA, contralateral PIA, and DCIA. We surmise that the LTA does not anastomose with its counterpart because of its unidirectional, oblique, and craniocaudal course. Postoperative flap images and microangiographic examination revealed that the extended LTAP flap represents a good model for the study of the physiology of the extended flap through inclusion of anatomical, dynamic, and potential vascular territories.

Choke vessels normally provide little blood flow. Disruption of blood flow to a region of skin can cause an adjacent cutaneous perfusion territory to expand via choke vessels as a compensatory mechanism for the decreased blood flow. Over the first 72 h after vascular disruption, a transient vasospasm is followed by a rapid dilation. After 72 h, dilation continues with hyperplasia in the cells lining the vessel walls. On postoperative day 7, choke vessel diameter and cell size increase and adopt a tortuous character.

CD11b and ICAM-1 are involved in inflammation and arteriogenesis. Arteriogenesis requires inflammation; CD11b—a cell surface protein expressed by monocytes and granulocytes during inflammation—is involved in cell adhesion and migration through blood vessel wall; furthermore, it is a subunit of Mac-1. Therefore, arteriogenesis is dependent on the ICAM-1 and Mac-1 pathways.[15] CD11b and ICAM-1 are indicators of arteriogenesis-associated monocyte adhesion and infiltration. In our study, although we demonstrated increased ICAM-1 immunoreactivity in the dynamic territory, no statistically significant changes in the immunoreactivity within the potential territory were observed. There were also no significant differences between the dynamic and potential territories in CD11b immunoreactivity.

VEGF promotes angiogenesis and is paramount for vascular development. Similarly, VEGFR2 is considered the main receptor to regulate angiogenesis.[16] In this study, increased VEGFR2 immunoreactivity was observed in the dynamic territories. We believe that an extended TAP flap provides baseline immunohistochemical and microangiographic data for the analysis of a wide range of pharmacological,

mechanical, and other strategies[9] with the potential to improve flap survival rate.

It was reported that a thin flap of >15 cm long, a flap with less reliable perforator, a length-to-width flap ratio of 2.5:1, a long vascular pedicle, and patient's medical history were risk factors associated with vascular insufficiency of thoracodorsal artery perforator flaps. Furthermore, these studies suggested measures for flap viability improvement, including T-anastomosis (keeps the continuity of the recipient artery), the supercharge technique, and the muscle-sparing technique.[22] Lin *et al*. found that a length-to-width ratio of 3:1 was the safe upper limit if the flap was oriented according to the suprafascial courses of included perforators. In addition, a long vascular pedicle has been found to serve as a bypass around the injured zone in the reconstruction of traumatic defects.[23] There is no scientific evidence regarding T-anastomosis and its influence in decreasing arterial inflow to prevent venous congestion.[23,24] However, there is no standard method available to improve perforator flap viability. Our flap design can be a useful research tool to establish objective criteria to help define optimal flap design, thickness, width-length ratio, and length of vascular pedicle and to judge whether or not T-anastomosis and the muscle-sparing technique are necessary.

An extended LTAP flap encompasses true multiple territories and offers the following advantages: (1) its robust vascular supply facilitates creation of a large perforator flap on the dorsal region with a flap contraction rate lower than that of the abdominal region; (2) flap design is easy, and flap harvesting and manipulating do not require a microscope; and (3) multiple territories, as demonstrated by flap immunohistochemical analysis and angiography, may be useful for flap survival studies.

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Authors' Contributions: The authors T.G. analyzed statistical data and made the video; S.A.S. helped during the surgical procedures and took care of the rats; and A.A. planned the study, analyzed statistical data, and wrote the paper; C. İbrahim Başsorgun analyzed histopathological data.

Disclosure

None of the authors has a financial interest in any of the products, devices, or drugs mentioned in this manuscript. The authors have no financial interest to declare in relation to the content of this article. No external funding was received.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jss.2016.08.066.

REFERENCES

1. Mirzabeigi MN, Wang T, Kovach SJ, Taylor JA, Serletti JM, Wu LC. Free flap take-back following postoperative microvascular compromise: predicting salvage versus failure. *Plast Reconstr Surg*. 2012;130:579–589.
2. Adamson JE, Horton CE, Crawford HH, Ayers Jr WT. The effect of dimethyl sulfoxide on the experimental pedicle flap: a preliminary report. *Plast Reconstr Surg*. 1966;37:105–110.
3. Syed SA, Tasaki Y, Fujii T, Hirano A, Kobayashi K. A new experimental model: the vascular pedicle cutaneous flap over the dorsal aspect (flank and hip) of the rat. *Br J Plast Surg*. 1992;45:23–25.
4. Syed SA, Tasaki Y, Fujii T, Murakami R, Kobayashi K. Cutaneous vascular anatomy of the thoracic region of the dorsum and its role in flap design in the rat. *Ann Plast Surg*. 1992;29:420–424.
5. Ahmed S, Pierce J, Reid M, Thomson JG, Restifo RJ. A new experimental model: the vascular pedicle cutaneous flap over the mid-dorsum of the rat. *Ann Plast Surg*. 1997;39:495–499.
6. Taylor GI, Minabe T. The angiosomes of mammals and other vertebrates. *Plast Reconstr Surg*. 1992;89:181–215.
7. Syed SA, Restifo RJ. An economical axial-pattern flap necrosis model. *Plast Reconstr Surg*. 1997;99:263–264.
8. Kayikcioglu A, Akyurek M, Safak T. The importance of vascular territories in designing new experimental skin flaps. *Plast Reconstr Surg*. 1998;101:1155–1157.
9. Hallock GG. The complete nomenclature for combined perforator flaps. *Plast Reconstr Surg*. 2011;127:1720–1729.
10. Zhuang Y, Hu S, Wu D, Tang M, Xu da C. A novel in vivo technique for observations of choke vessels in a rat skin flap model. *Plast Reconstr Surg*. 2012;130:308–317.
11. Yang D, Morris SF. An extended dorsal island skin flap with multiple vascular territories in the rat: a new skin flap model. *J Surg Res*. 1999;87:164–170.
12. Kayano S, Nakagawa M, Nagamatsu S, Koizumi T, Akazawa S. Why not perforator flap training models in rats? *J Plast Reconstr Aesthet Surg*. 2010;63:e134–e135.
13. He Z, Gao W, Li J, et al. A perforator-based dorsal flap's experimental research in the rat. *Zhonghua Zheng Xing Wai Ke Za Zhi*. 2014;30:40–44. Chinese.
14. Bali U, Gungor M, Yoleri L. Lateral thoracic artery perforator-based flap: a new experimental model. *J Surg Res*. 2016;200:738–742.
15. Watt SM, Gullo F, van der Garde M, et al. The angiogenic properties of mesenchymal stem/stromal cells and their therapeutic potential. *Br Med Bull*. 2013;108:25–53.
16. Williams BA, Currie RW, Morris SF. Impact of arteriogenesis in plastic surgery: choke vessel growth proceeds via arteriogenic mechanisms in the rat dorsal island skin flap. *Microcirculation*. 2009;6:235–250.
17. Mittermayr R, Morton T, Hofmann M, Helgerson S, van Griensven M, Redl H. Sustained (rh)VEGF(165) release from a sprayed fibrin biomatrix induces angiogenesis, up-regulation of endogenous VEGF-R2, and reduces ischemic flap necrosis. *Wound Repair Regen*. 2008;16:542–550.
18. Ozkan O, Koshima I, Gonda K. A supermicrosurgical flap model in the rat: a free true abdominal perforator flap with a short pedicle. *Plast Reconstr Surg*. 2006;117:479–485.

19. Tatlidede S, McCormack MC, Eberlin KR, Nguyen JT, Randolph MA, Austen Jr WG. A novel murine island skin flap for ischemic preconditioning. *J Surg Res*. 2009;154:112–117.
20. Wei FC, Jain V, Suominen S, Chen HC. Confusion among perforator flaps: what is a true perforator flap? *Plast Reconstr Surg*. 2001;107:874–876.
21. Hallock GG, Rice DC. Cranial epigastric perforator flap: a rat model of a true perforator flap. *Ann Plast Surg*. 2003;50:393–397.
22. Kim JT, Kim YH. Initial temporary vascular insufficiency in latissimus dorsi and thoracodorsal perforator flaps. *Plast Reconstr Surg*. 2009;124:408–418.
23. Lin CT, Chen LW. Inclusion of extra perforators—a single and efficient measure to prevent vascular insufficiency in unreliable thoracodorsal artery perforator flaps. *J Plast Reconstr Aesthet Surg*. 2012;65:342–350.
24. Kim JT. Two options for perforator flaps in the flank donor site: latissimus dorsi and thoracodorsal perforator flaps. *Plast Reconstr Surg*. 2005;115:755–763.