EXPERIMENTAL PHARMACOLOGY

Rus.

UDC: 615.275.4 **DOI: 10.18413/2313-8971-2017-3-3-3-8**

Dovgan A.P.[1], Urojevskaya J.S., Khavansky A.V.

POSSIBLE WAYS OF PHARMACOLOGICAL CORRECTION OF ISCHEMIC DAMAGE TO THE LIVER WITH THE AGONIST OF PERIPHERAL IMIDAZOLINE RECEPTORS C7070

MAKS-Med Clinic "Garmonia Zdorovia", 32, Beregovaya str., 125284, Moscow, Russia
Corresponding author, [1]e-mail: dovgan@bsu.edu.ru

Abstract

Introduction: We glad to introduce several variants of pharmacological correction of ischemic hepatic injury by imidazoline I_2 receptor agonist C7070.

Materials and methods: The experiment was carried out on 70 rats of both sexes, divided into 7 groups (n = 10): an intact group; Pseudo-operated animals (autopsy of the abdominal wall without ligation of the liver vessels); Ischemia / reperfusion group without drug correction; Animals undergoing ischemia / liver reperfusion + Metformin (50 mg / kg); Animals undergoing ischemia / liver reperfusion + Moxonidine (1 μg / kg); Animals undergoing ischemia / liver reperfusion + C7070 (1 mg / kg). For the evaluation, the coefficients calculated from the level of hepatic transaminases (ALT, AST), as well as morphometric ratios of the area of necrosis and deep ischemia of the liver, were used for the evaluation according to the histological examination.

Results and discussion: The indicated agonists of peripheral imidazoline I_2receptors (C7070) significantly reducesischemically-reperfusion injury of the liver, in comparison with the preparations of moxonidine and metformine. Indirect sign of imidazoline activating mechanism of C7070 is decreasing of the hepatoprotective effect of C7070 by the preliminary administration of imidazoline receptor blocker BU-224. The coefficients for ALT / AST for C7070, moxonidine and metformin were 72.8 / 62.13, respectively; 44.99 / 34.20 and 36.88 / 21.02. The coefficients of the morphological hepatoprotective activity of the preparations were: C7070 – 82.61, moxonidine – 72.33, metformin – 38.96.

Conclusions: The imidazoline receptor agonists significantly and significantly reduce the functional and morphological manifestations of liver ischemia / reperfusion.

Keywords: Liver ischemia, liver reperfusion, diabetes mellitus, C7070, moxonidine, metformin, imidazoline receptor agonists.

Introduction

Ischemia may be considered as a trigger moment and part of pathway of numerous pathological conditions [1].

The development of new effective medicines for diabetes mellitus treatment is one of the most actual problems nowadays. As known, this development is enable without studying of pharmacokinetic [2].

In case of diabetes mellitus and a metabolic syndrome the fatty dystrophy develops. It can rise to hepatic necrosis [3].

Modern drugs of metabolic syndrome and diabetes mellitus treatment do not protect liver effectively.

Remembering this, we are able to assert, that studying of additional pharmacological correction of standard therapy by biguanides is scientifically interests [4].

Materials and methods

The studying of hepatic anti-ischemic activity was conducted by D.A. Lopatin method [5].

In the experiment were included 50 both sex adult rats, divided in 5 groups: intact group, pseudo-operated group, control group (moderated pathology without therapy); and3 groups with treatment by C7070 (1 mg/kg), moxonidine (1 μg/kg) and metformine (50 mg/kg).

The studying drugs were administrated before moderating a pathology.

The animals were narcotized by inroperitoneal administration of chloralhydrate in dose 300 mg/kg.

Narcotized animal was subjected to the median laporotomy through the lineaalba. Ligamentum hepatica major was stupid stood and pinched by atraumatic clamp for 15 minutes. Then, content of abdominal cavity was replaced back. A surgical wound was sawed in layers.

During 3 days studying drugs were administrated into the animals per os.

3 days after the end of experiment animals were euthanized by diethyl ether with subsequent taking blood from the heart. The blood was analyzed for ALT, AST.

There was none death during the experiment.

ALT and AST were chosen as biochemical markers of ischemic injury [6].

Histological sections were selected as morphological markers of ischemic injury [7].

All differences, detected by comparing of stated parameters, received in different groups were considered as statistically significant in case of $p<0.05$.

Results and discussion

Moderating of 15 minute ischemia with subsequent reperfusion caused to the increasing of ALT and AST level by 3rd day in 5 times (tab. 1). Pseudo-operated animals did not different from intact group. The same time, morphometric metering of ischemic injury area and necrosis area was 0.387±0.014 and 0.207±0.021 respectively (tab. 2).

Table 1

The influence of agonists of imidazoline receptors at the level of ALT and AST in modeling of ischemia/reperfusion of the liver (M±m, n=10)

Animal group	ALT (U/ml)	AST (U/ml)
Intact	102.89±8.82	284.14±19.36
Pseudo-operated	110.27±21.96*	289.80±16.29*
Ischemia/Reperfusion (I/R)	526.90±17.97**	1045.16±80.02**
I/R+C7070 (1 mg/kg)	143.27±16.93[1]	395.85±33.31[1]
I/R +Moxonidine (1 μ/kg)	289.86±15.27[1]	687.71±28.37[1]
I/R +Metformine (50 mg/kg)	332.56±22.05[1]	825.49±22.46[1]
I/R +C7070 (1 mg/kg)+BU224 (1 mg/kg)	300.45±19.44[1]	798.59±21.34[1]

Note: * – $p>0.05$ incomparing with intact group, ** – $p>0.05$ incomparing with pseudo-operatedgroup, [1] – $p>0.05$ incomparing with ischemia/reperfusion group.

Table 2

The influence of agonists of imidazoline receptors in the area of ischemic damage to the liver and area of the zone of necrosis of liver tissue by modeling of ischemia/reperfusion of the liver (M±m, n=10)

Animal group	Ischemia injury area, mm^2	Necrosis area, mm^2
Intact	n/a	n/a
Pseudo-operated	n/a	n/a
Ischemia/Reperfusion (I/R)	0.387±0.014	0.207±0.021
I/R+C7070 (1 mg/kg)	0.058±0.029*	0.046±0.013*
I/R +Moxonidine (1 μ/kg)	0.090±0.025*	0.075±0.015*
I/R +Metformine (50 mg/kg)	0.238±0.052*	0.125±0.020*
I/R +C7070 (1 mg/kg)+BU224 (1 mg/kg)	0.159±0.031*	0.104±0.008*

Note: * – $p>0.05$ incomparing with ischemia/reperfusion group.

Using of studying drugs statistically significant decrease the level of ALT and AST in experimental animals. Maximal activity had C7070 (1 mg/kg), the level of ALT and AST were 143.27±16.931 and 395.85±33.311 respectively (tab. 1).

The same time C7070 significantly decreased ischemia injury area and necrosis area till 0.058±0.029 and 0.046±0.013 respectively.

Metformine and moxonidine decreased biochemical and morphometrical markers of ischemia injury, but they were worse than C7070 (tab. 1-2).

For the ease evaluation we offer to compare the degree of AST and ALT level decreasing in different animal groups. It is possible with help of simple math formula 1.

$$К_{ALT} = 100 - \frac{ALT\ (exp)}{ALT\ (ctrl)} * 100\%, \qquad (1)$$

ALT (exp) – ALT level in blood of experimental animals,

ALT (ctrl) – ALT level in blood of control (I/R) animals.

The same formula (formula 2) may be useful for AST studying.

$$К_{AST} = 100 - \frac{AST\ (exp)}{AST\ (ctrl)} * 100\%, \qquad (2)$$

AST (exp) – AST level in blood of experimental animals,

AST (ctrl) – AST level in blood of control (I/R) animals.

With the help of previous formulas we can take next data of functional activity of studing drugs (tab.3).

Table 3

Hepatoprotective activity C7070, moxonidine and Metformin in modeling of ischemia/reperfusion of the liver according biochemical research (M±m; n=10)

Hepatoprotective coefficient, U.	C7070 (1 mg/kg)	Moxonidine (1 μ/kg)	Metformine (50 mg/kg)
$К_{ALT}$	72.81±1.71*	44.99±1.23	36.88±1.02
$К_{AST}$	62.13±1.34*	34.20±1.21	21.02±1.49

Note: * – p>0.05 incomparing with moxonidine and metformine groups.

Based on these data we are able to speak about maximal anti-ischemic activity of imidazoline I_2 agonist (C7070) through the all studied in frames of this studying drugs.

There are not enough data for the full assessment of anti-ischemic activity of studying drugs.

The coefficient of morphological hapetoprotection was chosen as additional data for full assessment of anti-ischemic activity for the studying drugs. This coefficient include data of ischemic injury area and necrosis area.

The coefficient was calculated by next formula (formula 3).

$$К = 100\% - (\frac{Mi(exp)+Mn(exp)}{Mi(ctrl)+Mn(ctrl)} * 100\%), \qquad (3)$$

Mi (exp) – mean area of ischemic injury of liver in experimental animals;

Mn (exp) – mean necrosis area in liver of experimental animals;

Mi (ctrl) –mean area of ischemic injury of liver in control (I/R) animals;

Mn (ctrl) – mean necrosis area in liver of experimental animals.

Thus we receive next data of morphological anti-ischemic activity of studied drugs. (tab. 4)

Table 4

Hepatoprotective activity C7070, moxonidine and Metformin when modelirovanii ischemia/reperfusion of the liver according moramerica research (M±m; n=10)

Animal group	Coefficient of hepatoprotective activity, U.
И/Р+С7070 (10 мг/кг)	82.61±3.22*
И/Р+Moxonidine (1 мг/кг)	72.33±1.04
И/Р+Metformine (50 мг/кг)	38.96±5.69

Note: * – p<0.05 in comparing with. 2 и 3.

So, agonist of peripheral imidazoline receptor (I_2) C7070 has maximal anti-ischemic activity among all studied drugs.

Received data are able to be explained by different mechanism of action.

The base of metformine action is a decreasing of glucose production. Itcorrelatedwiththedecreasingofglycemialevel.Metformine plays a role in increasing of peripheral insulin effects, decreasing gluconeogenesis and oxidation of free fat acids in liver, increasing of anaerobes way of glucose metabolism with the lactate formation, decreasing of lipolisis. In range of in vivo and in vitro studies was detected activating influence of metformine on the cell ferment AMP-kinase, playing a role in glucose transport by GLUT4 and oxidation of free fat acids. Probably, betterment of glycemic profile by metformine therapy may be related to the same aspects of its mechanism of action. Besides, dimethylbiguanide demonstrated an ability to decrease the rigidity of cell membranes, which often detected at patient with diabetes and can contribute the development of its complications [8].

Metformine asctivates AMPK – hepatic ferment, playing a role in insulin signalization, in total energy balance in organism and in glucose and lipid metabolism, including liver. Activation of AMPK is necessary for inhibition metformine effect to hepatic gkuconeogenesis [9].

Resuming of previous, it can be argued that anti-ischemic action of metformine based on the accumulation of hepatocytes energy reserves and deceleration of in spending generated current nutrients.

Moxonidine as an agonist of central (I_1) imidazoline receptor involved in redistribution of hepatic blood flow according to opening of collateral vessels, started from a. gastricasinistra, free from the blocking. Also, additional activity can due to the central control by moxonidine on the opening of hepatic vessels in moment of reperfusion. It can't be excluded the influence of moxonidine to the peripheral imidazoline receptors [10].

The agonist of peripheral imidazoline (I_2) receptors C7070 releases its hepatoprotective action by mechanisms, like in case of skin flap ischemia. Obliviously, its influence to the preservation of mitochondria using ATPase canals, presented on the external and internal mitochondrial membranes. Slowing and blocking of avalanche ferric ion current decrease the oxidative stress with all its manifestations during the reperfusion of liver [11].

Activation of the imidazoline receptors results to the rise of arachnid acid synthesis and inhibition of Na^+/H^+ ion exchange canals. It seems, that imidazoline receptors belong to the neurocytokine receptors [12]. Activation of the central imidazoline (I_1) receptors leads to the decreasing of the blood pressure and slows a heartbeat. These all are the result of braking influence to the peripheral sympatic nervous system.

During the time we know the new generation of the imidazoline receptors agotists there were many pre-clinical and clinical trials of effectiveness of these drugs.

So, Mukaddam-Daher in his trial shows, that intravenous administration of moxonidinerised rat diuresis and Na/K excretion. This effect was blocked by administration of imidazolinereseptors antagonist epharoxan and decreased by effect of α-andrenoblocker – jobichinin [13].

Central imidazoline (I_1) receptors of hypothalamic area are involved into the glycemic level regulation, that was shown at the experiment with the selective I_1 receptor agonist agmantine, which causes the decreasing of glucose level in blood. The same action has moxonidine. Besides, it is assumed, that imidazoline receptors can be located in pancreas. Activation of these receptors is able to leads to increasing insulin secretion [14].

Using of moxonidine at Zucker line rats caused decreasing the level of hypothalamic neuropeptid Y, that may be one of the probably mechanism explaining the decrease in body mass during therapy with this drug [15].

It should be noted that not all of these effects can be explained by the activation of central I_1–receptors. Apparently, some of them still mediated by α_2–adrenergic receptors. In addition, a certain contribution is made by the peripheral action of drugs.

Representatives of the Kharkov pharmaceutical schools, by contrast, drew attention to the ability of agonists of peripheral imidazoline receptors influence glycemic control

[16]. According to their research, this group of drugs in its hypoglycemic action is not inferior to Metformin. But the unwanted side effects they have much less. Moreover, unlike Metformin, agonists peripheral imidazoline receptors do not cause the development of hypoproteinemia and hyperlactacidemia.

In addition to its other localizations, imidazoline receptors located on the membranes of adipocytes — fat cells. Stimulation of these receptors leads to increased lipid metabolism.

In the treatment of moxonidine (0.4 mg/day) for 8 weeks in 20 patients with hypertension there was a significant decrease in blood pressure, but the levels of total lipids, oxidized low-density lipoprotein and the ratio of different subtypes of lipoproteidov low density did not change significantly [17]. In an 8−week study, involving 51 patients with hypertension and hypercholesterolemia did not affect the level of blood lipids and rilmenidine [18].

The history of biguanide derived from guanidine – thing of the past. In the Middle ages for the treatment of patients with diabetes used an extract from the root of the French lilac. The drug Galegaofficinalis contained a substance "guanidine" which was aimed at reducing the clinical symptoms of diabetes.

Since, at this time established a significant correlation of insulin resistance with the activity of the sympathetic nervous system, it is logical that you need a "universal" tool that would control and glycemia, and insulin resistance and SNS activity.

Besides, do not forget that the role of endothelial cells in the development of cardiovascular disease was an important discovery for understanding pathogenesis including diabetic vascular lesions [19].

Modern hypoglycemic medicines may have positive pleiotropic effects on the pancreatic tissue. However, their activity is insufficient to prevent the development spotswoode diabetes complications [20].

In view of the foregoing, it becomes apparent the advantage of agonists of peripheral imidazoline receptors (IR2) as drugs that affect the early survival of patients with diabetes mellitus type II.

Conclusion

1.Theimidazoline I_2 receptors agonist C7070 at a dose of 1 mg/kg at 4.5 times prevents the increase of ALT and AST and at 2.5 times reduces areaof ischemic injury and necrosis in the modereating of a 15-minute ischemia of the liver.Hepatoprotective effect of C7070 50% decreased by antagonist of peripheral imidazolinereceptors BU224 (1 mg/kg)

2. Moxonidin andmetformine also has a hepatoprotective effect and their coefficients are 36.88% to 44.99% for moxonidine (ALT and AST) and 34.20/21.02 for Metformin (ALT/AST). Factors histological hepatoprotective activity amounted to 72.33 and 38.96 for moxonidine and metformine respectively.

3. On hepatoprotective activity of the drug was inferior C7070 (72.81/62.13/82.61 ALT/AST/histology, respectively).

Acknowledgement

A study produced in the framework of the collaborative work on the state contract 10 Dec 2014 №14411.2049999.19.109 "Preclinical studies of anti-diabetic drugs – agonist imidazoline receptors" by “Pharma2020”.

Conflicts of interest

The authors have no conflict of interest to declare.

References

1. Kolmykov DI, Alehin SA. Ischemia/reperfusion effect on pancreatic volumetrical blood flow velocity. *Research result: pharmacology and clinical pharmacology*. 2015;1(1):42-46. doi: 10.18413/2500-235X-2015-1-4-51-56.[Full text]

2. Buzov AA, Kulikov AL, Avtina TV, Pokrovskii MV, Osipova OA. Development and validation of methods of quantitative determination of the new antidiabetic drug in the blood plasma of rats by high performance liquid chromatography with mass spectrometric detection. *Research result: pharmacology and clinical pharmacology.* 2016;2(1):52-57. [eLIBRARY]

3. Chigunadze AL, Artyushkova EB, Mishustin VN, et al. Experimental justification of new way of pharmacological correction for contact frostbite using DSLET opioid peptide and serotonin adipinate to enhance surgycal treatment. *Research result: pharmacology and clinical pharmacology*. 2016;2(2):3-19. doi: 10.18413/2313-8971-2016-2-2-3-19. [eLIBRARY] [Full text]

4. Ragulina VA, Kostina DA, Dovgan AP, Burda YE, Nadezhdin SV. Nuclear factor kappa B as a

potential target for pharmacological correction endothelium-associated pathology. *Research result: pharmacology and clinical pharmacology.* 2017;3(1):114-124 doi: 10.18413/2500-235X-2017-3-1-114-124. [eLIBRARY] [Full text]

5. Zhernakova NI, Alechin SA, Lomykov DI, Dolzhikov AA, et al. Preconditioning for ischemic and reperfusion injury of the liver. *Scientific bulletins of BSU. Medicine. Pharmacy. [Nauchnye vedomosti belgorodskogo gosudarstvennogo universiteta. Seriya: medicina. Farmaciya]*. 2012;(4-1(123)):157-162. (In Russian) [Full text] [eLIBRARY]

6. Abrashova TV, Gushchin YA, Kovaleva MA, Rybakova AV, Selezneva AI, Sokolova AP, Khodko SV. Directory. Physiological, biochemical and biometric indicators of the norm of experimental animals. SPb: LEMA; 2013. 116 p. (In Russian) [Full text]

7. Bivalkevich NV. Regularities of structural and functional reorganization of the liver in the formation of diets-induced non-alcoholic fatty liver disease in rats [dissertation]. [Vladivostok]; 2015. 127 p. (In Russian) [Full text]

8. Zilov AV, Terekhova AL. Metformin – 50 years in clinical practice. *The attending physician*. [Lechashchij vrach]. 2008;3:16-20. (In Russian) [Full text] [eLIBRARY]

9. Kirpichnikov DI, McFarlane SI, Sowers JR. Metformin: an update. *Ann Intern Med*. 2002;137(1):25-33. [PubMed]

10. Chan CK, Head GA. Relative importance of central imidazoline receptors for antihypertensive effects of moxonidine and rilmenidine. *JHypertens* 1996;14(7):855-64. [PubMed]

11. Free radicals and antioxidants in chemistry, biology and medicine. International Scientific and Practical Conference; 2013; Novosibirsk: NGPU; 2013. 172 p. (In Russian) [Full text]

12. Ernsberger P. The II-Imidazoline Receptor and Its Cellular Signaling Pathways. *ArmNYAcadSci.* 1999;881:35-53. [Full text]

13. Amann K, Nichols C, Tornig J, et al. Effect of ramipril, nifedipine, and moxonidine on glomerular morphology and podocyte structure in experimental renal failure. *Nephrol Dial Transplant.* 1996;11(6):1003-1011. [PubMed]

14. Bing C, King P, Pickavance L, et al. The effect of moxonidine of feeding and body fat in obese Zucker rats; role of hypothalamic NPY neurons. *Br J Pharmacol.* 1999;127(1):35-42. [PubMed]

15. Bauduceau B, Mayaudon H, Dupuy O. Rilmenidine in hypertensive type 2 diabetic: a controlled pilot study versus captopril. *JCardivascRisk.* 2000;7(1):57−61. [PubMed]

16. Kalinkin NV. Endothelial dysfunction as one of the possible pathogenetic mechanisms of anthracycline heart damage. *Ukranian cardiologic journal. [Ukrainskij kardiologicheskij zhurnal]*. 2000;5-6:67-71. (In Russian) [Full text]

17. Kondratieva L.V. Metformin is a test of time. *Russian Medical Jornal.* 2007;27:2098. (In Russian) [Full text]

18. Starostina EG. Place of metformin in therapy of type 2 diabetes. *Voronezh Society of Endocrinologists and Diabetologists*. Available date: *http://www.voed.ru/art_029.htm.* (In Russian) [Full text]

19. Wang G.W., Klein J.B., Kang Y.J. Metallothionein inhibits doxorubicin-induced mitochondrial cytochrome c release and caspase-3 activation in cardiomyocytes. *J. Pharmacol. Exp. Ther*. 2001;298(2):461-468 [PubMed]

20. Molchanova OV, Pokrovskaya TG, Povetkin SV, Reznikov KM. Endothelioprotective property of the combination of the thioctic acid and rosuvastatin shown in the endothelial dysfunction models. *Research result: pharmacology and clinical pharmacology.* 2016;2(1):9-15. [Full text] [eLIBRARY]

Author Contributions

Dovgan Anton Pavlovich, Radiologist MAKS-Med Clinic "Garmonia Zdorovia", e-mail: dovgan@bsu.edu.ru. The author performed significant contribution to the concept and design of the work; collection and analysis of data for publication.

Urojevskaya Janna Sergeevna, Pediatrician MAKS-Med "Garmonia Zdorovia", e-mail: personanongrataj@mail.ru. The author performed interpretation of data for publication.

Khavansky Anatoly Viacheslavovich, Therapeutic MAKS-Med "Garmonia Zdorovia", e-mail: anatoly_khav@mail.ru. The author performed statistical data processing and evaluation of the cytokine profile.

Received: July, 03, 2017
Accepted: August, 30, 2017
Available online: September, 27, 2017