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Protective Effect of Asialized Erythropoietin in Modelling of Retinal Ischemia-Reperfusion

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ABSTRACT

An important task of pharmacology is to find effective means to improve the microcirculation of the retina and increase its resistance to ischemia. The aim of the study was to evaluate the retinoprotective effect of asialized erythropoietin on a model of retinal ischemia-reperfusion in rats. The study was performed on a retinal ischemia-reperfusion model, in which intraocular pressure (IOP) was increased to 110 mm Hg for 30 minutes. The retinoprotective effect of asialized erythropoietin at a dose of 0.4 mg/kg and 2.4 mg/kg compared with recombinant erythropoietin at a dose of 50 IU/kg was evaluated by the level of retinal microcirculation using laser Doppler flowmetry (LDF) and electroretinography (ERG) after 72 hours of reperfusion. The use of asialized erythropoietin at doses of 0.4 mg/kg and 2.4 mg/kg resulted in an increase in the level of retinal microcirculation to 718.1 ± 7.1 p.u. and 746.8 ± 9.3 p.u., the b/a coefficient was 2.17 ± 0.09 and 2.41 ± 0.12, respectively, compared with the group without treatment. Thus, asialized erythropoietin can be used as a retinoprotective agent.

Key words: Retinal ischemia-reperfusion, Pharmacological preconditioning, Asialized erythropoietin, ATP-dependent potassium channels

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INTRODUCTION

Retinal ischemia plays a leading role in the loss of work capacity and disability of the population. Ischemic lesions occur due to acute or chronic blood supply disorders caused by other diseases such as atherosclerosis, hypertension, diabetes mellitus, and damage to the central retinal artery. Worldwide, there is a significant increase in ischemic retinal diseases due to the high prevalence of the above diseases. The number of retinopathies associated with damage to the vascular tract of the eyeball is close to 49-53% of the total number of diseases of the retina and optic nerve [1-5].

That is why the main task of pharmacology is the search and study of active pharmacological agents aimed at the prevention [6,7] and correction [8-10] of ischemic processes occurring in various organs and tissues. the search for new substances and ways to increase the resistance of tissues to ischemia remains relevant, despite the rapid development of experimental pharmacology [11-14], the spread of peptide drugs [15-19], the improvement of targeted synthesis methods that allow the creation of highly selective drugs [20-22].

Pharmacological preconditioning is one of the ways to increase tissue resistance to ischemia. It is based on the mechanism of activation of endogenous protective functions of the body with the introduction of an exogenous agent. As a result, there is an increase in tissue resistance to subsequent episodes of ischemia [4, 23,24].

One of these substances with a preconditioning effect is asialized erythropoietin, a derivative of recombinant erythropoietin [25,26]. Due to the complete absence of sialic acids in asialized erythropoietin, there is a short-term binding of this molecule to the heterodimeric erythropoietin receptor and the implementation of cytoprotective (non-erythropoietin) effects. The expected pharmacological effect can be confirmed by evaluating the microcirculation and electroretinogram.

The objective of this work was to study the effect of pharmacological preconditioning with Asialized erythropoietin on electrophysiological and microcirculatory retinal changes in modelling ischemia-reperfusion of the eye.

MATERIALS AND METHODS

Animals: Experiments were conducted on 90 white Wistar rats, sexually mature males, weighing 230 ± 20 g without external signs of acute or chronic diseases. Before the start of the experiment, the rats passed the quarantine regime for 14 days. Retinal ischemia-reperfusion modelling was performed under anaesthesia (chloral hydrate, 300 mg/kg of animal body weight, intraperitoneally) by applying mechanical pressure (110 mm Hg) to the anterior chamber of the eye for 30 minutes. The absence of ocular blood flow was a confirmation of the formation of ischemia [27].

Design of the Experiment: Nine series of experiments were performed to study the protective properties of asialized erythropoietin.

Group 1–Group of intact animals (n=10).

Group 2-Retinal ischemia-reperfusion model (control) (n=10).

Group 3-Correction with asialized erythropoietin (asialoEPO) at a dose of 0.4 μg/kg (n=10).

Group 4 - correction with asialoEPO at a dose of 2.4 μg/kg (n=10).

Group 5 - correction with recombinant erythropoietin (EPO) at a dose of 50 IU/kg (comparison drug).

Group 6-Control+glibenclamide (blocker of ATP-dependent potassium channels) (n=10).

Group 7-Correction with asialoEPO at a dose of 0.4 μg/kg +glibenclamide (n=10).

Group 8-Correction with asialoEPO at a dose of 2.4 μg/kg +glibenclamide (n=10).

Group 9-Correction with erythropoietin+glibenclamide (n=10).

asialized erythropoietin (Proteinovy Kontur LLC) was injected at a dose of 0.4 μg/kg and 2.4 μg/kg once intraperitoneally 30 min before modelling retinal ischemia. Recombinant erythropoietin ("Epokrin" epoetin alpha; FSUE "State Research Institute of High-Purity Drugs" of the Federal Medical and Biological Agency of St. Petersburg, Russia) was administered at a dose of 50 IU/kg once intraperitoneally 30 minutes before modelling retinal ischemia. Glibenclamide ("Maninil" Berlin-Chemi AG) was administered at a dose of 5 mg/kg once 60 minutes before modelling retinal ischemia.

Laser Doppler Flowmetry. To assess the level of microcirculation in the rat retina, a Biopac-systems MP-150 laser Doppler flowmeter and a TSD-144 needle-type sensor (USA) were used after 72 hours of reperfusion. The value of the microcirculation level in the group of rats was calculated as the average of the values obtained in each animal in the group [27].

Electroretinography. An electroretinographic study (ERG) was performed after evaluating the microcirculation. The functional retinal activity was evaluated by estimating the amplitudes of the a-and b-waves of the ERG, followed by calculating the b/a coefficient. The study was conducted according to the method described earlier [19].

Statistical Data Processing. For all data, descriptive statistics were used, and the data were checked for normal distribution. Distribution type was determined by using the criterion of Shapiro-Wilk. In case of normal distribution, the average value (M) and standard error of the mean (m) were calculated. Inter-group differences were analyzed using the Student's t-test. The differences are considered significant at $p<0.05$. The calculations were performed using statistical programs Microsoft Excel 7.0.

RESULTS

LDF results: The results of assessing the level of microcirculation in the rat retina at 72 hours of reperfusion after modelling retinal ischemia and its correction with asialized erythropoietin at a dose of 0.4 μg/kg and 2.4 μg/kg and recombinant erythropoietin at a dose of 50 IU/kg are presented in Table 1.

Table 1: Results of assessing the level of microcirculation in the rat retina at 72 hours of reperfusion (M ± m), p.u. (perfusion units).

Experimental group (n=10)	Microcircula-tion level
Intact	761.3 ± 9.6y
Ischemia-reperfusion model	344.6 ± 10.2x
AsialoEPO correction, 0.4 μg/kg	718.1 ± 7.1xy
AsialoEPO correction, 2.4 μg/kg	746.8 ± 9.3y
EPO correction, 50 IU/kg	710.7 ± 8.6xy
Ischemia-reperfusion model+glibenclamide 5 mg/kg	355.2 ± 9.1x
Correction with asialoEPO, 0.4 μg/kg+glibenclamide 5 mg/kg	361.3 ± 12.4x
Correction with asialoEPO, 0.4 μg/kg + glibenclamide 5 mg/kg	371.0 ± 11.7x
Correction with EPO, 50 IU/kg+glibenclamide 5 mg/kg	356.7 ± 10.8x
Note: x – $p<0.05$ compared to intact; y – $p<0.05$ compared to retinal ischemia-reperfusion model	

The level of microcirculation in the retina of intact rats was 761.3 ± 9.6 p.u. The level of microcirculation after modeling ischemia in the control group for 72 hours of reperfusion was 344.6 ± 10.2 p.u., which is significantly lower than the value in the group of intact animals (p<0.05).

Against the background of the administration of asialiated erythropoietin at a dose of 0.4 μg/kg, the level of microcirculation in the retina at 72 h after the pathology simulation was 718.1 ± 7.1 p.u., which exceeds this indicator in the group with the pathology model by 2 times and has a significant difference from the control group (p<0.05) and from the group of intact animals (p<0.05).

Against the background of correction of the pathology with asialized erythropoietin at a dose of 2.4 μg/kg, the level of microcirculation in the retina at 72 h of reperfusion significantly increases to 746.8 ± 9.3 p.u. and has a significant difference from the group with a pathology model (p <0.05). At the same time, the achieved values of microcirculation during the correction of pathology with asialized erythropoietin in high doses (2.4 μg/kg) are close to the values in the group of intact animals and do not have significant differences.

After correction of the pathology with erythropoietin at a dose of 50 IU/kg the level of microcirculation in the group increases to 710.7 ± 8.6 p.u. and significantly differs both from the values in the control group (p <0.05) and from the group of intact animals (p <0.05).

Administration of glibenclamide in the groups with correction of ischemic damage prevented an increase in the level of microcirculation due to the blockade of ATP-dependent potassium channels, which confirms the presence of preconditioning properties of asialized erythropoietin (0.4 μg /g and 2.4 μg/kg) and recombinant erythropoietin (50 IU/kg) in the retinal ischemia-reperfusion model.

Results of the ERG: The results of evaluating the ERG of the rat retina after 72 hours of reperfusion after modeling retinal ischemia and its correction with asialized erythropoietin at a dose of 0.4 μg/kg and 2.4 μg/kg and recombinant erythropoietin at a dose of 50 IU/kg are presented in Table 2.

Table 2: The values of the b/a coefficient after 72 h of reperfusion in the simulation of retinal ischemia and in the correction of pathology (M ± m), r.u.

Experimental group (n=10)	b/a
Intact	2.63 ± 0.12y
Ischemia-reperfusion model	1.11 ± 0.06x
AsialoEPO correction, 0.4 μg/kg	2.17 ± 0.09xy
AsialoEPO correction, 2.4 μg/kg	2.41 ± 0.12y
EPO correction, 50 IU/kg	2.11 ± 0.06xy
Ischemia-reperfusion model+glibenclamide 5 mg/kg	1.10 ± 0.09x
Correction with asialoEPO, 0.4 μg/kg + glibenclamide 5 mg/kg	1.13 ± 0.08x
Correction with asialoEPO, 2.4 μg/kg + glibenclamide 5 mg/kg	1.18 ± 0.10x
Correction with EPO, 50 IU/kg+glibenclamide 5 mg/kg	1.11 ± 0.10x

Note: R.U. – relative units; x – p<0.05 compared to intact; y – p<0.05 compared to retinal ischemia-reperfusion model

DISCUSSION

Hemodynamic disturbances in the retinal blood flow system after 72 hours of reperfusion after modelling the pathology led to electrophysiological changes in the retina characteristic of ischemia, which confirms the adequacy of the chosen model of pathology. At the same time, the suppression of the a-wave was less significant and was not observed in all animals after a given period of reperfusion. The reduction of the b-wave usually develops before the suppression of the a-wave, which occurs because of a deterioration in the trophism of neurons in the inner nuclear layer of the retina with retinal circulation disorders.

The electrophysiological b/a coefficient in the group of intact animals was 2.63 ± 0.12 r.u. The b/a coefficient in the control group decreased by 2 times compared with intact animals and amounted to 1.11 ± 0.06 r.u., which significantly differs from the value in the group of intact animals (p <0.05).

When modelling retinal pathology and correction of asialoEPO at doses of 0.4 μg/kg and 2.4 μg/kg 30 minutes before the induction of ischemia after 72 h of reperfusion, the b/a coefficient increases dose-dependently to 2.17 ± 0.09 r.u. and 2.41 ± 0.12 r.u. accordingly, which indicates the functional safety of the retinal layer after modelling the pathology.

At the same time, the values achieved during the correction of the pathology with asialoEPO at high doses significantly differed from the ischemia-reperfusion group (p<0.05) and were statistically insignificant in comparison with the group of intact animals.

The activity of the study drug at a high dose significantly exceeded the retinoprotective properties of recombinant EPO (p <0.05), against the background of the use at a

dose of 50 IU/kg 30 minutes before ischemia, after 72 h of reperfusion, the b/a coefficient was 2.11 ± 0.06 r.u.

The use of the K+ATP channel blocker glibenclamide (5 mg / kg) in groups of correction with asialoEPO and recombinant EPO led to a decrease in the b/a coefficient to values significantly different from those of the group of intact animals.

CONCLUSION

As a result of the studies, it can be concluded that there is a retinoprotective effect of asialized erythropoietin at doses of 0.4 μg/kg and 2.4 μg/kg in the rat retinal ischemia-reperfusion model. The retinoprotective effect of asialized erythropoietin is confirmed by a significant increase in the level of microcirculation and the b/a coefficient of the electroretinogram of experimental animals after 72 h of reperfusion compared to the control group.

The administration of glibenclamide prevented the correction of ischemic retinal damage due to the blockade of ATP-dependent potassium channels, which confirms the presence of preconditioning properties of asialized erythropoietin in the retinal ischemia-reperfusion model.

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