

Derivatives of 5-hydroxynicotinic Acid: New Compounds with Cardioprotective Action

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Abstract

Introduction: The search for new compounds with cardioprotective activity among the derivatives of 5-hydroxynicotinic acid is promising. **Objectives:** The objective of this study is to study the cardioprotective effects of the derivatives from 5-hydroxynicotinic acid SSC-77 (K-5-hydroxynicotinic acid) and SSC-497 (Mg-5-hydroxynicotinic acid). **Methods:** The cardioprotective effect of SSC-77 and SSC-prisocorubicin (20 mg/kg, intraperitoneally for 48 h) pathology was assessed by the results of the functional test with high-frequency stimulation (480 bpm). The research of myocardial resistance to ischemia/reperfusion injury was studied according to hypo-reperfusion model on an isolated rat heart on the record of pressure in the left ventricle. The isoenzyme creatine phosphokinase (KFK-MB) and lactate dehydrogenase (LDH) were determined for the complex evaluation of myocardial damage in the flowing off perfusate from isolated hearts. The activity of lipid peroxidation (LPO) was assessed by the content of malondialdehyde (MDA) and diene conjugates (DC). **Results:** SSC-77 (27.6 mg/kg/day) and SSC-497 (58.1 mg/kg/day) show a cardioprotective effect using the doxorubicin pathology model, which is expressed in the decrease of diastolic dysfunction coefficient (S_{DTT}) to 2.1 ± 0.2 r.u. and 3.3 ± 0.1 r. units, respectively, as compared with the control group 8.3 ± 0.1 r. un. Using the model of hypo-reperfusion, the substances SSC-77 (10^{-6} mol/l) and SSC-497 (10^{-6} mol/l) prevent the decrease of contractility indices on the 5th and 20th min during the reperfusion period in comparison with the control where the fall made 50%. The cardioprotective effect was confirmed by 47% and 39% decrease concerning the levels of KFK-MB marker damage by 39% and 47% and LDH by 21.8% and 19.6%, respectively, in the series with SSC-77 and SSC-497 in comparison with the control group, as well as by the prevention of LPO products MDA and DC accumulation in the ventricular myocardium. **Conclusion:** The derivatives of 5-hydroxynicotinic acid SSC-77 and SSC-497 reduce diastolic dysfunction, prevent the decrease of cardiac functional activity after ischemia/reperfusion, reduce the irreversible damage of cardiomyocytes, and have antioxidant properties.

Key words: Doxorubicin cardiopathy, isolated heart of rats, magnesium 5-hydroxynicotinate, potassium 5-hydroxynicotinate

INTRODUCTION

An excessive activation of free radical oxidation reactions is a typical pathological process that occurs in a variety of diseases, including myocardial ischemia/reperfusion.^[1,2]

Modern pharmacotherapy of cardiovascular diseases as a supplementary therapy can recommend natural antioxidants (vitamin E, C, A and carotenoids, resveratrol, and L-carnitine), and more often, synthetic ones such as trimethylhydrazinium propionate

(mildronate) derivatives, as well as heterocyclic analogs of aromatic phenols, the derivatives of 3-hydroxypyridine (mexicor, emoxipin, and mexidol) with a number of other

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pharmacological effects important for complex treatment of the cardiovascular system (endothelioprotective, preconditioning, and antihypoxic).^[3-6]

In this regard, the purpose of this study was to study the cardioprotective effects of new 5-hydroxynicotinic acid derivatives.

MATERIALS AND METHODS

The object of the study is two new derivatives of 5-hydroxynicotinic acid (laboratory cipher of the developer SSC-77 and SSC-497), and the structural formula of substances is presented in Figure 1.

The experiments were carried out on 90 mature Wistar rats of both sexes with the weight of 220 ± 20 g. All animal manipulations were performed in compliance with the “European Convention for the Protection of Vertebrates Used for Experiment or Other Scientific Purposes” (Directive 2010/63/EU). All experiments were approved by the local Ethics Committee (Minutes No. 11-2016 issued on January 24, 2016).

The study of cardioprotective activity of substances SSC-77 (27.6 mg/kg/day) and SSC-497 (58.1 mg/kg/day) was studied using the doxorubicin cardiomyopathy model (20 mg/kg, intraperitoneally within 48 h), and the heart of isolated rats was used according to Langendorf.^[2]

The drug meksikor (“MiraxBioPharma”) was administered at the dose of 85.72 mg/kg/day.^[7] The modeling of hypo/

reperfusion was performed on a rat heart isolated by Langendorf.^[8] The damage markers and the level of peroxidation were evaluated by conventional methods.^[9,10]

RESULTS AND ITS DISCUSSION

Doxorubicin cardiomyopathy was characterized by the decrease of myocardial contractility [Table 1].

The conduct of the functional test with high-frequency stimulation revealed the development of the “diastole defect” [Figure 2b], and S_{ITTI} increased up to 8.3 ± 0.3 r.u. in comparison with intact animals, 1.4 ± 0.1 r.u., i.e., in 8 times [Figure 2a].

The substances such as SSC-77 (27.6 mg/kg/day) and SSC-497 (58.1 mg/kg/day) and comparison drug Mixicor (85.72 mg/kg/day) prevented the decrease of contractility in the sample with high-frequency stimulation. S_{ITTI} SSC-77 and SSC-497 amounted to 2.1 ± 0.2 r.u. and 3.3 ± 0.1 r. units, for Mixicor the amount made 5.3 ± 0.3 units, respectively.

During the study of the cardioprotective activity using the model of hypo and reperfusion, it was found that with 10 times perfusion decrease (ischemic hypoperfusion), the pronounced drop in heart beat rate and contractility indicators occurred for the first 5 min. During the 20th min of heartbeat rate (HBR) hypoperfusion, the left ventricular pressure (LVP), $+dP/dt_{\text{max}}$ and $-dP/dt_{\text{max}}$ were below the initial values. The restoration of the initial volume of perfusion (reperfusion) was accompanied by the development of reperfusion arrhythmias, which resulted in fibrillation in 3 cases out of 10.

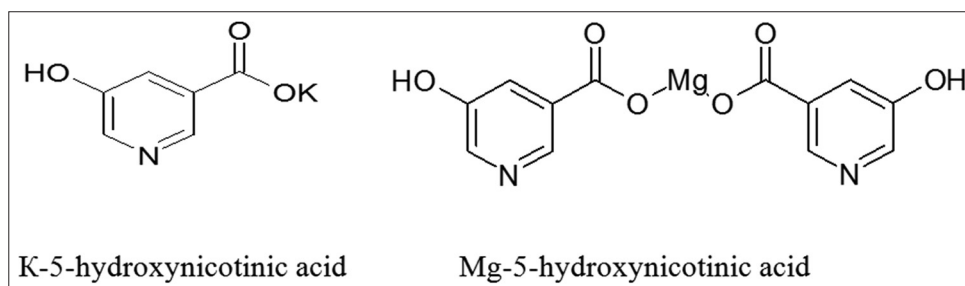


Figure 1: Chemical structures of 5-hydroxynicotinic acid derivatives

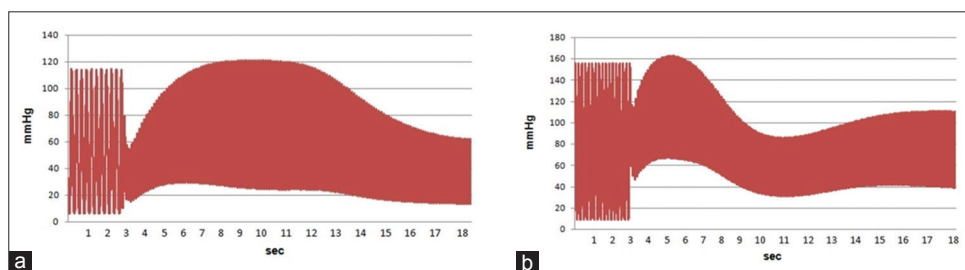


Figure 2: (a and b) A loading test with submaximal electric stimulation of the rat heart isolated according to Langendorf. The dynamics of pressure in the left ventricle (mmHg) with the imposition of the rapid contraction rhythm for the heart (480 beats per minute) for 15 s. The concentrations of Ca^{2+} in the perfusate of 5 mmol/l. Intact group (a). Doxorubicin (20 mg/kg) once in 48 h (b)

At the 5th min of reperfusion, LVP, $+dp/dt_{max}$ and $-dp/dt_{max}$ remained below the baseline level. A similar trend was maintained at the fall of contractility parameters, and further until the 20th min of reperfusion, where LVP, $+dp/dt_{max}$ and $-dp/dt_{max}$ made less than half of the initial value [Table 2].

The addition of substances SSC-77 and SSC-497 into perfused solution, statistically significant changes in LVP, $+dp/dt_{max}$, $-dp/dt_{max}$ and HBR were not discovered during hypoperfusion by the 20th min as compared to control. During reperfusion period, both substances SSC-77 and SSC-497 prevented the drop of contractility indicators, and on the 5th and 20th min, they differed a little from baseline values, whereas in the control, their drop was more than 50% [Table 2]. Besides, unlike the control fibrillation did not develop during an aqueous experiment, and the number

of reperfusion arrhythmias decreased noticeably [Table 2]. The comparison drug mexicor showed the cardioprotective effect, but at the same time, when perfusion was restored, contractility was not restored completely, and fibrillation developed in 2 cases out of 10.

The ability of SSC-77 and SSC-497 to prevent the damage of cell membranes was assessed by the changes in the activity of KFK-MB and lactate dehydrogenase (LDH) in perfusate during the reperfusion period [Figure 3].

SSC-77 and SSC-497 contributed to KFK-MB reduction by 47% and 39% and LDH reduction by 21.8% and 19.6% in the series with SSC-77 and SSC-497, respectively, relative to the control group [Figure 3].

Table 1: The effect of SSC-77 (27.6 mg/kg/day) and SSC-497 (58.1 mg/kg/day), the drug mexicor (85.72 mg/kg/day) on the indices of contractile heart function among the rats with doxorubicine cardiomyopathy

Groups of animals	LVP	$+dp/dt_{max}$	$-dp/dt_{max}$	HBR
Intact animals	87.3±9.2*	1423±122.2*	-1265.2±173.2*	248±32.1
Control doxorubicin	64.5±11.2**	1025.7±114.3**	-931.1±159.4**	247±29.4
Doxorubicin+SSC-77 (27.6 mg/kg/day)	85.2±9.4*	1434.7±124.3*	-1254.9±119.4*	232±29.4
Doxorubicin+SSC-497 (58.1 mg/kg/day)	92.1±7.4*	1356±109.2*	-1207.4±137.3*	231±26.9
Doxorubicin + mexicor (85.72 mg/kg/day)	86.3±10.4*	1276±119.4*	-1159.2±149.3*	228±22.9

LVP: Left ventricular pressure (mmHg), $+dp/dt_{max}$: The maximum contraction rate (mmHg/s), $-dp/dt_{max}$: Maximum relaxation rate (mmHg/s), HBR: Heartbeat rate (bpm). Doxorubicin was administered intraperitoneally 48 h before the experiment. SSC-77 (27.6 mg/kg/day) and SSC-497 (58.1 mg/kg/day), the mexicor preparation (85.72 mg/kg/day) was administered twice at the interval of 24 h, intramuscularly.

** $P<0.005$ in comparison with a group of intact animals; * $P<0.005$ in comparison with the control group ($M\pm m$; $n=10$)

Table 2: Cardioprotective effect of SSC-77 (10^{-6} mol/l) and SSC-497 (10^{-6} mol/l), the drug mexicor (10^{-6} mol/l) at the hypo-reperfusion in the isolated rat heart (in % of baseline level)

Indices	Group of animals	Result	Hypoperfusion period		Reperfusion period	
			5 min	20 min	5 min	20 min
HBR, beats per minute	Control	224.3±8.4	-64.6±3.2	-58.3±2.3	-16.6±10.3	-10.8±16.1
	SSC-77	262.0±7.1	-38.9±3.4*	-54.5±9.3	+3.4±12.1*	+3.8±17.8
	SSC-497	245.2±10.6	-37.6±8.3*	-47.3±8.8*	-19.2±17.6	-5.4±6.7*
	Mexicor	221.4±8.8	-55.3±2.9	-56.8±5.2	-7.9±2.2*	-5.1±8.1*
LVP, mm of mercury column	Control	120.0±9.8	-65.8±7.5	-69.3±9.1	-19.8±8.1	-19.5±14.1
	SSC-77	118.8±6.1	-79.7±10.3	-61.5±7.8	-4.0±12.6*	-1.5±18.0*
	SSC-497	114.2±3.2	-80.4±5.3*	-40.7±2.3*	+12.9±11.0*	+18.2±11.2*
	Mexicor	104.0±11.1	-66.0±7.7	-63.2±8.2	-23.0±13.4	-3.8±2.0*
$+dp/dt_{max}$, mm of mercury column	Control	2498±4.7	-76.7±2.5	-78.8±1.5	-19.8±14.1	-15.0±19.0
	SSC-77	2609±6.8	-75.6±8.9	-76.7±16.1	-12.3±13.4	+7.8±9.2*
	SSC-497	2551±2.2	-69.4±7.6	-65.0±11.2	-6.7±8.5*	-1.5±10.7*
	Mexicor	2325±6.5	-77.6±2.0	-77.8±5.0	-22.2±7.8	-1.4±12.0*
$-dp/dt_{max}$, mm of mercury column	Control	1346±1.8	-71.6±2.0	-74.8±10.2	-36.6±10.4	-55.2±12.1
	SSC-77	1545±2.3	-70.1±2.7	-68.5±9.5*	-9.6±8.6*	+3.9±16.0*
	SSC-497	1414±1.9	-68.0±4.1	-64.5±3.1*	-5.3±7.2*	+11.1±12.7*
	Mexicor	1168±5.7	-68.5±1.8	-72.5±6.7	-18.8±5.5*	-13.5±6.1

LVP: Left ventricular pressure (mmHg), $+dp/dt_{max}$: The maximum contraction rate (mmHg/s), $-dp/dt_{max}$: Maximum relaxation rate (mmHg/s), HBR: Heartbeat rate (bpm). * $P<0.05$ in comparison with the control group ($M\pm m$; $n=10$)

Similar changes in the level of POL products were detected against the background of Mexicor application [Figures 4 and 5].

Thus, the derivatives of 5-hydroxynicotinic acid SSC-77 and SSC-497 exhibited cardioprotective activity in the models of doxorubicin pathology and hypo-reperfusion injury of isolated rat hearts. The most informative for doxorubicin pathology was the results of the sample with high-frequency

stimulation and the prevention of contractility decline in hypo-reperfusion. The acknowledgment of cardioprotective activity SSC-77 and SSC-497 resulted in the decrease of damage marker levels KFK-MB and LDH, the accumulation of malondialdehyde, and DK lipid peroxidation (LPO) products [Figures 6].

It is known that different active forms of oxygen (O_2^- , H_2O_2 , and OH) have a different ability to initiate subsequent

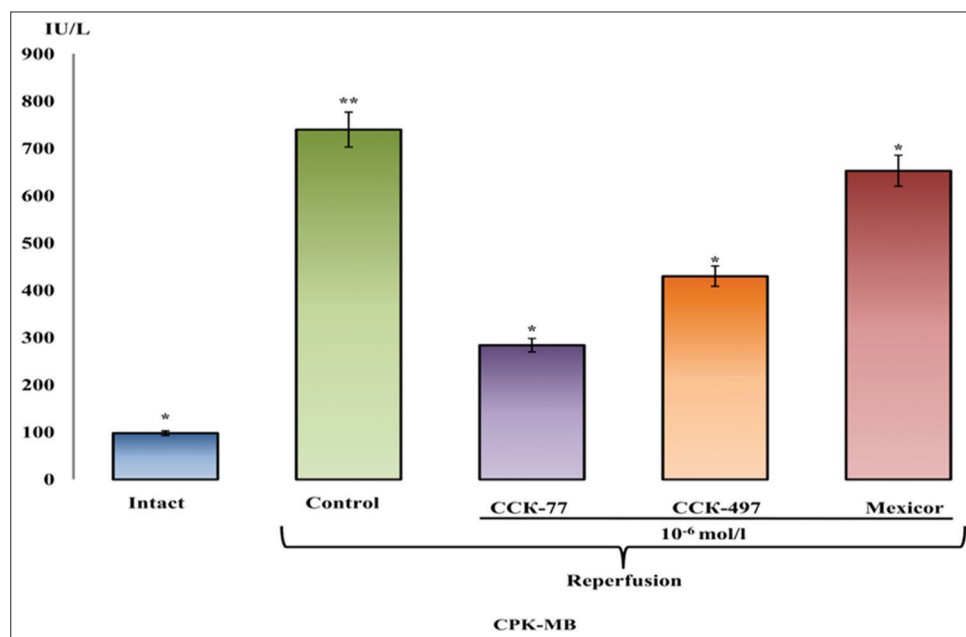


Figure 3: The content of creatine phosphokinase in perfusate in the groups with SSC-77 (10^{-6} mol/l) and SSC-497 (10^{-6} mol/l), with Mexicor (10^{-6} mol/l) during reperfusion. * $P < 0.05$ as compared with the control. ** $P < 0.05$ as compared with the group of intact animals

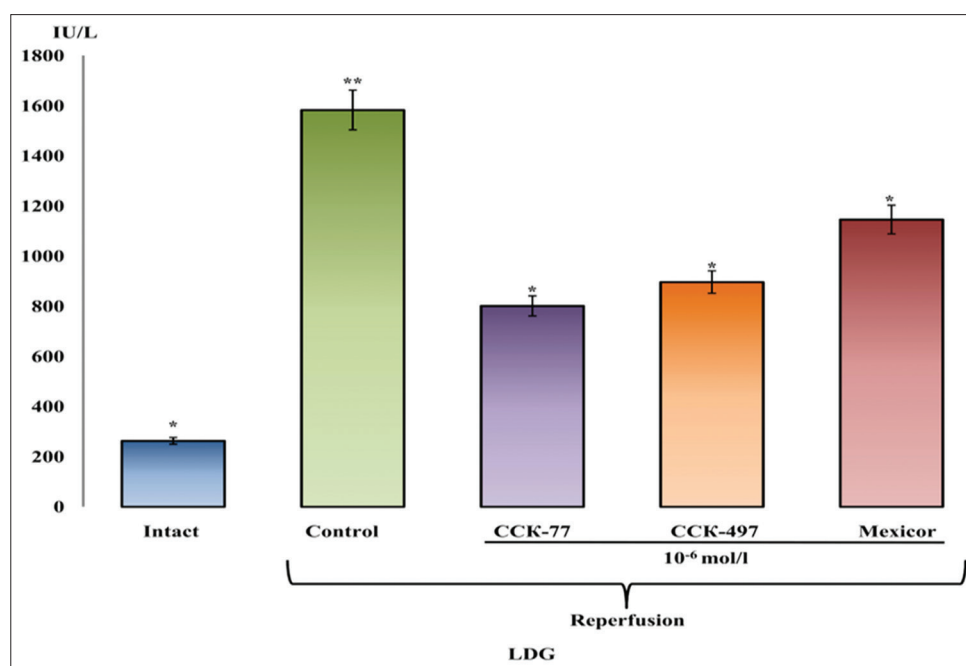


Figure 4: Lactate dehydrogenase content in perfusate within SSC-77 (10^{-6} mol/l) and SSC-497 (10^{-6} mol/l) groups, Mexicor preparation (10^{-6} mol/l) during reperfusion. * $P < 0.05$ as compared with the control. ** $P < 0.05$ as compared with the group of intact animals

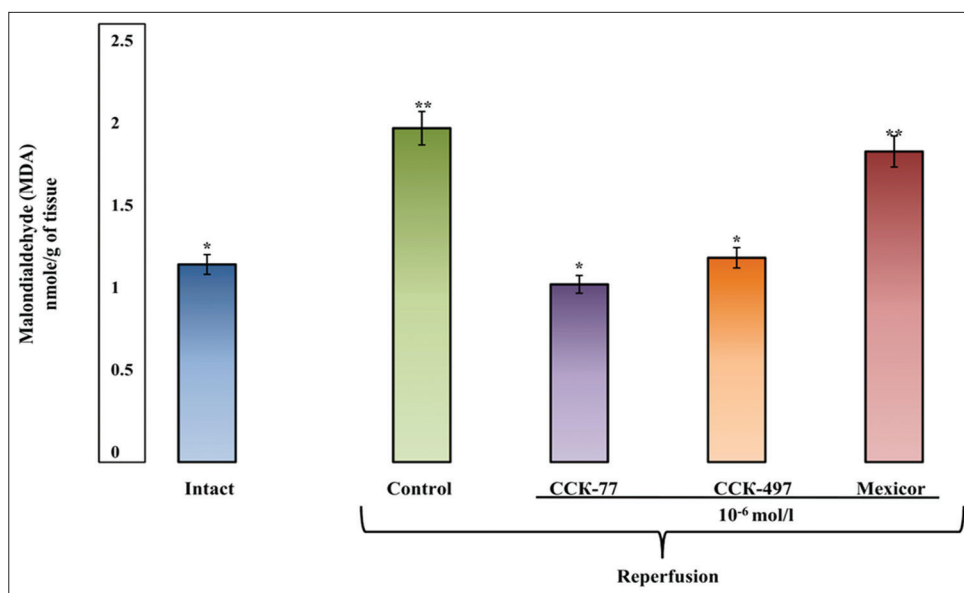


Figure 5: The content of malonic dialdehyde in the groups with SSC-77 (10^{-6} mol/l) and SSC- $(10^{-6}$ mol/l), with mexicor (10^{-6} mol/l) during reperfusion. * $P < 0.05$ AS compared with the control. ** $P < 0.05$ as compared with the group of intact animals

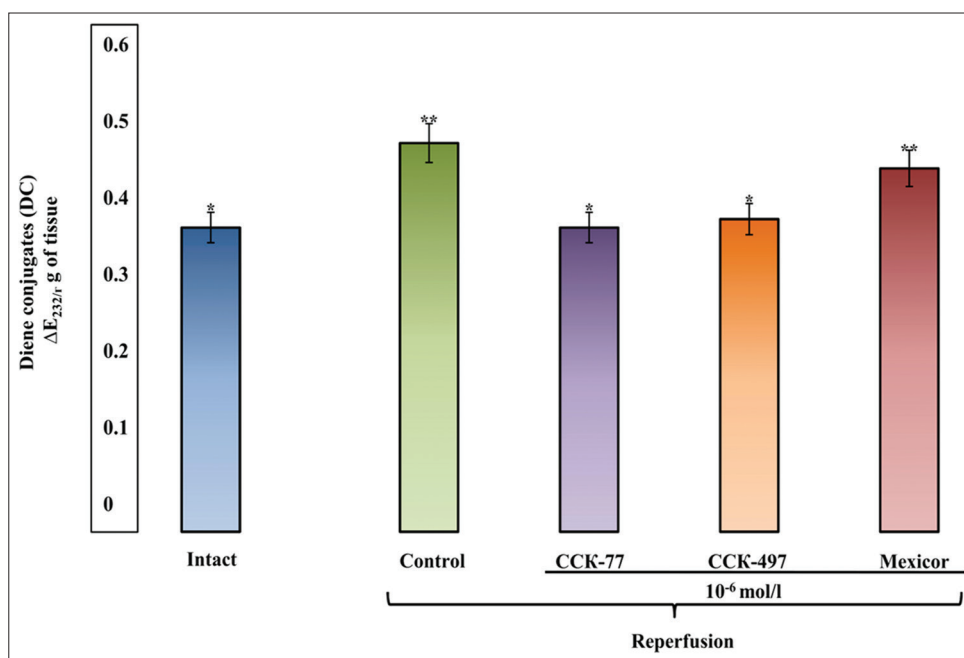


Figure 6: The content of diene conjugates in the groups with SSC-77 (10^{-6} mol/l) and SSC- $(10^{-6}$ mol/l), with mexicor (10^{-6} mol/l) during reperfusion. * $P < 0.05$ as compared with the control. ** $P < 0.05$ as compared with the group of intact animals

free-radical reactions (FRR). The superoxide anion radical (O_2^-) has the lowest activity and the hydroxyl radical (OH), which is formed in the Huber-Weiss reaction with the participation of superoxide dismutase and ferrous ions, has the highest activity. One of the alleged causes of doxorubicin cardiomyopathy is related to the effect on iron metabolism: Anthracyclines are bound to Fe^{2+} ions, which leads to the development of a hydroxyl radical and promotes the release of Fe^{2+} ions from ferritin, exacerbating oxidative stress.^[11]

Therefore, if cell cytoplasm demonstrates the conditions for chelating or the oxidation of ferrous ions Fe^{2+} in a catalytically

inactive state of Fe^{3+} ions, thus leading to the decrease of current hydroxyl radical concentration, this will create the conditions for micromolar AOS concentrations in cell cytoplasm.^[12,13] The substances with antioxidant activity play an important role in the regulation of free-radical mechanisms. As an example of such a scheme, it can be assumed that SSC-77 and SSC-497 have the property of Fe^{2+} chelator and Fe^{2+} oxidizer in Fe^{3+} , respectively.^[14] Besides, the control over the concentration of Fe^{2+} can have a certain value in the regulation of FRR: LPO, inactivation of proteins, and nucleic acids. It is well known that, during the emergence and the development of a number of inflammatory diseases, the activation of FRR

is observed. This circumstance gave the right to call such diseases “free-radical pathologies.” The activation of FRR in the development of free radical pathologies is caused by two main factors: The increase of primary and secondary radical initiators and FRR participant (FRR initiation stage) levels and the appearance of FRR catalysts, mainly Fe^{2+} ions (oxidation chain branching stage). Thus, the inhibition of FRR can be achieved both by the capture of free radicals and by the elimination of catalytically active Fe^{2+} ions. The latter is, especially important in pathologies, which are characterized by the violation of blood vessel integrity: Strokes, gastric bleeding, and wounds. The derivatives of 5-hydroxynicotinic acid with the property of Fe^{2+} chelation and Fe^{2+} oxidation in Fe^{3+} can inhibit the catalysis of FRR, thereby inhibiting free radical oxidation.

The second point of substance application on the doxorubicin pathology model is glutathione. The content of glutathione-disulfide (GSSG) form in a body within norm in mammalian tissue and blood is maintained at the levels which are many times lower than for GSN. Oxidative stress can lead to a significant accumulation of GSSG in the liver and its release into blood. The increase of GSSG content in blood plasma, in its turn, can cause the oxidation of the thiol groups of proteins for the basolateral membranes of tissue cells and their inactivation. The basic mechanisms causing the depletion of glutathione system functionality in doxorubicin model of pathology is the inhibition of glutathione enzyme recovery rate from an oxidized form (glucose-6-phosphate dehydrogenase), which leads to the critical drop of restored glutathione, and then to the reduction of glutathione-dependent enzyme activity of antiradical defense, the activation of free radical processes, and the death of cardiomyocytes. As a directed pharmacological protection of cardiomyocytes, the use of drugs whose action is directed to the correction of the glutathione system state is pathogenetically justified.^[15,16] One of such trends may be the compensation of some part of antioxidant load that can be found on glutathione systems. The proposed derivatives of 5-hydroxynicotinic acid SSC-77 and SSC-497 can be used as such preparations.

SUMMARY

The derivatives of 5-hydroxynicotinic acid SSC-77 and SSC-497 reduce diastolic dysfunction, prevent the reduction of heart functional activity after ischemia/reperfusion, reduce irreversible damage of cardiomyocytes, and possess antioxidant properties.

CONCLUSIONS

1. The derivatives of 5-hydroxynicotinic acid SSC-77 and SSC-497 possess cardioprotective activity on doxorubicin cardiomyopathy model, expressed in the prevention of “diatol defect.”

2. 5-hydroxynicotinic acid SSC-77 and SSC-497 possess cardioprotective activity on hypo-reperfusion model of isolated rat hearts reducing the level of KFK-MB, LDH, as well as the content of malondialdehyde and diene conjugates.

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