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Address: 1 Kraljice Natalije Street, 11000 Belgrade, Serbia
☎ +381 11 4092 776, Fax: +381 11 3348 653
E-mail: office@srpskiarhiv.rs, Web address: www.srpskiarhiv.rs

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Anna A. Peresypkina†, Victoria O. Gubareva, Elena A. Levkova,
Anna S. Shabelnikova, Mikhail V. Pokrovskii

Pharmacological correction of retinal ischemia/reperfusion by minoxidil

Фармаколошка корекција ретиналне исхемије-реперфузије миноксидалом

Belgorod State University, Belgorod, Russia

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† **Correspondence to:**
Anna A. PERESYPKINA
85 Pobeda Street, 308015 Belgorod, Russia
E-mail: **peresypkina_a@bsu.edu.ru**

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SUMMARY

Introduction/Objective The objective of this paper was to increase the effectiveness of pharmacological correction of retinal ischemia-reperfusion by using minoxidil.

Methods The research was carried out on 180 Wistar rats. The modification of the retinal ischemia-reperfusion model was used, in which, increase in intraocular pressure is carried out by mechanical pressure (110 mm Hg) to the front chamber of the eye for 30 min. Protective effects of minoxidil in a dose 0.5 mg/kg on the model of retinal ischemia-reperfusion were estimated by the changes in the level of retinal microcirculation (laser Doppler flowmetry), electroretinogram amplitude, morphometry of retinal layers after 1 hour and 72 hours of reperfusion.

Results Minoxidil in a dose 0.5 mg/kg of rat mass improves retinal microcirculation, its electrophysiological state after 1 hour and 72 hours of reperfusion, and, prevents the development of degenerative changes in the retina caused by ischemic damage to a greater extent than recombinant erythropoietin in a dose 50 IU/kg and sildenafil in a dose 0.5 mg/kg in monotherapy. The protective effects of minoxidil were eliminated by the preliminary administration of glibenclamide in a dose 5 mg/kg, which indicates the presence of the preconditioning effect of minoxidil, realized through ATP-dependent potassium channels.

Conclusion Minoxidil in a dose 0.5 mg/kg of rat mass protects the retina from ischemic/reperfusion injury. Protective effects of minoxidil is carried out by preconditioning action, as evidenced by the lack of positive effects with administration of glibenclamide.

Keywords: ischemia-reperfusion; retina; minoxidil; erythropoietin; sildenafil; ATP-dependent potassium channels

САЖЕТАК

Увод/Циљ Циљ студије јесте да се побољша ефикасност фармаколошке корекције исхемије-реперфузије мрежњаче миноксидилом.

Методе Истраживање је спроведено на 180 пацова врсте Вистар. Коришћена је модификација модела исхемије-реперфузије, при чему се повећање интраокуларног притиска вршило механичким притиском (110 mm Hg) на предњу комору ока у трајању од 30 минута. Заштитне ефекте миноксидила у дози од 0,5 мг/кг процијењене су на основу промена микроциркулације мрежњаче (ласерска доплер-флоуметрија), амплитуде електроретинограма, морфометрије слојева мрежњача након 1 и 72 сата од реперфузије.

Резултати Миноксидил у дози од 0,5 мг/кг масе пацова побољшава микроциркулацију мрежњаче, њено електрофизиолошко стање после 1 и 72 сата реперфузије и спречава развој дегенеративних промена слојева мрежњаче изазваних исхемијом више него монотерапија рекомбинантним еритропоетином у дози од 50 IU/кг и силденафилом у дози од 0.5 мг/кг. Заштитно дејство миноксидила елиминише се давањем глибенкламида у дози од 5 мг/кг, што доказује прекондиционирани ефекат код миноксидила, који је остварен кроз АТП-зависне калијумове канале.

Закључци Миноксидил у дози од 0,5 мг/кг штити мрежњачу од исхемије / реперфузионе повреде. Заштитни ефекат миноксидила се реализује посредством прекондиционирања, што доказује недостатак позитивних ефеката на примену глибенкламида.

Кључне речи: исхемије-реперфузија; ретина; миноксидил; еритропоетин; силденафил; АТП зависни канали калијума

INTRODUCTION

Local circulatory disorders in the branches of retinal artery are observed in diabetic retinopathy, hypertensive retinopathy, degenerative diseases of the retina, optic nerve atrophy vascular origin, traumatic eye injury, ischemic neuropathy [1, 2, 3].

Studying the way of how to improve tissue tolerance to ischemia is an actual problem of modern experimental and clinical pharmacology. Up to now, the treatment of ischemic retinal conditions was done by use of angioprotectors, antioxidants, fibrinolytics, anticoagulants and others. As the authors note, due to the instability and short-term effects after using these drugs in combination with other drugs and physiotherapy treatments is necessary to seek out a more effective way to

improve blood circulation and increase resistance to ischemic retinal tissue having a specific orientation [4].

Thus, an important task is to find new, specific and highly effective means for correcting of retinal ischemia.

Therefore, the objective of the study is to increase the effectiveness of pharmacological correction of retinal ischemia-reperfusion by using minoxidil.

METHODS

Experiments were carried out on 180 Wistar rats weighing 250 ± 25 g. For the study, the rats were taken with no external signs of disease, passed quarantine regime.

Ethical principles of handling laboratory animals are observed in accordance with the «European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes. CETS No. 123».

Minoxidil, 0.5 mg/kg, was administered intragastrically (i/g) once 1 hour before ischemia-reperfusion modeling.

Recombinant erythropoietin (EPO) was administered intraperitoneally (i/p) once in a dose 50 IU/kg 30 min before pathology modeling for the purpose of preconditioning [5] as a reference drug.

Animals received i/p injection of sildenafil in a dose 0.5 mg/kg once 30 min before pathology modeling.

Glibenclamide was administered in a dose 5 mg/kg i/g once 1 hour before ischemia-reperfusion modeling.

Ischemia-reperfusion injury of the retina was simulated under anesthesia (chloral hydrate, 300 mg/kg of animal body weight, i.p.) by applying mechanical pressure (110 mm Hg) to the anterior eye chamber for 30 min [4].

The experiment included 2 series of animals (with an assessment of the parameters after 1 hour and 72 hours of reperfusion), 9 groups in each series, 10 rats in each group.

Measuring the level of retinal microcirculation of rats were carried out by laser Doppler flowmetry (LDF) [6] after 1 hour and 72 hours of reperfusion. Registration is carried out by means of hardware and software Biopac-systems MP-150 and the needle-type sensor TSD-144 (USA) with AcqKnowledge 4.2 program. After animal anesthesia assessment of microcirculation level was carried out in ten points on the circumference of the eyeball, the recording duration of the microcirculation level readings at one point was 20 seconds. From the microcirculation level results at every point the average value has been calculated, which was taken as the indicator of the microcirculation level in the retina of the experimental animal. Value of microcirculation in the animal group was calculated as the average of the values obtained from each experimental animal in group.

To perform electroretinography, (ERG) after 1 hour and 72 hours of reperfusion rats were kept in the dark for 30 min [7], then, anesthetized (chloral hydrate, 300 mg/kg, i/p), and fixed on the table

isolated from the electromagnetic radiation. Strobe flash of white light that was connected to the stimulator STM200 by Biopac System, Inc. (USA), placed behind the animal; ERG registration was carried out in response to a single stimulation. Evoked biopotentials were run at a frequency 1-1000 Hz, amplified, averaged, and, presented graphically on the screen using the Biopac-systems MP-150 with a computer program AcqKnowledge 4.2 (USA). ERG recording was carried out for 0.5 sec in each rat in the groups. To assess the degree of retinal ischemia, the ratio of the amplitudes of the a- and b- waves of ERG - the coefficient b/a was evaluated [7]. From 10 values received, the mean was derived for each group, which is introduced into the protocol.

After the LDF and ERG, eyes with surrounding tissues were subject to enucleation in both series of experiments. Eyes with immediately adjacent tissues were fixed in 10% formalin solution for histological research. After fixation, the eyes were cut into two parts in the meridian direction strictly through the center and both halves were poured into paraffin by a standard procedure. Sections for standard histological examination were stained with hematoxylin-eosin. A descriptive study of histological preparations was performed under a microscope Axio Scope A1 (Carl Zeiss Microimaging GMbH, Germany). The morphometric studies were performed on the microscope Mikmed-5, JSC "LOMO" with use the program Micro-Analysis View [8].

RESULTS

After the pathology modeling after 1 h and 72 h of reperfusion, microcirculation was measured in the retina by LDF, electrophysiological condition of the retina by ERG was determined, the extirpation of animals were carried out and enucleation of eyes for morphological studies.

After the pathology modeling, microcirculation level measurement was performed after 1 h and 72 h of reperfusion by LDF. The results obtained after 1 h of reperfusion, presented in table 1.

The level of microcirculation after ischemia modeling in the control group reached 1155.0±51.9 PU after 1 h of reperfusion, which was significantly higher than the value in the group of intact animals ($p < 0.05$).

With the correction of pathology by MIN, microcirculation level in the retina after 1 h of reperfusion decreased to 751.3±21.8 PU, which was significantly different from the control group ($p<0,05$).

Table 1. The level of retinal microcirculation after 1 h and 72 h of reperfusion (M ± m), PU.

	The experimental groups	Level of microcirculation after 1 h of reperfusion, PU (n = 10)	Level of microcirculation after 72 h of reperfusion, PU (n = 10)
1	Intact	738.9±37.6	743.9±5.0
2	Control (ischemia)	1155.0±51.9*	353.3±11.7*
3	Control + MIN	751.3±21.8†	739.5±14.1†
4	Control + EPO	798.5±12.3†	724.0±4.1†
5	Control + SIL	832.3±20.1†	711.5±15.3^{y}
6	Control + Glib	1135.8±31.2*	359.4±10.3*
7	Control + MIN + Glib	1149.8±18.6*	361.1±10.9*
8	Control + EPO + Glib	1148.3±15.3*	372.3±13.4*
9	Control + SIL + Glib	1151.2±31.9*	360.3± 2.1*

* - $p<0,05$ compared with intact rats; † - $p<0,05$ compared with control group; MIN – minoxidil; EPO – recombinant erythropoietin; Glib - glibenclamide; SIL – sildenafil; PU – perfusion units

With the correction of pathology by EPO, microcirculation level in the group is reduced to 798.5±12,3 PU, and, was significantly different from the values in the control group ($p<0.05$).

Introduction of glibenclamide, a blocker of ATP-sensitive potassium channels, prevented reduction of microcirculation in groups with correction by MIN; EPO; SIL; that confirms the preconditioning action of this drugs in studied doses on retinal ischemia-reperfusion model on rats after 1 h of reperfusion.

The level of microcirculation after the pathology modeling in the control group after 72 h of reperfusion was 353.3±11.7 PU, that was significantly lower than in the group of intact animals ($p<0.05$). In group with correction by MIN this rate increased to 739.5±14.1 PU ($p<0.05$), that was significantly different from the values in the control group.

Correction of the modeled pathology by EPO led to increase of microcirculation level in group to 724.0 ± 4.1 PU, that was significantly different from the values in control group ($p<0.05$).

Introduction of glibenclamide in groups with MIN-correction; EPO-correction; SIL-correction, prevented improving of microcirculation level after 72 h of reperfusion.

After the pathology modeling and measuring of microcirculation level in the retina, ERG on evoked potential was performed. The results obtained after 1 h and 72 h of reperfusion are shown in table 2.

Table 2. Results of evaluation of electrophysiological retinal function after 1 h and 72 h of reperfusion (M ± m).

	The experimental groups	Ratio b/a after 1 h of reperfusion (n=10)	Ratio b/a after 72 h of reperfusion (n=10)
1	Intact	2.6 ± 0.09^{y}	2.5 ± 0.10^{y}
2	Control (ischemia)	2.0 ± 0.09*	1.2 ± 0.04*
3	Control + MIN	2.5 ± 0.06†	2.4 ± 0.09†
4	Control + EPO	2.5 ± 0.10†	2.3 ± 0.06†
5	Control + SIL	2.4 ± 0.09*†	2.3 ± 0.09†
6	Control + Glib	2.0 ± 0.08*	1.3 ± 0.04*
7	Control + MIN + Glib	2.1 ± 0.09*	1.3 ± 0.06*
8	Control + EPO + Glib	2.1 ± 0.09*	1.2 ± 0.07*
9	Control + SIL + Glib	2.0 ± 0.08*	1.2 ± 0.08*

* - p<0,05 compared with intact rats; † - p<0,05 compared with control group; MIN – minoxidil; EPO – recombinant erythropoietin; Glib - glibenclamide; SIL – sildenafil.

Ratio b/a in control group was 2.0±0.09 after 1 h of reperfusion, which was significantly different from the values in the group of intact animals ($p<0.05$). In the group of animals with correction by MIN ratio b/a was 2.5±0.06 after 1 h of reperfusion, which was significantly different from the group's ratio with retinal ischemia and approached the values in the group of intact animals ($p<0.05$). Increase of this indicator in the group with the correction by EPO to 2.5±0.10, by SIL – up to 2.4±0.09 after 1 h of reperfusion, confirms the saving of retinal electrophysiological function after pathology modeling.

The coefficient b/a in the control group after 72 h of reperfusion was 1.2±0.04, that was significantly different from that of the group of intact animals. In the group of animals with correction by MIN ratio b/a was 2.4 ± 0.09, which was significantly different from that of the group with retinal ischemia ($p<0.05$) and approached the values in the group of intact animals. The increase of this indicator in the group with the correction by EPO to 2.3±0.06, SIL – up to 2.3±0.09 confirms the maintaining of electrophysiological retinal function after pathology modeling.

Introduction of glibenclamide in groups with correction by MIN; EPO; SIL in monotherapy decreased the ratio b/a to values significantly different from the group of intact rats, indicating the blockade of the ATP-dependent potassium channels.

Decrease in ratio b/a in animals with ischemia (control) due to inhibition of the positive b-wave of ERG indicates violation of electrophysiological function of bipolar and Muller cells with the possible contribution of the horizontal and amacrine cells. Saving the electrophysiological function of the photoreceptor layer is confirmed by the absence of changes in the negative a-wave (Table 2).

During the morphometric analysis of the thickness of inner nuclear layer and a layer of photoreceptors, the increase of thickness of the inner nuclear layer was determined up to 25.9 ± 0.6 μm in the control group after 1 h of reperfusion, which is significantly different from the values in the group of intact rats ($p<0.05$) (Table 3).

Table 3. Morphometric values of retinal layers of experimental animals after 1 h and 72 h of reperfusion (M ± m).

		1 h of reperfusion (n = 10)		72 h of reperfusion (n = 10)	
	The experimental groups	**The thickness of the inner nuclear layer, (μm)**	**The thickness of layer of photoreceptors, (μm)**	**The thickness of the inner nuclear layer, (μm)**	**The thickness of layer of photoreceptors, (μm)**
1	Intact	23.5±0.8†	38.4±0.8	23.8±1.0†	38.1±1.2
2	Control (ischemia)	25.9±0.6*	39.1±0.7	20.3±0.8*	36.9±0.9
3	Control + MIN	23.7±0.6†	38.4±0.9	23.5±0.5†	37.9±0.9
4	Control + EPO	23.8±0.6†	38.6±0.9	23.3±0.7†	38.0±1.0
5	Control + SIL	24.0±0.7†	39.1±0.6	22.7±0.6†	37.3±0.7
6	Control + Glib	25.8±0.6*	39.2±0.6	20.6±0.6*	36.9±0.9
7	Control + MIN + Glib	25.7±0.6*	39.0±0.5	20.3±0.5*	37.2±1.0
8	Control + EPO + Glib	25.3±0.4*	38.6±0.6	20.5±0.5*	37.6±1.1
9	Control + SIL + Glib	25.7±0.5*	39.0±0.7	20.5±0.8*	38.0±1.2

* - $p<0,05$ compared with intact rats; † - $p<0,05$ compared with control group; MIN – minoxidil; EPO – recombinant erythropoietin; Glib - glibenclamide; SIL – sildenafil.

In groups with MIN-correction the thickness of inner nuclear layer was 23.7±0.6 μm after 1 h of reperfusion, that differs significantly from the values of control group ($p<0.05$). Prior administration of EPO reduces the thickness of the inner nuclear layer to 23.8±0.6 μm, which was significantly different from the control group and approaches the values in the group of intact animals ($p<0.05$). In groups with SIL-correction, the thickness of inner nuclear layer was 24.0±0.7 μm after 1 h of reperfusion, that also differs significantly from the values of control group ($p<0.05$).

Prior administration of glibenclamide in groups with MIN-correction; EPO-correction; SIL-correction, after 1 h of reperfusion, led to an increase of the thickness of the inner nuclear layer – group values were 25.7±0.6 μm; 25.3±0.4 μm; 25.7±0.5 μm respectively.

The inner nuclear layer thickness was 20.3±0.8 μm after 72 h in the control group, which is significantly different from the group of intact animals ($p<0.05$). In the group of animals with MIN, EPO, the inner nuclear layer thickness after 72 h of reperfusion were 23.5±0.5 μm; 23.3±0.7 μm respectively, that significantly different from the values of the group with ischemia. In the group with

SIL the inner nuclear layer thickness was 22.7±0.6 μm, that also differs significantly from the values of control group ($p<0.05$).

DISCUSSION

The search of new methods of retinoprotection for possible reduction of the damaging effect of ischemia, formed in various systemic diseases, is an urgent task of pharmacology and ophthalmology. Segment of drugs for the treatment of vascular diseases of the eye such as complication from hypertension, diabetes, and others, is expedient to expand due to an increase in morbidity and lack of funds for targeted correction of ischemic lesions of the eye vessels.

Based on the fact that electrophysiological studies often have a decisive importance in the early and differential diagnosis of retinal disorders [9], to study the correction of functional changes in the retina, researcher must conduct a comprehensive analysis, including electroretinography and microcirculation research. Analysis of the dynamics of retinal electrogenesis allows to evaluate the nature and topography of retinal disorders, as well as to identify the most labile hypoxic retinal structure and their reaction to the correction by the medications.

The most pronounced retinal protective action was observed in groups with pharmacological preconditioning by minoxidil in a dose 0.5 mg/kg after 1 h and 72 h of reperfusion.

A single i/g injection of minoxidil in a dose 0.5 mg/kg, 60 min before modeling of retinal ischemia/reperfusion, significantly reduced the level of retinal microcirculation after 1 h of reperfusion, and saved the retinal electrophysiological activity, that also confirmed by morphometric of retinal layers. Found that minoxidil prevents the retinal layers' damages caused by ischemic injury to a greater extent than recombinant erythropoietin in a dose 50 IU/kg and sildenafil in a dose 0.5 mg/kg in monotherapy after 1 h of reperfusion and degenerative changes of retinal layers after 72 h of reperfusion.

Prior administration of glibenclamide in a dose 5 mg/kg eliminated the positive effects of minoxidil, erythropoietin, sildenafil, that confirms the implementation of retinal protection by preconditioning with the participation of ATP-dependent potassium channels.

CONCLUSION

Minoxidil in a dose 0.5 mg/kg protects the retina from ischemic/reperfusion injury better than recombinant erythropoietin in a dose 50 IU/kg and sildenafil in a dose 0.5 mg/kg in monotherapy.

Protective effects of minoxidil is carried out by preconditioning action as evidenced by the lack of positive effects with administration of glibenclamide.

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