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ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

Pharmacological correction of retinal ischemia/ reperfusion by minoxidil

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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. A modification of the retinal ischemia-reperfusion model was used, in which an increase in intraocular pressure is carried out by mechanical pressure (110 mmHg) to the front chamber of the eye for 30 minutes. Protective effects of minoxidil at 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 at 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 at a dose of 50 IU/kg and sildenafil at a dose of 0.5 mg/kg in monotherapy. The protective effects of minoxidil were eliminated by the preliminary administration of glibenclamide at a dose of 5 mg/kg, which indicates the presence of the preconditioning effect of minoxidil, realized through adenosine triphosphate-dependent potassium channels.
Conclusion Minoxidil at a dose of 0.5 mg/kg of rat mass protects the retina from ischemic-reperfusion injury. Protective effects of minoxidil are carried out by a preconditioning action, as evidenced by the lack of positive effects with the administration of glibenclamide.
Keywords: ischemia-reperfusion; retina; minoxidil; erythropoietin; sildenafil; ATP-dependent potassium channels

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 were 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 at a dose of 50 IU/kg 30 minutes before pathology modeling for the purpose of preconditioning as a reference drug [5].

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

Glibenclamide was administered at a dose of 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 mmHg) to the anterior eye chamber for 30 minutes [4].

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

The measuring of the level of retinal microcirculation of rats was carried out by laser Doppler flowmetry (LDF) after 1 hour and 72 hours of reperfusion [6]. Registration was carried out by MP150 data acquisition and analysis systems and the TSD144 needle-type sensor, with AcqKnowledge 4.2 software (BIOPAC Systems, Inc., Goleta, CA, USA). After animal anesthesia, assessment of microcirculation level was carried out at 10 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 was calculated, which was taken as the indicator of the microcirculation level in the retina of the experimental animal. The value of microcirculation in the animal group was calculated as the average of the values obtained from each experimental animal in the group.

To perform electroretinography (ERG), after 1 hour and 72 hours of reperfusion, rats were kept in the dark for 30 minutes, then, anesthetized (chloral hydrate, 300 mg/kg, i/p), and fixed on the table isolated from electromagnetic radiation [7]. Strobe flash of white light that was connected to the STM200 stimulator (Biopac Systems, Inc.) and placed behind the animal; ERG registration was carried out in response to a single stimulation. Evoked biopotentials were run at a frequency of 1–1000 Hz, amplified, averaged, and presented graphically on the screen using the MP150 data acquisition and analysis system and the aforementioned software. The ERG recording was carried out for 0.5 seconds 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 was introduced into the protocol.

After the LDF and ERG, eyes with surrounding tissues were subjected 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 according to the standard procedure. Sections for the standard histological examination were stained with hematoxylin-eosin. A descriptive study of histological preparations was performed under a Axio Scope A1 microscope (Carl Zeiss Microscopy GMbH, Jena, Germany). The morphometric studies were performed on the Mikmed-5 microscope with the use of the Micro-Analysis View software (LOMO, JSC, Saint Petersburg, Russia) [8].

RESULTS

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

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

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

No.	Experimental groups	Level of microcirculation after 1 hour of reperfusion, PU (n = 10)	Level of microcirculation after 72 hours of reperfusion, PU (n = 10)
1	Intact	738.9 ± 37.6	743.9 ± 5.0
2	Control (ischemia)	1,155.0 ± 51.9*	353.3 ± 11.7*
3	Control + MIN	751.3 ± 21.8y	739.5 ± 14.1y
4	Control + EPO	798.5 ± 12.3y	724.0 ± 4.1y
5	Control + SIL	832.3 ± 20.1y	711.5 ± 15.3y
6	Control + Glib	1,135.8 ± 31.2*	359.4 ± 10.3*
7	Control + MIN + Glib	1,149.8 ± 18.6*	361.1 ± 10.9*
8	Control + EPO + Glib	1,148.3 ± 15.3*	372.3 ± 13.4*
9	Control + SIL + Glib	1,151.2 ± 31.9*	360.3 ± 12.1*

Glib – glibenclamide; SIL – sildenafil; PU – perfusion units; MIN – minoxidil; EPO – recombinant erythropoietin;
*p < 0.05 compared to intact rats;
yp < 0.05 compared to the control group

The level of microcirculation after ischemia modeling in the control group reached 1,155.0 ± 51.9 PU after 1 hour of reperfusion, which was significantly higher than the value in the group of intact animals ($p < 0.05$).

With the correction of pathology by minoxidil (MIN), microcirculation level in the retina after 1 hour of reperfusion decreased to 751.3 ± 21.8 PU, which was significantly different from the control group ($p < 0.05$).

With the correction of pathology by EPO, microcirculation level in the group was 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 adenosine triphosphate-sensitive (ATP-sensitive) potassium channels, prevented the reduction of microcirculation in groups with correction by MIN, EPO, sildenafil (SIL); this confirms the preconditioning action of these drugs in studied doses on retinal ischemia-reperfusion model on rats after 1 hour of reperfusion.

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

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

Introduction of glibenclamide in groups with MIN-correction, EPO-correction, and SIL-correction prevented the improvement of the microcirculation level after 72 hours 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 hour and 72 hours of reperfusion are shown in Table 2.

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

No.	Experimental groups	Ratio b/a after 1 hour of reperfusion (n = 10)	Ratio b/a after 72 hours of reperfusion (n = 10)
1	Intact	2.6 ± 0.09y	2.5 ± 0.10y
2	Control (ischemia)	2.0 ± 0.09*	1.2 ± 0.04*
3	Control + MIN	2.5 ± 0.06y	2.4 ± 0.09y
4	Control + EPO	2.5 ± 0.10y	2.3 ± 0.06y
5	Control + SIL	2.4 ± 0.09*y	2.3 ± 0.09y
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*

MIN – minoxidil; EPO – recombinant erythropoietin; Glib – glibenclamide; SIL – sildenafil;
*p< 0.05 compared to intact rats; yp < 0.05 compared to the control group

The b/a ratio in the control group was 2.0 ± 0.09 after 1 hour of reperfusion, which was significantly different from the values in the group of intact animals (p < 0.05). In the group of animals with the correction by MIN, the b/a ratio was 2.5 ± 0.06 after 1 hour 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). An 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 hour of reperfusion, confirms the saving of retinal electrophysiological function after the pathology modeling.

The b/a coefficient in the control group after 72 hours of reperfusion was 1.2 ± 0.04, which was significantly different from that of the group of intact animals. In the group of animals with the correction by MIN, the b/a ratio 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 the pathology modeling.

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

A decrease in the b/a ratio in animals with ischemia (control) due to inhibition of the positive b-wave of ERG indicates a 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.

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

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

Prior administration of glibenclamide in groups with the MIN-correction, EPO-correction, and SIL-correction, after 1 hour 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, and 25.7 ± 0.5 μm, respectively.

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

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

No.	Experimental groups	1 hour of reperfusion (n = 10)		72 hours of reperfusion (n = 10)	
		Thickness of the inner nuclear layer [μm]	Thickness of the photoreceptor layer [μm]	Thickness of the inner nuclear layer [μm]	Thickness of the photoreceptor layer [μm]
1	Intact	23.5 ± 0.8y	38.4 ± 0.8	23.8 ± 1.0y	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 ± 0.6y	38.4 ± 0.9	23.5 ± 0.5y	37.9 ± 0.9
4	Control + EPO	23.8 ± 0.6y	38.6 ± 0.9	23.3 ± 0.7y	38.0 ± 1.0
5	Control + SIL	24.0 ± 0.7y	39.1 ± 0.6	22.7 ± 0.6y	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

MIN – minoxidil; EPO – recombinant erythropoietin; Glib – glibenclamide; SIL – sildenafil;
*p < 0.05 compared to intact rats; yp < 0.05 compared to the control group

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, would be 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, to study the correction of functional changes in the retina, researcher must conduct a comprehensive analysis, including electroretinography and microcirculation research [9]. Analysis of the dynamics of retinal electrogenesis allows to evaluate the nature and topography of retinal disorders and to identify the most labile hypoxic retinal structure, as well as their reaction to the correction by the medications.

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

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

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

CONCLUSION

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

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

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Фармаколошка корекција ретиналне исхемије/реперфузије миноксидалом

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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 *mmHg*) на предњу комору ока у трајању од 30 минута. Заштитни ефекти миноксидила у дози од 0,5 *mg/kg* процењене су на основу промена микроциркулације мрежњаче (ласерска доплер-флоуметрија), амплитуде електроретинограма, морфометрије слојева мрежњаче после једног и 72 сата од реперфузије.

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

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

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