Research Article

Correction of retinal ischemia/reperfusion by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in experiment

Anna A. Peresypkina[1]*, Elena A. Levkova[1], Vladimir I. Yakushev[1], Natalia D. Bunyatyan[2], Pavel A. Galenko-Yaroshevsky[3], Mikhail I. Churnosov[1]

ABSTRACT

Introduction: Studying the way of how to improve tissue tolerance to ischemia is an actual problem of pharmacology. Up to now, the treatment of ischemic retinal conditions was done by use of angioprotectors, antioxidants, fibrinolytics, anticoagulants, and others. Due to the instability and short-term effects after using these drugs and physiotherapy treatments is necessary to seek out a more effective way to improve blood circulation and increase resistance to ischemic retinal tissue. **Research tasks:** The aim of this study is to increase the effectiveness of pharmacological correction of retinal ischemia-reperfusion using agonist of imidazoline receptors Type I, II, 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid. **Methods:** To investigate the fundus of experimental animals, a direct ophthalmoscopy was used. To zoom a lens, Osher MaxField 78D model OI-78M has been used. Electroretinography (ERG) was performed at once after the ophthalmoscopy. To assess the degree of functional damage to the retina, we evaluated the ratio of amplitudes of a- and b-waves - the coefficient b/a. For all data, the descriptive statistics were used, and data are checked for normal distribution. Distribution type was determined using the criterion of Shapiro–Wilk. Between groups, differences were analyzed by parametric (t-Student criterion) or non-parametric (Mann–Whitney test) methods, depending on the type of distribution. Differences were determined at 0.05 significant level. **Results:** The protective effect of agonist of imidazoline receptors Type I, II, 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid, in doses 10 mg/kg and 50 mg/kg on the retinal ischemia-reperfusion model on Wistar rats was studied. In the experiment, it was found that the 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 50 mg/kg prevents the development of neuronal damage in the retina caused by intraocular pressure increase to 110 mmHg within 30 min by applying the mechanical pressure to the anterior chamber of the eye to a greater extent than in a dose 10 mg/kg. The detected protective effects were confirmed by the results of ophthalmoscopy and ERG after 72 h of reperfusion. **Conclusion:** Results of ocular fundus studies revealed the most pronounced protective effects of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 50 mg/kg on the model of retinal ischemia-reperfusion in Wistar rats, which is reflected in the restoration of the optic disc. Correction of retinal ischemia-reperfusion by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 50 mg/kg leads to higher values of the coefficient b/a of ERG after 72 h of reperfusion compared to the group with pathology correction by the same drug in a dose 10 mg/kg, which indicates the restoration of the electrophysiological state of the retina.

KEY WORDS: 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid, Electroretinography, Ophthalmoscopy, Retinal ischemia-reperfusion

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, and ischemic neuropathy.[1-3]

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The search for innovative molecules[4,5] is an important task of pharmacology. Moreover, their study should be carried out on pharmacological targets,[6,7] *in vivo* models.[8,9]

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 the use of angioprotectors, antioxidants, fibrinolytics, anticoagulants, and others. As the authors note, due

[1]Department of Pharmacology, Belgorod State University, Belgorod, Russia, [2]FSBI "Scientific Center for Expertise of Medical Applications" of the Ministry of Health of the Russian Federation, 8(2), Petrovsky boulevard, Moscow, Russia, [3]Depatment of Pharmacology, Kuban State Medical University, Krasnodar, Russia

***Corresponding author:** Anna A. Peresypkina, Department of Pharmacology, Belgorod State National Research University 85, Pobedy Street, Belgorod - 308015, Russia. E-mail: peresypkina_a@bsu.edu.ru

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to the instability and short-term effects after using these 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.[10] Thus, an important task is to find new, specific, and highly effective means for correcting of retinal ischemia.

Imidazoline receptors are located on the membranes of mitochondria and are actively associated with antioxidant and monoamine oxidase enzyme systems. In this regard, the correction of mitochondrial activity can largely level out ischemic and reperfusion damage to the retina.

In connection with the foregoing, it should be noted the relevance of the study of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid as a protective agent on the model of retinal ischemia-reperfusion.

MATERIALS AND METHODS

Experiments were carried out on Wistar rats weighing 250 ± 25 g. 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." Manipulations on rats were carried out under general anesthesia with intraperitoneal (i/p) administration of an aqueous solution of chloral hydrate in a dose 300 mg/kg of rat weight.

3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid, 10 mg/kg and 50 mg/kg, was administered intragastrically (i/g) once 1 h before ischemia-reperfusion modeling.

Ischemia-reperfusion injury of the retina was simulated under anesthesia by applying mechanical pressure (110 mmHg) to the anterior eye chamber within 30 min.[10]

To study the fundus in experimental animals, direct ophthalmoscopy was used after 72 h of reperfusion (Bx a Neitz ophthalmoscope, Japan). To expand the pupil, eye drops Irifrin 2.5% were used. The ophthalmoscope was approached to the rat's eye and sent a beam of light at a distance of 0.5–2 cm to obtain a clear picture of the fundus. To increase the image, the lens Osher MaxField 78D model OI-78M was used.[11]

Electroretinography (ERG) was performed immediately after ophthalmoscopy. For this purpose, the animals were kept in the dark within 30 min,[12] then anesthetized and fixed on a table. The corneal silver electrode was placed on the cornea, the reference needle electrode EL452 was placed subcutaneously (s/c) in the skull region, and the ground needle electrode EL450 was placed in the base of the tail. A stroboscope with a flash of white light, connected to the stimulator STM200 by Biopac System, Inc. (USA), was placed behind the animal back, and the ERG was recorded in response to a single stimulation. The induced biopotentials were amplified, averaged, and presented graphically on the screen with the help of Biopac-systems MP-150 and program AcqKnowledge 4.2 (USA). To evaluate the degree of development of functional retinal damage, the ratio of the amplitudes of the b- and a-waves of the ERG, the coefficient b/a, was estimated.[13] From the ten values obtained in each group, the average was output, which was recorded in the protocol.

For all data, the descriptive statistics were applied: the data were checked for the normality of the distribution. The type of distribution was determined by the Shapiro–Wilk criterion. In the case of a normal distribution, the average value (M) and the standard error of the mean (m) were calculated. In cases of abnormal distribution, the median (Me) and the quarterly range were calculated. Intergroup differences were analyzed by parametric (Student's *t*-test) or non-parametric (Mann–Whitney test) methods, depending on the distribution type. Differences were determined at 0.05 significant level. Statistical analysis is performed using the software Statistica 10.0.

We used our own modification of the model of retinal ischemia-reperfusion, in which the increase in intraocular pressure (IOP) is due to mechanical pressure (110 mmHg) on the anterior chamber of the eye.[13]

The experiment included 4 groups, 10 rats in each group: The 1st group - a group of intact animals; the 2nd - a group with retinal ischemia-reperfusion (control); the 3rd - with the correction of pathology by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 10 mg/kg; and the 4th - with the correction by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 50 mg/kg.

RESULTS AND DISCUSSIONS

In accordance with the study protocol, after an IOP increase and 72 hour of reperfusion, anesthesia of animals was performed. Further, an ophthalmoscopy and assessment of the electrophysiological retinal state were performed.

Example of ophthalmoscopy on intact animal is shown in Figure 1.

Example of ophthalmoscopy on Wistar rat with modeling of retinal ischemia after 72 h of reperfusion is shown in Figure 2.

Example of ophthalmoscopy in the group with correction by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl

cyclopentanecarboxylic acid in a dose 10 mg/kg after 72 h of reperfusion is shown in Figure 3.

In the group with correction by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 50 mg/kg, the following pattern was observed: Optic disc is round, pink, lies in the plane of the retina, the boundaries are clear. The veins and arteries are straight, the caliber is uniform, and there is no crimp. The general background is pink. The picture of the fundus is close to normal.

The results of evaluation of electrophysiological retinal function after 72 h of reperfusion in experimental groups are presented in Table 1.

Thus, the results of fundus studies and ERG in experimental groups revealed pronounced protective properties of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 50 mg/kg, exceeding its effect in a dose 10 mg/kg, consisting in a reduction in the development of neuronal damage in the retina on the model of retinal ischemia-reperfusion, which were noted in the control group, and an increase in the coefficient b/a in the groups with the correction of pathology, which is caused by the restoration of the positive wave b on the ERG and indicates the preservation of the electrophysiological function of the retina.

Through the stimulation of I1 and I2 imidazoline receptors, 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid manifests antioxidant, antiatherogenic, reparative, and cerebroprotective effects. By means of cerebroprotective, nootropic activity, it prevents the development of severe consequences of acute cerebral circulation impairment according to the ischemic type, restores all phases of memory, contributes to the preservation of the histo- structure of the brain in conditions of cerebral ischemia of different genesis.

The search of new methods of retinoprotection for a possible reduction of the damaging effect of ischemia, formed in various systemic diseases, is an urgent task of pharmacology and ophthalmology.[10] Segment of drugs for the treatment of vascular and neuronal 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.

In connection with the foregoing, the study of the protective properties of the 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid on the model of retinal ischemia-reperfusion in the experiment was topical.

Proceeding from the fact that the data of electrophysiological studies are often of decisive importance in the early and

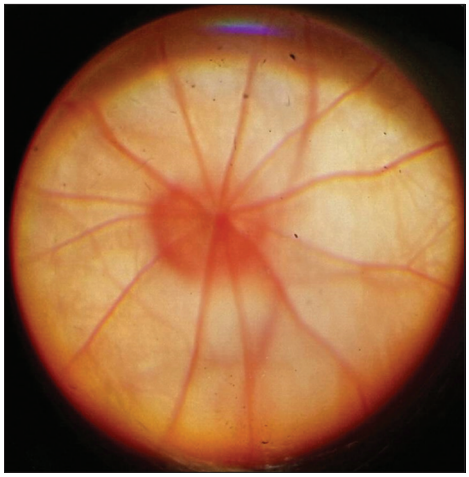

Figure 1: Example of ophthalmoscopy on intact Wistar rat. Optic disc is circular or oval shape and stands out from the fundus in pink. The boundaries of disc are clear. It lies in the plane of the retina. From the middle of the disc, exit the central vessels of the retina. Retinal blood vessels do not have anastomoses. The veins and arteries are straightforward, caliber is uniform, not crimped. The general background is pink

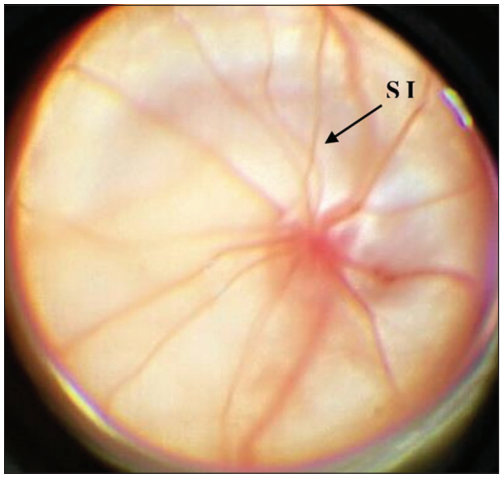

Figure 2: Example of ophthalmoscopy on Wistar rat with modeling of retinal ischemia-reperfusion. Optic disc is edematous, edema extends to the retina. Blurring boundaries of disc. Veins are congested. Arteries are narrowed. Vessel caliber is uneven. Retina is p alely (ischemic). Symptom Salus-Hun I (arrow + S I)

differential diagnosis of retinal disorders,[14] a complex analysis including ophthalmoscopic, electroretinographic, and microcirculatory studies is needed to study the correction of functional changes in the retina.

CONCLUSION

Results of ocular fundus studies revealed the most pronounced protective effects of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 50 mg/kg on the model of retinal ischemia-reperfusion in Wistar rats, which is reflected in the restoration of the optic disc.

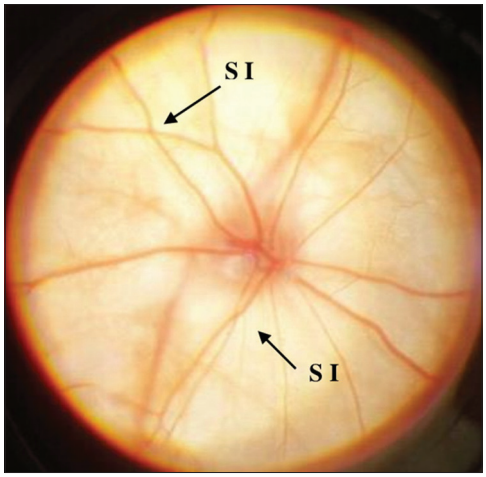

Figure3: Example of ophthalmoscopy in the group with correction of retinal ischemia-reperfusion by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 10 mg/kg. Optic disc is circular or oval shape and stands out from the fundus in pale-pink. The boundaries of disc are clear. It lies in the plane of retina. Viens are congested. Arteries are narrowed. Vessel caliber is uneven. Retina is palely (ischemic). Symptom Salus-Hun I (arrow + S I)

Table 1: Results of ERG after 72 h of reperfusion (M±m; n=10), r.u.

Experimental groups	b/a
Intact	2.5±0.10^{y}
Control	1.2±0.04*
Correction by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid, 10 mg/kg	2.0±0.15^{y}
Correction by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid, 50 mg/kg	2.4±0.10^{y}

*P<0.05 compared with the group of intact animals, ^{y}P<0.05 compared with the control group

Correction of retinal ischemia-reperfusion by 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentanecarboxylic acid in a dose 50 mg/kg leads to higher values of the coefficient b/a of ERG after 72 h of reperfusion compared to the group with pathology correction by the same drug in a dose 10 mg/kg, which indicates the restoration of the electrophysiological state of the retina.

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