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CHARACTERISTICS OF MECHANISMS OF THE DISTANT STIMULATING EFFECT OF SKIN FLAP AUTOGRAFT ON MICROVASCULAR PERFUSION IN LOCAL AND SYSTEMIC MICROCIRCULATION DISORDERS

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Objective: to study the characteristics of mechanisms of the distant stimulating effect of full-thickness skin autograft (FTSG) on microvascular perfusion in local and systemic microcirculation disorders. **Materials and methods.** The experiment was carried out on 87 white male rats, divided into 5 groups: 1) control; 2) animals with local microcirculation disorders induced by sciatic nerve transection and neuroraphy; 3) animals with systemic microcirculation disorders caused by alloxan-induced diabetes; 4) animals that underwent FTSG after sciatic nerve transection and neurography; 5) animals that underwent FTSG in alloxan-induced diabetes. Laser Doppler flowmetry (LDF) was used to study microcirculation of the dorsal skin of the rear paw. Serum concentrations of vasoactive substances, including catecholamines (CA), histamine, and vasculoendothelial growth factor (VEGF) in the experimental animals were measured. A morphological study of the tissues of the autograft site was carried out on day 42 of the experiment. **Results.** On day 42 of the experiment, FTSG normalized perfusion in local and systemic microcirculation disorders. FTSG decreases CA level in nerve injury, and to a greater extent in alloxan-induced diabetes. Serum histamine increase under FTSG was more pronounced in rats with nerve injury. Serum VEGF in rats with nerve injury and FTSG increased, which was not observed in alloxan-induced diabetes. Histological assay of the autograft site revealed degenerative changes in the epidermis and dermis of the autotransplant in both experimental models of microcirculatory disorders. Eosinophilic infiltration of the autograft site was more pronounced in nerve injury than in alloxan-induced diabetes. **Conclusion.** FTSG has a distant stimulating effect on microcirculation, which manifests itself in the same degree in both local and systemic microcirculation disorders. The distant stimulating effect of FTSG on microcirculation is multicomponent in nature and includes a set of regulatory reactions, whose severity differs in local and systemic microcirculatory disorders.

Keywords: autograft, microcirculation, regeneration, skin flap.

INTRODUCTION

The physiological basis for maintaining homeostasis in tissues and organs of living organisms is transcapillary metabolism in the microcirculatory bloodstream, while the normal functioning of microcirculation is associated with the metabolic activity of cells [1]. In addition, microcirculatory disorders are the main link in the pathogenesis of various pathological processes. They also play an important role in sanogenesis, providing trophic support for reparative processes [2]. Systemic and local metabolic disorders affect the state of the microcirculatory bloodstream, which leads to an even greater aggravation of dysmetabolism in organs and tissues [3, 4]. Under normal and disease conditions, the body's own cells and tissues are able to influence the state of microcirculation through synthesis and release of bioactive substances that regulate microcirculation at the local and systemic levels [5].

The current level of scientific knowledge determines the process of regeneration of body tissues and organs as a complex of interconnected sequential local and general reactions of the body, which proceed with involvement of many regulatory systems with different levels of organization [6]. However, it should be noted that different body cells and tissues have different regenerative potential and intensity of production of bioactive substances stimulating reparative processes. So, some researchers have demonstrated the acceleration of regenerative processes under the influence of placenta-based drugs [7, 8]. Despite the effectiveness of allotissues, including tissues of the fetoplacental complex, in regenerative medicine, they are associated with the risk of infectious and immune complications. Therefore, a more promising method for stimulating regenerative processes is the use of autogenous tissue, particularly skin. It has been shown that the subcutaneous adipose tissue is rich in bioactive substances providing a high regenerative potential [9]. It was experimentally shown that autotransplantation of adipose tissue stimulates microcirculation, and also positively influences systemic changes in metabolic

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processes [10, 11]. In addition, skin cell populations include leukocyte cells that produce various bioactive substances [12].

It has been previously demonstrated that autologous transplantation of a full-thickness skin autograft (FTSG) into the interscapular region has a distant stimulating effect on microcirculation in the dorsal skin of the rear paw in intact white rats, in animals with sciatic nerve injury, and also under conditions of experimental alloxan-induced diabetes [13, 14]. It has been shown that FTSG's mechanism of action is multicomponent in nature and includes a complex of changes in the histology of the autotransplantation zone, concentrations of vasoactive substances in the blood, and mechanisms of microcirculatory modulation [13, 14]. At the same time, previous studies did not take into account the dependence of FTSG's biostimulating effect on prevalence of microcirculatory bloodstream lesions. For instance, differences in the degree of the effect in local and systemic vascular bed lesions were not established, which is critically important for determining the limitations of this method in systemic microcirculatory disorders. Besides, there are currently no data on the influence of the etiology of vascular lesions on this effect, which may contribute to identifying additional restrictions for practical application of the method, since in some cases, changes in metabolic processes that cause microcirculation dysfunction can quantitatively and qualitatively change the nature of regulatory reactions in FTSG, including modulation of inflammatory response, vasomotor activity, and others. As indicated above, the mechanism of the distant stimulating effect of FTSG is complex and includes many regulatory reactions, whose interactions and hierarchy remain unclear. For instance, available data is not enough to unequivocally determine which regulatory reactions have a trigger value and which are secondary. It is not clear which of them are universal, and which are strictly specific for a certain method of modeling microcirculatory disorders. In this regard, it is of scientific and practical importance to identify the dependence of the complex of regulatory reactions during biostimulation by the body's own tissues on the nature and extent of damage to the microcirculatory system, which determined the direction of this study, the purpose of which was to study the features of FTSG's distant stimulating effect on perfusion of the skin microcirculation system in local and systemic microcirculatory disorders.

MATERIALS AND METHODS

The studies were performed on 87 white nonlinear rats. The experiment was carried out in accordance with the recommendations of the Ethics Committee of the Razumovsky Saratov State Medical University (Protocol No. 1 dated February 5th, 2019). All manipulations were carried out under general anesthesia by intramuscular injection of xylazine (Interchemie, Netherlands) at 1 mg/kg dose and zoletil (VirbacSanteAnimale, France) at 0.1 mL/kg dose. In the course of the experiment, 5 groups of animals were formed: 1) control, which included 10 intact rats; 2) comparative group, consisting of 27 rats with local microcirculatory disorders caused by sciatic nerve transection and neurorrhaphy; 3) comparative group of 20 rats with systemic microcirculatory disorders caused by alloxan-induced diabetes mellitus; 4) experimental group, which included 20 rats that received FTSG in the interscapular region simultaneously with sciatic nerve neurorrhaphy; 5) experimental group, consisting of 10 rats, which received FTSG on day 21 after alloxan injection.

As a model of local microcirculation dysfunction, denervation hypersensitivity, which occurs and develops during transection and delayed sciatic nerve neurorrhaphy and is accompanied by microvascular vasospasm and microcirculation deterioration, was used [15]. Sciatic nerve transection was performed at the level of the middle third of the thigh. Neurorrhaphy was performed 21 days after nerve transection by applying epineural suturing using atraumatic needles and 7/0 USP suture material.

Systemic microcirculation disorders were modeled by development of alloxan-induced diabetes in animals, which has a direct β-cytotoxic effect on the pancreatic islet and causes absolute insulin deficiency that is characteristic of type 1 diabetes [16]. Alloxan (100 mg/kg of animal weight) was injected subcutaneously once. Development of diabetes mellitus was verified by a statistically significant ($p < 0.05$) increase in glycated hemoglobin levels by 1.7 times to 9.3% (6.9; 12.1) compared to the intact animals.

FTSG in the experimental groups was performed on day 21 from the beginning of the experiment (sciatic nerve transection or alloxan administration, respectively). A full-layer skin flap, 0.1% of the animal's body surface area, was excised in the interscapular region, and treated alternately in 3% hydrogen peroxide, 70% ethyl alcohol and 0.9% saline solution. In the wound formed during the excision of the flap, a subcutaneous pocket was formed where the processed autograft was placed. The operating wound was tightly sutured [17].

Microcirculation in the dorsal skin of the posterior paw using LAKK-OP (NPP "Lazma", Russia) on day 42 of the experiment in the comparative and experimental groups. LDF-grams of intact animals were used as a control. Microcirculation state was assessed by the value of perfusion index in perfusion units.

Blood was collected from animals on day 42 of the experiment by puncture of the right heart sections using VACUETTE blood tubes (Greiner Bio-One, Austria) with a coagulation activator and a separating polyester gel to obtain serum, as well as with 3.2% sodium citrate for obtaining plasma and making smears.

The serum catecholamine (CA) level as the main vasoconstrictor substances was determined by microscopy using μ-Vizo 101 microvisor (LOMO, Russia) of smears stained with silver nitrate and eosin by Mardar and Kladienko cytochemical method (1986) with preliminary exposure in a potassium dichromate solution to obtain adrenochromes adsorbed on red blood cell. Serum CA levels were expressed in arbitrary units, defined as the amount of silver-impregnated adrenochrome granules adsorbed on 100 red blood cells of a smear.

Blood concentrations of vasodilator substances, including histamine and vascular endothelial growth factor (VEGF), were determined by enzyme immunoassay using semi-automated readers StatFax4200 (Awareness Technology, USA) and Epoch (BioTekInstruments, USA), as well as VEGF Rat reagents (RnDSystems, USA) and Histamine-ELISA (IBL International, Germany).

The animals were withdrawn from the experiment by an overdose of anesthesia drugs immediately after blood sampling. The tissues of the FTSG zone were harvested and fixed in 10% formalin for histology. Skin samples from intact animals were used as controls. 5μm-thick histological sections were stained with Mayer's hematoxylin (OOO Biovitrum, Russia) and eosin (Biovitrum, Russia). Microscopy of the FTSG zone preparations was performed using the AxioImager Z2 system (CarlZeiss, Germany). Quantitative calculation of cell populations of the autograft dermis was performed at a magnification of 40× objective lens.

Statistical processing was done using the Statistica 10.0 software package. Most of the data obtained did not conform to the law of normal distribution. So, they are presented as median and interquartile range. The groups were compared using the Mann–Whitney U test and the certainty index of the difference p, whose critical level was taken to be 0.05.

RESULTS

A study of microcirculation of the dorsal skin of the rear paw in the group of animals that underwent transection and deferred sciatic nerve neurorrhaphy showed a significant decrease in the perfusion index relative to the control values on day 42 of the experiment. The animals that underwent FTSG simultaneously with neurorrhaphy showed an improved microcirculation state on day 42 of the experiment, as evidenced by the significant increase in perfusion index by 44% compared to its value in animals in the corresponding comparison group. The perfusion index in this group did not differ from the values observed in the control group (Fig.).

It was shown that animals with experimental diabetes by day 42 after alloxan injection showed a statistically significant decrease in perfusion of the dorsal skin of the rear paw compared to the control group, indicating a reduction in microcirculation. In animals that underwent FTSG against the background of alloxan-induced diabetes, a statistically significant increase (by 43%) in this indicator was observed on day 42 of the experiment relative to the group of animals with impaired carbohydrate metabolism, indicating improved microcirculation. For the two experimental groups, it was found that the level of perfusion was statistically higher in animals that underwent FTSG against the background of systemic microcirculatory disorders (Fig.).

In studying the role of changes in the sympathetic regulation of vascular tone in realization of the distant stimulating effect of FTSG, it was found that in rats

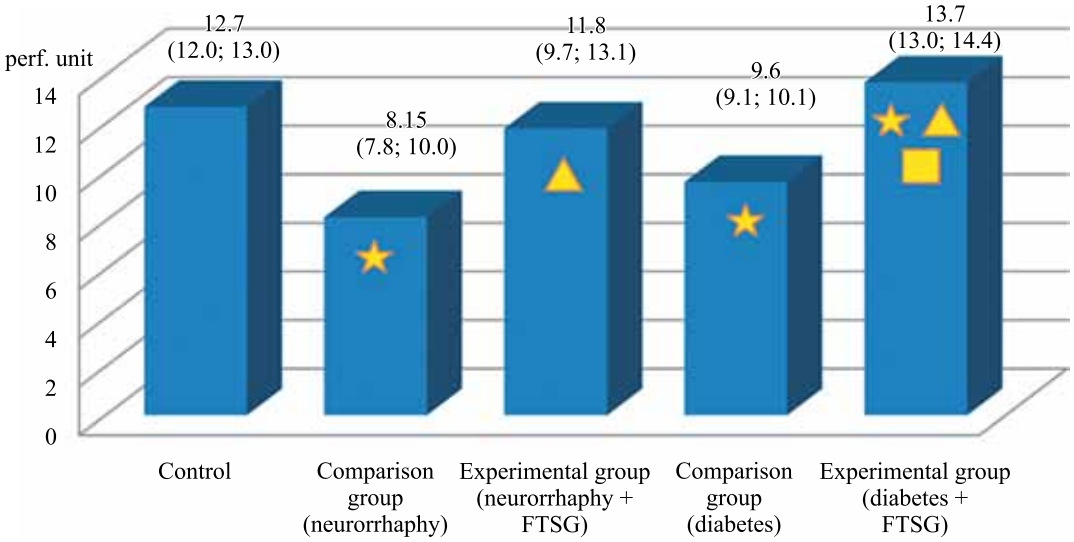

Fig. Changes in the perfusion index of the dorsal skin of the rear paw with FTSG autotransplantation in sciatic nerve injury and alloxan-induced diabetes. Statistically significant differences ($p < 0.05$) are indicated by: a star (relative to the control), a triangle (relative to the corresponding comparison group), and a square (comparison of the experimental groups between each other)

that underwent neurorrhaphy, there was a statistically significant increase in blood catecholamine levels on day 42 after surgery relative to the control. In this group, we found that FTSG reduced catecholamine levels by about 1.5 times as compared to the group of animals that did not receive FTSG against the background of neurorrhaphy (Table 1).

At the same time, animals with systemic microcirculatory disorders induced by alloxan-induced diabetes also showed a significant increase in serum catecholamine levels by day 42. FTSG in rats with alloxan-induced diabetes almost halved the serum catecholamine levels relative to the corresponding comparison group. Consequently, FTSG limits the activity of the sympathetic nervous system more pronounced in systemic microcirculatory disorders occurring against the background of impaired carbohydrate metabolism (Table 1).

The study of serum histamine levels revealed that transection and neurorrhaphy does not cause significant changes in histamine concentrations relative to the control. At the same time, in the experimental group, which underwent FTSG, plasma histamine levels on day 42 of the experiment exceeded both the control values and the values of the corresponding comparison group. It was found that FTSG, performed against the background of impaired carbohydrate metabolism, increases the blood histamine levels on day 42 of the experiment, which exceeds both the control values and the values of the comparison group by an average of 2.5 times (Table 1). This being said, blood histamine levels in animals that underwent FTSG against the background of alloxan-induced diabetes were significantly lower than in rats with FTSG against the background of sciatic nerve injury and neurorrhaphy.

It was found that FTSG, performed simultaneously with neurorrhaphy of the injured sciatic nerve, increases serum VEGF concentrations on day 42 of the experiment, which exceeds the control values by 1.8 times (Table 1). Alloxan was found to cause a significant increase in plasma VEGF levels in rats. Moreover, the FTSG animals also had increased VEGF levels relative to the control values, which did not differ significantly from that in the rats of the corresponding comparison group (Table 1).

Microscopy of tissue preparations from the autograft area revealed skin flap thinning accompanied by epidermis degradation. At the same time, there is a change in the composition of the cellular populations of the autograft dermis, manifested by increased number of fibroblasts, lymphocytes and macrophages. A distinctive feature of preparations in this group is the increased number of eosinophils, averaging up to 6 cells in the field of view (Table 2).

Morphological analysis of the preparations in animals with alloxan-induced diabetes revealed that by day 42 of the experiment, the autograft had thinned. The epidermis was absent in 20% of rats in this group. The thickness of the hypodermis was sharply reduced in all animals. Full-blooded arterial and venous vessels were observed in the dermis and hypodermis. Degenerating hair follicles were present in the dermis. The dermis of the autograft contained a large number of fibroblasts and was moderately infiltrated with leukocytes. Leukocyte infiltration was dominated by lymphocytes and macrophage cells. Animals with impaired carbohydrate metabolism had single eosinophils in the autograft dermis, but they were significantly less in number than in rats with sciatic nerve injury (Table 2).

DISCUSSION

The mechanism of local microcirculatory disorders in sciatic nerve injury is due to, on one hand, denerva-

Table 1

Changes in serum concentrations of vasoactive substances after FTSG autotransplantation in rats with sciatic nerve injury and alloxan-induced diabetes

Group / Indicator	Control	Comparison group (neurorrhaphy)	Experimental group (neurorrhaphy + FTSG)	Comparison group (diabetes)	Experimental group (diabetes + FTSG)
Serum catecholamine levels, units	22 (16; 25)	105 (95; 110) $p_1 = 0.000161$	65 (60; 70) $p_1 = 0.000176$ $p_2 = 0.021572$	56 (49; 60) $p_1 = 0.000001$	30 (26; 33) $p_1 = 0.001236$ $p_2 = 0.000001$ $p_3 = 0.000003$
Histamine, mg/L	1.7 (0.6; 2.1)	1.34 (1.19; 2.1) $p_1 = 0.885234$	3.16 (2.59; 3.69) $p_1 = 0.000939$ $p_2 = 0.001764$	1.1 (0.6; 2.2) $p_1 = 0.732678$	2.8 (2.2; 3.2) $p_1 = 0.025348$ $p_2 = 0.021828$ $p_3 = 0.023271$
VEGF, pg/mL	9.4 (7.3; 15.7)	8.6 (4.65; 9.96) $p_1 = 0.222082$	16.97 (9.96; 28.5) $p_1 = 0.004041$ $p_2 = 0.045501$	85.2 (79.6; 97.5) $p_1 = 0.000453$	83.3 (78.9; 86.3) $p_1 = 0.001033$ $p_2 = 0.286423$ $p_3 = 0.001033$

Note. p_1 – significance of differences relative to the control group; p_2 – significance of differences relative to the comparison group; p_3 – significance of differences when the two experimental groups are compared to each other.

Table 2

Dynamics of cell populations of the autograft dermis in sciatic nerve injury and alloxan-induced diabetes

Group / Indicator	Control	Comparison group (neurorrhaphy + FTSG)	Experimental group (alloxan-induced diabetes + FTSG)
Fibroblasts	42 (27; 52)	104 (60; 182) $p_1 = 0.000077$	119 (97; 165) $p_1 = 0.000028$ $p_2 = 0.394742$
Fibrocytes	18 (13; 23)	21 (14; 32) $p_1 = 0.558185$	27 (21; 44) $p_1 = 0.034962$ $p_2 = 0.183147$
Neutrophils	0 (0; 0)	1 (0; 4) $p_1 = 0.005414$	0 (0; 1) $p_1 = 0.291698$ $p_2 = 0.044686$
Eosinophils	0 (0; 0)	3 (2; 8) $p_1 = 0.000016$	1 (0; 2) $p_1 = 0.010519$ $p_2 = 0.000292$
Lymphocytes	0 (0; 2)	13 (5; 19) $p_1 = 0.000034$	20 (14; 27) $p_1 = 0.000017$ $p_2 = 0.077135$
Monocytes / histiocytes / macrophages	0 (0; 0)	1 (1; 3) $p_1 = 0.001393$	2 (0; 3) $p_1 = 0.047679$ $p_2 = 0.394742$

Note. p_1 – significance of differences relative to the control; p_2 – significance of differences relative to the comparison group.

tion hypersensitivity of the pre-capillary vessels of the microcirculatory bloodstream, characterized by increased expression of alpha-1 adrenergic receptors on the membrane of smooth muscle cells and their sensitivity to catecholamines [15]. On the other hand, traumatic injury to the nerve trunk is accompanied by activation of the sympathetic nervous system and, as evidenced by obtained data, by increased serum CA levels. Together, these two factors lead to increased tone of smooth muscle cells of the precapillary vessels and vasoconstriction of small arteries and arterioles, which is manifested by decreased perfusion of the dorsal skin of the rear paw of the operated limb. Recent studies indicate that alloxan administration in mice is also accompanied by increased expression of alpha-1 adrenergic receptors in the smooth muscle cells of the skin vessels during 2–4 weeks of development of alloxan-induced diabetes. Using laser speckle contrast imaging, it was shown that in these animals, norepinephrine reduces arterial and venous blood flow in the dorsal skin without subsequent recovery, which reflects a persistent vasospastic response caused by vascular hypersensitivity to catecholamines [18]. Results obtained in the course of this study indicate that the level of circulating catecholamines increases in alloxan-induced diabetic rats. Elevated blood catecholamine levels in alloxan-induced diabetes may be associated with the effect of hyperglycemia on the activity of enzymes providing synthesis and degradation of these biogenic amines. Thus, under experimental conditions, it was shown that hyperglycemia in diabetic mice increases the expression of tyrosine hydroxylase in renal mesangial cells, accompanied by increased consumption of tetrahydrobiopterin and increased production of catecholamines. Moreover, there was reduced activity of monoamine oxidase, which prevented the destruction of catecholamines [19]. Consequently, in diabetes and in nerve injury, microcirculatory disorders are caused, on one hand, by increased levels of circulating catecholamines, and, on the other hand, by increased expression of alpha-1 adrenergic receptors on the membrane of smooth muscle cells, causing development of vascular hypersensitivity to the action of circulating catecholamines. That is, the observed microcirculatory disorders in rats with traumatic sciatic nerve injury and animals with alloxan-induced diabetes are not only similar, but also have homologous developmental mechanisms. However, in the case of nerve injury, microcirculatory disorders are predominantly local, while in alloxan-induced diabetes, they are systemic.

FTSG in the interscapular region against the background of both nerve injury and alloxan-induced diabetes has a distant stimulating effect on the microcirculation of the dorsal skin of the rear paw in white rats. The perfusion index of the foot skin surface in experimental animals of the experimental groups increased on average by 43–44% relative to the corresponding comparison groups, indicating the same degree of severity of the distant stimulating effect of FTSG both in local and in systemic microcirculatory disorders. Earlier studies indicate that realization of the distant stimulating effect of FTSG on microcirculation in intact rats, under conditions of sciatic nerve injury and in carbohydrate metabolism disorders, is associated with decreased neurogenic vascular tone [14, 17]. The dynamics of serum catechola-

mine levels presented in this work is consistent with the previously obtained data and indicates that FTSG in the interscapular region, against the background of local and systemic microcirculatory disorders, limits the activity of the sympathoadrenal system. Limitation of the influence of the sympathetic system on the microcirculatory bloodstream vessels under the influence of autologous transplantation may explain the maintenance of adequate perfusion in the distal limb in rats of the experimental groups. The mechanism of limiting the activity of the sympathoadrenal system under FTSG influence may be due to the detected degenerative changes in the skin flap, which may be accompanied by formation of peptide fragments acting as cytomedines. As peptide regulators, cytomedines can exert distant effects, including on the nervous system [20]. A similar mechanism for limiting the effects of the sympathetic nervous system on the vascular bed has been described for peptide drug SEMAX, whose action was characterized by decreased expression of alpha-adrenergic receptors under experimental conditions in white rats [21]. The data obtained in the course of this work indicate that limitation of the influence of the sympathetic nervous system on microcirculation in FTSG is more pronounced in systemic microcirculatory disorders caused by absolute insulin deficiency.

It was noted earlier that FTSG performed against the background of sciatic nerve neurorrhaphy has an effect not only on the blood levels of circulating catecholamines, but also on the levels of other biogenic amines, including those with vasodilatory activity. For instance, it increases plasma histamine levels [13]. The results of this work demonstrate that FTSG exerts a similar effect on plasma histamine levels after alloxan injection. Meanwhile, the average value of the histamine levels in the group of animals that received FTSG after alloxan administration was 12% lower than the values in the experimental group that underwent autotransplantation against the background of sciatic nerve injury.

One of the possible mechanisms for realization of the distant stimulating effect of FTSG on microcirculation under normal carbohydrate metabolism with impaired innervation of the limb is the ability to prolong the production of growth factors, particularly VEGF and neurotrophin 3 [13, 22]. The sources of growth factors can be fibroblasts [23] and leukocyte infiltration cells of the autograft. For example, eosinophils [24], mast cells [25], and macrophages [26] secrete VEGF, which stimulates angiogenesis, causing vascular dilatation and an increase in their permeability. However, this study found that FTSG does not implement this mechanism of action in conditions of alloxan-induced diabetes in rats. The blood levels of this factor in the experimental group significantly exceeds the control values, but does not differ from the comparative group of rats with alloxan-induced diabetes. In systemic microcirculatory disorders caused by experimental diabetes, high plasma VEGF levels are associated with systemic endothelial alteration by hyperglycemia, which is a compensatory mechanism for vascular wall repair [27]. In this regard, the initially high level of VEGF in rats with alloxan-induced diabetes is likely to block its production by autograft cells through a negative feedback mechanism.

The above described biochemical mechanisms of realization of the distant stimulating effect of FTSG on microcirculation are closely interrelated with histomorphological restructuring of the autograft. Histomorphological changes in the autograft are stereotypic and include degradation of the epidermis, thinning of the dermis and hypodermis, and mild leukocyte infiltration. Minor differences between the experimental groups lie in the number of eosinophils in the composition of cell populations of the autograft dermis. Their presence in autograft tissues is associated with the release of histamine by mast cells, which is involved in distant vasodilating effect [28]. In animals, against the background of impaired carbohydrate metabolism, there was a lower number of eosinophils in the autograft region compared to animals with neurorrhaphy. These histological examination results are consistent with biochemical data reflecting a less pronounced increase in plasma histamine levels under FTSG influence in animals with alloxan-induced diabetes. The authors in [29] have shown that alloxan in rats significantly reduces the content of mast cells in the skin. Given the less pronounced increase in plasma histamine levels in FTSG under carbohydrate metabolism disorders, the small number of eosinophils in the autograft composition in rats with alloxan-induced diabetes is probably due to decreased intensity of mast cell degranulation.

CONCLUSION

FTSG has a distant stimulating effect on microcirculation, which manifests itself to the same extent under conditions of both local and systemic microcirculatory disorders, and does not have an obvious dependence on the etiological factor inducing them. The data obtained from comparative analysis of FTSG effects on two models of vascular alteration in white rats indicate that local structural and functional changes in the autograft are stereotypic. Therefore, they can be considered triggering, while biochemical changes in the concentrations of regulatory substances have an unequal degree of severity depending on the etiology and prevalence of microcirculatory disorders. Differences in the blood levels of bioactive substances allow to isolate universal and variable responses in the complex of FTSG regulatory mechanisms. Universal reactions include changes in the balance of biogenic amines in the blood, in particular, changes in the blood levels of catecholamines and histamine. It should also be noted that correction of vasoconstrictor activity using FTSG is more pronounced in systemic microcirculatory disorders induced by alloxan-induced

diabetes. In contrast, realization of the FTSG effect on production of vasodilator substances, in particular histamine, is more pronounced in local microcirculatory disorders in the denervated limb. Variable responses depend on the etiology of simulated microcirculatory disorders; in particular, changes in the production of growth factors, including VEGF, are specific for the microcirculatory effects of FTSG in limb denervation.

Thus, the relationship between structural and functional tissue changes in the field of autotransplantation and microcirculation regulation factors circulating in systemic blood flow determine a compensatory change in the balance of regulatory mechanisms, which provides a stable implementation of the distant stimulating effect of FTSG on microcirculation, regardless of the prevalence and etiology of its injury.

The authors declare no conflict of interest.

REFERENCES

1. *Mironov SP, Krupatkin AI, Golubev VG, Panov DE.* Diagnostika i vybor taktiki lecheniya pri povrezhdeniyah periphericheskih nervov. *Vestnik travmatologii i ortopedii im. N.N. Priorova*. 2005; 2: 33–39.
2. *Shchanicyn IN, Ivanov AN, Bazhanov SP, Ninel' VG, Puchin'yan DM, Norkin IA.* Stimulyaciya regeneracii periphericheskogo nerva: sovremennoe sostoyanie, problem i perspektivy. *Uspekhi fiziologicheskih nauk*. 2017; 48 (3): 92–112.
3. *Inanc M, Tekin K, Kiziltoprak H, Ozalkak S, Doguizi S, Aycan Z.* Changes in Retinal Microcirculation Precede the Clinical Onset of Diabetic Retinopathy in Children With Type 1 Diabetes Mellitus. *J Ophthalmol*. 2019; 207: 37–44. doi: 10.1016/j.ajo.2019.04.011.
4. *Stacenko ME, Turkina SV, Shilina NN, Kosivcova MA, LopushkovaYuE, Vinnikova AA i dr.* Osobennosti sostoyaniya mikrocirkulyacii u bol'nyh hronicheskoj serdechnoj nedostatochnost'yu i saharnym diabetom vtorogo tipa. *Volgogradskij nauchno-medicinskij zhurnal.* 2015; 1 (45): 35–39.
5. *Kuo L, Hein TW.* Vasomotor regulation of coronary microcirculation by oxidative stress: role of arginase. *Front Immunol.* 2013; 19 (4): 237.
6. *Mihajlusov RN.* Faktory rosta – perspektivnye tekhnologii vozdejstviya na ranevoj process. *Harkivs'ka hirurgichna shkola*. 2014; 5 (68): 90–98.
7. *Gromova OA, Torshin IYu, Volkov AYu*. Elementnyj sostav preparata Laennek i ego klyuchevaya rol' v farmakologicheskom vozdejstvii preparata. *Plasticheskaya hirurgiya i kosmetologiya*. 2010; 4: 1–7.
8. *Öhnstedt E, Lofton Tomenius H, Vågesjö E, Phillipson M.* The discovery and development of topical medicines for wound healing. *Expert Opinionon Drug Discovery*. 2019; 14: 485–497. doi: 10.1080/17460441.2019.1588879/.
9. *Nozdrin VI, Belousova TA, Al'banova VI, Lavrik OI.* Gistofarmakologicheskie issledovaniya kozhi (nash opyt). M., 2006. 376.
10. *Hocking SL, Stewart RL, Brandon AE*. Subcutaneous fat transplantation alleviates diet-induced glucose intolerance and inflammation in mice. *Diabetologia.* 2015; 58 (7): 1587–600.
11. *Foster MT, Shi H, Softic S et al.* Transplantation of non-visceral fat to the visceral cavity improves glucose tolerance in mice: investigation of hepatic lipids and insulin sensitivity. *Diabetologia*. 2011; 54 (11): 2890–2899.
12. *Hramcova YuS, Artashyan OS, Yushkov BG, Volkova YuL, Nezgovorova NYu.* Vliyanie tuchnyh kletok na reparativnuyu regeneraciyu tkanej s raznoj stepen'yu immunologicheskoj privilegirovannosti. *Citologiya*. 2016; 58 (5): 356–363.
13. *Ivanov AN, Lagutina DD, Gladkova EV, Matveeva OV, Mamonova IA, Shutrov IE i dr.* Mekhanizmy distantnogo stimuliruyushchego dejstviya autotransplantacii kozhnogo loskuta pri povrezhdenii periphericheskogo nerva. *Rossijskij fiziologicheskij zhurnal im. I.M. Sechenova*. 2018; 11: 1313–1324.
14. *Ivanov AN, Popyhova EB, Stepanova TV, Pronina EA, Lagutina DD*. Izmenenie pokazatelej mikrocirkulyacii pri autotransplantacii polnoslojnogo kozhnogo loskuta na fone eksperimental'nogo saharnogo diabeta u krys. *Regionarnoe krovoobrashchenie i mikrocirkulyaciya.* 2019; 4: 72–80.
15. *Ivanov AN, Norkin IA, Ninel' VG, Shchanicyn IN, Shutrov IE, Puchin'yan DM.* Osobennosti izmenenij mikrocirkulyacii pri regeneracii sedalishchnogo nerva v usloviyah eksperimenta. *Fundamental'nye issledovaniya*. 2014; 4 (2): 281–285.
16. *Dzhafarova RE*. Sravnitel'noe issledovanie razlichnyh modelej alloksan-inducirovannogo saharnogo diabeta. *Kazanskij medicinskij zhurnal*. 2013; 94 (6): 915–919.
17. *Ivanov AN, Shutrov IE, Norkin IA*. Autotransplantaciya polnoslojnogo kozhnogo loskuta kak sposob biostimulyacii mikrocirkulyacii v usloviyah normal'noj i narushennoj innervacii. *Regionarnoe krovoobrashchenie i mikrocirkulyaciya.* 2015; 3 (55): 59–65.
18. *Feng W, Shi R, Zhang C, Liu S, Yu T, Zhu D*. Visualization of skin microvascular dysfunction of type 1 diabetic mice using *in vivo* skin optical clearing method. *J Biomed Opt*. 2018; 24 (3): 1–9.
19. *Marco GS, Colucci JA, Fernandes FB, Vio CP, Schor N, Casarini DE*. Diabetes induces changes of catecholamines in primary mesangial cells. *Int J Biochem Cell Biol.* 2008; 40 (4): 747–754.
20. *Anohova LI, Pateyuk AV, Kuznik BI, Kohan ST*. Sravnitel'noe vliyanie polipeptidov endometriya i timalina na nekotorye pokazateli immuniteta i gemostaza v opytah *in vitro* i *in vivo*. *Byulleten' VSNC SO RAMN*. 2011; 6: 156–159.
21. *Gorbacheva AM, Berdalin AB, Stulova AN, Nikogosova AD, Lin MD, Buravkov SV i dr.* Izmenenie simpaticheskoj innervacii hvostovoj arterii krysy pri eksperimental'nom infarkte miokarda; vliyanie peptida "SEMAKS". *Byulleten' eksperimental'noj biologii i mediciny*. 2016; 4: 462–467.
22. *Ivanov AN, Shutrov IE, Ninel' VG, Korshunova GA, Gladkova EV, Matveeva OV i dr.* Vliyanie autotransplantacii kozhnogo loskuta i pryamoj elektrostimulyacii

sedalishchnogo nerva na regeneraciyu nervnyh volokon. *Citologiya.* 2017; 7: 489–497.

23. *Chakroborty D, Sarkar Ch, Lu K, Bhat M*. Activation of dopamine d1 receptors in dermal fibroblasts restores vascular endothelial growth factor-a production by these cells and subsequent angiogenesis in diabetic cutaneous wound tissues. *Am J Pathol*. 2016; 186 (9): 2262–2270. doi: 10.1016/j.ajpath.2016.05.008.
24. *Panagopoulos V, Zinonos I, Leach D, Evdokiou A*. Human peripheral blood eosinophils induce angiogenesis. *The International Journal of Biochemistry & Cell Biology*. 2005; 37 (3): 628–636. doi: 10.1016/j.biocel.2004.09.001.
25. *Lee AJ, Ro MJ, Park JI, Jang JH, Kim JH*. The synthesis of VEGF in allergen-stimulated mast cells is through a leukotriene B4 receptor-2-dependent signaling pathway. *J Immunol*. 2016; 1: 196.
26. *Monastyrskaya EA, Lyamina SV, Malyshev IYu.* M1 i M2 fenotipy aktivirovannyh makrofagov i ih rol' v immunnom otvete i patologii. *Patogenez*. 2008; 4: 31–39.
27. *Iskakova SS, Zharmahanova GM, Dvoracka M.* Mesto angiogeneza v razvitii saharnogo diabeta i ego oslozhnenij (obzor literatury). *Vestnik KazNMU*. 2014; 2 (2): 303–307.
28. *Swartzendruber JA, Byrne AJ, Bryce PJ.* Cutting edge: histamine is required for IL-4-driven eosinophilic allergic responses. *J Immunol*. 2012; 188 (2): 536–540.
29. *De F Carvalho V, Campos LV, Farias-Filho FA, Florim LT, Barreto EO, Pirmez C et al.* Suppression of allergic inflammatory response in the skin of alloxan-diabetic rats: relationship with reduced local mast cell numbers. *Int Arch Allergy Immunol.* 2008; 147 (3): 246–254.

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