



Doxorubicin-associated Cardiomyopathy: New Approaches to Pharmacological Correction Using 3-(2,2,2-trimethylhydrazinium) Propionate Derivatives

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Abstract

Introduction: The search for new compounds with cardioprotective activity amongst the 3-(2,2,2-trimethylhydrazinium) propionate derivatives looks promising.

Research objectives: to study cardioprotective effects of the 3-(2,2,2-trimethylhydrazinium) propionate derivatives.

Methods: The cardioprotective effect of the derivatives (nicotinate, 5-hydroxynicotinate) of 3-(2,2,2-trimethylhydrazinium) propionate and reference medicine meldonium in the case of doxorubicin (DOX) (20 mg/kg, intraperitoneally for 48 hours) cardiomyopathy was evaluated by the results of a functional test with high-frequency stimulation (480 bpm).

To provide integral validation for the development of the simulated pathological processes, biochemical and morphological studies of the heart were carried out. For a biochemical evaluation of myocardial damage in the homogenisate, the isoenzyme creatinine kinase MB (CK-MB) and lactate dehydrogenase (LDH) were determined.

Results: The derivatives nicotinate and 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate exert a cardioprotective effect on a doxorubicin pathology model, which is expressed in a decreased coefficient of diastolic dysfunction (S_{DTP}) to the level of 5.8 ± 0.1 ru and 4.6 ± 0.2 ru in comparison with that in the control group 8.3 ± 0.1 ru and reference medicine meldonium 6.5 ± 0.1 ru, respectively.

The cardioprotective effect was confirmed by decreased levels of markers of damage to CK-MB and LDH and a decreased diameter of cardiomyocytes compared to those in the control group.

Conclusion: The derivatives of 3-(2,2,2-trimethylhydrazinium) propionate (nicotinate, 5-hydroxynicotinate) 3-(2,2,2-trimethylhydrazinium) propionate reduce diastolic dysfunction and irreversible damage to cardiomyocytes in case of doxorubicin-associated cardiomyopathy.

Keywords

nicotinate of 3-(2,2,2-trimethylhydrazinium) propionate, 5-hydroxynicotinate of 3-(2,2,2-trimethylhydrazinium) propionate, meldonium, doxorubicin cardiopathy, isolated rat heart.

Introduction

Most anthracycline drugs, in particular DOX, are still widely used chemotherapeutic agents (Shuykova et al. 2012, Xiong et al. 2018). However, the use of anthracyclines is limited due to the chances of developing severe cardiomyopathy, which is accompanied by progressive systolic dysfunction of the left ventricle (LV) leading to irreversible congestive heart failure (Cappetta et al. 2017).

Modern concepts consider several possible mechanisms underlying the cardiotoxicity of doxorubicin: oxidative and nitrosative stresses, dysregulation of intracellular calcium level, pathological changes in the performance of mitochondria, selective inhibition of expression of cardiomyocyte-specific genes, which ultimately leads to the activation of cell death mechanisms (Štěrba et al. 2013, Renu et al. 2018, Mantawy et al. 2018).

DOX-induced cardiotoxicity can be overcome in various ways. However, substances with cardiotropic effects are looked for amongst various classes of chemical and pharmacological groups (Popelova et al. 2008, Zhao et al. 2018, Pecoraro et al. 2018, Gu et al. 2017).

This is the reason that the purpose of this study was defined as the study of the cardioprotective effects of new 3-(2,2,2-trimethylhydrazinium) propionate derivatives.

Materials and methods

The object of the study was two new derivatives of 3-(2,2,2-trimethylhydrazinium) propionate, (the laboratory codes of the development institution are CTK-733 and CTK-735; the structural formulae of the substances are shown in Fig. 1).

The experiments were performed on 50 mature Wistar rats of both sexes weighing 220 ± 20 g. All activities with the animals were carried out in compliance with “The European Convention for the Protection of Vertebrate Animals used for Experiment or Other Scientific Purposes” (Directive 2010/63/EU). All the experiments were approved by the local Ethics Committee (Minutes No. 9-2016 of 17 March 2017).

The cardioprotective activity of substances CTK-733 (189 mg/kg/day) and CTK-735 (199.1 mg/kg/day) was stu-

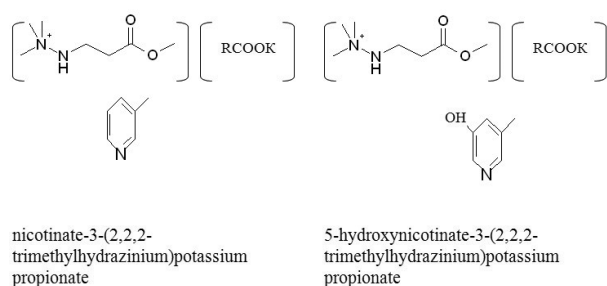


Figure 1. Chemical structures of 3-(2,2,2-trimethylhydrazinium) propionate derivatives.

died in the model of DOX-induced cardiotoxicity (20 mg/kg, intraperitoneally for 48 hours) by imposing on the heart a submaximal stimulation rate (480 beats per minute) and hypercalcium perfusion (5 mmol/l) in an isolated Langendorff rat heart (Danilenko and Pokrovskii 2014). Reference medicine meldonium (Mildronata manufactured by GRIN-DEX, Latvia) was administered at a dose of 90 mg/kg/day.

Biochemical markers of damage were assessed by the conventional methods.

The micropreparations study, photorecording and morphometry were carried out using a Leica DM 4000B microscope equipped with a video recording system and software for image archiving and analysing – Leica Application Suite Version 3.8.0. The diameters of the cardiomyocytes were measured in strictly longitudinal sections.

Results and discussion

DOX-induced cardiotoxicity was characterised by a decreased myocardial contractility. The principal difference in the area under the curve of the end-diastolic pressure build-up when imposing a high rhythm of contractions at 480 bpm for 11 seconds with a background of increased Ca^{2+} concentration up to 5 mmol in the perfusate in the intact group and with a background of DOX in the control group, logically led to the necessity of introducing the S_{TTI} factor, which is quite indicative and informative and which can be used for screening innovative molecules.

Conducting a functional test with high-frequency stimulation revealed the development of “the diastole defect” (Fig. 2b) and S_{TTI} increased to 8.3 ± 0.3 ru when compared with the intact animals, 1.4 ± 0.1 ru, i.e. an eight-fold increase (Fig. 2a).

The tested substances CTK-733 (189 mg/kg/day) and CTK-735 (199.1 mg/kg/day), reference medicine meldonium (90 mg/kg/day), prevented the reduction of contractility when sampling at a high-frequency stimulation S_{TTI} to the level of 5.8 ± 0.1 ru and 4.6 ± 0.2 ru, respectively. The S_{TTI} value of meldonium amounted to 6.5 ± 0.1 ru.

The cardioprotective effect of the derivatives was confirmed by decreased levels of markers of damage CK-MB and LDH, as well as a decreased diameter of cardiomyocytes (Table 1).

Thus, the derivatives of 3-(2,2,2-trimethylhydrazinium) propionate – CTK-733 (189 mg/kg/day) and CTK-735 (199.1 mg/kg/day) – exerted a cardioprotective effect in a model of doxorubicin myocardiopathy, which on the basis of functional, biochemical and morphological parameters, exceeded the reference medicine meldonium (90 mg/kg).

The comparative dynamic evaluation of the parameters of the contractile activity of the hearts of the groups under study made it possible to establish that the use of both CTK-733 (189 mg/kg/day) and CTK-735 (199.1 mg/kg/day) promoted the stability of the contractile apparatus of the cardiac muscle, which convincingly demonstrates their pronounced cardioprotective effect. The ability of CTK-733 (189 mg/kg/day) and CTK-735 (199.1 mg/kg/

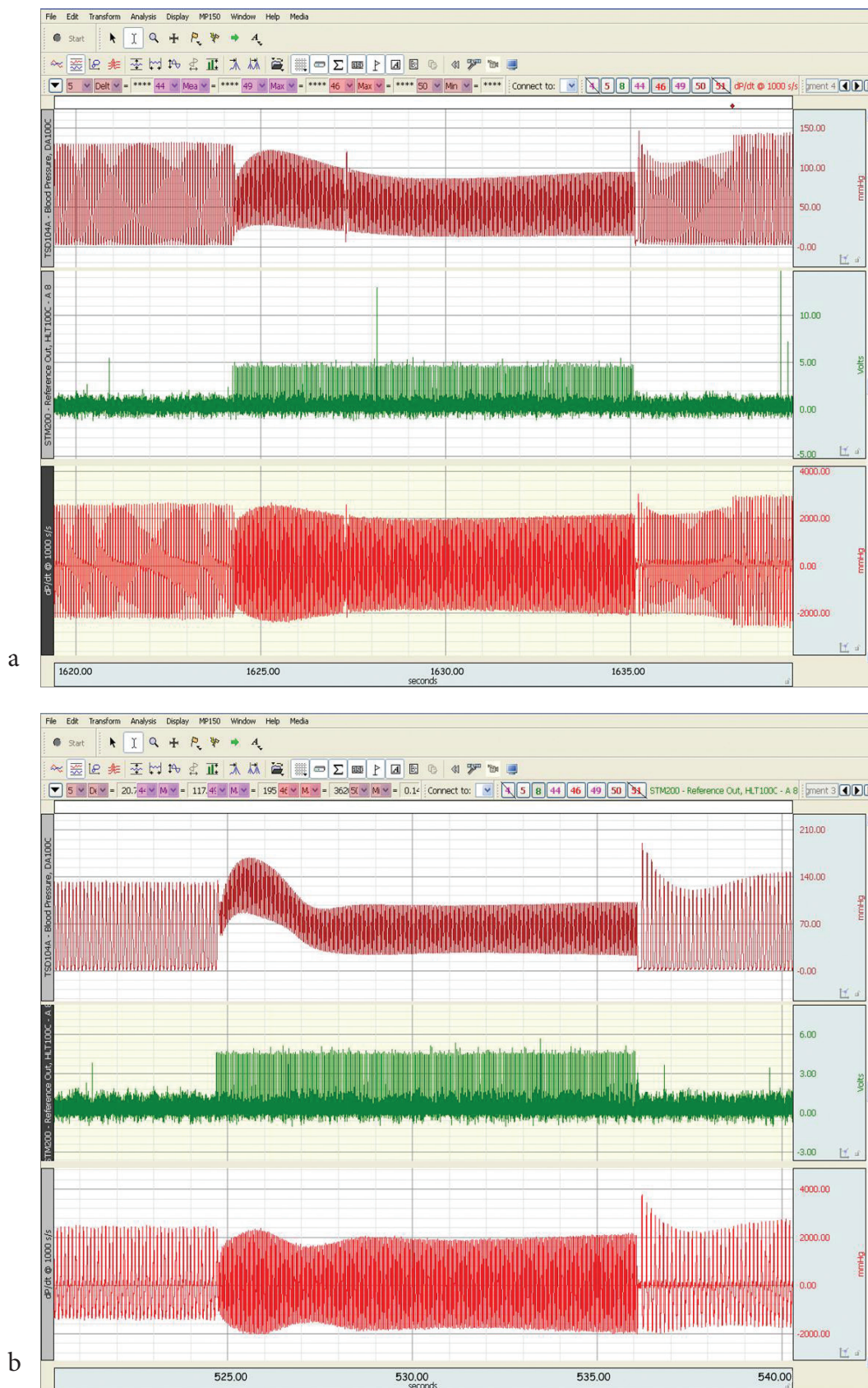


Figure 2. Loading test with submaximal electrostimulation of the isolated Langendorff rat heart. Key: The dynamics of pressure in the left ventricle (mm Hg) with the cardiac stimulation (480 beats per minute) for 11 seconds. Concentrations of Ca^{2+} in the perfusate 5 mmol/l. Intact group (a). Doxorubicin (20 mg/kg) once per 48 hours (b). From top to bottom: scale 1 – left ventricular pressure (LVP in mm Hg); scale 2 – cardiac pacing (480 beats per minute for 11 seconds); scale 3 – the rate of change of the LDVP ($+dP/dt_{\text{max}}$, $-dP/dt_{\text{max}}$, mm Hg/sec)

Table 1. Effect of CTK-733 (189 mg/kg/day), CTK-735 (199.1 mg/kg/day) and meldonium (90 mg/kg/day) on functional, biochemical and morphological indices in case of DOX-induced cardiotoxicity (M±m; n = 10)

Group	S _{ITT} (ru)	CK-MB (IU/L)	LDH (IU/L)	diameter of cardiomyocytes (µm)
Intact animals	1.4±0.1*	98.0±11.8*	263.0±24.9*	8.1±0.3*
DOX (20 mg/kg) control	8.3±0.3**	740.0±13.6**	1583.0±30.6**	17.3±0.4**
DOX (20 mg/kg) + CTK-733 (189 mg/kg/day)	5.8±0.1*	592.1±9.3*	1347±23.5*	13.9±0.1*
DOX (20 mg/kg) + CTK-735 (199.1 mg/kg/day)	4.6±0.2*	464.3±10.1*	988.7±25.8*	10.5±0.2*
DOX (20 mg/kg) + meldonium (90 mg/kg/day)	6.5±0.3*	618.4±7.2*	1311.8±19.7*	14.8±0.3*

Key: The coefficient of diastolic dysfunction StITT (ru), creatine kinase CK-MB (IU/L), lactate LDH (IU/L), ** – p<0.05 compared to the group of intact animals; * – p<0.05 compared to the control.

day) to prevent damage to cell membranes was also assessed by the positive dynamics of changes in the activity of CK-MB and LDH and the diameter of cardiomyocytes.

Anthracycline cardiomyopathy is a multifactorial process that leads to apoptosis and death of cardiomyocytes. Disturbance of metabolism is an important cause for the development of DOX cardiomyopathy (Huang et al. 2018, McLaughlin et al. 2017). The heart requires a large amount of energy to provide contractility. In the isolated models of cardiomyocytes, DOX was shown to reduce concentrations of intracellular adenosine triphosphate (ATP) and phosphocreatine (PK) by more than 50% within 24 hours (Menna and Salvatorelli 2017). More than 90% of ATP used by cardiomyocytes is produced in the process of mitochondrial respiration. The prolonged influence of doxorubicin on isolated myocardial mitochondria leads to a rapid disruption of oxidative mitochondrial processes (Kankeu et al. 2017). There are several practical pharmacological strategies for preventing the development of left ventricular (LV) dysfunction and cardiac failure (CF) caused by anthracyclines. They are ACE inhibitors, ARB inhibitors and BB, as well as using dexrazoxane as a cardioprotector (ESC 2017). However, despite numerous clinical and experimental studies, the approaches used for cardioprotection of toxic lesions with anthracyclines are not without shortcomings and require new promising pharmacological approaches. When treating with doxorubicin, an increase in the level of fatty acids is observed in the serum. Doxorubicin inhibits the oxidation of palmitate, a long-chain fatty acid. A possible mechanism is supposed to be damage to Carnitine palmitoyl transferase 1 and/or depletion of L-carnitine stocks. A reduced rate of glycolysis may be due to the effect of DOX on maintaining the level of glucose and/or on the cells' ability to stimulate its production. Meldonium, by suppressing the synthesis and re-absorption of carnitine, reduces the transport of long-chain fatty acids in the mitochondrion and prevents damage to mitochondrial membranes due to the accumulation of fatty acid metabolites in them. As a result of limited transport and oxidation of fatty acids, there appears to be an alternative way for energy production in the mitochondria by oxidation of glucose. Besides, meldonium activates the two most important enzymes of aerobic gly-

colysis – hexokinase and pyruvate dehydrogenase, which involve the pyruvate formed from sugars into the Krebs cycle, preventing the formation of lactate. Under the influence of meldonium, the activity of these enzymes increases and their biosynthesis is induced, resulting in preventing the development of acidosis, which is harmful to cells. The metabolic pathway in doxorubicin cardiomyopathy is also discussed (Schaupp et al. 2015). It is well known that the introduction of new functional groups into a drug molecule leads to a change in its pharmacological effect, which can show in an expanded spectrum of its therapeutic effect, improved or weakened therapeutic effect and the appearance of new properties. In order to analyse the possibility of enhancing the cardioprotective effects detected in meldonium, essentially different functional groups with respect to the chemical structure of a basic molecule were introduced into a molecule of 3-(2,2,2-trimethylhydrazinium) propionate, namely the residue of substituted and unsubstituted nicotinic and 5-hydroxynicotinic acid.

Nicotinic acid was chosen as an object to be introduced into the base molecule for a reason. It exerts an unusually wide range of effects for small (10-200 mg/day) and large (over 1000 mg/day) doses and is already widely used in practical medicine. Moreover, a pharmacological composition containing a hydroxyl group that enhances the antioxidant properties of the molecule exerted the maximum cardioprotective effect in case of doxorubicin-associated cardiomyopathy. The reason for this is that one of the alleged causes of DOX-induced cardiotoxicity is related to the effect on iron metabolism: anthracyclines bind to Fe²⁺ ions, which leads to the formation of a hydroxyl radical and promotes the release of Fe²⁺ ions from ferritin, exacerbating oxidative stress even further.

Therefore, if in the cell cytoplasm, there arise the conditions for chelation or oxidation of ferrous (Fe²⁺) ions into the catalytically inactive state of Fe³⁺ ions, leading to a decrease in the effective concentration of hydroxyl radicals, this will create the conditions for achieving micromolar concentrations of ROS in the cytoplasm of cells. The substances possessing an antioxidant effect play an important role in regulating free-radical mechanisms. As an example of such a scheme, it can be assumed that CTK-

733 and CTK-735 possess the property of the chelator Fe^{2+} and the oxidant Fe^{2+} in Fe^{3+} . In addition, control over the concentration of Fe^{2+} can be important in regulating free-radical reactions: lipid peroxidation, inactivation of proteins and nucleic acids. Derivatives of 3-(2,2,2-trimethylhydrazine) propionate, having the chelating property of Fe^{2+} and oxidising property of Fe^{2+} to Fe^{3+} , can inhibit free radical oxidation catalysis and thereby inhibit free radical oxidation, which in turn reduces cardiotoxicity from DOX (Montaigne et al. 2012).

Thus, meldonium and its derivatives CTK-733 and CTK-735 demonstrated high efficacy and safety when

treating DOX-induced cardiotoxicity. A similar pathogenesis of metabolic disturbances under the influence of DOX and chronic cardiac insufficiency, as well as the results of experimental studies on the use of meldonium and its derivatives CTK-733 and CTK-735 to treat and prevent cardiotoxic effects of doxorubicin, suggest their high efficacy.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Author contribution

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