The Influence of Imidazoline Receptor Agonists on the Ophthalmoscopic and Morphometric Parameters in Retina in Simulated Retinal Ischemia-reperfusion

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Abstract

Introduction: An important task of experimental and clinical pharmacology and ophtalmology is to find highly effective drugs for correcting retinal ischemia.

Objective: Improving the pharmacological correction efficiency of the retinal ischemia-reperfusion with use the new substances related to imidazoline receptor ligands.

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 of the eye for 30 min. The protective effects of the studied imidazoline receptor ligands: sodium salt of C7070, potassium salt of C7070 were evaluated based on the results of semi-quantitative assessment of the eye fundus condition during ophthalmoscopy and counting the specific number of neuronal nuclei in the inner nuclear layer.

Results and Discussion: The most pronounced protective 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 semi-quantitative assessment of the eye fundus condition and the specific number of neuronal nuclei in the inner nuclear layer. When the pathology is corrected with sodium salt of C7070 in a dose of 10 mg/kg, the semi-quantitative assessment of the eye fundus condition in the group decreased by 71.4% ($p < 0.05$), specific number of neuronal nuclei in the inner nuclear layer increased by 74,6% ($p < 0.05$) compared with the group without correction.

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

Keywords: Imidazoline Receptor Ligands, Sodium Salt of C7070, Potassium Salt of C7070, Retinal Ischemia-Reperfusion Model, Rats.

Introduction

Nonarteritic anterior ischemic optic neuropathy (NAION), acute ischemia of the anterior segment of the optic nerve, occurs 6 times more often compared with developed against the background of systemic vasculitis. The prevalence of the disease is 2.3-10.2 per 100 000 population over 50 years [1].

In the base of ischemic optic neuropathy is an acute violation of arterial blood circulation in the system of vessels feeding the optic nerve [2]. Biochemical, hemodynamic disorders that develop in atherosclerosis, hypertension, diabetes mellitus, blood diseases, arterial (brachiocephalic, etc.) occlusions play a leading role in the development of this pathology [3].

Studying the way of how to improve retinal 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, and others. Due to the instability effects after using these drugs it is necessary to seek out more effective ways to improve blood circulation in retina and increase resistance to ischemic retinal tissue having a specific orientation [4, 5]. Therefore, an important task is to find specific and highly effective agents for the treatment of retinal ischemia.

Imidazoline receptors (IR) of type II are a new target for the correction of neurological disorders [6]. IR of type II are widely distributed in the brain, and their agonists are potential neuroprotectors [7, 8].

IR of type III regulate the K^+ and Ca^{2+} concentrations in cells and are associated with the activation of ATP-dependent potassium channels [9], which is expected to have a positive effect in the correction of ischemia, in particular, in the retina.

To study the pharmacological activity of new biologically active substances [10], as well as study the new effects of already known drugs [11], it is necessary to conduct experimental studies *in vitro* and *in vivo* [12].

Thus, it is important to study the possibilities of pharmacological correction of retinal ischemia with use the imidazoline receptor ligands in vivo.

Objective of the study is improving the pharmacological correction efficiency of the retinal ischemia-reperfusion in vivo with use the imidazoline receptor ligands: sodium salt of C7070, potassium salt of C7070.

Materials and Methods

Experiments were carried out on 60 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 ***experimental groups*** were included:

The first (n = 10) – an intact group;

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

The third (n = 10) – a group with correction of the retinal ischemia-reperfusion 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 potassium salt of C7070 in a dose of 10 mg/kg + glibenclamide in a dose of 5 mg/kg;

The sixth (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.

Retinal ischemia-reperfusion was simulated under general anesthesia with use the chloral hydrate in a dose of 300 mg/kg, intraperitoneally (i.p.) by applying mechanical pressure up to 110 mm Hg, to the anterior eye chamber for 30 min [13].The potassium salt of C7070 (the potassium salt of 3-(1H-benzimidazol-2-il)-1,2,2-trimethyl cyclopentancarbonic acid) with 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 injection 60 min before the retinal ischemia simulation. The sodium salt of C7070 (the sodium salt of 3-(1H-benzimidazol-2-il)-1, 2, 2-trimethyl cyclopentancarbonic acid) with laboratory code Na^+C7070 («JSC Experimental Plant VladMiVa», Belgorod, Russia) was injected i.g. in a dose of 10 mg/kg as a single injection 60 min before the retinal ischemia simulation. Glibenclamide was administered in a dose of 5 mg/kg i.g. once 1 hour before retinal ischemia-reperfusion simulation.

Ophthalmoscopy. The rats were anesthetized to perform ophthalmoscopy after 72 hours of reperfusion. To dilate the pupils, Irifrin eye drops 2.5% were used. The ophthalmoscope (Bx a Neitz, Japan) was brought closer to the rat's eye and a beam of light was directed at the eye from a distance of 0.5–2.0 cm to obtain a clear eye fundus image. An Ocular Osher MaxField 78D, model OI-78M (Volk Optical Inc, Mentor, OH, USA), was used to magnify and obtain photos of the eye fundus (Peresyp RRP).

For further statistical processing, the degree of changes in the eye fundus caused by ischemia-reperfusion detected by ophthalmoscopy was ranked (Table 1).

Table-1: Method of Semi-quantitative Assessment of Changes in the Eye Fundus Caused by Retinal Ischemia-Reperfusion, points

Features of eye fundus changes	Score
Norm. The optic disc (OD) is round or oval, and against the background of the fundus its colour is pale pink. The boundaries of OD are clear. It is located in the plane of the retina. Out of the middle of the OD come the central vessels of the retina. Retinal vessels have no anastomoses. Veins and arteries are straight, the caliber is uniform, with no tortuosity. The overall background is pink.	0
Correction of neuronal damage. The OD is round or oval, and against the background of the fundus its colour is pale pink. The boundaries of OD are clear. It is located in the plane of the retina. There are spasm of arteries (filamentous arteries), dilation and tortuosity of veins, microaneurysms.	1
Correction of vascular damage. Retinal vessels have no anastomoses. Veins and arteries are straight, the caliber is uniform, with no tortuosity. Obvious OD decoloration. Edema of the OD and peripapillary retina. The boundaries of OD are not clear.	2
Retinal ischemia-reperfusion model. Obvious OD decoloration. Edema of the OD and peripapillary retina. The boundaries of OD are not clear. There are spasm of arteries (filamentous arteries), dilation and tortuosity of veins, microaneurysms, hemorrhages. The overall background is pale.	3

The eyes were completely removed along with the adjacent structures for morphometric examination and were fixed by immersion in 10% formalin solution. After fixing, the eyes were sectioned into two parts through the centre and both halves were processed into paraffin by routine methods. Also in the meridian direction, serial sections were made with a thickness of 5-7 μm, which were stained with haematoxylin and eosin. The stages of histological processing were performed using Leica equipment (Germany). For microscopy, morphometry and archiving, the prepared microslides were scanned using Mirax Desk, a computerised archiving and image analysis system. Image analysis and morphometry were carried out by a Pannoramic Viewer 1.15.4. The quantitative data were recorded in MS Excel spreadsheets.

Calculation of the *specific number of neuronal nuclei in the inner nuclear layer* was carried out by Avtandilov G. point test on standard computer images obtained by the system for scanning and archiving images Mirax Desk and the program "Pannoramic Viewer" 1.15.4. A computer version of the 0.3 mm^2 grid projected on the image was used [8].

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

Semi-quantitative Assessment of the Eye Fundus Condition. After 72 hours of reperfusion in all experimental groups rats were anesthetized for the study of the eye fundus image. An example of an eye fundus of rat in intact group is shown in figure 1 A with the following description: OD is round, and against the background of the fundus its colour is pink. The boundaries of OD are clear. It is located in the plane of the retina. Out of the middle of the OD come the central vessels of the retina. Retinal vessels have no anastomoses. Veins and arteries are straight, the caliber is uniform, with no tortuosity. The overall background is pink.

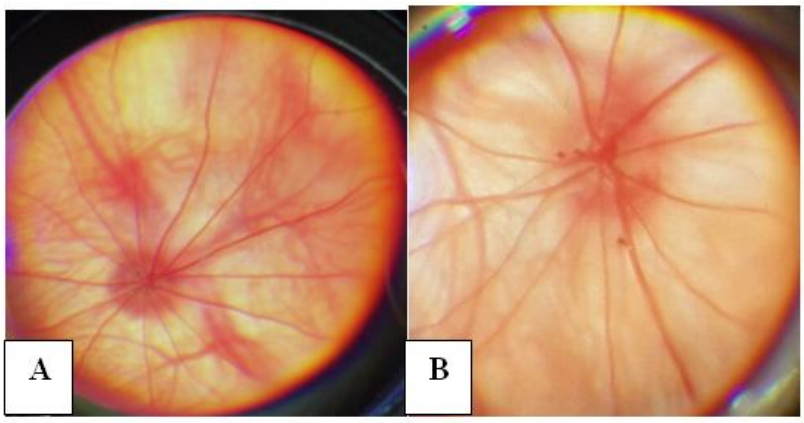

Figure-1: Eye fundus images of Wistar rats: A – from intact group; B – with the simulated retinal ischemia-reperfusion.

Example of the eye fundus of the laboratory rat with the simulated retinal ischemia-reperfusion is shown in figure 1 B with the following description: Obvious OD decoloration. Edema of the OD and peripapillary retina. The boundaries of OD are not clear. There are spasm of arteries, dilation of veins, hemorrhages. The overall background is pale.

Ophthalmoscopic image with the correction of retinal ischemia-reperfusion by potassium salt of C7070 in a dose of 10 mg/kg is shown in figure 2 A and its description is close to normal.

The observed protective effect in the eye fundus of laboratory rats with the correction by K^+C7070 may be associated with the activation of ATP-dependent potassium channels, both in retinal vessels and in neurons, inhibition of Na^+/H^+ exchange channels in retinal neurons, in turn leading to the suppression of apoptosis mechanisms.

When the sodium salt of C7070 in a dose of 10 mg/kg was administered to rats, for the correction of the simulated pathology the alignment of the retinal vessels caliber is observed, but the veins are plethoric, microaneurysms are not observed. The OD is slightly

increased in size, pink. The boundaries of OD are clear (Fig. 2 B).

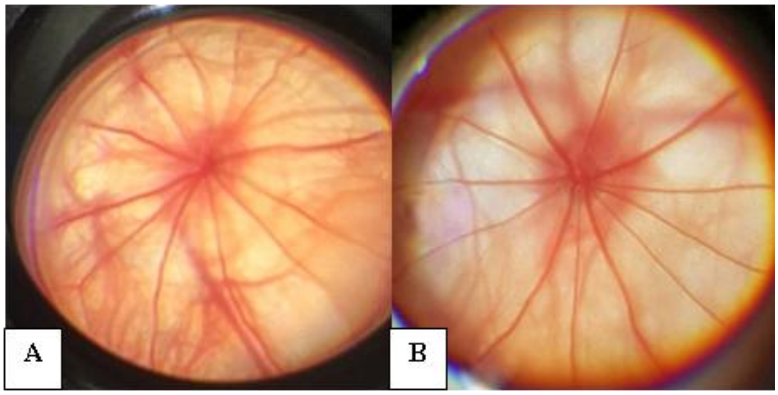

Figure-2: Eye fundus images of Wistar rats with correction of retinal ischemia-reperfusion: A – by potassium salt of C7070 in a dose of 10 mg/kg; B – by sodium salt of C7070 in a dose of 10 mg/kg.

The observed protective effect of Na^+C7070 during ophthalmoscopy on the model of retinal ischemia-reperfusion probably has a similar mechanism with K^+C7070, but the renoprotective effect of Na^+C7070 in a dose of 10 mg/kg is less pronounced than that of K^+C7070 in a dose of 10 mg/kg, as partially vascular changes on the background of retinal ischemia are preserved.

In the experimental groups with the injection of a potassium channels blocker glibenclamide in correction of retinal ischemic damage by K^+C7070 in a dose of 10 mg/kg and Na^+C7070 in a dose of 10 mg/kg, the spasm of the retinal arteries is observed. The state of OD is close to normal (Fig. 3).

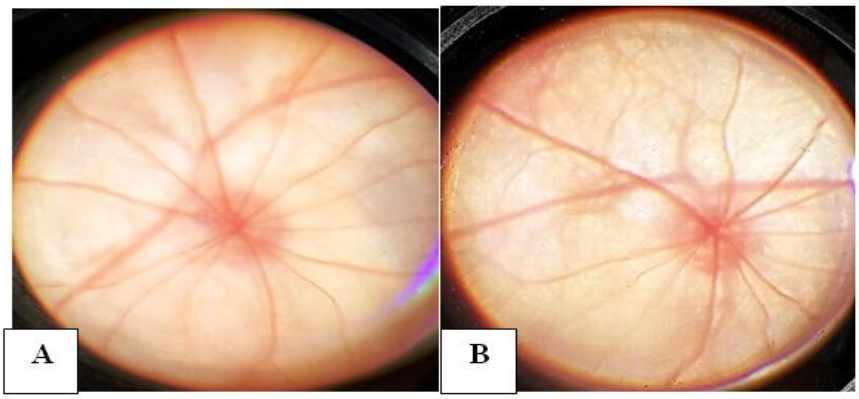

Figure-3: Eye fundus images of Wistar rats with the injection of glibenclamide in a dose of 5 mg/kg in correction of retinal ischemia-reperfusion: A – by potassium salt of C7070 in a dose of 10 mg/kg; B – by sodium salt of C7070 in a dose of 10 mg/kg.

The results of the semi-quantitative assessment of the eye fundus state in the experimental groups are presented in Table 2.

Table-2: Influence of potassium salt of C7070; sodium salt of C7070 on the results of assessment of changes in the eye fundus when correcting retinal ischemia-reperfusion (M±m; n=10), points.

Experimental Groups	Score
1. Intact	0.2 ± 0.2
2. Retinal ischemia-reperfusion model	2.8 ± 0.3*
3. Ischemia-reperfusion + K^+C7070, 10 mg/kg	0.2 ± 0.2 ^{y}
4. Ischemia-reperfusion + Na^+C7070, 10 mg/kg	0.8 ± 0.2 *^{y}
5. Ischemia-reperfusion + K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	1.1 ± 0.1*^{y}
6. Ischemia-reperfusion + Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	1.2 ± 0.2*^{y}

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

Based on the obtained data of the semi-quantitative assessment of the eye fundus state on the model of retinal ischemia-reperfusion in rats, it follows that the retinoprotective effect is in descending order: potassium salt of C7070 (10 mg/kg) > sodium salt C7070 (10 mg/kg).

The observed protective effects of the studied substances, consisting in the correction of retinal artery spasm, may be associated with the activation of ATP-dependent potassium channels in the retinal vessels. The neuroprotective effect of the substances is probably related both to the inhibition of Na^+/H^+ exchange channels and to the activation of ATP-dependent potassium channels in retinal neurons, which in turn leads to the inhibition of the mechanisms of apoptosis in the retina.

Counting the Specific Number of Neuronal Nuclei in the Inner Nuclear Layer. The results of the counting the specific number of neuronal nuclei in the inner nuclear layer in the experimental groups are presented in table 3.

Table-3: Influence of potassium salt of C7070; sodium salt of C7070 on the results of the countingthe specific number of neuronal nuclei in the inner nuclear layer when correcting retinal ischemia-reperfusion (M±m; n=10), absolute units.

Experimental Groups	Specific number of neuronal nuclei, absolute units
1. Intact	12.2 ± 0.8^{y}
2. Retinal ischemia-reperfusion model	6.3 ± 0.6*
3. Ischemia-reperfusion + K^+C7070, 10 mg/kg	11.9 ± 0.8^{y}
4. Ischemia-reperfusion + Na^+C7070, 10 mg/kg	11.0 ± 0.7^{y}
5. Ischemia-reperfusion + K^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	10.5 ± 0.6^{y}
6. Ischemia-reperfusion + Na^+C7070, 10 mg/kg + glibenclamide, 5 mg/kg	$9.4 \pm 0.5^{*y}$

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

When modeling retinal pathology in the group, the mean specific number of neuronal nuclei in the inner nuclear layer significantly reduces by 48% ($p < 0.05$) in comparison with the group of intact animals. Against the background of the injection of K^+C7070 in a dose of 10 mg/kg, as well as Na^+C7070 in a dose of 10 mg/kg, this indicator significantly increases by more than 74% ($p < 0.05$) in comparison with the group without correction, and does not significantly different from the intact group. With the injection of glibenclamide in the group with correction of ischemia by K^+C7070 this indicator significantly increases by 67% ($p < 0.05$) in comparison with the mean value in the group without correction, but slightly decreases than value in the group with the injection of K^+C7070 without glibenclamide. On the background of glibenclamide injection in the group with the correction by Na^+C7070 this indicator significantly decreases in comparison with the value of the group of intact animals, by 23% ($p < 0.05$), significantly decreases in comparison with the mean value of the group with the injection of Na^+C7070 ($p < 0.05$), but significantly increases in comparison with the mean in the group without correction.

Discussion

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 [14].

It is known that activation of IR of type II reduces the voltage-dependent activation of Ca2+ channels in neurons innervating the blood vessels [15].

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 [16].

In the future it would be interesting to study the distribution of potassium salt of C7070, sodium salt of C7070 in the eye tissue with use high performance liquid chromatography with mass spectrometric detection [17] and possibility of correction the retinal microcirculation by studied substances with use laser Doppler flowmetry [18-22].

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^+ exchange 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 [23-25].

Conclusion

Probably, ATP-sensitive potassium channels make a great contribution to the realization of neuroretinoprotective effects of the studied IR-ligands: sodium salt of C7070, potassium salt of C7070 since their blockade by glibenclamide leads to partial elimination of the positive dynamics of ophthalmoscopic and morphometric parameters in retina during the correction of retinal ischemia-reperfusion by the studied substances.

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