Рус. Eng.

UDC: 612.172.1:615.2 **DOI: 10.18413/2500-235X-2015-1-4-25-31**

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PHARMACOLOGICAL PROTECTION OF THE ISCHEMIC MYOCARDIUM BY DERIVATIVES OF 3-(2,2,2-TRIMETHYLHYDRAZINIUM) PROPIONATE AND EVALUATION OF THEIR ANTIOXIDANT ACTIVITY

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Abstract. The paper presents results of a study of antioxidant (in vitro) and anti-ischemic effect of a number derivatives of 3-(2,2,2-trimethylhydrazinium) propionate. It was revealed that among other compounds 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate had the most pronounced antioxidant activity. The effect of 3-(2,2,2-trimethylhydrazinium) propionate and its derivatives on the necrotized area of the myocardium of the left ventricle in the course of simulation of myocardial infarction with coronary occlusion in rabbits showed that pharmacological preconditioning by 3-(2,2,2-trimethylhydrazinium) propionate and its derivatives could be considered as prevention of a syndrome of ischemia/reperfusion injury in case of myocardial infarction. Thus it was demonstrated that both 3-(2,2,2-trimethylhydrazinium) propionate (40 mg/kg) and its derivatives, namely nicotinate (84.1 mg/kg), 5-bromonicotinate (105.5 mg/kg), 5-hydroxynicotinate (88.5 mg/kg) and glycine (71.0 mg/kg) had anti-ischemic activity expressed in effective decrease of the area of necrotized infarction and reduction of the level of Tn I in the blood plasma as compared to the reference substance, i.e. Mildronate®.
Keywords: 3-(2,2,2-trimethylhydrazinium) propionate, ischemia/reperfusion, antioxidant activity, preconditioning.

Introduction. The current theories suggest that myocardial ischemia resistance may be improved through preconditioning resulting from short episodes of ischemia/reperfusion or hypoxia, short-term hypothermia and other moderate stressful actions which are able to activate endogenic defense mechanisms [6].

At that alongside with the ischemic preconditioning there is a pharmacological one which from the clinical point of view seems to be more preferable since it does not involve such complicated technological solutions and potential danger of ischemic episodes for the altered myocardium. Description of the phenomenon of pharmacological training often contains references to a well-known myocardial cytoprotective agent, i.e. mildronate (3-(2,2,2-trimethylhydrazinium) propionate) which holds the leading place among the similar agents [2, 8, 10]. When endothelial, inducible NO-synthase and ATP-sensitive potassium channels are blocked the anti-ischemic effects of mildronate are neutralized [3].

The medicine is efficient in the course of treatment of ischemic events; it induces the preconditioning effect in cells and ensures pronounced protective effect in case of myocardial ischemic and reperfusion injury by promoting more intensive oxygen uptake by the myocardial tissue [3]. Its antioxidant effect allows reducing generation of reactive oxygen intermediates and peroxy radicals in blood and tissues thus preventing cellular structures injury [1, 7]. It is common knowledge that when additional functional groups are introduced into a molecule of a medicinal substance its

pharmacological activity spectrum becomes wider. At the same time it is important that such additional properties would not affect the medicinal substance efficiency but vice versa would strengthen its major effect.

Due to the above we've stated study of antioxidant and anti-ischemic activity of 3-(2,2,2-trimethylhydrazinium) propionate Mildronate® and its derivatives (nicotinate, 5-bromonicotinate, 5-hydroxynicotinate, glycinate of (2,2,2-trimethylhydrazinium) propionate) as a **purpose of our investigation**.

Materials and methods. There were studied 4 chemical derivatives of 3-(2,2,2-trimethylhydrazinium) propionate synthesized by "All-Russian scientific center for security of biologically active substances" ("ARSC BAS"), Kupavna city, namely nicotinate, 5-bromonicotinate, 5-hydroxynicotinate, glycinate of 3-(2,2,2-trimethylhydrazinium) propionate and reference substance Mildronate® (produced by JSC «Grindeks»).

In order to determine antioxidant activity (AOA) of Mildronate® and its derivatives there were selected test portions of the stated samples with the weight of 0.05g, 0.049g, 0.051g, 0.05g and 0.053g correspondingly. The test portions were placed into measuring flasks with the capacity of 25 ml and diluted with water up to the necessary volume. For ensuring better dissolution and homogenization of the substances there was used an ultrasonic bath UZV 1/100-TN-RELTEC. The obtained extractions were studied for gross antioxidant activity.

Measurement of AOA was made with the aid of the device «Color Jauza-01-AA» with volt-amperometric detector under the conditions of constant voltage of 1.3 V in the direct-current mode (DC AD). The solution of orthophosphoric acid with the molar concentration of 0.0022 mol/L was used as an eluent. The eluent feed rate made 1.2 cm^3/min. Data processing was performed by means of «ADKD» software. Amperometric method of antioxidants total weight concentration measurement is based on change of current intensity in a cell occurring at the surface of an electrode as a result of antioxidant molecules oxidation under the conditions of a definite voltage which after amplification will be converted into a digital signal.

Study of a survival rate of ischemic myocardium was made in 36 laboratory rabbits with the weight of 2-2.5 kg. The studied compounds: 3-(2,2,2-trimethylhydrazinium) propionate Mildronate® and its derivatives (nicotinate, 5-bromonicotinate, 5-hydroxynicotinate, glycinate of 3-(2,2,2-trimethylhydrazinium) propionate) in the dose of 40 mg/kg for 3-(2,2,2-trimethylhydrazinium) propionate (taking into consideration molar weight of 3-(2,2,2-trimethylhydrazinium) propionate and based on the amount of substance of 0.274 mol/kg) and the doses of derivatives correspondingly ACC (average curative concentration) 733 – 84.1 mg/kg, ACC 734 – 105.5 mg/kg, ACC 735 – 88.5 mg/kg, ACC 61K – 71.0 mg/kg were first dissolved in a sterile normal saline solution and administered to the anesthetized animals (chloral hydrate in the dose of 300 mg/kg) into the marginal vein of the rabbit ear (the rabbit is under controlled breathing conditions) 30 minutes before ligation of the left descending coronary artery. Upon expiration of 60 minutes of coronary occlusion the ligature was removed and myocardium reperfusion was performed over the period of 90 minutes. After that blood sampling from the right ventricle was made into a disposable vacuum test-tube containing anticoagulant in order to determine a specific cardiac marker Troponin I (TnI). The level of Troponin was determined by means of an immunofluorescent device Triage MeterPro (Biosite, USA). The dimensions of necrotic zone necrosis were determined upon expiry of 2 hours. Myocardium cross sections were made at 0.8 cm intervals starting from the level of 0.8 cm lower the ligature location. The myocardium sections were placed into a container with phosphate buffer (pH 7.4) and triphenyl tetrazolium chloride in the dose of 1 mg/ml. The rate between the weight of tissue sections and the buffer made 1:9. Weighing bottles were placed into an incubator and were incubated at the temperature of 37^0C within 15 minutes until red formazan would form. Calculation of the areas of intact and necrotized left ventricle myocardium was made for each of the four sections by means of a pixel-by-pixel analysis via Adobe Photoshop 9.0 program.

Results of study and their discussion.

Results of antioxidant activity study

Mildronate® medicine and all of its derivatives except for 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate do not have AOA in vitro.

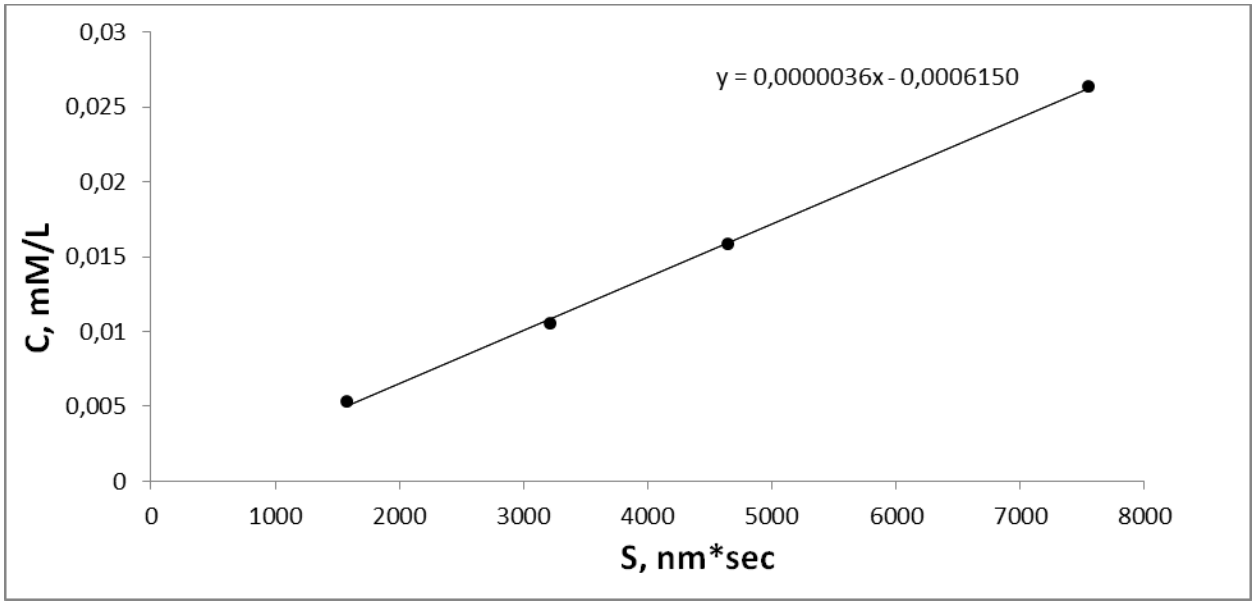

Figure 1. Calibration plot of dependence between the output signal and the ascorbic acid concentration.

5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate has the highest antioxidant activity. In terms of ascorbic acid it makes 9.2 mg (A.A.)/L. Ascorbic acid with the initial concentration of 1 g/L was used as a reference substance for the calibration plot construction. The obtained data were used for constructing a plot of dependence between the output signal (peak area) and concentration (Fig.1). The ready plot was further used for determination of the weight concentration of the antioxidant.

Electric current on the electrode surface is amplified as a result of oxidation of 3-(2,2,2-trimethylhydrazinium) propionate derivative, i.e. 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) potassium propionate. The occurring electrical currents are rather small, they fall within the range from 10^{-6}–10^{-9}A. These analogue signals are amplified and further converted into a digital signal by means of an analogue-to-digital converter, the digital signal is displayed on a computer screen (Fig. 2). Root mean square deviation makes less than 1%.

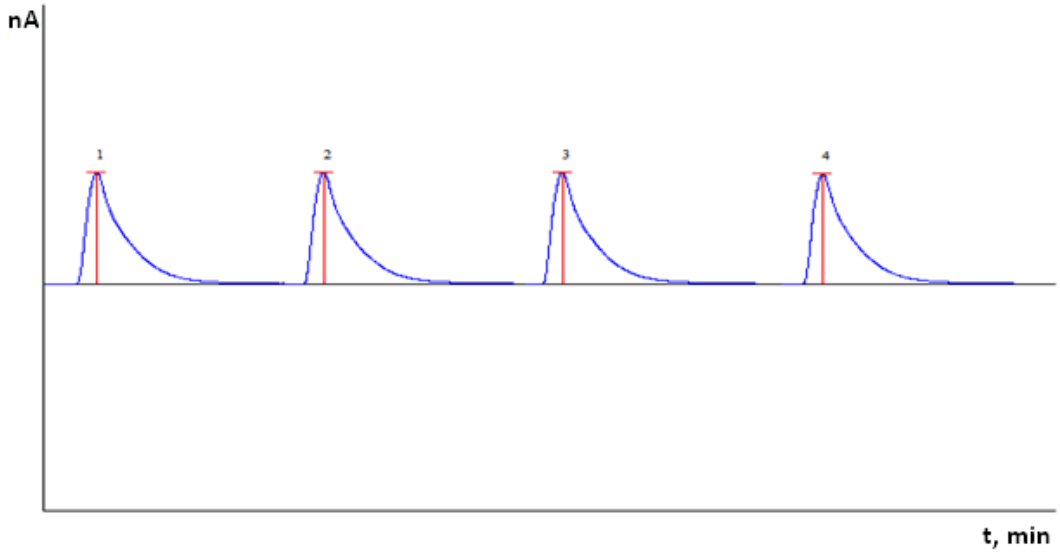

Figure 2. The signals recorded during four successive administrations of the solution of 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate (199 mg / kg) with the aid of a device «Color Jauza-01-AA»

Results of anti-ischemic effect study

Ligation of the descending branch of the left coronary artery in rabbits resulted in myocardium necrosis development, the size of necrosis made 27.3±1.2% of the total area of the myocardium. When the reference preparation Mildronate® in the dose of 40 mg/kg was administered before coronary artery occlusion modeling the area of necrotized

myocardium decreased up to 20.2±1.0%. The most intensive protective effect was observed in case of administration of 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate compound (88.5 mg/ kg) which ensured proved 2-fold reduction of the necrotized myocardium area, i.e. up to 13.1±1.4%. All of the rest derivatives also induced reliable decrease of the necrosis zone size but to the lesser degree (Table 1). Troponin I (TnI) being a specific myocardial infarction marker is found in the blood plasma several minutes after myocardial infarction and correlates with the infarction size. In the course of determining of the TnI concentration in our experiment the least concentration was observed in case of administration of 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate compound, i.e. 6.04±1.6 ng/ml.

Table 1

The effect of Mildronate® (40 mg/kg) and its derivatives administered intravenously on the necrosis zone size and the level of Troponin I in case of acute coronary occlusion (60 min) with further reperfusion (90 min) in anesthetized rabbits (M±m; in % of the mass of the left ventricle; n=6)

Group	Necrosis %	Intact myocardium %	Level of Troponin I (ng/ml)
Reference group (myocardium infarction)	27.3±1.2	72.7±1.2	16.26±1.9
CO/reperfusion + Mildronate (40 mg/ kg)	20.2±1.0*	79.8 ± 0.5	12.2±1.1*
CO/reperfusion + Nicotinate of 3-(2,2,2-trimethylhydrazinium) propionate (84.1 mg/ kg)	17.9±1.3*	82.1±0.7	10.95±1.8*
CO/reperfusion + 5-bromonicotinate of 3-(2,2,2- trimethylhydrazinium) propionate (105.5 mg/ kg)	16.0±0.8*	84.0±1.2	7.7±1.5*
CO/reperfusion + 5-hydroxynicotinate of 3-(2,2,2- trimethylhydrazinium) propionate (88.5 mg/ kg)	13.1±1.4*	86.9±1.3	6.04±1.6*
CO/reperfusion + glycinate of 3-(2,2,2-trimethylhydrazinium) propionate (71.0 mg/ kg)	17.2±1.4*	82.8±1.1	10.15±1.1*

Remark: CO/reperfusion – coronary occlusion of the descending branch of the left coronary artery in the area of the ear (60 min) with the following reperfusion (90 min); * - $p \leq 0.05$ when compared to the reference group

For all other preparations TnI concentration correlates with the infarction size in a definite group (Table 1).

It has been established that preventive medication of the derivatives of 3-(2,2,2-trimethylhydrazinium) propionate (nicotinate, 5-bromonicotinate, 5-hydroxynicotinate, glycinate of 3-(2,2,2- trimethylhydrazinium) propionate) in the relevant doses resulted in reduction of necrosis area. The results of experiments with administration of the studied compounds are indicative of their pronounced anti-ischemic activity which is higher than that of the reference substance, i.e. Mildronate®.

During the recent years there has been actively developed and investigated the methods of myocardial protection from ischemic and reperfusion injury. These methods give an opportunity to restrict the area of necrosis, to preserve myocardial functions, to prevent cardiac insufficiency development and to improve clinical results in the patients with coronary disease and other diseases mediated by epithelial dysfunction.

It is common knowledge that when additional functional groups are introduced into a molecule of a medicinal substance its pharmacological effect may change which can be manifested through widening of the spectrum of its therapeutic action, strengthening or weakening of pharmacological effect, appearing of new properties, addition or potentiation of the effects contributed by the contained components. In order to analyze the possibility to strengthen anti-ischemic, endothelio- and cardioprotective effects of Midronate

essentially different functional groups with respect to chemical structure of a basic molecule were introduced in a molecule of 3-(2,2,2-trimethylhydrazinium) propionate, namely the residue of substituted and non-substituted nicotinic acid as well as glycine aminoacid.

Introduction of a new functional group into a molecule of 3-(2,2,2- trimethylhydrazinium) propionate supposes finding out new properties and strengthening anti-ischemic effect nevertheless it's logical that depending on the introduced component the range of activity of the resulting substances may vary. Introduction of the group of 5-hydroxynicotinate into a molecule of 3-(2,2,2-trimethylhydrazinium) propionate results in strengthening of antioxidant activity of the investigated substance and increase of anti-ischemic activity as compared to other substances.

Despite of numerous experiments and clinical studies pharmacotherapy of cardiac disease needs research and practical application of new preparations [4, 5].

Data obtained during our experiments confirm promising outlook of the further in-depth study of the derivatives of 3-(2,2,2-trimethylhydrazinium) propionate (nicotinate, 5-bromonicotinate, 5-hydroxynicotinate, glycinate of 3-(2,2,2-trimethylhydrazinium) propionate) as potential cardiopharmacological preparations.

Conclusions:

1. 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate exhibits moderate antioxidant activity as confirmed by amperometric methods of investigations by means of the «Color Jauza-01-AA» device.

2. The derivatives of 3-(2,2,2-trimethylhydrazinium) propionate, i.e. nicotinate, 5-bromonicotinate, 5-hydroxynicotinate, glycinate of 3-(2,2,2-trimethylhydrazinium) potassium propionate have anti-ischemic effect in the model of myocardium infarction with coronary occlusion more intensive than that of the reference substance (Mildronate). This index varies depending on a functional group introduced in a molecule structure, it has the maximum value with 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate in the dose of 88.5 mg/kg.

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