Rus.

UDC: 615.275.4 **DOI: 10.18413/2313-8971-2017-3-4-78-88**

Anton P. Dovgan

LIGAND OF PERIPHERAL IMIDAZOLINE RECEPTORS BASED ON AMIDES OF HETEROCYCLIC ACIDS C7070: EFFECT ON ISHEMIZED TISSUES

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

Abstract

Introduction: In this regard, the study of pleiotropic hepatoprotective properties of the agonist of peripheral imidazoline receptors C7070 seems interesting from an applied point of view.

Materials and Methods: Models of a skin flap on a feeding leg, ischemia-reperfusion of the liver and rat heart isolated from Langendorff (ischemia-reperfusion and doxorubicin cardiomyopathy) were used.

Results and Discussion: The I2 agonist C7070 at a dose of 10 mg/kg 4.5-fold prevents the increase in ALT and AST (332.56 ± 22.05/825.49 ± 22.46 ALT/AST 526.90 ± 17.97/1045.16 ± 80.02 units/l in control) and 2.5 times reduces the areas of ischmeic damage and necrosis (0.058 ± 0.029/0.046 ± 0.013 mm^2) in the modeling of 15-minute ischemia liver. Moxonidine and metformin had a hepatoprotective effect: 44.99 and 36.88 for moxonidine (ALT and AST) and 34.20 / 21.02 for metformin (ALT / AST). The coefficients of histological hepatoprotective activity: 72.33 and 38.96 (moxonidine and metformin).

C7070 (10.0 mg/kg) has a pronounced dermatoprotective activity and prevents the formation of necrosis on days 3 and 7 of the pathology by 40%. The dermatoprotective activity of metformin (50 mg/kg) from 3 to 10 days decreases from 81% to 92%. The dermatoprotective activity of moxonidine (1 μg/kg) was maximal on the 7^{th} day and was 76%.

In the isolated rat heart, the C7070 showed a protective effect in ischemia-reperfusion and on the model of doxorubicin cardiomyopathy. The S_{TTi} index: 8.3, 1.5; 7.9 and 7.8 U. in control, C7070, moxonidine and metformin.

Keywords: C7070, metformin, moxonidine, ischemia, reperfusion, liver, skin flap, isolated heart, according to Langendorf.

Introduction

Ischemia is a trigger and a pathogenesis stage simulteniously [1].

Diabetes mellitus and a metabolic syndrome lead to fat hepatic dysthrophy transferring into the necrosis of hepar [2].

A drug therapy of diabetes mellitus and metabolic syndrome have neither hepatoprotective effects [3].

In this case, a study of additional pharmacological correction during the biguanide therapy looks very interesting.

Imidozoline receptors are localized in CNS (in the reticular formation cores, rostral ventrolateral area of oblong brain) – type I. Another type of imidazoline receptors – type II – able to be finded on the peripheral tissues (hepar, kidneys, pancreas) [4]. The last one was also founded based in mitochondrial membranes. One more type of imidazoline receptors was described as receptors in sympatic nerve endings. The activation of these receptors decreases the noradrenaline production [5]. I1−receptors was also detected on the platelet's membranes.

Rilmenidine and moxonidine are high selected agonists of I1-receptors. Their affinity to the I1-receptors more than to the α_2-adrenoreceptors. Both of them has pronounced hypotesive effect. It should be considered as a sympatolitic activity of the I1-agonists [6].

Imidozoline receptors were detected in smooth muscles of airways in the experiments on the dogs [7].

Imidazoline receptors agonists have same pharmacokinetic parameters. It was noticed in number of studies, that their effect to the imidazoline receptors continuing the whole day, despite their short elimination period. Most numbers of scintiests consider this property in connection with accumulation of these drugs in brain and other fat-containing tissues [8, 9, 10, 11].

Intolerance to glucose, overweight, dyslipiaemia and arterial hypertension are syndicated to the "metabolic syndrome" [12].

Probably, hyperinsulinaemia is able to be related to the arterial hypertencion development. Insuline increases sympatic nervous system activity. This leads to the increasing of cardiac output and periferial vascular resistence [13, 14]. Agonists of central imidazoline receptors (I1) have a sympatolitic effect [15, 16]. Agonists of peripheral (I2) imidazoline receptors decreases the tissue's insuline resistence, decreasing the level of insulin [16].

The studies of central imidazoline receptors agonists are more common, than the peripheral one [17].

For example, placebo-controlled study by Haenni A., (1999) [16] in 72 patients with AH and overweight moxonidine 8-week therapy leaded to the reliable insulin sensitivity. This effect was detected in glucose intolerance group only. The group of patients without glucose intolerance the modification of insulin sensitivity was not detected.

The basic role in pathogenesis of diabetes mellitus (II type) owned to the two independent pathophysiologycal processes: the resistance to the insulin and decreasing of its secretion by β-cells of pancreas [18]. The insulin resistance plays important role in metabolic syndrome development. Also, the insulin resistance is accompanied by hyperinsulinaemia – independent factor of heart ischemic disease evolution [19].

Pharmacological activity of metfrormin has not limited by positive influence to the carbohydrate metabolism.

Around at 2/3 patients with hyperlypoproteinaemia IIB and IV types without diabetes metformin decreases triglyceride level over 30%; decreasing of hypercholesterinaemia is less pronounced [9, 12].

Modern hypoglycemic drugs are able to exert positive pleotropic effects to pancreas. But their activity is not enough to prevent diabetes's complications.

In the view of foregoing, the vantage of peripheral imidazoline receptors agonists, as a medicines affecting early diabetic patient's survival, becomes obvious.

The purpose of this study was detection of vascular complication's protection of diabetic patients.

Materials and Methods

White Wistar rats were chosen for this study as standard animals for specific activity studies according to National pre-clinic studies manual (2012). There were used 180 rats both sexes.

Doses of medicines

Every used dosage was derived with help of the interspecific recount of doses according to human minimal therapeutic doses.

The minimal therapeutic dose for C7070 was detected as 100 mg for adult human. Using the formula, the dose 10 mg/kg for rats was obtained.

Metformin has 500 mg as a minimal therapeutic dose for human. According to formula, 50 mg/kg was selected as rat dose.

For moxonidine were obtained the rat dose 20 μg/kg (minimal therapeutic dose for human – 0.2 mg)

Data for minimal therapeutic doses were detected in instructions for use of the medication.

During the using of langenorff models the equivalent doses of medicines were added into the prfusate.

Pharmacological methods.

For **the study of ischemised skin flap survival** 40 both sex rats were used. The

animals were divided into the 4 groups (n=10): contoll group; metformin (50 mg/kg) group; moxonidine (20 µg/kg) group; C7070 (10 mg/kg) group.

Method of E.V. Kizhaev (1986) in P.A. Galenco-Yaroshevski was used in this model [20].

The first one was the formation of skin flap on the anterior abdominal wall. Then, studying medicines were administrated into the animals per os. Calculation of necrosis area at 3rd, 7th and 10th days with help of computer modeling was the third stage of this experiment.

The study of hepatotropic activity of these medicines was carried out using the D.A. Lopatin [21] method.

60 both sex rats were operated in this model. The animals were divided into the 6 groups (n=10): intact groups; pseudooperated animals (without vascular ligation); control group, metformin (50 mg/kg) group; moxonidine (20 µg/kg) group; C7070 (10 mg/kg) group.

All animals survived this experiment.

ALT and AST were chosen as biochemical markers of hepatic function. The structural changes evaluated with the help of hepatic histology.

The study of anti-ischemic cardio protective activity was made using isolated heart by Langendorff [22, 23, 24, 25, 26]. The animals were divided into the 4 groups (n=10): contoll group; metformin (50 mg/kg) group; moxonidine (20 µg/kg) group; C7070 (10 mg/kg) group.

The next functional indexes for heart work were chosen: left ventricular pressure (LVP), heartbeat (HB), the power of heart systole and diastole (+dp/dt and – dp/dt). All the indexes were registered by Biopac Inc. system hardware using the ACQ Knowledge software. Parameters were detecting during the whole experiment [27, 28, 29, 30, 31].

The model of doxorubicine cardiomyopathy was designed and patented by group of scientists in Center of Pre-Clinic and Clinic Trials of Belgorod State National Research University (including the author of this article).

50 both sex animals were divided into the 5 groups (n=10): intact groups; control group (doxorubixine 20 mg/kg); doxorubixine (20 mg/kg) + metformin (50 mg/kg) group; doxorubixine (20 mg/kg) + moxonidine (20 µg/kg) group; doxorubixine (20 mg/kg) + C7070 (10 mg/kg) group.

In 3 days before the modeling all experimental animals started administration of studying medicines per os. After an hour doxorubicine (20 mg/kg) was administrated into the rats intraperitoneal.

Results and Discussion

1. The influence of peripheral imidazoline receptors agonists at skin flap survival.

Intragastric administration of studying drugs leads to the increasing of skin flap survival. The agonist of peripheral imidazoline receptors (C7070) had the biggest anti-ischemic activity (tab. 1).

Table 1

THE influence of C7070 (10 mg/kg), moxonidine (20 µg/kg) and metformin (50 mg/kg) at the degree of skin flap necrosis at rats. (%) (M±m)

Drugs and doses	Skin flap necrosis's degree, %		
	3rd day	7th day	10th day
Control	30.9±2.8	65.7±2.6	84.5±3.2
C7070 (10 mg/kg)	21.5±3.1*	41.4±3.2*	68.4±2.7*
Moxonidine (20 µg/kg)	24.8±2.9*	50.5±3.0*	77.0±2.7*
Metformin (50 mg/kg)	25.4±2.4*	59.2±2.2*	78.3±2.9*

Note: *–p>0.05 in comparison with control group

Agonist of peripheral imidazoline receptors C7070 has more powerful dermatoprotective activity in comparing with moxonidine and metformin.

This difference is able to be controlled by influence of C7070 at the vascular tonus. From the other side, we can expect this difference due to mitochondrial protection of C7070. It leads to maintaining of work capacity of skin cells.

But, during the time, the difference between studing drugs' effects increasing from 5% at 3rd day to 10% at 10th day.

2. The influence of peripheral imidazoline receptors' agonist at hepar damage due to ischemia-reperfusion.

All animals survived the experiment.

The levels of AST and ALT were measured in blood of experimental animals after their euthanasia (3 days after modeling).

The next numbers were detected in biochemical blood analysis (tab. 2).

So, laporotomy has no significant impact to the ALT and AST levels. 15 minutes of hepatic ischemia and following reperfusion reliably increase the ALN and AST levels.

Using studying drugs decrease hepatic ferments' level. But even peripheral imidazoline receptors agonist C7070 was not able to return ferment level back to the control group numbers.

Despite this, C7070 had the highest anti-ischemic activity among all studied medicines in this experiment.

Hepatic ferments' levels are not enough to evaluate anti-ischemic activity of these drugs. That's why the rat liver was excise 3 days after modeling to describe its histological image.

Ischemic damage area and ischemic necrosis area was chosen as histology markers of structural damage (tab. 3).

Table 2

The influence of C7070 (10 mg/kg), moxonidine (20 μg/kg) and metformin (50 mg/kg) at the mean values of ALT and AST in blood of experimental animals (U/ml) (M±m)

Animal group	ALT, U/ml	AST, U/ml
Intact	102.8±8.8	284.1±19.3
Pseudo-operated	110.2±21.9*	289.8±16.2*
Control	526.9±17.9**	1045.1±80.0**
C7070 (10 mg/kg)	143.2±16.9[1]	395.8±33.3[1]
Moxonidine (20 μg/kg)	289.8±15.2[1]	687.7±28.3[1]
Metformine (50 mg/kg)	332.5±22.0[1]	825.4±22.4[1]

Note: *–p>0,05 in comparison with intact group; **– p<0,05 in comparison with psedo-operared group; 1 – p<0,05 05 in comparison with control group.

Table 3

The influence of C7070 (10 mg/kg), moxonidine (20 μg/kg) and metformine (50 mg/kg) to the hepatic damage area, mm^2 (M±m)

Animal group	Iscemic damage area, mm^2	Iscemic necrosis area, mm^2
Intact	n/a	n/a
Pseudo-operated	n/a	n/a
Control	0.387±0.014	0.207±0.021
C7070 (10 mg/kg)	0.058±0.029*	0.046±0.013*
Moxonidine (20 μg/kg)	0.090±0.025*	0.075±0.015*
Metformin (50 mg/kg)	0.238±0.052*	0.125±0.020*

Note: *–p>0.05 in comparison with control group

Histological examination did not detected significant sings of ischemic damage or necrosis in intact or pseudo-operated animal group.

Thus, peripheral imidaazolinee receptors' agonist C7070 has the highest hepatoprotective properties in frames of this study.

3. The influence of peripheral imidazoline receptors' agonist C7070 to the functional ischemic isolated heart damage. (by Langendorff)

It was detected, that decreasing of perfusion in 10 times (ischemic hypoperfusion) all selected parameters (HB, LVP, $+dP/dt_{max}$, $-dP/dt_{max}$) were significant decreased by 5^{th} minute. They didn't back to the normal level by 20^{th} minute.

During the reperfusion these parameters were less than start level by 5^{th} minute of experiment. The same image we had by 20^{th} minute of reperfusion, when most of parameters had less than half of start level (tab. 4). Besides, the recovery of perfusion volume (reperfusion) leaded to the reperfusion arrhythmias (30% fibrillations in cases).

Thus, moxonidine and metformin haven't significant influence to the heart muscle working. The same time, peripheral imidazoline receptors' agonist C7070 allowed cardiomyocytes to contract with the almost same power and frequency as heart without ischemia.

Metformin and moxonidine have a central and common activity to the whole organism. Peripheral imidazoline receptors' agonist C7070 has the action realized on the cells' level. That's why this medicine is able to work in isolated heart. Transmembrane ion current control and ATP'ase channels normalization allows C7070 significant decrease the heart ischemic damage.

In this connection, the question about decreasing of cardiotoxic drugs effects by C7070 is very interesting.

The most socially significant of them are antineoblastome antracycline drugs [32, 33].

Doxorubicine cardiomyopathy model has been used in this experiment.

This model uses the comparison of control animal group (doxorubicine 20 mg/kg 2 days before modeling) with intact group. And comparison of experiment groups with control.

The coefficient of AUC in time of 15" stimulating count was derived and patented specially for this model.

The stimulation of dilated by doxorubicine heart with help of electric stimulator leads to diastolic dysfunction. It express in increasing of minimal left ventrical pressure in time of electric stimulation. So, it leads to the increasing AUC level. The coefficient S_{TTi} shows the degree of diastolic dysfunction. Its meaning inversely to the cardioprotective activity of the drug.

The results of experiment are able in fig. 1.

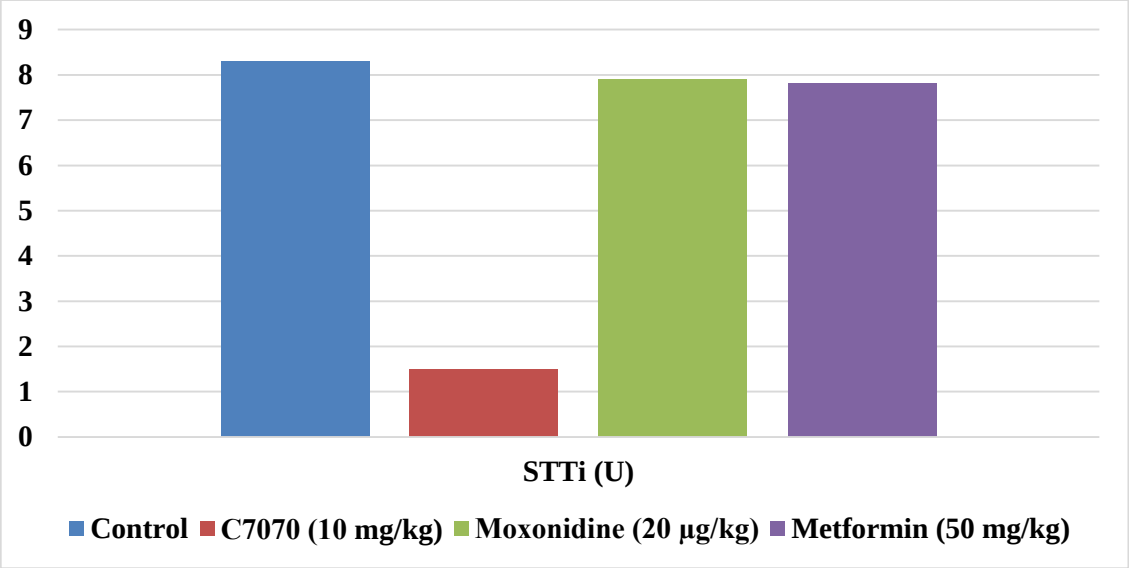

Fig. 1. The coefficient S_{TTi} for C7070 (10 mg/kg), moxonidine (20 μg/kg) and metformine (50 mg/kg) in modeling of doxorubicine (20 mg/kg) cardiomyopathy

Table 4

The influence of C7070 (10 mg/kg), moxonidine (20 μg/kg) and metformine (50 mg/kg) to the heart function measures (M±m, n=10)

Animal group			Control	Moxonidine, 20 μg/kg	Metformin, 50 mg/kg	C7070, 10 mg/kg
Start level		LVP	129.0±2.4	127.4±3.7	130.1±1.1	125.1±1.1
		+dp/dt	2728.0±38.4	2694.3±46.1	2697.7±39.4	2799.3±20.0
		-dp/dt	1346.4±41.2	1342.1±25.0	1331.1±36.1	1235±31.2
		HB	168.1±22.4	179.2±11.8	165.4±18.9	160.1±15.3
Hypo perfusion time	5’	LVP	50.75±7.6	48.47±4.1	50.81±5.2	65.84±3.4*
		+dp/dt	1114.0±12.4	1181.2±21.2	1102.5±15.8	1276.4±11.7*
		-dp/dt	613.1±11.2	625±1.2	617±7.6	656±4.1*
		HB	76.6±2.4	78.1±4.3	74.1±3.7	85.6±1.1*
	20’	LVP	28.2±1.4	23.48±4.2	26.94±3.1	39.63±2.3*
		+dp/dt	448.8±12.4	441.6±22.1	442.4±15.8	629.8±14.4*
		-dp/dt	306.7±9.8	307.8±6.7	309.5±7.1	378.4±11.2*
		HB	213.1±7.2	211.8±6.4	215.6±8.2	280.4±4.5*
Reperfusion time	5’	LVP	101.9±2.5	115.9±4.9	128.7±7.1*	126.8±5.8*
		+dp/dt	2079.4±84.5	1991.5±86.1	1987±74.9	2564±81.2*
		-dp/dt	1137.1±25.8	1195.0±29.7	989±15.3*	1215±20.8*
		HB	191.7±9.8	195.8±8.3	171.5±7.4*	161.9±9.1*
	20’	LVP	101.3±8.6	115.4±7.5	125.8±5.1*	129.5±4.3*
		+dp/dt	2121.1±30.8	2095.4±35.6	2184.6±29.3	2859±38.5*
		-dp/dt	1137.0±25.4	1184.4±21.3	1121.1±36.4	1279±30.1*
		HB	181.8±10.8	185.4±9.6	169.5±11.2	160.2±3.6*

Note: LVP – left ventrical pressuare, “+dp/dt” – heart systolic power; “-dp/dt” – heart diastolic power; HB – heartbeat. *–p>0,05 in comparison with control group

Conclusion

Thus, peripheral imidazoline receptors' agonist C7070 had the highest anti-ischemic properties among all medicines included in this study.

All differences are able to be explaned by their mechanisms of action.

Metformin's mechanism of action based on decreasing of gluconeogenesis. Also, metformine plays one of the main roles in insulin peripheral effects increasing, anaerobe glucose metabolic way increasing, lipless decreasing.

In number in vivo and in vitro studies the metformin influence to the cell AMP-kinase was detected. This enzyme plays a role in glucose transport through the membranes with help of GLUT4 enzyme. Besides, metformin has showed the ability to cell membrane rigidity decreasing. It is one of the frequent condition able to lead to the diabetes's complications [20].

Metformin actives AMP-activated proteinkinase (AMPK) – hepatic enzyme of insulin signalization. AMPK also playing a role in common energy balance and glucose and lipid metabolism. AMPK activation is necessary stage for metformin inhibition effect to hepatic gluconeogenesis [14].

Resuming the foregoing, anti-ischemic metformin action based on energy reserves cumulating and decreasing of current nutrial reserves spending.

Moxonidine is a central imidazoline receptors' agonist. It takes a part in redistribution of hepatic bloodstream through the collateral vessels from a. gastrica sinistra. They were free from flow reducing. Also, moxonidine's anti-ischemic activity is able to base on vessels' opening in reperfusion moment [35].

There were enough number of pre-clinic and clinic trials of imidazoline receptors agonists.

For example, Mukaddam-Daher in his trial detected the increasing of diuresis, Na and K excretion at rats in moxonidine group. The effect was blocked by efaroxan and decreased by α-adrenoblocker himbine [11].

Central I1-receptors of hypothalamic zone include in glycemia level control. It was showed in experiment with selective I1 agonist – agmantin – reduce of glucose blood level. The same action has a moxonidine too. Besides, imidazoline receptors are able to locate in pancreas and control the insulin secretion [35].

The using of moxonidine at Zucker rats leaded to hypothalamic neuropeptid Y level decreasing. It is able to be one of the weight-reducing mechanisms of this drug [15].

Probably, some part of these actions realize through the α_2-adrenoreceptors. The peripheral actions of these drugs are able to lead these changes too.

Peripheral imidazoline receptors' agonist C7070 realize its action through the mitochondrial protection. The influence to the ATP-channels of external and internal mitochondria membranes. Slowing and blocking of avalanche current of iron ions leads to the oxidative stress decreasing [.

Imidazoline receptors activation leads to the arachnid acid synthesis increasing and Na^+/H^+ ion change inhibition [17].

Scientists from Kharkov paid their attention to the peripheral imidazoline receptors' agonists ability to glycemia control. According to their studies, anti-diabetic effects of these drugs not less than metformin. But, C7070 has more favorable toxic profile in comparison with biguanides [39]. Besides, imidazoline receptors' agonists don't lead to the hypoproteinaemia and hyperlactacidaemia progression [40].

Modern anti-diabetic drugs can lead some pleiotropic effects to the pancreas. But their activity is not enough to prevent the complications.

So, imidazoline receptors' agonists advantage as drugs with vascular prevent properties becomes obvious.

Resuming the foregoing, it is able to combine all mechanisms of action into one scheme (fig. 2).

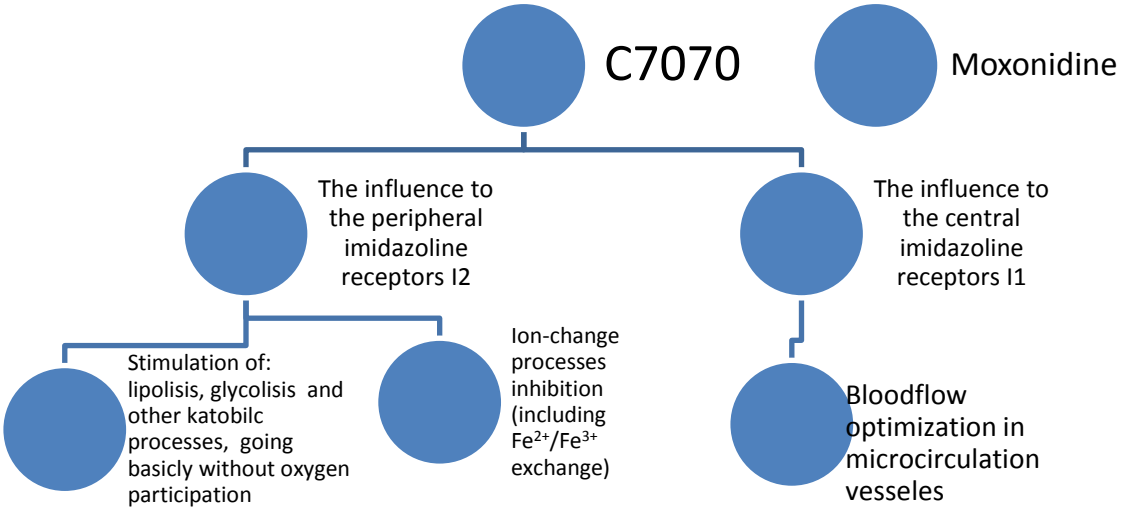

Fig. 2. The differences between C7070 and moxonidine mechanisms of action

Findings

Peripheral imidazoline receptors' agonist C7070 (10 mg/kg) has pronounced dermatoprotective activity and prevents the skin flap necrosis progression by 3rd and 7th day to 40%.

Peripheral imidazoline receptors' agonist C7070 (10 mg/kg) shows 4.5 times less increasing of ALT and AST (332.56±22.05/ 825.49±22.46 U/ml ALT/AST in C7070 group versus 526.90±17.97/1045.16±80.02 U/ml in control group) and decreases in 2.5 times ischemic damage and ischemic necrosis area (0.058±0.029 и 0.046±0.013 mm^2 respectively) in modeling of 15-minute hepatic ischemia.

Dermatoprotective activity of metformin (50 mg/kg) decreases from 81% at 3rd day to 92% at 10th day.

Dermatoprotective activity of moxonidine (20 μg/kg) was maximal by 7th day – 76%.

Moxonidine and metformine had a hepatoprotective activities too.

Metformin decreases the level of ALT/AST to 332.5±22.0/825.4±22.4 U/ml and ischemic damage/necrosis area to 0.238±0.052/0.125±0.020 mm^2.

Moxonidin parameters were: 289.8±15.2/687.7±28.3 U/ml for AST/ALT and 0.090±0.025/0.075±0.015 mm^2 for ischemic damage/necrosis areas 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 C7070" by "Pharma2020".

Conflicts of Interest

The authors have no conflict of interest to declare

References

1. 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.: Publishing house "LEMA",* [*SPRAVOCHNIK. Fiziologicheskie, biohimicheskie i biometricheskie pokazateli normy ehksperimental'nyh zhivotnyh.]* 2013. 116 p. (in Russian) [Fulltext]

2. Zhernakova NI, Alechin SA, Lomykov DI, Dolzhikov AA. Preconditioning for ischemic and reperfusion injury of the liver.

Scientific bulletins of BSU. Medicine. Pharmacy. [Nauchnye vedomosti belgorodskogo gosudarstvennogo universiteta. Seriya: medicina. Farmaciya]. *2012;17(*4-1(123)):157-162. (in Russian) [Fulltext][eLIBRARY]

3. 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]: Vladivostok State Medical University; 2015. 127 p (in Russian) [Fulltext]

4. Ciubotariu D, Nechifor M. Involvement of the imidazoline system in drug addiction. *Rev Med ChirSoc Med Nat Iasi*. 2012;(116(4)):1118-22. [PubMed]

5. Dupuy O, Bauduceau B, Mayaudon H. Efficacy of rilmenidine, a selective I1 imidazoline receptor binding agent in diabetic hypertensive patients. *Am J Hypertens.* 2000;(13(6Pt2)):123S-126S. [PubMed]

6. Chan CK, Head GA. Relative importance of central imidazoline receptors for antihypertensive effects of moxonidine and rilmenidine. *JHypertens.* 1996;(14):55-64.[Fulltext]

7. Ernsberger P. The II-Imidazoline Receptor and Its Cellular Signaling Pathways. *ArmNYAcadSci.* 1999;(881):35−53.[Fulltext]

8. Lee IT, Hung YJ, Chen JF, Wang CY, Lee WJ, Sheu WH. Comparison of the efficacy and safety profiles of two fixed-dose combinations of antihypertensive agents, amlodipine / benazepril versus valsartan / hydrochlorothiazide, in patients with type 2 diabetes mellitus and hypertension: a 16-week, multicenter, randomized, double-blind, noninferiority study . *ClinTher.* 2012;(34(8)):1735-50. doi: 10.1016 / j.clinthera.2012.06.014. [PubMed]

9. Bakris GL, Sarafidis PA, Weir MR, Dahlöf B, Pitt B, Jamerson K, Velazquez EJ, Staikos-Byrne L, Kelly RY, Shi V, Chiang YT, Weber MA; ACCOMPLISH Trial investigators. Renal outcomes with different fixed-dose combination therapies in patients with hypertension at high risk for cardiovascular events (ACCOMPLISH): a prespecified secondary analysis of a randomized controlled trial. *Lancet*. 2010;(375 (9721)):1173-81. doi: 10.1016 / S0140-6736 (09) 62100-0 [PubMed]

10. Everett BM, Glynn RJ, Danielson E, Ridker PM; Val-MARC Investigators. Combination therapy versus monotherapy as initial treatment for stage 2 hypertension: a prespecified subgroup analysis of a community-based, randomized, open-label trial. *ClinTher*. 2008;(30(4)):661-72. [PubMed]

11. 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):1003-1011.[abstract]

12. De Luca N, Izzo R, Fontana D, Iovino G, Argenziano L, Vecchione C, Trimarco B. Haemodynamic and metabolic effects of rilmenidine in hypertensive patients with metabolic syndrome X. A double-blind parallel study versus amlodipine. *J Hypertens*. 2000;(18(10)):1515-22. [PubMed]

13. Zilov AV, Terekhova AL. Metformin – 50 years in clinical practice. *The attending physician*. 2008;(3):16-19.[Full text][eLIBRARY]

14. Kirpichnikov DI, McFarlane SI, Sowers JR., Metformin: an update. *Ann Intern Med*. 2002;(2.137(1)):23-25.[PubMed]

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

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

17. Gasparik V, Greney H, Schann S, Feldman J, Fellmann L, Ehrhardt JD, Bousquet P. Synthesis and biological evaluation of 2-aryliminopyrrolidines as selective ligands for I1 imidazoline receptors: discovery of new sympatho-inhibitory hypotensive receptors effects in metabolic syndrome. *J Med Chem*. 2015;(22;58(2)):878-87. doi: 10.1021 / jm501456p. [PubMed]

18. Starostina EG. Place of metformin in therapy of type 2 diabetes. *Voronezh Society of Endocrinologists and Diabetologists.*

[Voronezhskoe obshchestvo Endokrinilogov I Diabetologov]. 2012 (in Russian) [Full text]

19. Kondratieva LV. Metformin is a test of time. *Russian Medical Jornal*. [*Russkii Medicinskii Zhurnal]*. 2007;(27):2098. (in Russian) [Fulltext]

20. Zelenskaya AV. Dermatorotective effects of combined therapy by reamberine and roxod in diabeted rats with exogenic hypercholesterineaemia. [dissertation]. [Staraya Kupavna]: Russian center of biologically active substances safty; 2014. 183p (in Russian) [Fulltext]

21. Lopatin DA, Pokrovsky MV, Loktionov AL, Konoplya AI, Denisyuk TA, Cepeleva SA. A method of correction of ischemic disorders caused by reperfusion injury of the liver. Russian Federation patent RF 2012100863/14. 2013 Apr 20 [Fulltext]

22. Danilenko LM, Pokrovsky MV, Novikov OO. Pharmacological preconditioning of e- (2,2,2-trimethylhydrazinium) propionate, new ways of realization of its anti-ischemic effects. *Scientific bulletins of the Belgorod State University. Series: Medicine. Pharmacy. [Nauchnye vedomosti belgorodskogo gosudarstvennogo universiteta. Seriya: medicina. Farmaciya]*. 2012;(20(22-3)):13-19. (in Russian) [FullText]

23. Danilenko LM, Pokrovsky MV, Korolev AE. Mitochondrial ATP-dependent potassium channels as a point of application of action in the case of distant re-conditioning. *Scientific bulletins of the Belgorod State University. Series: Medicine. Pharmacy. [Nauchnye vedomosti belgorodskogo gosudarstvennogo universiteta. Seriya: medicina. Farmaciya]*. 2010;(22(12-2)):15-18. (in Russian) [FullText]

24. Pokrovskiy MV, Artyushkova EB, Pokrovskaya TG. Methods of Experimental Modeling of Endothelial Dysfunction. *Allergology and Immunology*. *[Allergologia I immunologia]*. 2008;(9(3)):327 (in Russian) [eLIBRARY]

25. Chernomortseva ES, Pokrovskii MV, Pokrovskaia TG. Experimental study of cardioprotective and endothelioprotective action of macrolides and azalides. *Experimental and clinical pharmacology* [*Eksperimental'naia I klinicheskaia farmakologiia]*. 2009;(72(2)):29-31. (In Russian) [PubMed]

26. Kalvins IYa. Synthesis and biological activity of the new Mildronate bioregulator. *Experim. and clinical. pharmacotherapy*. 1991;(19): 7-14. [Fulltext]

27. Artyushkova EB, Pokrovskiy MV, Artyushkova EV. Endothelio- and cardio-protective effects of meldonium and thymetazidine in L-NAME induced endothelial dysfunction in the experiment. *Kursk scientifically-practical Herald "Persons and his health" [Kurskii nauchno-prakticheskii vestnic "Chelovek I ego Zdorov'e]*. 2010;(3):5-10. (in Russian) [FullText]

28. Belous AS, Pokrovskii MV, Pokrovskaya TG. Correction of endothelial dysfunction with impaza preparation in complex with enalapril and losartan during modeling of NO deficiency. *Bulletin of Experimental Biology and Medicine [Bulleten' experimenta;noi biologii I mediciny]*. 2009;(148(3)): 511-513. (in Russian) [PubMed]

29. Pokrovsky MV, Danilenko LM, Inderova AV. Endothelial and cardioprotective action in a series of 3- (2,2,2-trimethylhydrazinium) propionate derivatives. *Modern problems of the science of education [Sovremennye problem nauki I obrazovania]*. 2012;(20(4)):60. (in Russian) [FullText]

30. Danilenko LM, Pokrovsky MV, Novikov OO. Pharmacological preconditioning of e- (2,2,2-trimethylhydrazinium) propionate, new ways of realization of its anti-ischemic effects. *Scientific bulletins of the Belgorod State University. Series: Medicine. Pharmacy. [Nauchnye vedomosti belgorodskogo gosudarstvennogo universiteta. Seriya: medicina. Farmaciya]*. 2012;(20(22-3)):13-19. (in Russian) [FullText]

31. Pokrovskii MV, Pokrovskaya TG, Gureev VV. Correction of endothelial dysfunction by L-arginine under experimental pre-eclampsia conditions *Experimental and clinical pharmacology* [*Eksperimental'naia I klinicheskaia farmakologiia]*. 2012;(75(2)):14-16. (in Russian) [PubMed]

32. Kalinkin NV. Endothelial dysfunction as one of the possible pathogenetic mechanisms of anthracycline heart damage. *Ukranian*

cardiologic journal. 2000;(5-6):67-71. [Fulltext]

33. Wang GW, Klein JB, Kang YJ. Metallothionein inhibits doxorubicin-induced mitochondrial cytochrome c release and caspase-3 activation in cardiomyocytes. *J. Pharmacol. Exp. Ther*. 2001;(298):461-468 [PubMed]

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

35. Li JX. Imidazoline I2 receptors: An update. *PharmacolTher*. 2017;(oct,178):48-56. doi: 10.1016 / j.pharmthera.2017.03.009. [PubMed]

36. Sjakste N, Baumane L, Boucher JL, Dzintare M. Effects of gamma-butyrobetaine and Mildronate on nitric oxide production in lipopolysaccharide-treated rats. *Basic. Clin.PharmacolToxicol*. 2004(94 (1)):46-50. [PubMed]

37. Korokin MV, Pashin EN, Bobrakov KE, Pokrovskiy MV. Studying endothelioprotection and coronary action of derivatives 3-oksipiridin. *Kuban Research Medical Bulletin.[Kubanskii Issledovatel'skii Medicinskii Bulleten']*. 2009;(4):104-108. (in Russian) [eLIBRARY]

38. Danilenko LM, Kharitonova OV, Pokrovsky MV. Endotheliazation Mildronate and its derivatives in modeling L-NAME induced nitrogen oxide. *Scientific statement BSU. Ser. Medicine. Pharmacy. [Nauchnye vedomosti belgorodskogo gosudarstvennogo universiteta. Seriya: medicina. Farmaciya]* 2011;(22/2(16)):58-62. (in Russian) [eLIBRARY]

39. Butenko AK. Substantiation of the creation of new combined antidiabetic drugs based on the results of the clinical approbation of diakamf. *Drugs to human. Modern problems of making, research and approbation of drugs. Proceedings of XXV conference with international activity; 2008; Kharkov: NPhU (UA)*; 2008:318-324. [Fulltext]

40. Lowry JA, Brown JT. Significance of the imidazoline receptors in toxicology. *ClinToxicol (Phila)*. 2014;(52(5)):454-69. doi: 10.3109 / 15563650.2014.898770. [PubMed]

Contributions

Anton P. Dovgan, Radiologist MAKSMed Clinic "Garmonia Zdorovia", e-mail: dovgan@bsu.edu.ru.

Received: October, 02, 2017
Accepted: November, 30, 2017
Available online: December, 30, 2017