Retinoprotective Effects of Non-selective Imidazoline Receptor Agonists

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Received: 16-05-2019, **Revised:** 11-06-2019, **Accepted:** 15-07-2019, **Published online:** 23-08-2019

How to cite this article: Elena A. Levkova, Anton L. Pazhinsky, Victoria V. Bashuk, Elizaveta Yu. Dubovtsova, Alexander A. Dolzhikov, Evgeniya A. Beskhmelnitsyna, Daria S. Vain and Vladimir V. Gureev (2019) Retinoprotective Effects of Non-selective Imidazoline Receptor Agonists, Journal of International Pharmaceutical Research *46*(4): 209-214

Abstract

Introduction: Current drug therapy of ophtalmic diseases associated with retinal ischemia is not always successful enough that ensures the relevance of the search of new effective drugs.

Objective: Improving the pharmacological correction efficiency of the retinal ischemic damage in the experiment using non-selective imidazoline receptor agonists.

Materials and methods: The retinal ischemia-reperfusion model in rats was used, in which an increase in intraocular pressure is carried out by mechanical pressure to the front chamber. The retinoprotective effects of the nonselective imidazoline receptor agonists: the sodium salt of C7070, the potassium salt of C7070 and C7070, treated with carbon dioxide, were estimated by the changes in the ratio of the amplitudes of the b- and a-waves of electroretinogramm – the b/a coefficient. Measuring the retinal microcirculation level was carried out using laser Doppler flowmetry.

Results and Discussion: The most pronounced retinoprotective effect is demonstrated by potassium salt of C7070 in a dose of 10 mg/kg, which is expressed in reaching the target values of the b/a and the retinal microcirculation level. When the pathology is corrected with sodium salt of C7070 in a dose of 10 mg/kg, the microcirculation level increases by 70.4% ($p < 0.05$), b/a increased by 94% ($p < 0.05$) compared with the group without correction. With the correction of retinal ischemia by C7070, treated with CO_2, in a dose of 10 mg/kg, the microcirculation level increased by 12% ($p > 0.05$), b/a increased by 84% ($p < 0.05$), compared with the group without correction.

Conclusion: The obtained data give an experimental substantiation of the pharmacological correction possibility of retinal ischemia by imidazoline receptor agonists.

Keywords: Imidazoline Receptor Ligands, C7070, Retinal Ischemia-Reperfusion Model, Rats.

Introduction

Retinal ischemia may have different etiology: central retinal artery (CRA) occlusion and occlusion of its branches, carotid arteries atherosclerosis, glaucoma with normal intraocular pressure (IOP), endocrine ophthalmopathy, surgical operation and so on. CRA occlusion is observed in 57% of cases, its branches occlusion – in 38%. Acute occlusion of retinal arteries in 91.2% of cases occurs against the background of the cardiovascular system diseases [1].

Local circulatory disorders in the retinal arterial system are observed in diabetic, hypertensive retinopathy (neuroretinopathy), optic nerve atrophy vascular origin, anterior ischemic optic neuropathy etc. [2, 3, 4, 5, 30].

Studying the way of how to improve retinal tissue tolerance to ischemia is an actual problem of modern pharmacology and ophthalmology. 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 [6, 7, 8, 31]. Thus, an important task is to find new, specific and highly effective means for correcting of retinal ischemia.

Imidazoline receptors (IR) of type II are a new biological target for the treatment of neurological disorders [9]. IR of type II are widely distributed in the brain, and their ligands may have therapeutic potential as neuroprotectors [10].

IR of type III perform their function by regulating the concentration of K^+ and Ca^{2+} in cells and is associated with the activation of ATP-dependent

potassium channels [11], which is expected to have a positive effect in the correction of ischemic conditions.

To study the pharmacological activity of new biologically active substances [12], as well as study the new effects of already known drugs [13], it is necessary to conduct experimental studies *in vitro* and *in vivo* [14].

In view of the above, it is important to study the possibilities of pharmacological correction of retinal ischemia using non-selective imidazoline receptor agonists in the experiment.

Objective of the study is improving the pharmacological correction efficiency of the retinal ischemic damage in the experiment using non-selective imidazoline receptor agonists.

Materials and Methods

Experiments were carried out on 80 rats Wistar weighing 225-275 g. For the study, the rats were taken with no external signs of disease, having passed the quarantine regime. Ethical principles of handling laboratory animals were observed in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, CETS No. 123.

The *following groups* were included in the experiment:

The first (n = 10) – an intact group;

The second (n = 10) – a group with the simulated retinal ischemia;

The third (n = 10) – a group with correction of the retinal ischemia by potassium salt of C7070 in a dose of 10 mg/kg;

The fourth (n = 10) – a group with the correction by sodium salt of C7070 in a dose of 10 mg/kg;

The fifth (n = 10) – a group with the correction by C7070, treated with CO_2, in a dose of 10 mg/kg;

The sixth (n = 10) – a group with the correction by potassium salt of C7070 in a dose of 10 mg/kg + glibenclamide in a dose of 5 mg/kg;

The seventh (n = 10) – a group with the correction by sodium salt of C7070 in a dose of 10 mg/kg + glibenclamide in a dose of 5 mg/kg;

The eighth (n = 10) – a group with the correction by C7070, treated with CO_2, in a dose of 10 mg/kg + glibenclamide in a dose of 5 mg/kg.

Retinal ischemic injury was simulated under general anesthesia (chloral hydrate, 300 mg/kg, intraperitoneally (i.p.) by applying mechanical pressure, 110 mm Hg, to the anterior eye chamber for 30 min. Mechanical pressure was carried out by a metal rod with an atraumatic surface placed in the cylinder with piston system with a calibration scale [15].

Potassium salt of C7070 (the potassium salt of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentancarbonic acid) under laboratory code K^+C7070 («JSC Experimental Plant VladMiVa», Belgorod, Russia) was injected intragastrically (i.g.) in a dose of 10 mg/kg as a single dose 60 min before the retinal ischemia simulation in a 1% starch solution.

Sodium salt of C7070 (the sodium salt of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentancarbonic acid) under laboratory code Na^+C7070 («JSC Experimental Plant VladMiVa», Belgorod, Russia) was injected i.g. in a dose of 10 mg/kg as a single dose 60 min before the retinal ischemia simulation in a 1% starch solution.

C7070 (3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentancarbonic acid) [16], treated with CO_2, under laboratory code C7070CO_2 («JSC Experimental Plant VladMiVa», Belgorod, Russia) was injected i.g. in a dose of 10 mg/kg as a single dose 60 min before the retinal ischemia simulation in a 1% starch solution.

Glibenclamide, ATP-dependent potassium channels blocker, was administered in a dose of 5 mg/kg i.g. once 1 hour before retinal ischemia-reperfusion modeling.

Electroretinography (ERG) in rats was performed according to the method previously published by us. To perform ERG, rats were kept in the dark for 30 min, then anesthetized (chloral hydrate, 300 mg/kg, i.p.). 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 (Biopac Systems, Inc., CA, USA) [17]. The duration of the flashes was 0.025 sec, intensity was 30 kV. ERG registration was carried out in response to a single stimulation. The ERG recording was carried out for 0.5 sec on each rat in the groups. To assess the degree of retinal ischemia, the ratio of the amplitudes of the b- and a-waves, the b/a coefficient was evaluated. The mean was derived for each group from ten values received and was introduced into the protocol [18].

Measuring the retinal microcirculation level in rats was carried out by *laser Doppler flowmetry* (LDF) [19]. 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., 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 sec. From the microcirculation level results at every point, the mean value was calculated, which was taken as the indicator of the microcirculation level in the retina of the experimental animal. The microcirculation value in the animal group was calculated as the mean of the values obtained from each experimental animal [20].

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. In cases of abnormal distribution, the median (Me) and the quartile range (QR) were calculated. Between-group 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 a 0.05 significance level. Statistical analyses were performed using Statistica 10.0 software.

Results

Evaluation of Electrophysiological Retinal Condition. Microcirculatory and neuronal damage of the retina after 72 hours of reperfusion after a long ischemic episode led to electrophysiological changes, namely, to a significant decrease in the amplitude of the b-wave, with relative preservation of the a-wave amplitude (table 1). Changes in the b-wave are pronounced due to damage, mainly, neurons of the inner nuclear layer.

Table-1: Influence of potassium salt of C7070, sodium salt of C7070 and C7070, treated with carbon dioxide, on the a- and b- waves amplitudes when correcting retinal ischemia-reperfusion (M ± m; n = 10), mV.

Experimental Groups	The a-Wave Amplitudes (n = 10)	The b-Wave Amplitudes (n = 10)
1. Intact	0.35 ± 0.03	0.88 ± 0.07 ^{y}
2. Retinal ischemia-reperfusion model	0.37 ± 0.03	0.44 ± 0.03 *
3. Ischemia-reperfusion + K^+C7070, 10 mg/kg	0.35 ± 0.02	0.84 ± 0.03 ^{y}
4. Ischemia-reperfusion + Na^+C7070, 10 mg/kg	0.36 ± 0.02	0.83 ± 0.05^{y}
5. Ischemia-reperfusion + C7070CO_2, 10 mg/kg	0.37 ± 0.03	0.81 ± 0.06^{y}
6. Ischemia-reperfusion + K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	0.35 ± 0.02	0.70 ± 0.06 ^{y}
7. Ischemia-reperfusion + Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	0.36 ± 0.04	0.65 ± 0.06*^{y}
8. Ischemia-reperfusion + C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg	0.37 ± 0.02	0.48 ± 0.04 *

* $p < 0.05$ compared to the intact; $^{y}p < 0.05$ compared to the retinal ischemia-reperfusion model.

In each group, the b/a coefficient was calculated, the values of which are presented in table 2.

Table-2: Influence of potassium salt of C7070, sodium salt of C7070 and C7070, treated with carbon dioxide, on the value of the b/a coefficient when correcting retinal ischemia-reperfusion (M ± m; n = 10), R.U.

Experimental Groups	Ratio b/a (n = 10)
1. Intact	2.51 ± 0.07
2. Retinal ischemia-reperfusion model	1.19 ± 0.05*
3. Ischemia-reperfusion + K^+C7070, 10 mg/kg	2.40 ± 0.11 ^{y}
4. Ischemia-reperfusion + Na^+C7070, 10 mg/kg	2.31 ± 0.06^{y}
5. Ischemia-reperfusion + C7070CO_2, 10 mg/kg	2.19 ± 0.09 ^{y}
6. Ischemia-reperfusion + K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	2.00 ± 0.12 *^{y}
7. Ischemia-reperfusion + Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	1.81 ± 0.09*^{y}
8. Ischemia-reperfusion + C7070CO_2, 10 mg/kg + glibenclamide, 5 mg/kg	1.30 ± 0.09*

R.U. – relative units; * $p < 0.05$ compared to the intact; ^{y} $p < 0.05$ compared to the retinal ischemia-reperfusion model.

When modeling retinal ischemia after 72 hours of reperfusion, the b/a decreased by 53% ($p < 0.05$) compared with the group of intact animals. With the injection of K^+C7070 in a dose of 10 mg/kg b/a significantly increased by more than 2 times compared with the group without correction ($p < 0.05$). In the group with the injection of Na^+C7070 in a dose of 10 mg/kg b/a increased by 94% compared with the mean value in the group without correction ($p < 0.05$). When administered to animals C7070CO_2 in

a dose of 10 mg/kg b/a in the group increased by 84% ($p < 0.05$), which is also significantly different from the mean value in the group without correction. Introduction of glibenclamide in the groups with correction of pathology by K^+C7070, Na^+C7070 the b/a increased by 68% and 52%, respectively, in comparison with the group with the model of retinal ischemia-reperfusion, which indicates partial preservation of retino protective properties against the background of blockade of ATP-sensitive potassium channels. In the group with the introduction of glibenclamide on the background of $C7070CO_2$ correction in a dose of 10 mg/kg, an increase in the b/a was prevented in comparison with the group without correction.

Evaluation of Retinal Microcirculation. After 72 hours of reperfusion, the microcirculation level in the retina was recorded by LDF. The results of the LDF are presented in table 3.

Table-3: Influence of potassium salt of C7070, sodium salt of C7070 and C7070, treated with carbon dioxide, on the level of retinal microcirculation (M ± m), perfusion units

Experimental Groups	Level of microcirculation, P.U. (n = 10)
1. Intact	743.9 ± 5.0
2. Retinal ischemia-reperfusion model	353.3 ± 11.7*
3. Ischemia-reperfusion + K^+C7070, 10 mg/kg	732.7 ±16.9 ^{y}
4. Ischemia-reperfusion + Na^+C7070, 10 mg/kg	602.1 ± 15.0 ^{*y}
5. Ischemia-reperfusion + $C7070CO_2$, 10 mg/kg	398.2 ± 11.6 ^{*y}
6. Ischemia-reperfusion + K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	450.4 ±14.2 ^{*y}
7. Ischemia-reperfusion + Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	428.5 ± 13.3^{*y}
8. Ischemia-reperfusion + $C7070CO_2$, 10 mg/kg + glibenclamide, 5 mg/kg	365.1 ± 10.3*

P.U. – perfusion units; * $p < 0.05$ compared to the intact; ^{y} $p < 0.05$ compared to the retinal ischemia-reperfusion model.

In the group with retinal ischemia-reperfusion model, the level of retinal blood flow is reduced more than in 2 times ($p < 0.05$) in comparison with the mean value in the group of intact animals. When correcting ischemia-reperfusion by K^+C7070 in a dose of 10 mg/kg, the level of retinal microcirculation increased more than 2 times ($p < 0.05$) compared with the group without correction and did not differ from the mean value of the norm, which suggested the restoration of microcirculation in the retina. When the pathology is corrected by Na^+C7070 in a dose of 10 mg/kg, the level of microcirculation increased by 70.4% ($p < 0.05$) compared with the group without correction, but did not reach the target values. When correcting $C7070CO_2$ in a dose of 10 mg/kg, the microcirculation level increased by 12% compared with the value in the group without correction and did not differ significantly from it. In the group with correction by K^+C7070 in a dose of 10 mg/kg and administration of glibenclamide in a dose of 5 mg/kg, the blood flow level in the retina significantly differs from the normal values, but is 27.5% higher than in the group without correction ($p < 0.05$). In the group with correction by Na^+C7070 in a dose of 10 mg/kg and administration of glibenclamide in a dose of 5 mg/kg, the level of blood flow in the retina is also significantly different from the norm and 21.3% higher than in the group without correction ($p < 0.05$). The introduction of glibenclamide in the group with correction by $C7070CO_2$ in a dose of 10 mg/kg was almost completely prevented the increase in the level of retinal blood flow in comparison with the model of pathology.

Discussion

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 [20, 28, 29]. 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.

Previous studies showed that 2-(2-benzofuranyl)-2-imidazoline (2-BFI), an immidazoline receptor ligand, dose-dependently protects rodent brains from cerebral ischemia injury. However, the molecular mechanisms remain unclear. In this study, was found that 2-BFI transiently and reversibly inhibits NMDA, but not AMPA currents, in a dose-dependent manner in cultured rat cortical neurons. 2-BFI also transiently and reversibly blocked NMDA receptor-mediated calcium entry to cultured neurons and provided long-term neuroprotection against NMDA toxicity in vitro. Collectively, these studies demonstrated a potential mechanism of 2-BFI-mediated neuroprotection and

indicated that 2-BFI is an excellent candidate for repositioning as a drug for stroke treatment [21, 27].

It is known that activation of IR of type II reduces the voltage-dependent activation of Ca^{2+} channels in neurons innervating the blood vessels [22].

As a result of a series of experiments on cell cultures of insulinoma RIN-5AH, it was shown that the insulin secretagogue function of pancreatic β-cells is increased by the action of imidazoline compounds even with selective blocking of I1R and I2R. This research has indicated the presence of another imidazoline-sensitive receptor and initiated the investigation of I3R. Since the addition of an activator of K^+-ATP-dependent diazoxin channels prevents insulinolysis, it is considered that I3R performs its function through regulation of K^+ and Ca^{2+} concentrations of Langerhans cells. Later, β-carboline Harmane was found as its selective agonist [23, 25, 26].

In the future interesting is the study of the influence of the studied imidazoline receptor agonists on the eNOs expression [24] in retinal vessels.

In connection with the foregoing, the proposed mechanism of neuroretinoprotection of IR-agonist of type II can be associated with the inhibition of Na^+/H^+ ion channels in the retinal neurons, the inhibition of NMDA receptors; type III – with the activation of ATP-sensitive potassium channels in blood vessels and retinal neurons.

Conclusion

Presumably, ATP-sensitive potassium channels make a great contribution to the realization of retinoprotective effects of the studied non-selective IR-agonists, since their blockade by glibenclamide leads to partial elimination of the positive dynamics of electrophysiological parameters of the retina (values of the amplitude of the b-wave and b/a) and partial elimination of the positive dynamics of blood flow in the retina during the correction of retinal ischemia-reperfusion by the studied substances.

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