EXPERIMENTAL STUDY

Anti-arrhythmic and cardio-protective effects of atorvastatin and a potent pyridoindole derivative on isolated hearts from rats with metabolic syndrome

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ABSTRACT

OBJECTIVES: Aims of this study were to investigate the anti-arrhythmic and cardio-protective effect of ator-vastatin and of a new pyridoindole derivative (SMe1EC2) on isolated and perfused hearts while following the Langendorff principles.

BACKGROUND: Metabolic syndrome is a widely distributed condition progressing to cardiovascular disease. Many of the metabolic syndrome patients take (HMG)-co-enzyme A (CoA) reductase inhibitors with potential cardio-protective effects. SMe1EC2 is a promising new drug, exerting many positive effects in experimental settings.

METHODS: Rats with induced metabolic syndrome were treated with atorvastatin (25 mg/kg) and SMe1EC2 (25 mg/kg and 0.5 mg/kg, respectively) daily for 3 weeks. After the treatment, the hearts were isolated and perfused according to Langendorff.

RESULTS: Both atorvastatin and SMe1EC2 improved cardiac function by elevating the left ventricular developed pressure (VLDP) and cardiac contractility. Both SMe1EC2-treated groups improved LVDP during reperfusion, significantly increased –dP/dt, and moderately elevated +dP/dt values. The treatment with both atorvastatin and SMe1EC2 (25 mg/kg) significantly reduced malignant arrhythmia in comparison to control group and group treated with SMe1EC2 0.5 mg/kg.

CONCLUSIONS: Owing to its anti-arrhythmic and cardio-protective effects, atorvastatin and SMe1EC2 could be of benefit to patients suffering from metabolic syndrome *(Tab. 3, Fig. 3, Ref. 41)*. Text in PDF *www.elis.sk*.

KEY WORDS: hyper-triacylglycerolemic rats, Langendorff, ventricular fibrillation, dyslipidemia.

Introduction

Atherosclerosis is an underlying cause of about 50 % of all deaths in westernized societies (1) while the subjects with potential atherosclerosis and cardiovascular disease are in many cases individuals with metabolic syndrome diagnosis. These patients have chronic elevation of triacylglycerols (TG), low-density lipoprotein cholesterol (LDL), very low-density lipoprotein cholesterol (VLDL), decreased high-density lipoprotein cholesterol (HDL), and impaired glucose metabolism. They are often overweight as a result of factors commonly linked to unhealthy lifestyle and diet, which is characteristic for a significant part of the population today

(2, 3, 4, 5). Negative lifestyle adaptations are irrefutably linked to the emergence of atherosclerosis and lead to major cardiovascular disease, complications and possible death (6, 7, 8, 9). The major cause of cardiovascular mortality is that of malign heart arrhythmias. They represent a major clinical problem while its management is unsatisfactory (10, 11). Ventricular and atrial arrhythmias may result in severe morbidity, coma and eventual death. More than half of the patients with ischemic heart disease die due to ventricular arrhythmias and ventricular tachyarrhythmias, which are the most common and most severe forms of heart dysrhythmias occurring in 84 % of patients with sudden cardiac death (12).

Patients suffering from metabolic syndrome, type II diabetes mellitus, and obesity may have decreased chances of survival at the onset of ventricular arrhythmia. In these patients, malign heart arrhythmias may occur more frequently and with increased duration, severity and eventual damage. The incidence of coronary heart disease is increased threefold in patients with metabolic syndrome with markedly increased cardiovascular morbidity (13). Metabolic syndrome has been identified in ~35 % of patients in the USA (14) and ranges from 8 % to 43 % in men and 7 % to 56 % in women across the world (15, 16). Dyslipidemia occurs in 52.9 % of adults in the USA (17) and 53.6 % in China (18). Elevated total cholesterol concentrations ($\geq$ 190 mg/dL) were found in 60.5 % of

women and 56.6 % of men in the German population (19). Metabolic syndrome is a global health problem concerning patients across many countries of western and eastern parts of the world.

Many of these patients receive treatment with drugs inhibiting the cholesterol synthesis, such as statins. Atorvastatin is a widely used 3-hydroxy-3-methylglutaryl (HMG)-co-enzyme A (CoA) reductase inhibitor taken by many patients in the effort to reduce progression of atherosclerosis (20). Besides the reduction of LDL cholesterol, statins have other effects. It has been suggested that these effects may provide cardiovascular protection, be of benefit at the onset of myocardial infarction, as well as decrease ischemic-reperfusion injury (21). Atorvastatin is thought to provide anti-arrhythmic protection and may reduce mortality (22, 23) by restoring optimal heart functions (24). Then a novel experimental drug SMe1EC2 was identified to exert many beneficial effects expressing potent anti-arrhythmic activity (25, 26) with unknown action. The anti-arrhythmic activity of SMe1EC2 was here re-evaluated on a matabolic syndrome rat model to obtain additional results regarding this potential drug.

Our hypotheses were as follows: 1) Metabolic disturbances associated in the metabolic syndrome will result in an increase in the occurrence of malign heart arrhythmias in comparison to treated animals; 2) Administration of atorvastatin and potent experimental drug SMe1EC2 will protect these animals from malign heart arrhythmias and decrease cardiac abnormalities after global ischemia.

Material and methods

Animals and animal model

All procedures involving animals were performed in compliance with the Principles of Laboratory Animal Care issued by the Ethical Committee of the Institute of Experimental Pharmacology and Toxicology and State Veterinary and Food Administration of Slovakia. Prague hereditary HTG rats (n = 40) were obtained from the breeding station Dobra Voda (Slovak Republic, reg. No. SK CH 24016). The animals were divided into four groups (ten animals per group). The laboratory rats were than divided into boxes, each box containing five animals. After a one-week quarantine, all the animals were kept in a housing room for 8 subsequent weeks. All rats in all four groups received high-fat, high-fructose diet (HFFD; 7.5 % lard, 1 % cholesterol and 10 % fructose). All animals received

the same diet for 8 weeks. After 5 weeks of diet, one group (HFFD-A) received 25 mg/kg of atorvastatin, another group (HFFD-SMe25) received 25 mg/kg of experimental pyridoindole derivative SMe1EC2, and the third group (HFFD-SMe0.5) received the latter drug but in a different dosage (0.5 mg/kg). The control group (HFFD-C) received physiological solution. The application of substances was oral with a gastrointestinal probe. The substances were applied daily in the morning for a period of 21 days (3 weeks). The same researchers conducted all the applications. At this point of time, the animals were housed in standardized conditions with 12/12-h light/dark cycle and at 40–60 % humidity, with chow and water *ad libitum*. In the first, fifth and eighth weeks of the diet, weight, arterial blood pressure and fasting blood glucose were assessed. Blood samples were taken from all the animals. After 5 weeks of the diet, all the animals developed metabolic syndrome assessed by means of elevated total cholesterol, LDL cholesterol, triacylglycerol level, glucose and atrial pressure (not published), and decreased HDL cholesterol (27).

Langendorff apparatus and experimental setting

The hearts of all laboratory rats were isolated according to Langendorff principles. After the animals had been transported into the laboratory, they were anesthetized with diethyl ether and immediately decapitated (to isolate the brain). The heart was then rapidly isolated and cannulated *in situ*. The same researcher conducted all the procedures. After successful cannulation and initiation of the perfusion, all hearts were put immediately on the Langendorff apparatus for retrograde perfusion. The apparatus was set to constant pressure (80 mm Hg). Krebs-Henseleit bicarbonate perfusion solution was prepared as follows (in mM): NaCl (118), KCl (4.7), $CaCl_2$ (2.5), NaH_2PO_4 (1.18), $NaHCO_3$ (25), and glucose (11.1) equilibrated with a gas mixture of 95% O_2 and 5% CO_2 at 37 °C, pH 7.4. Electrocardiograph electrodes were put on the right atrium and left ventricle of the heart and a latex balloon was inserted into the left ventricle immediately after placing the heart on the apparatus. After insertion of the latex balloon, the volume of the balloon was filled with water and the tubular balloon holder was connected to the pressure transducer *via* a tube. Left ventricular end-diastolic pressure (LVEDP) was set to give an approximate power of 0.5 kPa (~10 mm Hg) before starting the measurements. Tesla electro-manometers were used to monitor left ventricular pressure. The developed left ventricular pres-

Tab. 1. Baseline values of isolated hearts.

	HFFD-C	HFFD-A	HFFD-SMe25	HFFD-SMe0.5
Heart rate (HR) (*beats/min*)	280.1±22.3	282.2±35.5	259.1±6.8	300.4±29.5**
Left ventricular developed pressure (LVDP) (*kPa*)	11.5±3.8	14.9±3.0*	16.4±4.3**	15.5±2.3*
Left ventricular end-developed pressure (LVEDP) (*kPa*)	1.8±2.49	0.9±0.8	1.05±0.95	0.8±0.8
Coronary flow (*ml/min*)	15.1±6.0	16.3±3.2	16.9±7.3	15.8±2.3
–dP/dt (*kPa/s*)	207.4±73.4	281.1±64.1*	285.5±62.0*	316.2±48.4***
+dP/dt (*kPa/s*)	286.8±109.2	381.6±99.0*	417.9±108.8**	420.7±67.5**
–dP/dt/+dP/dt	0.73±0.05	0.75±0.06	0.69±0.07	0.75±0.03*

Values are presented as mean ± SD. LVDP: HFFD-C vs HFFD-A (p < 0.036), vs HFFD-SMe25 (p < 0.0043), vs HFFD-SMe0.5 (p < 0.011). HR: HFFD-SMe25 vs HFFD-SMe0.5 (p < 0.009). –dP/dt: HFFD-C vs HFFD-A (p < 0.015), vs HFFD-SMe25 (p < 0.012), vs HFFD-SMe0.5 (p < 0.0004). +dP/dt: HFFD-C vs HFFD-A (p < 0.04), vs HFFD-SMe25 (p < 0.0073), vs HFFD-SMe0.5 (p < 0.004). –dP/dt/+dP/dt: HFFD-SMe25 vs HFFD-SMe0.5 (p < 0.028).

Tab. 2. Baseline values of isolated hearts at the end of reperfusion after global ischemia.

	HFFD-C	HFFD-A	HFFD-SMe25	HFFD-SMe0.5
Heart rate (HR) (*beats/min*)	268.9±59.6	249.1±72.5	274.0±45.5	279.9±60.5
Left ventricular developed pressure (LVDP) (*kPa*)	10.8±3.5	11.9±3.3	13.5±1.5	13.9±2.1*
Left ventricular end-developed pressure (LVEDP) (*kPa*)	0.8±1.1	1.0±1.1	0.5±0.8	0.2±0.4
Coronary flow (*ml/min*)	17.7±6.4	14.6±3.8	16.9±6.4	13.6±2.4
−dP/dt (*kPa/s*)	215.8±72.7	221.5±48.8	249.1±83.3*	276.6±21.1*
+dP/dt (*kPa/s*)	288.3±103.2	317.1±90.8	326.7±99.1	378.5±44.8
−dP/dt/+dP/dt	0.75±0.07	0.71±0.08	0.75±0.05	0.73±0.03

Values are presented as mean ± SD. LVDP: HFFD-C vs HFFD-SMe0.5 (p < 0.036). −dP/dt: HFFD-C vs HFFD-SMe25 (p < 0.041), vs HFFD-SMe0.5 (p < 0.042).

sure (LVDP), LVEDP, heart rate (HR), coronary flow (CF), −dP/dt and +dP/dt were subsequently monitored with the BioLabF software throughout the experiment. After the setting, all hearts were kept stabilized for 15 minutes. Stabilization was followed by global ischemia lasting 20 minutes. The 20-minute ischemia was followed by 20-minute reperfusion. Then the stimulation electrodes were put on the myocardium and ascending stimulation followed with doses from 10 mA to 50 mA. The goal was to induce sustained VF (lasting 2 minutes) or ventricular tachycardia (VT). If sustained VF or VT developed during the reperfusion and persisted until the end of the reperfusion, the hearts were not conducted to cardio-stimulation. After the induction of sustained VF or VT (2 min), CF was stopped and sinus rhythm recovery was monitored. During the reperfusion, the ventricular premature beats (VPB), ventricular tachycardia (VT) and ventricular fibrillation (VF) were monitored.

Statistical analysis

Analytic software from the BioLabF was used to analyze the data. STATISTICA 10 software was used for the statistics. ANO-VA Fisher LSD test was used for data comparison. All data in tables is presented as mean±SD. All data infigures and in the text is presented as mean±SD. * p < 0.05, ** p < 0.01, *** p < 0.001.

Results

LVDP, −dP/dt and +dP/dt values were significantly increased in all treated groups in comparison to the control HFFD-C group before global ischemia (Tab. 1).

LVDP was significantly higher in the HFFD-SMe0.5 group in comparison to the HFFD-C group, while −dP/dt was higher in both SMe1EC2 treatments in comparison to HFFD-C after global ischemia (Tab. 2). The LVDP course in the reperfusion is demonstrated in Figure 1. Both SMe1EC2 drug dosages elevated LVDP in crucial moments of reperfusion. A moderately higher value of developed ventricular pressure of both SMe1EC2 treatments was observed during the whole reperfusion period. The values of −dP/dt were significantly elevated in the case of both dosages of SMe1EC2 drug treatment (Fig. 2). HR, CF, LVEDP, +dP/dt and −dP/dt/+dP/dt ratio did not differ significantly among
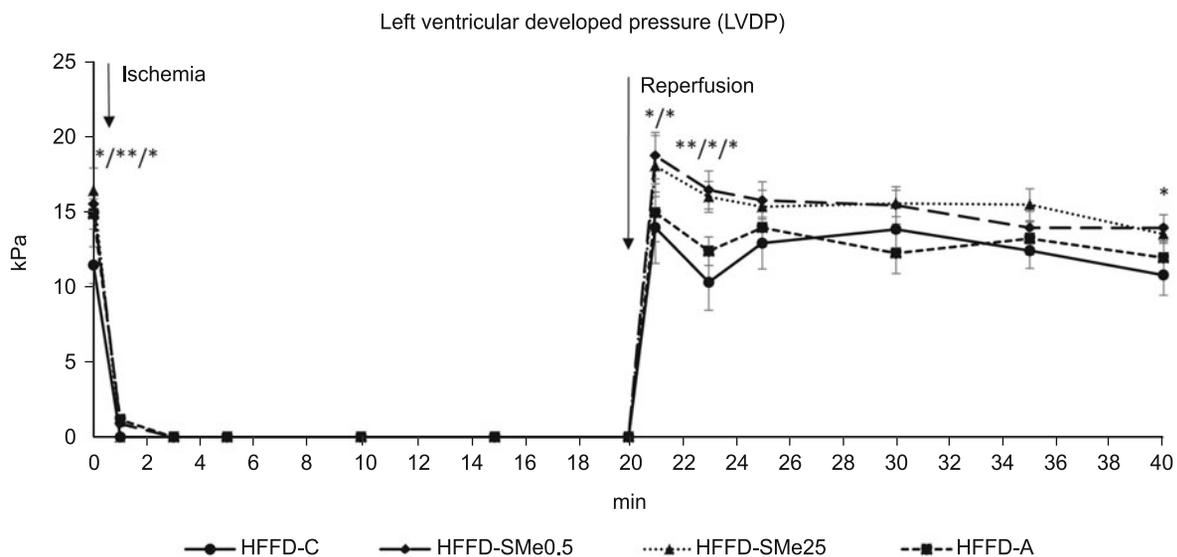

Fig. 1. LVDP during reperfusion. Values are presented as mean ± SEM. 0 min stabilisation: HFFD-C vs HFFD-A (p < 0.036), vs HFFD-SMe25 (p < 0.0043), vs HFFD-SMe0.5 (p < 0.011), 1st min reperfusion: HFFD-C vs HFFD-SMe0.5 (p < 0.025), HFFD-SMe0.5 vs HFFD-A (p < 0.037), 3rd min reperfusion: HFFD-C vs HFFD-SMe0.5 (p < 0.004), vs HFFD-SMe25 (p < 0.036), HFFD-SMe0.5 vs HFFD-A (p < 0.016), 20th min reperfusion: HFFD-C vs HFFD-SMe0.5 (p < 0.036).
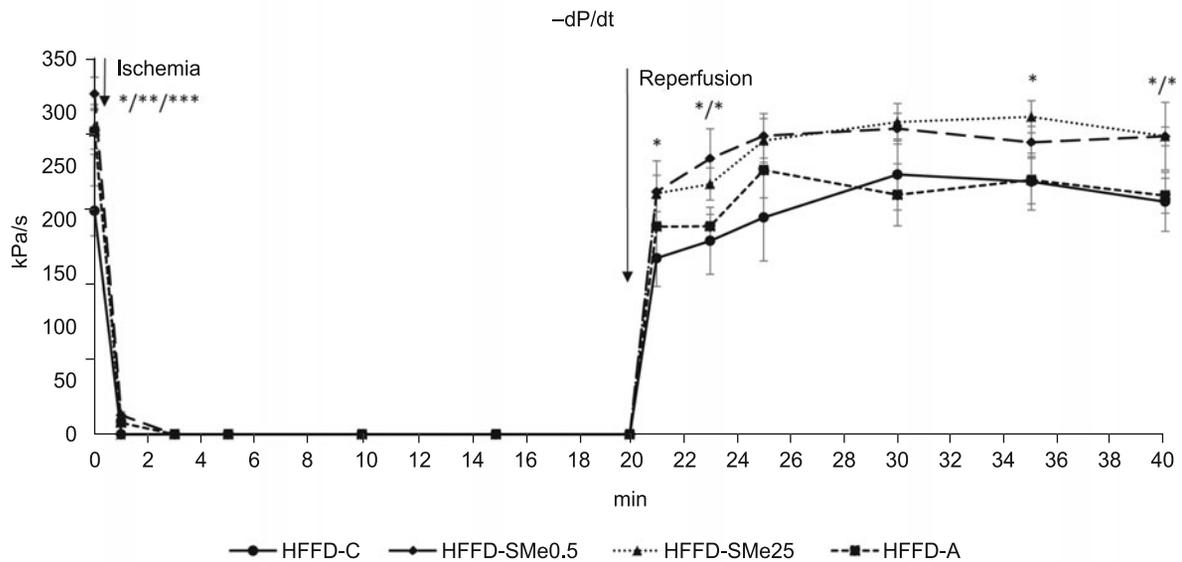
Fig. 2. –dP/dt during reperfusion. Values are presented as mean ± SEM. 0 min stabilisation: HFFD-C vs HFFD-A (p < 0.015), vs HFFD-SMe25 (p < 0.012), vs HFFD-SMe0.5 (p < 0.0004), 1st min reperfusion: HFFD-C vs HFFD-SMe0.5 (p < 0.041), 3rd min reperfusion: HFFD-C vs HFFD-SMe0.5 (p < 0.024), HFFD-SMe0.5 vs HFFD-A (p < 0.042), 15th min reperfusion: HFFD-SMe25 vs HFFD-A (p < 0.041), 20th min reperfusion: HFFD-C vs HFFD-SMe25 (p < 0.041), vs HFFD-SMe0.5 (p < 0.042).

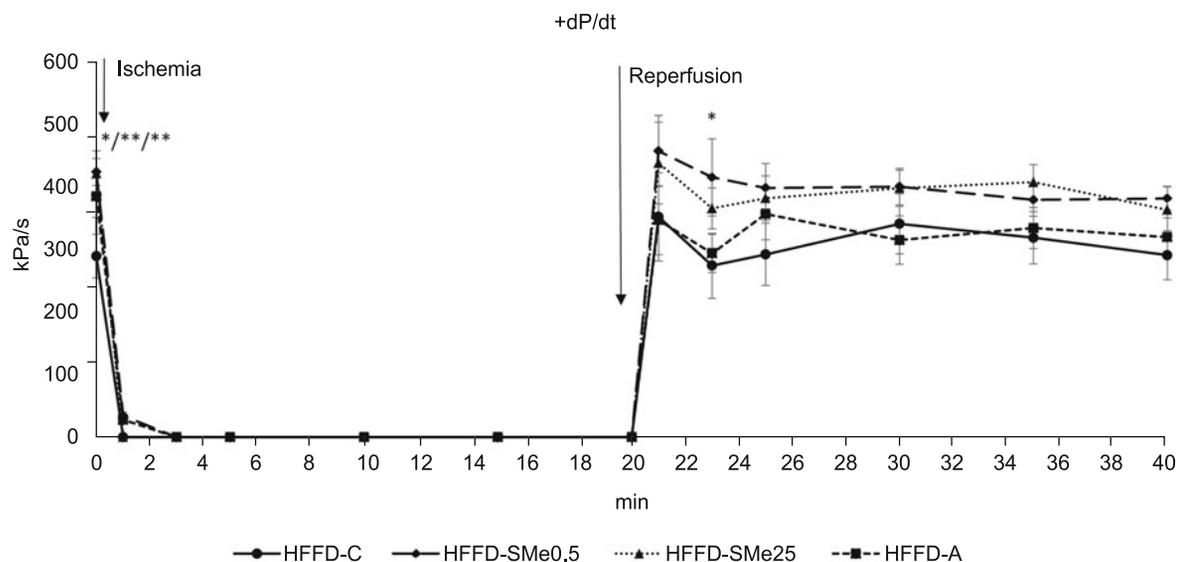Fig. 3. +dP/dt during reperfusion. Values are presented as mean ± SEM. 0 min stabilisation: HFFD-C vs HFFD-A (p < 0.04), vs HFFD-SMe25 (p < 0.0073), vs HFFD-SMe0.5 (p < 0.004), 3rd min reperfusion: HFFD-C vs HFFD-SMe0.5 (p < 0.042).

the groups, although +dP/dt values were moderately elevated in both SMe1EC2-treated groups (Fig. 3).

Ventricular arrhythmia (VA) appeared in 8 animals in the HFFD-C group, 4 cases of VA were observed in the HFFD-SMe25 group, while 5 animals had VA in the HFFD-A group, and 7 in the HFFD-C and HFFD-SMe0.5 group, respectively (Tab. 3).

Sustained ventricular fibrillation (SVF) was recorded in 4 animals in the control group, while 3 animals fibrillated in the HFFD-SMe25 group, 3 animals developed SVF in the HFFD-A group and 5 animals developed SVT in the HFFD-SMe0.5 group.

Sustained ventricular tachycardia (SVT) was observed in 2 animals in the HFFD-C and HFFD-SMe0.5 groups, while 1 animal developed SVT in each of the HFFD-SMe25 and HFFD-A groups.

Ventricular premature beats (VPB) were as follows: altogether 1,229 (123±30.6) VPB were recorded in the HFFD-C group, 1,674 (186±47.5) in the HFFD-SMe25 group, 2,070 (207±54.7) in the HFFD-SMe0.5 group and 2,308 (256±53.7) VPB were recorded in the HFFD-A group.

SVF was induced in all the animals from the HFFD-C (with a mean of 25±4.6 mA) and HFFD-A group (with a mean of

Tab. 3. Occurrence of ventricular arrhythmia in animals among the groups.

	HFFD-C	HFFD-A	HFFD-SMe25	HFFD-SMe0.5
Sustained ventricular fibrillation	4	3	3	5
Sustained ventricular tachycardia	2	1	1	2
Total cases of sustained ventricular arrhythmias	6	4	4	7
Total arrhythmia cases*	8	5	4	7

*sustained VF and VT together with transient (short) VF or VT cases.

27±4.0 mA). Two cases in the HFFD-SMe25 group and one case in the HFFD-SMe0.5 group were without SVF. Mean dosage for the induction of SVF for the HFFD-SMe25 group was 24±4.0 mA, while only 21±2.1 mA were sufficient for SVF induction for the HFFD-SMe0.5 group.

The difference in time to sinus rhythm recovery after 2 minute SVF following the stop flow was not significant among the groups. Means of 97±32.0 s were recorded in the HFFD-C group, 92±48.0 s in the HFFD-SMe25 group, while 116±22.0 s and 121±38.0 s were recorded for the HFFD-A and HFFD-SMe0.5 groups, respectively.

Discussion

All the HFFD rats in this experiment developed metabolic syndrome (for details see 27). Compared to healthy Wistar rats, the animals had increased levels of total cholesterol, LDL cholesterol and triacylglycerols, while the levels of HDL cholesterol were decreased. Beside dyslipidemia, HTG rats fed with high-fat diet had increased blood pressure (28), elevated glucose levels and higher scores in the glucose tolerance test. On the other hand, the rats were not obese. The originally designed model to simulate hypertriacylglycerolemia was adapted by the means of diet change to give rise to other metabolic abnormalities and establish the metabolic syndrome. Both drugs, atorvastatin and SMe1EC2, produced cardio-tonic effect. Beside the elevation of LVDP, the drugs in all treatments positively influenced –dP/dt and +dP/dt, while SMe1EC2 in lower dosage (HFFD-SMe0.5 group) had the most pronounced effect. The drugs failed to restore the baseline values of the heart physiology to output values (Tab. 1). The HFFD-SMe0.5 group succeeded to establish the highest LVDP among the groups, namely with significantly higher values than those in the HFFD-C group. Both dosages of SMe1EC2 positively influenced –dP/dt values after global ischemia.

As proposed in our hypotheses, the rats with metabolic syndrome were found to have increased chances of malignant arrhythmia occurrence (29) and untreated animals in this experiment were most susceptible to arrhythmia (the HFFD-C group). HFFD-A and HFFD-SMe25 groups had reduced numbers of malignant arrhythmia records, while the lower dosage of the experimental SMe1EC2 had no pronounced effect. Only 4 animals developed ventricular arrhythmia in the HFFD-SMe25 group in comparison to 8 animals in the HFFD-C group, while 5 animals developed ventricular arrhytmia in the HFFD-A group. Analysis and comparison of the total duration time of arrhythmia and the mean time of these arrhythmic episodes did not yield statistically significant differences among the groups. However the total and mean time of VT was lower in all the treatments in comparison to HFFD-C, while the total and

the mean count of extrasystoles was higher in all treatments. The arrhythmic threshold in the cardio-stimulation experiment did not give statistically significant differences, although the threshold in atorvastatin group was slightly higher than in other groups. The mean time to sinus rhythm establishment after the stopped flow in fibrillating hearts was not significantly improved by any treatment. Atorvastatin produced effects comparable with SMe1EC2 in the same dosage. It reduced the total numbers of cases of sustained arrhythmia and arrhythmia in the given experiment and produced cardio-protective effects, as in previous research (23, 26).

Similarly to other statins, atorvastatin is thought to have many beneficial pleiotropic effects (22, 30). It also has been found to protect against cardiovascular events and reduce morbidity (31), act as anti-inflammatory agent in higher concentrations in subjects with metabolic syndrome (32) and have antioxidative effects (33). Acute atorvastatin treatment protected the heart against ischemia-reperfusion injury following myocardial infarction in rats (34). In another experimental setting, atorvastatin was found to activate many pro-survival signalling pathways, which resulted in decreased infarction size and morbidity (35). As a result of these actions, preoperative administration of atorvastatin one week prior the coronary artery bypass grafting followed by postoperative atorvastatin treatment significantly decreased the incidence of atrial fibrillation (36). It may also be beneficial in diabetes mellitus treatment by reducing oxidative and inflammatory negative effects (37).

Anti-inflammatory and anti-oxidative effects with high free radicals scavenging activity is also known for SMe1EC2 (38, 39). Both atorvastatin and SMe1EC2 can thus protect the heart from excessive oxidative damage following ischemia/reperfusion injury. SMe1EC2 is thought to have many effects and could be used as a universal drug to partially treat all the metabolic syndrome symptoms (40) and affect arrhythmia development as previously reported (25), where decrease in arrhythmia occurrence was observed after direct perfusion with SMe1EC2 at a dose of 1 x 10⁻⁵ M 10 at onset of ischemia and during reperfusion.

Both atorvastatin and SMe1EC2 exerted cardio protective effects (22, 26, 31), established most probably by a complex action on numerous mechanisms affecting cell survival, signalling, oxidative and energy metabolism. In addition to lowering high cholesterol levels, atorvastatin treatment can protect patients suffering from metabolic syndrome, impaired lipid metabolism, and established cardiovascular disease from the occurrence of malignant life-threatening arrhythmia (23). SMe1EC2 showed cardio protective effects comparable with atorvastatin, and when considering also its other effects, it could be a beneficial drug for patients suffering from metabolic syndrome and cardiovascular disease in the future.

Learning points

Similarly to other statins, atorvastatin has many pleiotropic actions including anti-arrhythmic and cardio-protective effects. SMe1EC2 pyridoindole derivative is a potent drug with anti-arrhythmic effects as well as effects positively influencing the heart function.

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