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Andrey V. Voronkov, Olesya Yu. Gamzeleva[1]

THE EFFECT OF NEW FORMS FOR EXTERNAL APPLICATION ON THE VASODILATING FUNCTION OF THE ENDOTHELIUM AND THE CONCENTRATION OF ENDOTHELIAL NITRIC OXIDE SYNTHASES IN RATS WITH AN EXPERIMENTAL MODEL OF A PATHOLOGICAL SCAR AT EARLY HEALING TIMES

Pyatigorsk Medical and Pharmaceutical Institute – branch of Volgograd State Medical University, 11, Kalinin av., Pyatigorsk, 357532, Russia.
Corresponding author, [1]e-mail: lesya_gamz@mail.ru

Abstract

Introduction: According to statistical data, the tendency towards an increase in the number of patients with keloid and hypertrophic scars at the site of skin lesions remains in the world, which in most cases is due to the lack of effective methods for preventing this type of complications. Therefore, the development of new methods of pharmacological correction, which allow us to normalize the wound healing process at an early stage, seems very relevant. One of the promising areas for the search for substances suitable for the prevention of pathological scars is the study of natural compounds possessing endothelioprotective activity, since, according to modern ideas, it is the endothelial dysfunction that underlies the pathogenesis of this complication.

Materials and Methods: All studies were performed on 60 male rats of the Wistar line, divided into 6 experimental groups. New forms for external use based on flavonoids PMFI-92 and PMFI-93 and comparison preparations "Kontraktubeks", "Egallohit" were used as research objects. The effect of new PMFI-92 and PMFI-93 formulations on the vasodilating function of the endothelium of the skin of animals with chemical burn was introduced with the introduction of nitric oxide synthesis modifiers: acetylcholine (ACS), L-arginine, nitro-L-arginine methyl ester (L-NAME). The effect of local pharmacological support in this pathology on the level of endothelial (eNOS), neuronal (nNOS) and inducible (iNOS) nitric oxide syntheses was determined by the method of solid phase enzyme immunoassay (ELISA).

Results and Discussion: Application of the proposed new forms based on natural compounds PMFI-92 and PMFI-93 allows to improve the vasodilating function of the endothelium already at the early stages of wound healing, with an increase in the concentration of eNOS and nNOS, as well as a decrease in the concentration of iNOS.

Conclusion: The experimental data obtained by us testify to the pronounced positive effect of the local application of PMFI-93 and PMFI-92 compositions on the vasodilating function of the skin vessels, and also to restore the balance between the activities of various forms of nitric oxide synthases.

Keywords: Endothelial dysfunction, keloid and hypertrophic scars, PMFI-92, PMFI-93, eNOS, nNOS, iNOS.

Introduction

Skin lesions leading to the formation of pathological scars are one of the main causes of aesthetic and functional defects in an average of 10-40% of patients [1, 2, 3]. In connection with the widespread prevalence of this problem, various scientific approaches have been developed that explain the pathogenesis of

the formation of pathological scars [4, 5, 6, 7, 8, 9]. So, their formation is associated with a violation of microcirculation in the area of damage, with excessive intensity of inflammation, with the accumulation of excess collagen, etc. [4, 5, 6, 7, 8, 9]. In recent years, the greatest scientific interest is dysfunction of the endothelium of damaged vessels as a target of pharmacological action in the prevention of keloid and hypertrophic scars, since it is this pathological process that leads to a disruption in the metabolism of biologically active substances and mediators in the damaged surface [4, 5, 10]. In the modern pharmaceutical market, there are a number of drugs that affect these pathological mechanisms, with the exception of drugs capable of restoring the damaged endothelial function in the early stages of wound healing [4, 5, 11, 12, 13, 14]. So, to reduce the already formed shallow defect and prevent the formation of scars after uncomplicated damage, the gels Kontraktubeks and Egallohit use natural compounds [15, 16, 17, 18, 19]. At the same time, the search for ways of emergency prevention of the formation of a pathological scar is still relevant. In connection with the above, the creation of new forms for external application capable of restoring the endothelial function is very promising, and, in our opinion, it is expedient to consider natural compounds of the flavonoid series as objects for the creation of such preparations [4, 5, 20].

The purpose of this study was to study the effect of new forms for external use on the basis of the flavonoids PMFI-92 and PMFI-93 on the vasodilating function of the endothelium as one of the components of the endothelial function, as well as the indirect determination of the activities of various isoforms of nitric oxide synthases, since restoring the balance between eNOS, nNOS and iNOS can act as one of the possible mechanisms for restoring both the vasodilating and endothelial functions in general.

Materials and methods

In the experiment, three-month-old male Wistar rats weighing 220-250 g were used, without external signs of diseases, divided into 6 experimental groups of 10 animals each: 1 group – pseudo-operated animals (L/O), 2 group – negative control (NK), Group 3 – animals receiving the composition PMFI-92, 4 group – PMFI-93, 5 group – comparison preparation "Kontraktubeks", 6 group – preparation of comparison "Egallohit". For narcosis, chloral hydrate was used, which was administered intraperitoneally at a dose of 350 mg / kg, slaughter of animals was carried out under ether anesthesia. New forms for external use based on flavonoids PMFI-92 and PMFI-93 and comparison preparations "Kontraktubeks", "Egallohit" were used as research objects.

A chemical burn was modeled in rats on a pre-depilated area of the skin of the back by subcutaneous injection with 0.2 ml of glacial acetic acid. The investigated objects were applied to the damaged area of the skin 2 times a day for 1.0 g for 14 days, the registration of the indices was carried out on the 7th and 14th days of the experiment. The analysis of the vasodilating function of the endothelium was performed by ultrasound dopplerography with the intravenous administration of ASC (Sigma-Aldrich, USA) at a dose of 0.1 mg / kg; L-arginine (Sigma-Aldrich, USA) at a dose of 150 mg/kg and nitro-L-arginine methyl ester (L-NAME) (Sigma-Aldrich, USA) at a dose of 15 mg / kg [21]. The UZOP-010-01 sensor with an operating frequency of 25 MHz was located on the inside of the area of the skin of the rumen, the indices were recorded with the help of the MM-D-K-Minimax Doppler v.1.9 working computer program. (Saint-Petersburg, Russia). ELISA was performed on a microtiter plate reader Tecan Infinite F50 (Austria) using species-specific nitric oxide synthetase assays of Cloud Clone Corp (USA). All studies corresponded to the rules of laboratory practice (GLP) in pre-clinical studies and the International Recommendations of the European Convention for the Protection of Vertebrates (1997). The results were processed using the variational statistics methods using the parametric t-test of the Student (t-test) and the nonparametric U-test (Mann-Whitney test) using the Statistica 6.0 and StatPlus 2009 application packages, as well as Microsoft Office Excel 2007.

Results and Discussion

The initial velocity of blood flow in the vessels of the skin of the back of the L/O animals was 2.366 ± 0.242 cm / sec, while in the NC animals on the 7th day of the experiment this index was significantly lower (p <0.001) 5.54 times compared to the first group , which is an unfavorable prognostic indicator [21]. It should be noted that local pharmacological stimulation of wound healing already at this stage of the experiment led to some improvement in the analyzed index, with the highest initial blood flow rate recorded in rats receiving the composition PMMI-93. In this group of rats, it was 0.606 ± 0.198 cm/sec, which exceeded the similar result in animals with pathology (p <0.05) by 1.42 times, and significantly exceeded by 1.17 times the same index in animals that received " Egallohit », in which the blood flow velocity in the zone of damage prior to the introduction of analyzers was 0.52 ± 0.089 cm/s. At the same time, in the animals of group 4, the initial blood flow velocity did not differ from that in the group receiving "Kontraktubeks" (0.6 ± 0.080 cm/s). In the rats receiving PMFI-92 during the week, the initial blood flow velocity was 0.57 ± 0.154 cm/sec, which is 1.33 times higher than in the rats of group 1 (p<0.05), but was somewhat lower, than in animals, which during the week were applied PMFI-93 and Kontraktubeks. The results of the analysis of this indicator are clearly reflected in Figure 1.

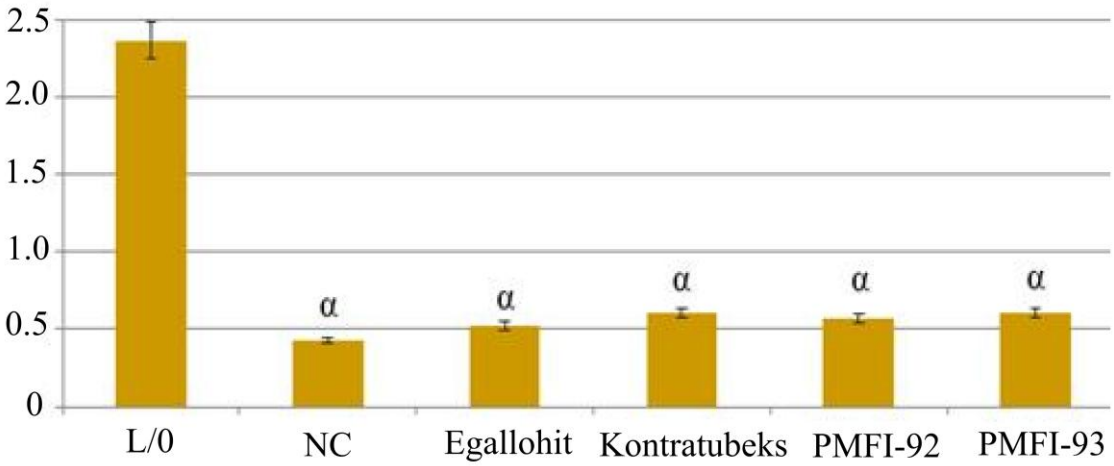

Fig. 1. Initial velocity of blood flow in the damaged area of the skin, cm/s
Note: α – is statistically significant in relation to the group of pseudo-operated animals (p <0.001), single-factor analysis with the Bonferroni amendment

An important diagnostic criterion for the state of the vasodilating function of the endothelium is the response of the vessels to intravenous administration of the ACX, which stimulates the production of nitric oxide and, accordingly, causes vasodilation [21]. Thus, in the animals of group 1, a classical response of the skin vessels to a given analyzer was observed, expressed in an increase in the average linear blood flow velocity by 47.61% (p <0.01) from its baseline level. Significantly less pronounced changes were recorded in group 2 – an increase in the blood flow velocity was only 8.14% of the baseline, which was 5.84 times (p <0.05) lower than in rats and rats, and indirectly suggests prevalence of vasoconstriction over vasodilation. In contrast to the control group of rats with a rumen model, the response to the administration of the ACX is preserved in animals receiving the composition PMFI-93, as evidenced by an increase in blood flow velocity by 17.39% of the baseline (p <0.05), which is 2, 14 times higher than in NK animals. In addition, the blood flow velocity of this group of rats was significantly different from the group of the comparison drug "Kontraktubeks", where the blood flow velocity increased by 16.87% compared to its baseline level, and was 1.18 times higher than in the group receiving

«Egallohit» (+ 14.76% of the initial level). In response to the introduction of the ACS, the blood flow velocity in the group of animals receiving PMFI-92 increased by 17.62% (p <0.05) from its baseline, the index was significantly different from the ND and the groups of animals that received the comparator. Changes in the rate of cutaneous blood flow with intravenous administration of the ACS in all experimental groups are shown in Figure 2.

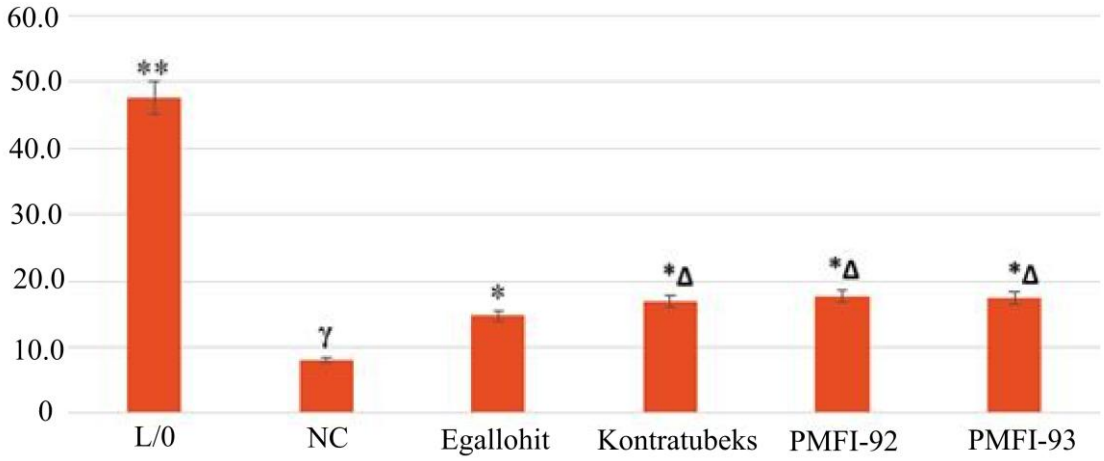

Fig. 2. Increase in blood flow velocity with intravenous injection of acetylcholine
Note: * – statistically significant with respect to the initial SC (p <0.05), ** – statistically significant with respect to the initial SC (p <0.01), γ – statistically significant in relation to the group of pseudo-operated animals (p < 0.05), Δ – statistically significant in relation to the group of negative control animals (p <0.05), single-factor analysis with the Bonferroni amendment

When L-arginine (substrate in the synthesis of nitric oxide synthesis) was administered to the animals of group No. 1, there were no significant changes in blood flow velocity, which agrees with available literature data [22]. A different reaction of the vessels to this analyzer was recorded in the group of NK rats, where the blood flow velocity increased by 31.78% (p <0.05) relative to its baseline level, which is described in the literature as the "L-arginine paradox" phenomenon and is one of the important markers of disturbance of the vasodilating function [21, 22]. Already at this period of the experiment, it is possible to note the significant differences between the animals that received PMFI-92 and PMFI-93 in this indicator. Thus, the administration of L-arginine to group No. 4 caused an unreliable increase in blood flow by only 8.06%, which is 3.94 times lower (p <0.05) than in Group 2. In turn, when L-arginine was administered to group 3, the rate of cutaneous blood flow increased (in comparison with the initial value) by 12.35%, which was statistically significant (p <0.05) less than those in negative control groups in 2, 56 times. In animals that received the comparative drugs "Kontraktubeks" and "Egallohit", the analyzed index had significantly higher values (p <0.05) than in groups No.3, No.4: an increase was observed in comparison with the initial value against L-arginine by 17.69% and 25.12% respectively (Fig. 3).

Following the introduction of L-arginine blockade of the nitric oxide synthesis system by intravenous administration of L-NAME, the blood flow velocity in the damaged area of the skin in L / O animals decreased by 35.28% (p <0.05). At the same time, in rats with a model of a pathological scar, the blood flow rate decreased by only 6.75% from its baseline level. The lack of influence of L-NAME on the indicator under study may be due to the already existing deficiency in eNOS activity as a result of the simulated pathology and also supports the theory of the "L-arginine paradox" [21, 22]. The response of the vessels to L-NAME in all animals receiving PMFI-93 was more significant and was expressed in a decrease in the linear velocity of cutaneous blood flow: in rats with CEPR – by 16.29% (p <0.05), which was more than the same indicator in rats NK in

2.41 times. Against the backdrop of L-NAME, a significant decrease ($p < 0.05$) in the rate of cutaneous blood flow was observed in rats receiving the PMFI-92 composition at 16.12% of the baseline, which did not differ significantly from animals of the PMFI-93 group. The use of comparator drugs led to a less pronounced vascular response ($p < 0.05$): in the animals treated with Kontraktubeks, the blood flow rate decreased by 15.27%, and in rats that received Egallohit 14.50% its initial value (Fig. 4).

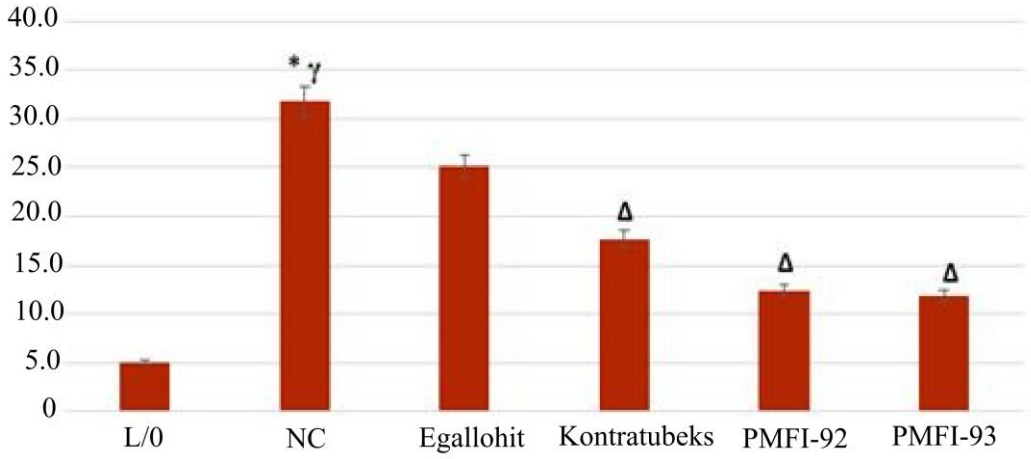

Fig. 3. Increase in blood flow velocity with intravenous administration of L-arginine
Note: * – statistically significant with respect to the initial SC ($p < 0.05$), ** – statistically significant with respect to the initial SC ($p < 0.01$), γ – statistically significant in relation to the group of pseudo-operated animals ($p < 0.05$), Δ – statistically significant in relation to the group of negative control animals ($p < 0.05$), single-factor analysis with the Bonferroni amendment

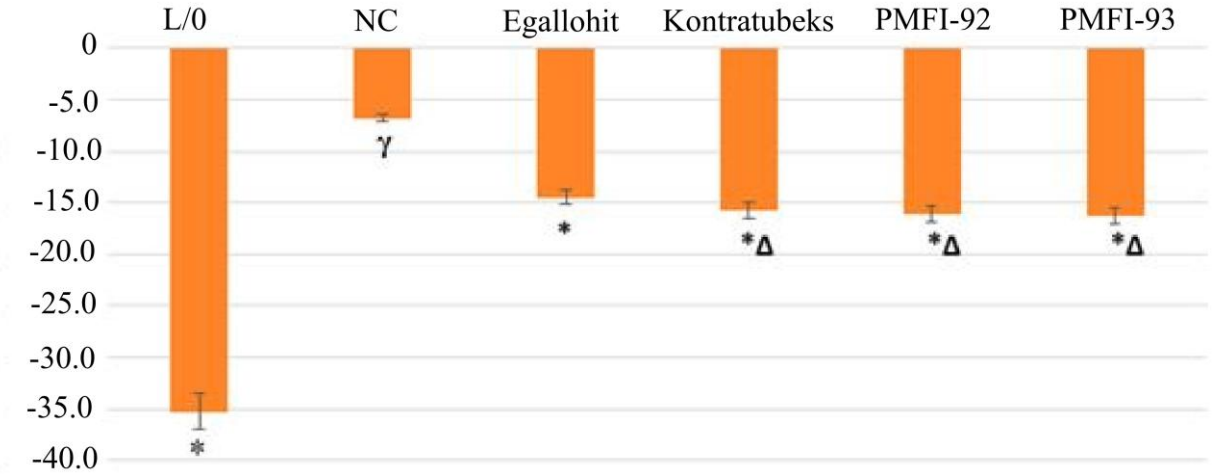

Fig. 4. The drop in blood flow velocity with intravenous injection of L-NAME
Note: * – statistically significant with respect to the initial SC ($p < 0.05$), ** – statistically significant with respect to the initial SC ($p < 0.01$), γ – statistically significant in relation to the group of pseudo-operated animals ($p < 0.05$), Δ – statistically significant in relation to the group of negative control animals ($p < 0.05$), single-factor analysis with the Bonferroni amendment

Thus, it can be concluded that the chemical burn provoked a negative change in the vasodilating function of the vascular endothelium on the 7th day of the experiment, expressed in the weakening of the response to the administration of ACX and L-NAME and the enhancement of the response to L-arginine, while the local pharmacological the support of experimental compounds allowed to correct the described violations. At the same time, at the

current stage of the experiment, new forms of PMFI-93 and PMFI-92 became the leading objects. The results obtained during the analogous course of the experiment on day 14 are presented in Table 1.

Table 1

Change in the rate of cutaneous blood flow with the introduction of analyzers 14 days after the reproduction of the pathology

Group of animals	Changes in blood flow velocity with the introduction of analyzers from the baseline, in %		
	Increase		Fall
	ATSX	L-arginine	L-NAME
Group 1	47.61±0.711**	5.00±2.063	35.28±11.022*
Group 2	8.55±1.631γ	22.73±5.673*γ	7.53±2.037γ
Group 3	18.64±1.881*Δ	9.44±1.258Δ	17.67±2.005*Δ
Group 4	19.49±2.746*Δ	7.89±2.375Δ	18.46±2.647*Δ
Group 5	18.52±2.290	14.04±1.349Δ	17.30±2.088*Δ
Group 6	15.53±1.660	17.78±10.278	15.77±1.653Δ

Note: * – statistically significant with respect to the initial blood flow velocity (p <0.05), single-factor analysis with Bonferroni correction; ** – statistically significant with respect to the initial blood flow velocity (p <0.01), single-factor analysis with Bonferroni correction; *** – statistically significant with respect to the initial blood flow velocity (p <0.001), single-factor analysis with Bonferroni correction; γ – statistically significant in relation to the group of pseudo-operated animals (p <0.05), single-factor variance analysis with Bonferroni amendment; Δ – statistically significant in relation to the group of negative control animals (p <0.05), single-factor analysis with the Bonferroni amendment; p is the level of the reliable difference.

At this stage of the study, we can note the preservation of pathological changes in the group of rats # 1, as well as the presence of recovery dynamics when using local pharmacological support. At the same time, after analyzing all the indicators listed above on the 7th and 14th day of the experiment, the objects of investigation can be arranged in the following order, reflecting the increase in the effect on the vasodilating function of the endothelium: "Egallohit" <"Kontraktubeks" <"PMFI-92" <"PMFI-93" .

Based on the well-known data that the process of improving the vasodilating function of the endothelium is primarily due to the restoration of the balance between the constitutive forms of eNOS, nNOS and the inducible form of iNOS, in the second stage of the studies we determined their concentration in the tissue homogenate of the damage zone on days 7 and 14 after the modeling of pathology [23, 24, 25, 26]. It was found that the compositions PMFI-93 and PMFI-92 are characterized by a unidirectional effect on vascular endothelial function, characterized by an increase in the concentration of constitutive NOS isoforms with a simultaneous decrease in iNOS content already on the 7th day of the experiment compared to animals not receiving pharmacological support. Thus, when PMFI-92 was used in the indicated time interval, the concentrations of eNOS and nNOS were significantly higher than those in group No. 2, respectively, by 1.31 and 1.02 times, and when PMFI-93 was applied, 1.25 and 1 , 14 times. The concentration of the inducible form of the enzyme, in turn, decreased by 1.29 times in comparison with the NK animals. As for the comparison drug "Egallohit", it caused only a significant change in the concentration of eNOS, which was less pronounced than in groups Nos. 3 and 4. In animals that received the reference preparation "Kontraktubeks", a significant increase in eNOS activity was noted, and a decrease in iNOS activity in the PMFI-92 and PMFI-93 groups was observed. It should be emphasized that a similar trend is observed on the 14th day of the experiment (Table 2).

Table 2

Determination of the concentrations of different isoforms of nitric oxide synthases at 7 and 14 days of the experiment

NOS	Concentration										
	Group 1	Group 2		Group 3		Group 4		Group 5		Group 6	
		7 days	14 days	7 days	14 days	7days	14 days	7 days	14 days	7 days	14 days
eNOS, ng/ml	97.33±2.577	76.80±0.615#	73.94±3.071#	100.45±1.004▲	264.69±9.964▲	96.34±0.695▲	126.58±5.581▲	93.74±0.947▲	98.98±1.116▲	84.56±1.461▲	84.99±2.715▲
nNOS, ng/ml	17.71±2.329	31.77±0.997#	26.97±0.389#	32.27±9.187▲	43.58±8.616▲	36.28±6.149▲	39.46±3.192▲	27.03±3.564	24.93±0.240	31.61±5.010	21.78±5.539
iNOSin plasma, ng/ml	14.16±0.448	31.89±4.587#	30.31±1.986#	24.79±1.672▲	24.03±2.857▲	24.78±4.299	24.75±3.566▲	21.88±4.259▲	22.05±2.347▲	35.64±2.503	36.20±4.977

Note: # – statistically significant in relation to the group of pseudo-operated animals (p <0.05), U – Mann-Whitney test; ▲ – statistically significant in relation to the negative control group (p <0.05), U – Mann-Whitney test.

Conclusion

Thus, the selected model of the pathological scar is accompanied by a persistent violation of the vasodilating function of the endothelium, as evidenced by the change in the response of the vessels of the skin to the administration of the ACS, L-NAME, L-arginine. In this group of animals there was a decrease in the concentration of eNOS with a simultaneous increase in the content of iNOS. Using PMFI-93 and PMFI-92 formulations on the basis of natural compounds, early in wound healing (7th day), it is possible to improve vasodilation and endothelial function, which is important for the prevention of the formation of pathological scars. In this case, the normalization of the vasodilatation process may be related to the enhancement of eNOS activity, and also to the inhibition of iNOS under the action of the listed formulations. The results of this study indicate the prospects of these compounds for further deeper study with the goal of creating on their basis an external dosage form with endothelioprotective activity for preventing the formation of pathological scars.

Conflicts of IIInterest

The authors have no conflict of interest to declare.

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Author Contributors

Andrey V. Voronkov, MD, associate professor of the Department of Pharmacology with the course of clinical pharmacology of the Pyatigorsk Medical and Pharmaceutical Institute, e-mail: prohor.77@mail.ru. The author analyzed the literature data, developed the design of the experiment, analyzed and interpreted the results.

Olesya Yu. Gamzeleva, postgraduate student, lecturer of the Department of Pharmacology with the course of clinical pharmacology of the Pyatigorsk Medical and Pharmaceutical Institute, e-mail: lesya_gamz@mail.ru. The author carried out the experimental part of the work, and also made a statistical analysis of the results.

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