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ORIGINAL ARTICLE

Monotherapy of experimental metabolic syndrome: II. Study of cardiovascular effects

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ABSTRACT

Metabolic syndrome belongs to the most important risk factors of cardiovascular diseases. The aim of this study was to investigate changes in cardiovascular system induced by high cholesterol and high fat diet (HCHF) in HTG rats and their influence by a pyridoindole antioxidant – SMe1EC2 (S). The effects of S were compared with those of atorvastatin (A). Male HTG rats were fed HCHF (1% cholesterol + 7.5% lard) for 4 weeks. S and A were administered p.o., 50 mg/kg b.w. Following experimental groups were used: Wistar rats (W), hypertriglyceridemic rats (HTG), HTG rats fed HCHF (CHOL), HTG+S (S-HTG), CHOL+S (S-CHOL), and CHOL+A (A-CHOL). Values of blood pressure (BP) and selected ECG parameters were monitored in conscious animals, functions of the isolated heart and aorta were analyzed *ex vivo*. At the end of the experiment, systolic (sBP) and diastolic (dBP) blood pressure was increased in HTG and CHOL. S and A decreased BP in all treated groups. Accordingly with BP changes, the aortic endothelial function of CHOL was damaged. Both S and A administration ameliorated the endothelium-dependent relaxation to values of W. PQ and QTc intervals were prolonged in CHOL, while the treatment with S or A improved ECG findings. Prodysrhythmogenic threshold was decreased significantly in CHOL and both treatments returned it to the control values. In conclusion, HCHF increased BP, impaired endothelial relaxation of the aorta and potentiated susceptibility of myocardium to dysrhythmias. The effect of S on the changes induced by HCHF diet was more pronounced than that of A.

KEY WORDS: metabolic syndrome; high-fat and high-cholesterol diet; SMe1EC2; atorvastatin, cardiovascular effects

Introduction

Metabolic syndrome (MetS) is the most important risk factor for cardiovascular diseases (CVD). Its prevalence in Slovakia is comparable with the prevalence in the European population and moves around 20.1% (National Cholesterol Education Program – NCEP/ATP III criteria) and 38.1% (International Diabetes Federation – IDF) (Galajda *et al.*, 2007). MetS is characterized by a cluster of interrelated metabolic factors such as hypertension, dyslipidemia (elevated LDL-cholesterol, triglycerides, decreased HDL-cholesterol), insulin resistance, impaired glucose tolerance (DM2T), central (abdominal) obesity, proinflammatory and prothrombotic state (Alberti *et al.*, 2009; Raal, 2009). Although the pathogenesis of the metabolic syndrome is not well understood, it is likely that it represents a complex of interplay between metabolic, genetic, and environmental factors. Inflammation and oxidative stress have been proposed as common ethiologic factors linking these processes (Watanabe *et al.*, 2008).

All of the individual components of the MetS are important risk factors for CVD, *e.g.* of coronary heart disease, ischemic cerebrovascular disease, and peripheral vascular disease as well as for DM2T (Lew & Garfinkel, 1979; Grundy, 2007; Raal, 2009). Combination of these factors creates cardiometabolic syndrom.

Currently, the most studies have correlated the individual symptoms of MetS with the risk to develop cardiovascular disease, however specific cardiac alterations induced by MetS have not been reported. The results obtained from other models of obesity described increased left ventricular dysfunction after ischemia *ex vivo* (du Toit *et al.*, 2005; Ooie *et al.*, 2005; Nduhirabandi *et al.*, 2011; Wensley *et al.*, 2013) and *in vivo* (Huang *et al.*, 2013). Our experimental model, using hereditary hypertriglyceridemic (HTG) rats, enables to study the consequences of metabolic and hemodynamic abnormalities separately. HTG rats have been developed as a genetic model of metabolic syndrome, manifested by hypertension, hypertriglyceridemia and hypercholesterolemia.

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Studies of Bezek *et al.* (2017) showed male HTG rats fed either standard or high fat and high cholesterol diet to have hypercholesterolemia and increased serum levels of total cholesterol (TC), triglyceride (TRG) and low-density lipoprotein cholesterol (LDL-C). Elevated plasma lipids belong to the major risk factors for atherosclerosis and coronary heart disease. Therefore, lipid lowering is one of the major approaches in prevention of cardiovascular diseases (Jain *et al.*, 2010).

The hypolipidemic activity of SMe1EC2 – a novel pyridoindole antioxidant was documented in the study of Bezek *et al.* (2017). The results of this study showed that administration of SMe1EC2 to rats with genetic metabolic syndrome reduced hypertension, hypercholesterolemia and hyperlipidemia in groups of HTG rats fed a control diet as well as those fed with high cholesterol and high fat diet. As SMe1EC2 is a strong antioxidant (Štolc *et al.*, 2006) its beneficial effects on cardiovascular system of HTG rats can be assumed, because oxidative stress is known to play very important role in cardiovascular disorders.

Atorvastatin is a lipid lowering drug which moreover possesses several pleiotropic properties. Recent evidence suggests a pleiotropic mechanism of action including vasoprotective, antiinflammatory, and antidysrhythmic properties that imply an immediate role for statin medications (Dougherty & Arora, 2012).

The aim of the study is to investigate the changes in the blood pressure, electrical activity of the heart and vascular reactivity induced by CHOL diet in HTG rats. The cardiovascular effect of a novel pyridoindole derivative – SMe1EC2 in experimental MetS will be compared with the effect of clinical reference drug – atorvastatin – a competitive inhibitor of acyl-CoA cholesterol acyltransferase.

Material and methods

The experiments were performed in compliance with the Principles of Laboratory Animal Care published in the Colection of Laws of the Slovak Republic (Z.z. SR No. 436/2012). The experimental design was approved by the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences and by the State Veterinary and Food Administration of the Slovak Republic.

Animals

Male Wistar rats and male hereditary hypertriglyceridemic rats (HTG) rats, aged 3–4 months weight 373.6±18.5 g, obtained from the breeding station Dobrá Voda, Slovakia, were used. The animals were housed under standard experimental conditions (temperature 21±2°C, relative humidity 55±10%, 12/12 hr light-dark cycle, food and water provided ad libitum).

Treatment

The animals were randomly divided into 6 experimental groups consisting of 8 animals. Male Wistar rats (W) group fed standard diet served as healthy controls without metabolic disturbances present. Male HTG groups of rats were fed either standard diet (HTG) or high cholesterol and high fat diet (1% cholesterol and 7.5% lard fat, CHOL). The animals were treated with $SMe1EC_2$ (S) or atorvastatin (A), both in a dose of 50 mg/kg/day p.o. for 4 weeks. Atorvastatin was dissolved in 0.5% methylcelulose. $SMe1EC_2$ was administered to hereditary hypertriglyceridemic rats fed standard diet (S-HTG) or to hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet (S-CHOL). Atorvastatin was added to hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet (A-CHOL).

Experiments *in vivo*

After 7 days of adaptation, 5 days lasting careful handling before *in vivo* measurements to produce repeatable results was realized. We focused on the blood pressure and standard ECG recordings in conscious animals.

Blood pressure measurements

Blood pressure in rats was measured by non-invasive technique by Power Lab approach using tail-cuff (NIBP Controller, ADInstruments, Spechbach, Germany). Animals were placed into perspex restraint cage and preheated to 35°C for 7–10 min (thermostat KBC G16/250, Zalimp, Warszawa, Poland). Warming of the tails by infrared lamp improved blood circulation in the tail during the measurements. The tail cuff was positioned at the proximal end of the tail and pulse transducer monitored caudal artery pulse. Blood pressure cycles were monitored and 5 consecutive spindles were used for offline analysis. Data analysis was performed by Chart 5 for Windows (ADInstruments, Spechbach, Germany).

ECG measurements

Standard lead ECG was recorded from conscious rats standing in the perspex restraint cage with plate electrodes (made at STU Bratislava, Slovakia) positioned on the bottom of the cage and connected to the ECG unit (EKG Praktik Veterinary ver. 6, Seiva, Prague, Czech Republic) and computer. Data collection and offline analysis were done by Seiva Database Veterinary program. The selected parameters PQ, QT intervals and QRS duration were analyzed from ECG recordings. To eliminate the effect of different heart frequencies, QT interval corrected to heart frequency (QTc) was evaluated. QTc interval (in ms) was calculated according to the formula:

$$QTc = \frac{QT}{\sqrt{\frac{RR}{200}}},$$

where QT is duration of QT interval in ms, RR is interval between R amplitudes of 2 following QRS complexes in ms, and factor 200 is a minimal heart rate of rats. The mean values obtained from the 5 consecutive complexes analysis were taken for the next calculations.

Ventricular dysrhythmias were analyzed according to the Lambeth`s Convention and grouped into the simple

dysrhythmias and life-threatening dysrhythmias (ventricular tachycardia VT, fibrillation VF). The incidence of individual episodes, as well as the duration of life-threatening dysrhythmias were detected.

Experiments *ex vivo*

Isolated heart according to the Langendorff

The formation of life-treatening dysrhythmias tachycardia and fibrillation is closely related to the fibrillatory treshold and dysrhythmias persistence is related to the myocardial inability to spontaneously terminate previously induced disturbances. Detection of these disturbances is possible in spontaneously beating isolated hearts perfused according to the Langendorff. Isolated hearts were retrogradely perfused via aorta at constant pressure mode 80 mmHg. To assign basal diastolic pressure of the left ventricle, a latex balloon was inserted into the left ventricular cavity, filled with water and adjusted to the value of 8–10 mmHg.

After 10 min lasting stabilization period, the fibrillation threshold was detected by steeply increased current intensity of stimulation by 5 mA/30 s from 10 mA to 50 mA. Myocardial susceptibility to persistent dysrhythmias was determined by induction of the 2 minute lasting sustained ventricular tachycardia (VT) or ventricular fibrillation (VF). Time to restore sinus rhythm by stop flow after sustained dysrhythmias was considered as the ability of myocardium to recovery electrical activity (Tribulova *et al.*, 2002; Liptak *et al.*, 2017).

Basic parameters of stimulation (Electrostimulator ST-3, Medicor, Budapest, Hungary) via a pair of stimulating electrodes attached to the epicardium of the right ventricle were set as current: 10 mA, train duration: 2 s, stimulation rate: 100 pps, delay: 0.1 ms, duration: 0.2 ms.

The system BioLab F ver.1 (Institute of Measurement Science, Slovak Academy of Sciences, Bratislava, Slovakia) was used for data collection and offline analysis.

Isometric tension measurements in isolated thoracic aorta

The aorta was rapidly removed from sacrificed animal, immersed into the physiological solution (PSS) and carefully cleaned of all fat and connective tissue. Rings of the aorta (approximately 2 mm long) were mounted in a tissue chamber containing PSS, gassed and maintained at 37 °C, and attached to an isometric force transducer. Rings were passively stretched to optimal length by imposing an optimal initial tension of 10 mN found in previous studies. After stabilization period (60 min), the experimental protocol was as follows: Rings were precontracted with 1 μmol/l phenylephrine and relaxant responses of the preparations to acetylcholine (10 nmol/l – 10 μmol/l) were tested at the plateau of the contraction. Responses to acetylcholine are expressed as percentages of phenylephrine-induced contraction.

Solutions and drugs

Composition of the Krebs-Henseleit solution used for isolated heart perfusion (in mmol/1): NaCl, 118; KCl, 4.75; $CaCl_2 \times 2H_2O$, 2.5; $MgSO_4 \times 7H_2O$, 1.2; KH_2PO_4, 1.18; $NaHCO_3$, 25.0; glucose, 11.1; saturated by the mixture of 95% O_2 + 5% CO_2, pH=7.4, temperature 37 °C.

Composition of the modified physiological solution (PSS) used for aortic rings (in mmol/1): NaCl, 122; KCl, 5.9; $NaHCO_3$, 15; glucose, 10; $MgCl_2$, 1.25; and $CaCl_2$, 1.25, saturated by the mixture of 95% O_2 + 5% CO_2, pH=7.4, temperature 37 °C.

Used chemicals were from Centralchem (Bratislava, Slovakia) and mikroCHEM (Pezinok, Slovakia). Methylcelulose was a kind gift from VÚLM (Modra, Slovakia).

The pyridoindole derivative SMe1EC2 (S) SMe1EC2 (2-ethoxycarbonyl-8-methoxy-2,3,4,4a,5,9b-hexahydro-1*H*-pyrido-[4,3b] indolinium chloride) was synthesized in the Institute of Experimental Pharmacology and Toxicology, Slovak Academy of Sciences, Slovakia. Atorvastatin was a generous gift from Saneca Pharmaceuticals Hlohovec, Slovakia.

Data calculation and statistic

Data are expressed as means ± S.E.M. and compared using Anova test with post hoc Bonferoni test. The difference was considered statistically significant at a level $p \leq 0.05$.

Results

In our experiments we focused on the characterization of the cardiovascular effects of high cholesterol and high fat diet administered to the HTG rats as well as on the effects of novel pyridoindole antioxidant – SMe1EC2 and a competitive inhibitor of acyl-CoA cholesterol acyltransferase – atorvastatin. We found significantly higher systolic and diastolic blood pressure in HTG rats compared to control normotensive Wistar rats. Four weeks of HTG rat treatment with high cholesterol and high fat diet (CHOL) increased significantly systolic and diastolic blood pressure in comparison to HTG animals without diet. Repeated 4 weeks lasting administration of SMe1EC2 decreased both systolic (sBP) and diastolic (dBP) blood pressure in HTG and CHOL groups. Atorvastatin in the same dose significantly decreased sBP in CHOL group but did not influence dBP of CHOL animals without treatment (Figure 1).

Analysis of selected ECG parameters showed a significant prolongation of both PQ and QTc intervals in CHOL rats compared to HTG rats (Figure 2). Both substances tested shortened duration of PQ interval in CHOL animals. We did not observe significant modification of the QRS complex duration. Administration of SMe1EC2 and atorvastatin had only tendency to shorten duration of QTc interval in CHOL rats, while SMe1EC2 significantly shortened the QTc interval duration in HTG rats.

Experiments on isolated perfused rat hearts were designed to detect myocardial susceptibility to stimulation induced life-threatening tachyarrhythmias (ventricular tachycardia/ventricular fibrillation – VT/VF). The prodysrhythmogenic threshold was significantly decreased in hearts isolated from CHOL rats compared to

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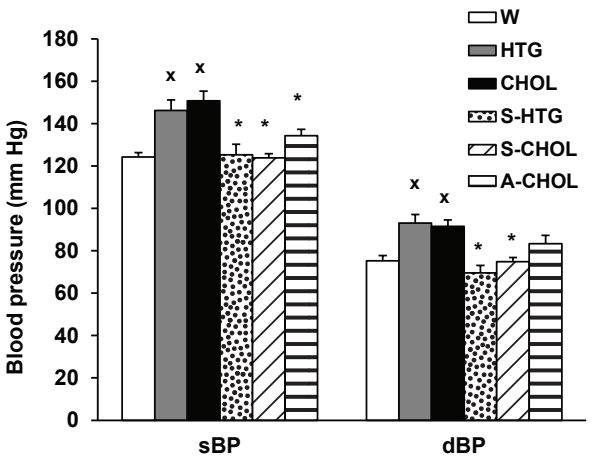

Figure 1. Systolic (sBP) and diastolic (dBP) blood pressure in conscious rats after 4 weeks lasting experiment. W – Wistar rats fed with standard diet; HTG – hereditary hypertriglyceridemic rats fed standard diet; CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet; S-HTG – hereditary hypertriglyceridemic rats fed standard diet administered SMe1EC2 50 mg/kg/day p.o.; S-CHOL – hereditary hypertriglyceridemic rats rats fed high cholesterol and high fat diet administered SMe1EC2 50 mg/kg/day p.o.; A-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered atorvastatin 50 mg/kg/day p.o. $^{x}p<0.05$ versus W, $^{*}p<0.05$ versus HTG. Data are means ± S.E.M. of 8 experiments.

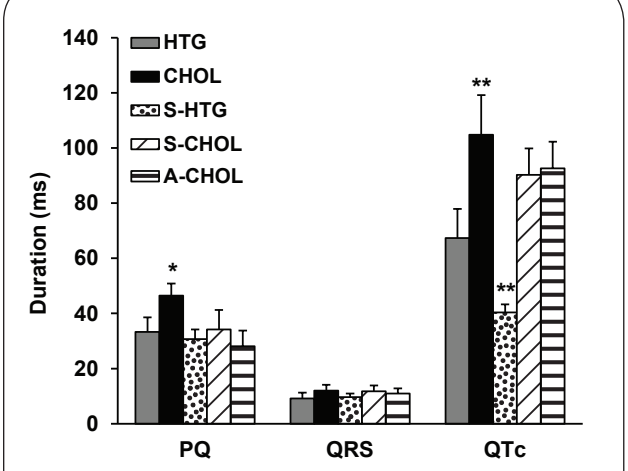

Figure 2. Selected ECG parameters recorded in conscious rats after 4 weeks lasting experiment – PQ interval, QRS interval and QTc interval in ms. HTG – hereditary hypertriglyceridemic rats fed standard diet (n=6); CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet (n=6); S-HTG – hereditary hypertriglyceridemic rats fed standard diet administered SMe1EC2 50 mg/kg/day p.o. (n=4); S-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered SMe1EC2 50 mg/kg/day p.o. (n=4); A-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered atorvastatin 50 mg/kg/day p.o. (n=5). $^{*}p<0.05$ versus HTG, $^{**}p<0.01$ versus HTG. Data are means ± S.E.M.

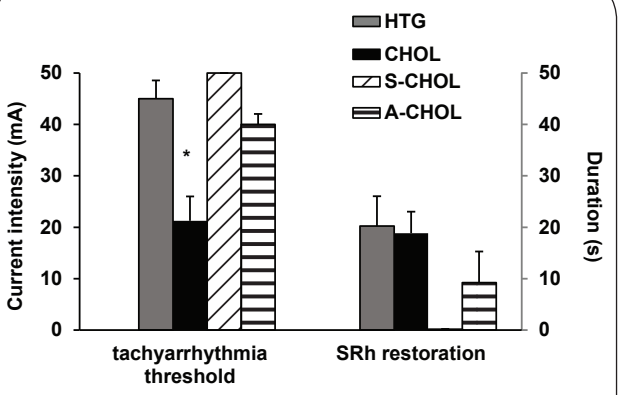

Figure 3. Tachyarrhythmia threshold (in mA) for persistent VT/VF induced by pacing of left ventricles at the end of experiments *ex vivo* (**part A**) and time to enforced restoration of sinus rhythm (SRh) by stop-flow (**part B**). HTG – hereditary hypertriglyceridemic rats fed standard diet; CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet; S-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered SMe1EC2 50 mg/kg/day p.o.; A-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered atorvastatin 50 mg/kg/day p.o. $^{*}p<0.05$ versus HTG. Data are means ± S.E.M. of 8 experiments.

HTG and mildly lowered in atorvastatin pretreatment of CHOL group. In SMe1EC2 treated group the stimulation exceeded maximal value (50 mA) without induction of dysrhythmias (Figure 3 part A). By application of stop-flow technique before termination of the experiment, time to enforced sinus rhythm (SRh) restoration was monitored. Time to sinus rhythm appearance was getting shorter in the order HTG > CHOL > A-CHOL. Since we did not elicit VT/VF in S-CHOL animals there was no reason to monitor time for SRh in this group (Figure 3, part B).

In hearts isolated from HTG rats the sustained tachyarrhythmias VT/VF were found in 67% of animals. The incidence of sustained VT/VF was 83% in CHOL rats and 80% in A-CHOL group, respectively. SMe1EC2 completely abolished the incidence of stimulation-induced sustained VT/ VF (Figure 4).

Further, changes in endothelium-dependent relaxation were studied in the aorta. Acetylcholine induced endothelium-dependent relaxation of the phenylephrine-precontracted aortic preparations which was significantly weaker in CHOL group in comparison to that in the control normotensive group. Administration of tested substances improved endothelium-dependent relaxation and reversed it to the control values. The effect of atorvastatin was more pronounced than the effect of SMe1EC2 (Figure 5.).

Discussion

Our results showed that high cholesterol and high fat diet (HCHF) administered to HTG rats had impact on the cardiovascular system of animals – BP was increased, endothelial relaxation of the aorta was impaired and the myocardium had a potentiated susceptibility to life-threatening dysrhythmias. The experimental model we used allow to study metabolic as well as cardiovascular

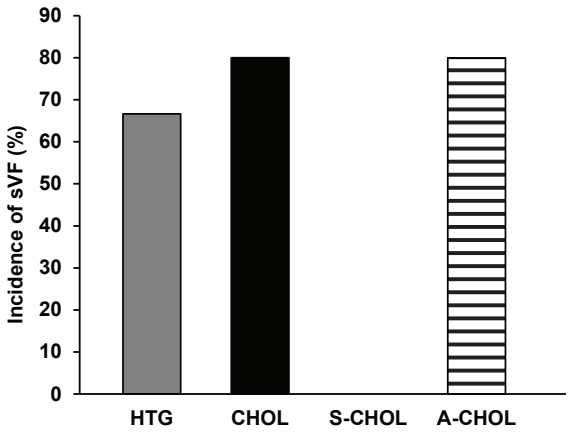

Figure 4. Induced sustained ventricular fibrillation detected at the end of the experiment *ex vivo* (in percentage of hearts in which ventricular fibrillation lasted ≥2 min). HTG – hereditary hypertriglyceridemic rats fed standard diet; CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet; S-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered SMe1EC2 50 mg/kg/day p.o.; A-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered atorvastatin 50 mg/kg/day p.o. *p<0.05 versus HTG. Data are means ± S.E.M. of 8 experiments.

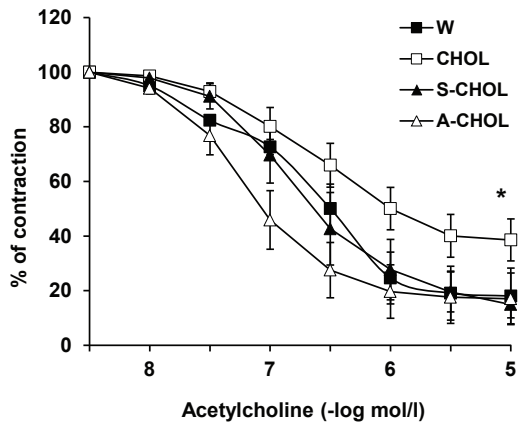

Figure 5. Endotelium-dependent relaxation induced by acetylcholine of the aortic preparations precontracted with phenylephrine (1 µmol/l). W – Wistar rats fed standard diet; CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet; S-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered SMe1EC2 50 mg/kg/day p.o.; A-CHOL – hereditary hypertriglyceridemic rats fed high cholesterol and high fat diet administered atorvastatin 50 mg/kg/day p.o.. *p<0.05 versus the other groups. Data are means ± S.E.M. of 8 experiments.

consequences of fatty diet in animals with genetic predisposition to MetS One of the important components of MetS is hypertension (Galajda *et al.*, 2007). In accordance with Klimes *et al.* (1995) we confirmed increase in blood pressure in hereditary hypertriglyceridemic animals which was even augmented in animals fed HCHF. Consistently with this finding the aortas displayed endothelial dysfunction. The involvement of vascular endothelium is of strategic importance in the regulation of vascular resistance and blood pressure. Due to its localization, endothelial cell layer is specially targeted in metabolic alterations. The endothelium is directly in contact with elevated concentration of glucose, insulin and triglycerides in blood suggesting that endothelial function alterations play an important role in cardiovascular diseases (Silva *et al.*, 2015). Endothelial dysfunction is the first step leading from decreased vascular dilatation up to hypertension and other cardiovascular disorders. This fact was confirmed not only in experimental (Liu *et al.*, 2017) but also in clinical studies (Reule *et al.*, 2017) and is in accordance with our findings.

Our results demonstrated an important impact of HCHF diet on electrical parameters of the heart of HTG rats – longer duration of PQ interval, QRS complex and QTc interval. The myocardial electrical and mechanical dysfunction is a background for cardiovascular complications of MetS (Yilmaz *et al.*, 2015). Patients with MetS displayed significant changes in QRS complex indicating depolarization sequence deterioration (Bacharova *et al.*, 2012). The repolarization abnormalities in patients with uncomplicated metabolic syndrome involved wider dispersion of ventricular repolarization time as well as increased QTc-min and QTc-max, and prolongation of both corrected QT interval (QTc) and QT dispersion (QTd) on electrocardiogram (Soydinc *et al.*, 2006). Prolongation of QT interval is a risk factor for development of cardiac rhythm disturbances.

Malignant dysrhythmias such as ventricular fibrillation and ventricular tachycardia are the most common dysrhythmias responsible for sudden cardiac death (Luu *et al.*, 1989). Kurl *et al.* (2016) described the 2.2–2.6 times higher risk of sudden cardiac death in male with identified MetS. Cardiac dysrhythmias are in relation to dyslipidemia, obesity, diabetes mellitus 2 type (DM2T) and lifestyle (Duflou *et al.*, 1995; Jouven *et al.*, 2001; Luscher *et al.*, 2003; Plourde *et al.*, 2014). Both, atrial and ventricular fibrillation are considered to be induced by abnormal impulse formation and/or by circuit movement – re-entry (Allesie *et al.*, 1984; Gray *et al.*, 1998; Witkowski *et al.*, 1998). However, the precise mechanisms through which MetS causes atrial fibrillation are not completely understood, but the syndrome has been associated with electrical remodeling of the atrium and sinoatrial nodes (Albarado-Ibañez *et al.*, 2013).

To study myocardial susceptibility to dysrhythmias we used the *ex vivo* model of cardiac burst pacing for initiation and persistence of malignant re-entry dysrhythmias (Merrilat *et al.*, 1990; Kihara & Morgan, 1991; Simor *et al.*, 1997; Tribulová *et al.*, 2002; Liptak *et al.*, 2017). Using this model we demonstrated the decreased stimulating threshold and increased incidence of sustained ventricular dysrhythmias. Moreover, we found that in comparison with HTG animals, the fatty diet significantly potentiated the initiation of ventricular tachyarrhythmias without

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significant changes in the time for restoration of sinus rhythm. The increased vulnerability to VF in hypertriglyceridemic rats compared to normotensive Wistar rats was detected also by Tribulová *et al.* (2006 and 2008).

At the present, the only drugs approved for treatment of risk factors of metabolic syndrom are those drugs that target the individual risk factors: lipid-lowering drugs, antihypertensive agents, hypoglycemic drugs, and antiplatelet drugs. A lipid lowering mechanism is one of the major approaches in prevention of cardiovascular diseases. Though drugs of various categories acting through the different mechanisms are available in the antihyperlipidemic therapy, problems and side effects associated with the currently available lipid lowering drugs persist (Jain *et al.*, 2010). Atorvastatin, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor, is a lipid-lowering drug which in addition to this effect possesses several pleiotropic properties including vasoprotective, antiinflammatory, and antiarrhythmic properties that imply an immediate role for statin medications (Dougherty & Arora, 2012).

Our experiments with HTG rats fed HCHF diet demonstrated the potential of atorvastatin treatment to decrease the systolic blood pressure and to protect endothelial function. Our findings are in agreement with beneficial effects of atorvastatine on endothelium-dependent relaxation of the superior mesenteric artery (SMA) in aged HTG rats (Sotnikova *et al.*, 2012). Among the other effects, atorvastatin improved endothelial function in aortas from diabetic rats (Simões *et al.*, 2016), showed the positive impact on morphological and functional parameters of the vascular wall (Mikhin *et al.*, 2016), and ameliorated oxidative stress and inflammatory reaction in rats with dyslipidemia (Zhang *et al.*, 2014).

In our experiments atorvastatin restored the prolonged duration of selected ECG parameters *i.e.*. PQ, QRS and QTc intervals to values detected in HTG rats. However, it should be taken into account that coadministration of atorvastatin to patients treated with another additional QT-prolonging drug could induce life-threatening torsade de pointes arrhythmia (Niedrig *et al.*, 2016).

Next we found out atorvastatin to increase the myocardial threshold for induction of tachyarrhythmias and to restore the sinus rhythm two-times faster than it was observed in non-treated HTG and HTG rats fed HCHF. However, atorvastatin only slightly reduced incidence of sustained tachyarrhythmias. Acute bolus administration of atorvastatin showed similar protective effects against arrhythmias induced by electrical pacing in HTG hearts (Benova *et al.*, 2015).Cardiovascular protective effects of atorvastatin were also confirmed by clinical studies (Horwich & MacLellan, 2007; Xu *et al.*, 2016).

Oxidative stress and inflammation are known to be involved in the pathogenesis of both metabolic syndrome and atrial and ventricular fibrillation (Tadic *et al.*, 2013). Thus, drugs with antioxidant properties can be a good choice for cardiovascular protection in MetS. SMe1EC2 meets these criteria as it showed intensive antioxidant properties (Zúrová-Nedelčevová *et al.*, 2006; Štolc *et al.*, 2006; Broskova & Knezl, 2011). Indeed, in conditions of in vitro ischemia/reperfusion injury of the rat heart, this antioxidant showed beneficial effect during reperfusion. Administration of SMe1EC2 significantly increased the left ventricular developed pressure, decreased pathologically elevated left ventricular end-diastolic pressure and potentiated recovery from the serious reperfusion induced dysrhythmias such as ventricular tachycardia, ventricular fibrillation and stunned myocardium (Broskova & Knezl, 2011). In addition to antioxidant properties, the hypolipidemic activity of SMe1EC2 was documented in groups of HTG rats fed a standard diet as well as in rats fed high cholesterol and high fat diet. SMe1EC2 administration decreased serum levels of TC and TRG, and plasma levels of IL-1 (Bezek *et al.*, 2017).

In the present experiments, administration of SMe1EC2 led to decrease of the elevated blood pressure of HTG animals fed HCHF diet. This beneficial effect was probably associated with amelioration of the aortic endothelium-dependent relaxation to acetylcholine to the control values of Wistar rats. The most important and original effects of SMe1EC2 were observed in the case of ECG parameters, where its administration led to adjustment of the increased values of PQ and QTc intervals to the control or even significantly lower values. Important effect of SMe1EC2 was found also in protection of the heart against induction of sustained VF/VT.

In summary, the present study shows for the first time cardioprotective effects of antioxidant SMe1EC2 in the model of HTG rats fed HCHF diet which are equal or even better that those of antilipidemic drug atorvastatin.

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