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Perforator flaps—how many perforators are necessary to keep a flap alive?

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Abstract

Perforator flaps are becoming increasingly important in reconstructive microsurgery because of their reduced donor-site morbidity. However, one drawback is partial necrosis caused by vasospasm or inconsistency of delicate perforator vessels. In this study we have evaluated the number and capacity of perforator vessels with respect to the size of a flap, and the influence of vascular endothelial growth factor (VEGF) on the capacity of perforators in a standard animal model. We realised an epigastric perforator flap 4 cm × 7 cm in 36 rats. In 3 control groups ($n = 6$ in each), flaps were raised based on 4, 2, or 1 perforator vessel(s), while all other perforators as well as the epigastric vessels were ligated. In three study groups ($n = 6$ in each), set up in the same way as the control groups, we also injected a single dose of VEGF into the wound area. After one week, all areas of necrosis were assessed planimetrically. We also evaluated the wounds by laser Doppler flowmetry preoperatively and after one week, and by histological and immunohistochemical examination. An increased number of perforators, together with VEGF, was associated with a significant reduction in the areas of necrosis. This observation was particularly true in flaps based on only one perforator. The inclusion of additional perforators has a more important role in the success of a flap than theoretical models suggest. Proangiogenetic factors may improve the viability of perforator flaps.

Keywords: Perforator flap; Epigastric flap model; Area of necrosis; Laser Doppler flowmetry; Animal model; Vascular endothelial growth factor

Introduction

Perforator flaps are based on cutaneous vessels with small diameters that branch off a main pedicle and perforate fascia or muscle to reach the skin.[1] These flaps are becoming more and more popular in reconstructive microsurgery because they are versatile and result in little morbidity at the donor site. The size of a perforator flap is judged by the size and number of perforators, the volume of tissue included, and the effects of vessels inside the flap. One common drawback of perforator flaps is partial necrosis of skin and fat as a result of inconsistency or frailty of perforator vessels, vasospasm, or other risk factors. If the blood supply of a perforator flap is based on more than one perforator, it is generally thought that it will provide greater versatility in design and reduce the risk of failure. We know of few studies that have focused on the influence of the number of perforators on survival of flaps, and most of the work is based on theoretical models.[2,3] Without evidence, the planning of the dimensions of a flap and the number of perforators to be included is mainly judged by the surgeon's experience.

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The present study was designed to investigate the influence of the number of perforators on the survival of perforator-based groin flaps in rats using clinical and non-invasive monitoring with laser Doppler flowmetry. We looked at whether the relations between the number of perforators and areas of necrosis on the flap were linear or exponential. We also investigated whether a single preoperative dose of vascular endothelial growth factor (VEGF) had any influence on the survival of the flap.

Materials and methods

Experiments were made according to current German regulations and guidelines for animal welfare and the international principles of care of laboratory animals. We used male Wistar rats (200–230 g), and all procedures were done under general anaesthesia (10% ketamine (1 ml/kg body weight) and 2% xylazine (0.25 ml/kg body weight)) and under aseptic conditions.

The anaesthetic was given as a single intraperitoneal injection and supplemented by quarter-doses as necessary.

Surgical technique

The epigastric flap was raised as a perforator flap based on the method described by Strauch and Murray.[4] To evaluate the influence of the capacity of perforators, an oversized flap model was selected to detect areas of low blood supply with a critically-perfused composite flap.[5] The size of the flap was marked with a standard 4 cm × 7 cm template. Subsequently it was raised completely based on the perforator vessels of the deep inferior epigastric system, while the pedicle of the superficial epigastric system was ligated or cauterised (Fig. 1).[6] After the flap had been raised it was sutured back into the wound bed with 6/0 monofilament interrupted sutures (Ethilon®, Ethicon, Norderstedt, Germany).

Experimental groups

Thirty-six rats were randomised from a computer-generated list and subdivided into 6 groups of 6 rats each. In the first 3 groups 4, 2, and 1 perforators, respectively, from the medial epigastric system were left intact, and all the others were cauterised and cut. In the other 3 groups, under identical conditions, but directly before the operation, VEGF 20 μl (hVEGF165, PeproTech, Hambourg, Germany) was injected into the area of the proposed wound bed.[7]

The necrotic areas of each flap were measured after 7 days using planimetry. Flaps were then harvested and the rats killed with a lethal injection of pentobarbital 200 mg/kg (Narcoren®, Rhone-Merieux, Laupheim, Germany).

Laser Doppler flowmetry

In addition to recording the colour of the flap and the degree of capillary refill, we measured tissue oxygen saturation (SO_2 in %), the haemoglobin concentration (Hb in arbitrary units (AU)), blood flow (AU), and velocity (AU) non-invasively using laser spectrophotometry (Lightguide O2C®, Lea Medizintechnik, Giessen, Germany). This technique has been described in detail by others.[8,9] Preoperatively we recorded a baseline measurement and compared it with the measurements taken after 7 days of wound healing. All measurements were taken from the central part of the flap and compared with the same area if possible. If the flap had necrosed, measurements were taken from the central part of the remaining tissue.

Measurement of necrotic areas

Results were documented using a CCD-camera (type COOLPIX 8700, Nikon, Düsseldorf, Germany) mounted in a perpendicular direction to the flap with a tripod. Pictures of the vital and necrotic areas were analysed (Fig. 1), and the total area of the flap and necrotic areas were calculated using NIH Image Software.

Histological examination

After 7 days the epigastric flaps were harvested after the necrotic area had been measured, and fixed in 4% formalin solution. The samples were then embedded in paraffin, sectioned at 5 μm and stained with haematoxylin and eosin for histological evaluation. For immunohistochemical examination an additional set of cross-sections was created as described above, and stained with endothelial cell markers CD 31 (cluster determinant 31) and vWF (von Willebrand factor), respectively. The technique has been described previously.[7]

Statistical analysis

Data were analysed with SPSS (SPSS for Mac, release 19.0.0.1, Armonk NY, IBM Corp). Differences in the area of necrosis between groups were evaluated using the Mann–Whitney *U* test, which was also used for evaluation of the results of immunohistochemical staining. Analysis was complemented by multiple linear regression analysis to evaluate the prognostic value of the number of perforators and the effect of VEGF on the rate of necrosis. For regression analysis we used method “enter”, because whether VEGF was given or not was independent of the number of perforators, so they were statistically independent variables used as covariates in the regression model. Generalised Estimating Equations Models were used to evaluate the laser spectrophotometry data. Two-tailed probabilities of less than 0.05 were accepted as significant. All observations were independently