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Healing and histostructure of vascularized fasciocutaneous flaps under the conditions of brain tissue lyophilizate microinjections

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Purpose To reveal a possible effect of subdermal injections of cerebrolysin on the parameters of healing and histomorphometric characteristics of the biological model of an extended fasciocutaneous flap with axial blood supply. **Materials and methods** A flap based on the superficial inferior epigastric artery (SIEA) and extended to the cranial side according to a template with an area of 18 cm^2 was formed in 29 rats. After 90 minutes of femoral artery clipping, it was re-planted at the site of SIEA origination. After the operation, microinjections of cerebrolysin (0.1 ml) were performed subdermally. Four of them were injected in the mezhangiosomal zone in group Cer 1, and six were uniformly distributed over the area of the flap in group Cer 2. Comparison groups (Comp 1 and Comp 2) had injections of saline in similar number and zones. Negative control group had flaps without injections. Conditional normal sites were contralateral areas of the skin. Twelve days after the operation, the animals are euthanized. The methods of the study were computer planimetry, histomorphometry, and immunohistochemistry. **Results** In groups Cer 1 and Cer2, when compared with the negative control and placebo, relative areas of the epidermal defects associated with partial flap necrosis or delayed wound healing (2.15 % and 0.23 % vs. 13.72 % and 11.33 %, respectively) were reliably reduced. In Cer 1, hypovascularization of the hypodermis, thinning of the epidermis and dermis were noted. In Cer 2, compared with the control and Cer 1, the largest thickness and capillarity of the dermis and hypodermis were revealed, as well as the thickness of the epidermis and the numerical density of the skin appendages. **Discussion** The pronounced decrease in polymorphic cell infiltration of the dermis and hypodermis in the experimental groups indicates that the angiogenesis in the dermis stimulated by subdermal microinjections of cerebrolysin occurred by inhibition of inflammatory reactions and was caused by the direct action on endothelial cells. **Conclusion** To optimize the healing of fasciocutaneous flaps with axial blood supply in cases where their area exceeds the territory of the blood supplying artery, it is advisable to use microinjections of cerebrolysin uniformly distributed over the entire area of the flap, including its angiosomal and interangiosomal zones.

Keywords: vascularized fasciocutaneous flap, cerebrolysin, angiogenesis

INTRODUCTION

Partial necrosis of skin flaps is one of the significant problems in reconstructive surgery [1]. The main cause of this complication is the deficiency of oxygen and nutrients in the sites that are remote from the vascular pedicle when the flaps with axial blood supply are used [2]. The lack of its effective therapy results in deterioration of functional and aesthetic outcomes as well as in repeated operations.

The most attractive strategy for solving this problem is therapeutic angiogenesis which can be induced by the use of molecular regulators, that is growth factors that control the division, growth, differentiation and metabolism of cells. The most effective among them is the vascular endothelial growth factor (VEGF) [3]. In the clinical settings, the genetic VEGF preparation is used for the treatment of critical limb ischemia [4]. However, it has high commercial costs.

A more accessible peptidergic preparation is cerebrolysin (porcine lyophilizate) that reacts with antibodies to several neurotrophic factors, as well as to insulin-like growth factor (IGF) of the first and second types [5]. Cerebrolysin reduces apoptosis caused by oxidative stress in various tissues [6]. IGF-1 enhances angiogenesis and reduces reperfusion lesions [7]. It is logical to assume that the use of this drug will optimize the survival of vascularized fasciocutaneous flaps, including the cases in which their area exceeds the territory of the artery that supplies blood.

Our study **purpose** was to reveal a possible effect of subdermal injections of cerebrolysin on the parameters of healing and histomorphometric characteristics of the biological model of an extended fasciocutaneous flap with axial blood supply.

MATERIAL AND METHODS

The experiments were carried out on 29 male Wistar rats (weight from 380 to 560 g, age range from 8 to 12 months) with observance of the requirements of the Order of the Ministry of Health of the Russian Federation

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No. 267 of 19.06.2003. Under general anesthesia in the operating conditions, a template that measured 3 x 6 cm was used to form and reposition the fasciocutaneous flap with the axial type of blood supply based on a. epigastrica inferior superficialis (SIEA). The incisions were made with a scalpel; the medial one was 0.5 cm lateral to the white line of the abdomen, the cranial one was along the lower edge of the rib arc, the caudal incision was parallel to the cranial one and distanced by 6 cm, and the lateral one was parallel to the medial incision and 3 cm away from it. The feeding pedicle (SIEA, accompanying veins and nerves) was isolated with surrounding tissues. The cranial portion of the flap referred to angiosomal a. thoracica lateralis and after the flap production was not perfused by it (**Fig. 1a**).

Simulation of a 1.5-hour ischemia similar to clinical operations of flap transfer and transposition was performed by clipping the feeding artery for 90 minutes. The rats were randomly assigned to 5 groups: a group of negative control without therapeutic intervention on the healing process (n = 10), two test groups consisting of Cer 1 with 4 subdermal microinjections of 0.1 ml of cerebrolysin performed an hour after the operation (n = 5) (**Fig. 1b**) and Cer 2 with 6 cerebrolysin microinjections, evenly distributed (**Fig. 1c**) over the area of the flap (n = 5), and also two comparison groups (Comp 1, n = 4; and Comp 2, n = 5) with similar injections of saline. Twelve days after the operation, the rats were euthanized.

The degree of flap contraction (% of the template area) was determined in scaled full-color digital images of the cutaneous surface of the flaps with computer-aided planimetry. The state of the flaps was assessed visually and stereologically (according to the results of calculating the percentage (%) of the epidermal defects in the flap area using dot-counting planimetry).

For histological examination, standard fragments of flaps in the experimental and control group animals were excised. A relative norm (positive control) was samples of the contralateral skin areas. Paraffin sections that were 5–7 microns thick were stained by Van Gieson and hematoxylin-eosin. A part was placed on adhesive-coated glasses (poly-L-lysine); the expression of ki-67 (proliferation marker) and vWF (endotheliocyte marker) was determined by the protocol of the manufacturing company using the Novolink Polymer imaging systems (Novocastra, UK). The preparations were studied with a stereomicroscope AxioScope.A1 supplied with a digital camera AxioCam (Carl Zeiss MicroImaging GmbH, Germany). Histomorphometry was performed using the computer program VideoTesT Master Morphology, 4.0. Statistical processing used the software program Attestat 9.3.1 (developed be I.P. Gaidyshev) with application of Mann-Whitney criteria and interval randomization criterion.

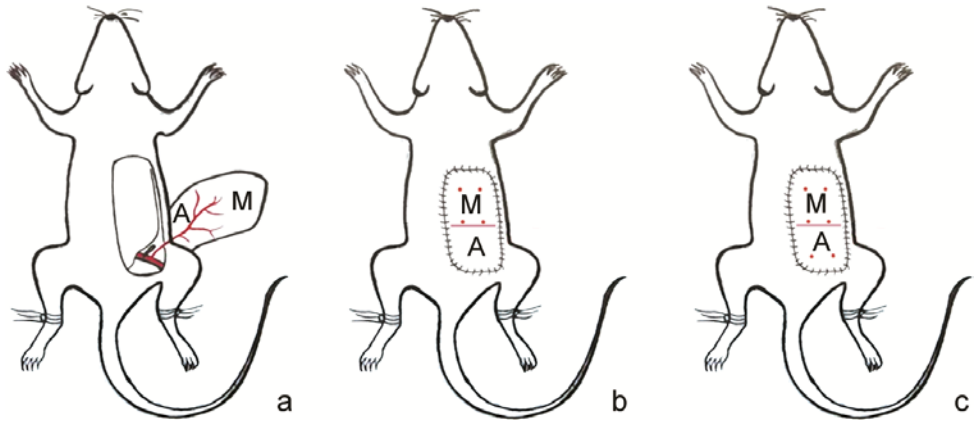

Fig. 1 Scheme of the experiment: **a** – clipping of the artery that feeds the extended flap with axial blood supply, A – angiosomal and M – interangiosomal parts of the flap. Topography of subdermal microinjections: **b** – in groups Cer 1 and Comp 1, **c** – in groups Cer 2 and Comp 2

RESULTS

Outcomes of the replantation of the extended SIEA flap of rats after an hour and a half of ischemia-reperfusion are shown in Figure 2.

By 12 days after the operation, complete flap and wound healing was noted only in one animal out of 10 in the control group and in three out of 10 in the experimental groups (**Table 1**). The frequency distribution of the healed and survived defects in the control group and comparison groups with the introduction of physiological saline was comparable. Necrosis of the interangiosomal zone was not observed in any animal of the experimental groups which is statistically significant from the groups without pharmacological intervention ($p < 0.001$ by the Barnard criterion).

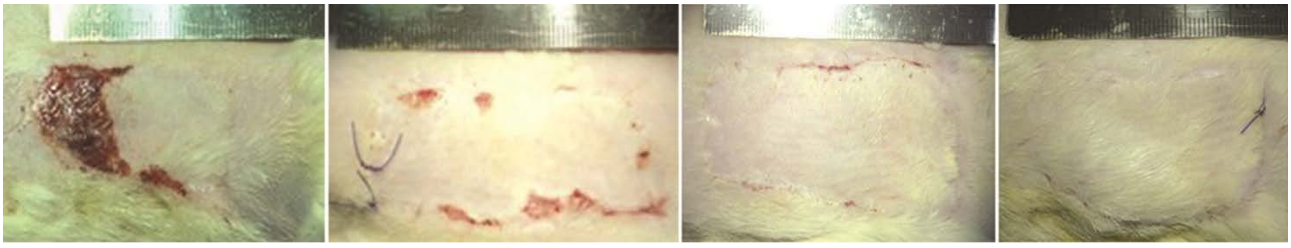

Fig. 2 Variants of the outcomes of the replantation of the extended murine SIEA-flap with an hour-and-a-half ischemia-reperfusion at 12 days after the operation: **a** – necrosis of the interangiosomal zone and marginal necrosis; **b** – marginal necrosis of the flap and incomplete wound healing; **c** – incomplete wound healing; **d** – complete survival and wound healing with restoration of flap fur cover

Table 1

Outcomes of replantation of the extended flap with axial blood supply at 12 days after the operation

Group	Complete survival and wound healing	Healing and survival defects		
		Partial wound healing	+ Marginal necrosis	+ necrosis of interangiosomal zone
Control (n = 10)	1	3	5	1
Comp 1+Comp 2 (n = 9)	0	3	5	2
Cer 1+Cer 2 (n = 10)	3	2	5	0

The relative area of epidermal defects in the control group was 13.72 ± 9.17 %. In the groups with physiological saline, it was 11.33 ± 3.68 %. In Cer 1 and Cer 2 it made 2.15 ± 0.85 % and 0.23 ± 0.17 %, respectively. The difference between the test groups, as well as from control and placebo groups was statistically significant ($p < 0.05$).

The histological study found that polymorphic cell infiltration of the dermis and hypodermis was significantly reduced in the experimental groups when compared with controls.

Similar to the controls, in the animals of group Cer 1, panniculus carnosum and subcutaneous adipose tissue was atrophic. Some areas of fatty tissue were replaced by maturing granulation tissue (**Fig. 3a**). There were areas of moderate polymorphic cell infiltration among the remaining adipocytes in which the content of mast cells was increased (**Fig. 3b**). The conduction part of the nerve trunks appeared in the state of Wallerian degeneration (**Fig. 3c**). The epidermis was thinned at the greater length of the flap due to the decrease in the number of layers and the vertical diameter of spiny cells (**Fig. 3d**). Group Cer 2 had a better retention of panniculus carnosum, subcutaneous fat, hair follicles and sebaceous glands (**Fig. 4a**). Foci of angiogenesis occurred in the subcutaneous adipose tissue of the angiosomal zone (**Fig. 4b**). Some nerve trunks contained conserved and regenerating axial cylinders along with degenerating fibers (**Fig. 4c**). The layers of the epidermis were well defined (**Fig. 4d**).

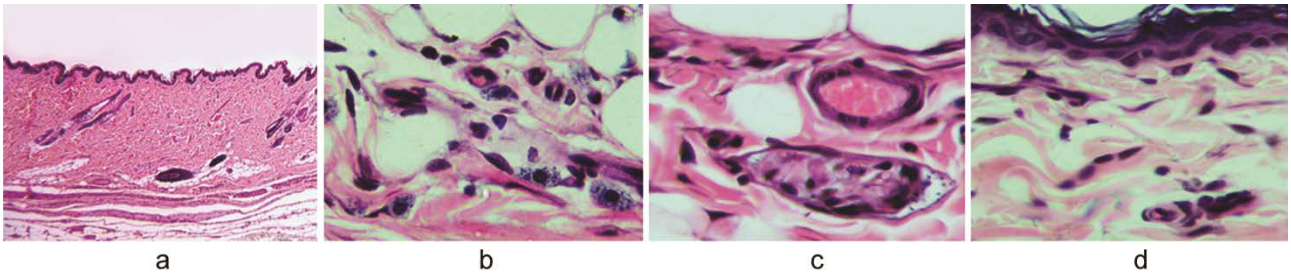

Fig. 3 Fragments of paraffin sections of survived flaps in the group Cer 1. Magnification 180× (**a**) and 500× (**b, c, d**). Staining with hematoxylin and eosin

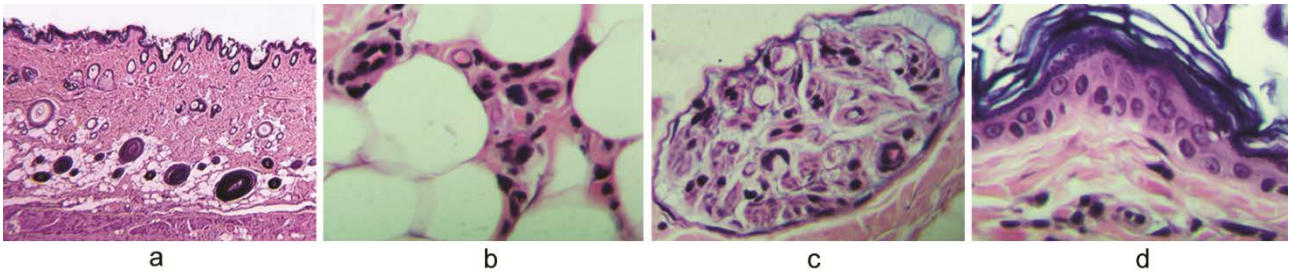

Fig. 4 Fragments of paraffin sections of survived flaps in the group Cer 2. Magnification 180× (**a**) and 500× (**b, c, d**). Staining with hematoxylin and eosin

The histomorphometric study (**Table 2**) found that the thickness of the epidermis was reduced by 27.3 and 36.4 % in the control group and in Cer 1 ($p < 0.05$), respectively, as compared with the contralateral skin areas. In the group Cer 2, the parameter was comparable with them. The thickness of the dermis was significantly increased only in the group Cer 2: in the interangiosomal zone by 13.4 % and in the angiosomal zone by 19 %. All the flaps differed from the contralateral skin areas and featured thinned hypodermis: in the control group by 42.7 %, in the group Cere 1 by 47.2 % in the interangiosomal zone and by 35.6 % in the angiosomal zones, but in the group Cer 2 by only 10.5 % and 17.9 %, respectively. The number of dermal papillae in flaps was reduced when compared with contralateral skin by 54.8 % in the controls. In Cer 1, this parameter did not differ from the contralateral skin in the interangiosomal zone but was reduced by 25.1 % in the angiosomal zone. In Cer 2, it was comparable with the contralateral skin. Cellularity of the dermis of the flaps in comparison with the contralateral areas of the skin was increased by 64.2 % in the control group and was comparable to them in the experimental groups. The numerical density of the skin appendages in the control group was reduced by 124.7 %, in Cer 1 group – by 126.2 % in the interangiosomal zone and by 103.3 % in the angiosomal region, and in Cer 2 group by 33.4 % and 57.8 %, respectively.

Capillarization of the dermis and hypodermis in the flaps of the control group was comparable to the skin of the contralateral areas. In group Cer 1, the capillarity of the dermis was increased by 26.1 %, and the capillarity of the hypodermis was reduced by 26.1 % in the interangiosomal zone and by 41.4 % in the angiosomal zone. In group Cer 2, capillarization of the dermis was enhanced by 112 %, capillarization of the hypodermis of the interangiosomal zone was comparable with the contralateral areas, and in the angiosomal zone it was increased by 11.7 %.

Table 2

Histomorphometric features of flap skin

Parameter	Control	Cer 1		Cer 2		Relative norm
		Zone M	Zone A	Zone M	Zone A	
Thickness (mcm)						
Epidermis	16.12 ± 0.61	13.99 ± 0.86	13.86 ± 0.78	18.79 ± 1.55	20.21 ± 1.16	22.14 ± 1.90
Dermis	543.43 ± 7.38	508.20 ± 73.23	604.72 ± 57.11	593.58 ± 59.25	627.96 ± 47.25	527.08 ± 19.22
Hipodermis	200.69 ± 3.40	185.17 ± 31.03	226.17 ± 44.51	314.33 ± 22.85	287.74 ± 23.66	351.03 ± 42.82
Number of sermal papillae						
(in 1 mm of section length)	5.89 ± 0.37	12.13 ± 0.81	9.60 ± 1.34	11.28 ± 0.77	12.27 ± 0.48	12.82 ± 0.40
Numeric density (mm^2)						
Dermis cells	2112.25 ± 354.05	1317.14 ± 7.88	1134.95 ± 46.35	1084.13 ± 76.61	1228.61 ± 69.04	1285.57 ± 110.93
Skin appendages	16.69 ± 1.95	16.58 ± 1.01	18.45 ± 1.55	28.12 ± 6.08	23.77 ± 3.64	37.5 ± 5.70
Dermis capillaries	34.87 ± 5.13	41.03 ± 7.07	41.03 ± 3.63	69.74 ± 9.54	68.72 ± 14.29	32.53 ± 2.69
Hypodermis capillaries	111.33 ± 9.18	84.10 ± 6.61	66.67 ± 5.38	107.69 ± 17.39	127.18 ± 13.70	113.85 ± 9.24

DISCUSSION

The results of the study proved that both schemes of using cerebrolysin used by us led to a significant decrease in the relative area of epidermal defects associated with a partial flap necrosis or delayed wound healing in comparison with the negative control and placebo groups. Anti-necrotic and regenerative effects were mainly associated with a protective effect on the capillaries of the papillary plexus which was shown by the numerical density of the papilla of the dermis that was significantly larger (1.6–2.1 times) than in the group without pharmacological intervention.

It is known that angiogenic factors can have both a direct and an indirect action on endothelial cells, stimulating inflammation, which, in turn, enhances angiogenesis [8]. The pronounced decrease in polymorphic cell infiltration of the dermis and hypodermis in the experimental groups leads to the conclusion that angiogenesis in the dermis stimulated by subdermal microinjections of cerebrolysin ran on the background of inflammatory response inhibition. Four cerebrolysin microinjections in the zone of the flap that was not supplied with blood (interangiosomal) caused hypovascularization of the hypodermis and, as a consequence, thinning of the epidermis and dermis. The second test with an increased dosage of the drug and a uniform distribution of six microinjections over the area of the flap provided protective and accelerating wound healing effects not only in the epidermis but also in the subcutaneous fat as well as in skin appendages. This was apparently mediated by a more pronounced angiogenic and neuroregenerative effect.

CONCLUSION

To optimize the survival of a fasciocutaneous flap that has axial blood supply, it is advisable to use microinjections of cerebrolysin uniformly distributed over the entire area of the flap if its area exceeds the territory of the blood supply artery, including its angiosomal and interangiosomal zones.

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