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Protective Effects of Recombinant Erythropoietin in Ischemia of the Retina: The Role of Mechanisms of Preconditioning

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Abstract: Recombinant erythropoietin in a dose 50 IU kg $^{-1}$ under the condition of half-hour total ischemia of the retina followed by reperfusion showed a pronounced protective action, expressed in the prevention of reduction of the microcirculation, electroretinography amplitude and change of the morphometric values of layers thickness of the retina. The mechanisms of pharmacological preconditioning are important to cardioprotective effects that confirmed by reduction of its effects under the condition of K_{ATP} channels blockade with glibenclamidum.

Key words: Ischemia/reperfusion of the retina, recombinant erythropoietin, ischemic preconditioning, glibenclamidum, reperfusion

INTRODUCTION

At the present time such drugs as vasodilators, fibrinolytic agents, antithrombotic agents, vasoprotective agents, biogenic stimulators, vitamins, immunomodulatory drugs, antioxidants are used for treatment of ischemic and reperfusion damage to the retina with includes diabetic and hypertensive retinopathy (Brodovsky *et al*., 2000). The treatment with these drugs has not always working, therefore expand the arsenal of drugs for treatment of eye diseases associated with ischemia is relevant problem.

Ischemia of the retina has a leading part in pathogenesis of the vascular disorders of fundus of the eye therefore, the problem of preventive measures and treatment of ischemic diseases of the retina is relevant for ophthalmology and pharmacology (Grozdanic *et al*., 2003).

This problem can be solved with using of pharmacological preconditioning which activates endogenous protection mechanism and reducing the severity of injuries by following ischemia for a long time.

In our opinion, recombinant erythropoietin is the most promising for researching. This pharmacological agent put in a strong performance in the earlier studies on the protective effects of preconditioning under the condition of ischemia/reperfusion of brain, heart, hind limb, kidney, placenta, liver (Kolesnik *et al*., 2011).

MATERIALS AND METHODS

The experiments were performed on the 140 white Wistar rats weighing 225-275 g. Simulation of the ischemia of the retina was performed by mechanic pressure (110 mm Hg) on the anterior eye during half-hour under anesthesia (aqua solution of chloral hydrate in dose 300 mg kg^{-1}). Measurement of the microcirculation in the retina was performed with Laser Doppler Flowmetry (LDF) and electrophysiological condition of the retina was performed with electroretinography at 1 and 72 h after reperfusion (Shabelnikova *et al*., 2014a, b). After it ophthalmectomy was performed in all animals for morphological examination (Peresypkina *et al*., 2014). Recombinant erythropoietin ("AEpocrin" GosNII OCHB (State Research Institute of especially pure biological products)) was administrated intraperitoneally in one dose 50 IU kg^{-1} for half-hour to simulation of the ischemia. Glibenclamidum ("Maniil" (Berlin-Chemie AG/Menarini group) was administrated intragastric in one dose 5 mg kg^{-1} for hour to simulation of the ischemia in order to confirm the effect of preconditioning (Peresypkina *et al*., 2014). Distant ischemic preconditioning was performed with application of tourniquet to proximal one-third of the thigh for 10 and 40 min before the simulation ischemia of the retina. Distant ischemic preconditioning episode was followed by half-hour reperfusion. Morphometry of the retina layers was performed with ImageJ 1.47 program.

RESULTS

Measurement of the microcirculation in the retina was performed with LDF at 1 and 72 h after reperfusion. The results after 1 h of reperfusion are in Table 1.

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Table 1: The level of microcirculation in the retina of rats after 1 and 72 h after reperfusion, p.u. (M±M)

Experimental groups	level of microcirculation after 1 h of reperfusion	level of microcirculation after 72 h of reperfusion
Intact rats (n = 10)	738.9±37.6^{y}	743.9±05.0^{y}
Control (n = 10)	1155.0±51.9*	353.3±11.7*
Correction by distant ischemic preconditioning (n = 10)	952.0±25.8*^{y}	638.5±15.8*^{y}
Correction by recombinant erythropoietin in a dose 50 IU kg^{-1} (n = 10)	798.5±12.3^{y}	724.0±04.1^{y}
Control+glibenclamidum, 5 mg kg^{-1} (n = 10)	1135.8±31.2*	359.4±10.3*
Correction by distant ischemic preconditioning+glibenclamidum, 5 mg kg^{-1} (n = 10)	1144.7±20.7*	361.7±13.9*
Correction by recombinant erythropoietin in a dose 50 IU kg^{-1}+glibenclamidum, 5 mg kg^{-1} (n = 10)	1148.5±14.3*	372.3±13.4*

*Significant difference from group of intact rats ($p<0.05$); ^{y}significant difference from control group ($p<0.05$)

Table 2: Electrophysiological condition of the retina after 1 h of reperfusion (M±M)

Experimental groups	b/a, c.u. after 1 h of reperfusion	b/a, c.u. after 72 h of reperfusion
Intact rats (n = 10)	2.6±0.09^{y}	2.6±0.09^{y}
Control (n = 10)	2.0±0.09*	1.3±0.05*
Correction by distant ischemic preconditioning (n = 10)	2.3±0.07*^{y}	2.4±0.08^{y}
Correction by recombinant erythropoietin in a dose 50 IU kg^{-1} (n = 10)	2.5±0.10^{y}	2.5±0.07^{y}
Control+glibenclamidum, 5 mg kg^{-1} (n = 10)	2.2±0.06*	1.2±0.05*
Correction by distant ischemic preconditioning+glibenclamidum, 5 mg kg^{-1} (n = 10)	2.1±0.08*	1.3±0.07*
Correction by recombinant erythropoietin in a dose 50 IU kg^{-1}+glibenclamidum, 5 mg kg^{-1} (n = 10)	2.2±0.09*	1.3±0.06*

The level of microcirculation in the retina in group of intact rats was 738.9±37.6 p.u. (perfusion unit). The level of microcirculation in the retina after 1 h of reperfusion in control group was 1155.0±51.9 p.u. that significantly higher than in group of intact rats $p<0.001$). The level of microcirculation in the retina in the course of the correction of ischemia of the retina by distant ischemic preconditioning after 1 h of reperfusion was down to 952.0±25.8 p.u. ($p<0.05$ compared with group of intact rats).

The level of microcirculation in the retina in the course of the correction of ischemia of the retina by recombinant erythropoietin was down to 798.5±12.3 p.u. and significantly differenced from control group ($p<0.001$). Administration of glibenclamidum in groups with course of the correction of ischemia of the retina by distant ischemic preconditioning and with correction by recombinant erythropoietin prevented a decreasing of the level of microcirculation in the retina. It confirms the presence of preconditional activity of recombinant erythropoietin in dose 50 IU kg^{-1} under the condition of ischemia of the retina after 1 h of reperfusion. The level microcirculation values after 72 h of reperfusion are in Table 1.

The level of microcirculation in the retina in group of intact rats was 743.9±5.0 p.u. The level of microcirculation in the retina under the condition of ischemia of the retina after 72 h of reperfusion in control group was 353.3±11.7 p.u. that significantly lower than in group of intact rats $p<0.001$). It indicates the formation of ischemia after 72 h of reperfusion. The level of microcirculation in the retina in the course of the correction of ischemia of the retina by distant ischemic preconditioning after 72 h of reperfusion increased to 638.5±15.8 p.u. ($p<0.05$ compared with group of intact rats) (Table 1).

The level of microcirculation in the retina in the course of the correction of ischemia of the retina by recombinant erythropoietin increased to 724.0±4.1 and approached to the level of the group of intact rats.

Administration of glibenclamidum in groups with course of the correction of ischemia of the retina by distant ischemic preconditioning and with correction by recombinant erythropoietin prevented increasing of the level of microcirculation in the retina. It confirms the presence of preconditional activity of recombinant erythropoietin in dose 50 IU kg^{-1} under the condition of ischemia of the retina after 72 h of reperfusion.

Electrophysiological condition of the retina. Electroretinography on evoked potential was performed after simulation of the ischemia and the measurement of the microcirculation level in the retina. The results of the electroretinography are in Table 2.

Coefficient b/a in control group was 2.0±0.09 c.u. and significantly differenced from group of intact rats. It increased in group with course of the correction of ischemia of the retina by distant ischemic preconditioning to 2.4±0.09 c.u. and in group with correction by recombinant erythropoietin to 2.5±0.10 c.u. that indicates saving electrophysiological function of the retina after simulation of ischemia. Administration of glibenclamidum in groups with course of the correction of ischemia of the retina by distant ischemic preconditioning and with correction by recombinant erythropoietin leaded to decreasing of the coefficient b/a compared with group of intact rats It confirms the presence of preconditional activity of recombinant erythropoietin in dose 50 IU kg^{-1} under the condition of ischemia of the retina after 1 hour of reperfusion.

Table 3: Morphometric research of the retinal layers in experimental animals

Experimental groups	1 h of reperfusion		72 h of reperfusion	
	Inner nuclear layer	layer of rods and cones	Inner nuclear layer	Layer of rods and cones
Intact rats (n = 10)	23.5±0.8$^{\gamma}$	38.4±0.8	23.8±1.0$^{\gamma}$	38.1±1.2
Control (n = 10)	25.9±0.6*	39.1±0.7	20.3±0.8*	36.9±0.9
Correction by distant ischemic preconditioning (n = 10)	24.0±0.5$^{\beta}$	38.4±0.9	21.7±0.4*$^{\gamma}$	37.8±0.8
Correction by recombinant erythropoietin in a dose 50 IU kg^{-1} (n = 10)	23.8±0.6$^{\gamma}$	38.3±0.9	23.3±0.7$^{\gamma}$	38.0±1.0
Control+glibenclamidum, 5 mg kg^{-1} (n = 10)	26.0±0.7*	39.1±0.6	20.5±0.4*	37.1±0.8
Correction by distant ischemic preconditioning+glibenclamidum, 5 mg kg^{-1} (n = 10)	25.8±0.6*	39.2±0.6	20.6±0.6*	36.9±0.8
Correction by recombinant erythropoietin in a dose 50 IU kg^{-1}+glibenclamidum, 5 mg kg^{-1} (n = 10)	25.7±0.6*	39.0±0.5	20.3±0.5*	37.0±0.9

*Significant difference from group of intact rats ($p<0.05$); $^{\gamma}$significant difference from control group ($p<0.05$)

Electroretinography after 72 h of reperfusion was performed after measurement of the microcirculation level in the retina. The results of the electroretinography are in Table 2.

Coefficient b/a in control group was 1.3±0.05 c.u. and significantly differenced from group of intact rats. It increased in group with course of the correction of ischemia of the retina by distant ischemic preconditioning to 2.4±0.08 c.u. and in group with correction by recombinant erythropoietin to 2.5±0.07 c.u. that indicates saving electrophysiological function of the retina after simulation of ischemia. Administration of glibenclamidum in groups with course of the correction of ischemia of the retina by distant ischemic preconditioning and with correction by recombinant erythropoietin leaded to decreasing of the coefficient b/a compared with group of intact rats. It confirms the presence of preconditional activity of recombinant erythropoietin in dose 50 IU kg^{-1} under the condition of ischemia of the retina after 72 h of reperfusion.

Decreasing of coefficient b/a in control group caused by depression of positive wave b of electroretinography. It indicates dysfunction of bipolar cells and mueller cells with the possible contribution of horizontal cells and amacrine cells. Electrophysiological function retention was confirmed with the absence of changes in the negative wave a.

Results of the morphological examination of the retina. The structure of the inner nuclear layer, outer plexiform layer and outer nuclear layer was preserved in group of animals with course of the correction of ischemia of the retina by distant ischemic preconditioning and in group with correction by recombinant erythropoietin. In the control group, there were outer plexiform layer rarefaction and separation of the layer of rods and cones from the retinal pigment epithelium. After administration of glibenclamidum distant ischemic preconditioning and recombinant erythropoietin didn't have a protective activity and morphological pattern of the retina in these groups didn't differ from the control group.

Inner nuclear layer and the layer of rods and cones were researched with morphometric study. The results of the morphometric study are in Table 3.

According to morphometric study, there were not significant differences in thickness of layer of rods and cones between experimental groups, that confirmed the absence of changes in the negative wave a.

The inner nuclear layer was the most sensitive to the ischemia/reperfusion. Increase in thickness of it after 1 h of reperfusion was associated with edema and its thinning after 72 h of reperfusion was associated with degenerative processes in the retina.

In the group of animals with correction by recombinant erythropoietin thickness of the inner nuclear layer after 1 h of reperfusion was 23.8±0.62 Fm and after 72 h of reperfusion it was 23.7±0.71 Fm and significantly differenced from group of intact rats.

In the group of animals with correction by distant ischemic preconditioning thickness of the inner nuclear layer after 1 h of reperfusion was 24.0±0.53 Fm and after 72 h of reperfusion it was 23.6±0.52 Fm and significantly differenced from group of intact rats.

Prior administration of glibenclamidum was associated with elimination of protective activity of distant ischemic preconditioning and recombinant erythropoietin. It confirms the presence of antiischemic activity of distant ischemic preconditioning and recombinant erythropoietin in dose 50 IU kg^{-1} under the condition of ischemia of the retina due to the preconditioning effect.

DISCUSSION

Taking into account the results of research we may be concluded that the correction of the ischemic damage of retina of the laboratory rats is associated with preconditioning activity of the distant ischemic preconditioning and recombinant erythropoietin in dose 50 IU kg^{-1}.

Proven ability of the recombinant erythropoietin to activate protein kinase suggests its triggered role in the

implementation of pharmacological preconditioning effects. This fact confirms the assumption that the recombinant erythropoietin is the trigger of the ischemic preconditioning and natural anti-ischemic mechanisms are realized by the activation of K_{ATP} channels, synthesis of nitric oxide and pharmacological preconditioning mimetic (Baker, 2005; Pain *et al*., 2000; Armstrong *et al*., 1995).

K_{ATP} channels take important part in cytoprotective effect of ischemic preconditioning and opening during ischemia. Initially, their activity was detected at sarcolemma and later at the mitochondrial level. All isoforms are inhibited by physiological concentrations of intracellular ATP and opening when the ATP concentration is significantly reduced, so it acts as sensors the availability of adequate oxygen and glucose (ATP source) (Danilenko *et al.,* 2010; Baines *et al*., 1999)

Absence of the protective effect in group with correction by distant is chemic preconditioning+ glibenclamidum and in group with correction by recombinant erythropoietin+glibenclamidum indicates retionoprotection associates with preconditioning activity and K_{ATP} channels are instrumental in preconditioning effect (Peresipkina *et al*., 2014).

Given the fact that results of the electrophysiological examination are often crucial in the early and differential diagnosis of retinal disorders for the study of the correction of degenerative changes in the retina an influence over the morpho-functional state of the retina a comprehensive analysis including electroretinography, morphological and microcirculatory examinations was performed.

CONCLUSION

The results of research indicate that the pharmacological preconditioning by the recombinant erythropoietin has anti-ischemic activity. It is confirmed by the significant increase of electroretinography coefficient b/a, the prevention of the microcirculation reduction, improving histologic pattern of the retina and preservation of the morphometric values. After 72 h of reperfusion morphometric values didn't have significant differences from the control group.

Prior administration of glibenclamidum was associated with elimination of protective activity of distant ischemic preconditioning and pharmacological preconditioning that led to the microcirculation reduction and decrease of electroretinography coefficient b/a, worsening of the histologic pattern of the retina due to the K_{ATP} channels blockade.

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