Effects of 4-week administration of simvastatin in different doses on heart rate and blood pressure after metoprolol injection in normocholesterolaemic and normotensive rats

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Abstract

Introduction: Statins and β1-adrenergic antagonists are well established in cardiovascular events therapy and prevention. The previous study showed that statins might impact on β-adrenergic signalling and blood pressure in a dose-dependent manner. The aim of the study was to evaluate the impact of 4-week administration of simvastatin given at different doses on the heart rate and blood pressure after injection of metoprolol in rats.
Material and methods: The experiments were performed in normocholesterolaemic and normotensive Wistar rats. Rats received simvastatin in doses of 1, 10 and 20 mg/kg body weight (bw) for 4 weeks. The control group received 0.2% methylcellulose. For the further estimation of the heart rate and blood pressure, metoprolol at 5 mg/kg bw or 0.9% NaCl was injected intraperitoneally.
Results: Simvastatin at doses of 1, 10 and 20 mg/kg bw did not influence the heart rate or blood pressure as compared to the control group. Metoprolol injection statistically significantly decreased the heart rate (439.29 ±14.03 min^{-1} vs. 374.41 ±13.32 min^{-1}; $p < 0.05$). In rats receiving simvastatin during the 4-week period after metoprolol injection, heart rate and blood pressure (mean, systolic, diastolic) were similar as compared to the group receiving metoprolol alone.
Conclusions: Simvastatin administration during a 4-week period in different doses did not influence the heart rate or blood pressure after metoprolol injection in normocholesterolaemic and normotensive rats.

Key words: rats, simvastatin, metoprolol, heart rate, blood pressure.

Introduction

Nowadays 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) inhibitors are the most important drugs used in the primary and secondary prevention of cardiovascular events. Their beneficial activity is dependent on limiting cholesterol synthesis; therefore current guidelines recommend aggressive cholesterol lowering with statins. Dose-dependent side effects of statins involving myopathy and alteration of cell membrane are observed, as well [1, 2]. On the other hand, cholesterol-independent pleiotropic effects of statins have been reported [3]. In some cases, to obtain target low-density lipoproteins (LDL)-C, statins are co-administered with other lipid-lowering agents [4]. From a practical viewpoint, monotherapy with HMG-CoA inhibitors is applied; the statin dosage used by patients might be enlarged,

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however. In therapy, statins are often applied with β1-blockers such as metoprolol. In the previous study it was shown that simvastatin influenced the heart rate and blood pressure after metoprolol administration [5]. Mülhäuser *et al.* showed that atorvastatin desensitized β1-adrenergic signalling by reducing isoprenylation of G-protein [6]. This interaction was dependent on both the drug concentration and drug administration period. In our previous study simvastatin after 2 weeks of administration to normocholesterolaemic and normotensive rats did not influence the heart rate or blood pressure after bolus injection of metoprolol [7, 8].

The aim of the study was to evaluate the influence of bolus injection of metoprolol after 4-week administration of simvastatin given at different doses on the heart rate and blood pressure.

Material and methods

Animals

The study was approved by the Ethics Committee of the Medical University of Lodz (Poland) – 43/ŁB300-Az/2006. The experiments were performed in 51 8-11-week-old anaesthetized Wistar rats, outbred males. A several-day adaptation period was scheduled prior to the beginning of the experiment. After the adaptation period, animals were divided into 8 groups receiving: 1) 0.2% methylcellulose, intragastrically (ig); 2) 0.2% methylcellulose (ig) and metoprolol at 5 mg/kg body wieght (bw) intraperitoneally (ip); 3) simvastatin at 1 mg/kg bw (ig); 4) simvastatin at 10 mg/kg bw (ig); 5) simvastatin at 20 mg/kg bw (ig); 6) simvastatin at 1 mg/kg bw (ig) + metoprolol at 5 mg/kg bw (ip); 7) simvastatin at 10 mg/kg bw (ig) + metoprolol at 5 mg/kg bw (ip); 8) simvastatin at 20 mg/kg bw (ig) + metoprolol at 5 mg/kg bw (ip). Simvastatin (Polfarmex, Poland series no. KY-SI-M20030102) or placebo (0.2% methylcellulose) were given ig over a 4-week period. Rats had free access to standard diet (granulated mix "LSK") and water. After administration of drugs or vehicle, heart rate and haemodynamic parameters were measured. The surgery was performed 24 h after administration of the last drug dose and 10 h after the last feed supply. For further surgical procedures, anaesthesia was initiated by an ip dose of pentobarbital sodium at 60 mg/kg bw. The anaesthesia was maintained by intraperitoneal bolus injections of pentobarbital sodium at 10 mg/kg bw, as needed. For the measurement of heart rate and blood pressure, catheters were implanted into the right carotid artery. The signals were provided by an Isotec pressure transducer connected to a direct current bridge amplifier (both Hugo Sachs Elektronik). After the haemodynamic stabilization period (about 15 min), an intraperitoneal single injection of metoprolol at

5 mg/kg bw or 0.9% NaCl (2 ml/100 g bw) was administered. After heart rate and blood pressure assessment, 0.25 ml of blood samples were taken for further lipid profile examination. Surgical procedures, heart rate and blood pressure recording were provided as described previously [7, 8]. The results of metoprolol injection were given as the absolute differences from the baseline of heart rate and as percent of change from the baseline.

Statistical analysis

All data are presented as means ± SD (standard deviation). Statistical comparisons between baseline conditions and metoprolol injection were done by paired Student's *t*-test. Comparisons among groups were performed using ANOVA. Post-hoc comparisons were performed using the LSD test. Normal distribution of parameters was checked by means of the Shapiro-Wilk test. If data were not normally distributed or the values of variance were different, ANOVA with Kruskal-Wallis and Mann-Whitney U test were used. All parameters were considered statistically significantly different if $p < 0.05$. The statistical analysis of heart rate and hemodynamic parameters was performed using Statgraphics 5.0 plus software.

Results

There were no significant differences among groups of rats considering age and lipid profile (Table I). The mean heart rate in the control group was 441.47 ±5.60 min^{-1}. In groups receiving simvastatin at 1, 10 or 20 mg/kg bw during the 4-week period, heart rate was comparable to the control rats. No differences among groups receiving simvastatin at 1, 10 or 20 mg/kg were observed, either. Administration of 0.9% NaCl did not influence the heart rate as compared to the initial values (99.55-101.21%). Metoprolol injection significantly decreased heart rate in control rats (85.27%). In rats receiving simvastatin at 1, 10 or 20 mg/kg bw for 4 weeks, metoprolol decreased heart rate as compared to rats receiving placebo (82.44% vs. 86.25%) (Table II).

Mean blood pressure in control rats was 94.01 ±4.34 mmHg and it was changed insignificantly after 0.9% NaCl intraperitoneal injection (93.46 ±4.43 mmHg). An insignificant influence of 0.9% NaCl injection on the systolic (99.17%) and diastolic blood pressure (99.08%) was observed as well. 4-week administration of simvastatin at 1, 10 or 20 mg/kg bw did not influence the mean systolic and diastolic blood pressure in normotensive and normocholesterolaemic rats. Intraperitoneal administration of 0.9% NaCl to rats receiving simvastatin did not influence the mean systolic and diastolic blood pressure, either. Intraperitoneal administra-

Table I. Body weight (bw), total cholesterol (TC), LDL cholesterol (LDL-C), HDL cholesterol (HDL-C), triglycerides (TG) in rats (mean ± SD)

	BW [g]	TC [mmol/l]	LDL-C [mmol/l]	HDL-C [mmol/l]	TG [mmol/l]
K_Na (9)	214 ±8.9	1.17 ±0.11	0.63 ±0.06	0.28 ±0.03	0.26 ±0.09
K_M (6)	210 ±14.0	1.27 ±0.20	0.76 ±0.09	0.24 ±0.09	0.27 ±0.06
S1_Na (6)	204 ±8.9	1.18 ±0.35	0.69 ±0.03	0.25 ±0.09	0.24 ±0.05
S1_M (6)	200 ±14.14	1.22 ±0.26	0.75 ±0.05	0.21 ±0.07	0.26 ±0.08
S10_Na (6)	202 ±11.2	1.25 ±0.01	0.75 ±0.10	0.25 ±0.03	0.25 ±0.09
S10_M (6)	210 ±14.0	1.21 ±0.07	0.68 ±0.07	0.24 ±0.05	0.29 ±0.05
S20_Na (5)	210 ±17.7	1.11 ±0.27	0.55 ±0.06	0.28 ±0.12	0.28 ±0.12
S20_M (7)	214 ±8.9	1.21 ±0.11	0.64 ±0.07	0.30 ±0.02	0.27 ±0.03

K_Na – control group receiving 0.9% NaCl injection, K_M – control group receiving metoprolol injection, S1_Na, S1_M – rats receiving 1 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S10_Na, S10_M – rats receiving 10 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S20_Na, S20_M – rats receiving 20 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, (n) – number of rats in group

tion of metoprolol to the control group and to rats receiving simvastatin at all doses had no impact on the blood pressure (Table III).

Discussion

Pleiotropic effects of statins involve improvement of endothelial function, stability of atherosclerotic plaques, decrease of oxidative stress and inflammation, and inhibition of thrombogenic response [3, 7, 9]. It has been reported that statins increased the endothelial production of nitric oxide (NO) and this effect was correlated with upregulation of endothelial NO synthase expression [10]. It was suggested that this effect might be intensified by the simultaneous inhibition of G proteins and reduction of endothelial NO synthase mRNA degradation [11]. Anti-hypertensive statin activity may result from drug impact on the decrease of vasoconstrictor endothelin-1 level [12]. 3-Hydroxy-3-methyl-glutaryl-CoA reductase inhibitors reduce the production of reactive oxygen species, such as superoxide anion, and hydroxyl radicals, as shown in experimental studies, and also this action may contribute to vasodilatory effects of statins [13]. Another possible mechanism of statin BP-lowering effect involves the downregulation of the angiotensin II-type 1 receptor. The angiotensin II-type 1 receptor is overexpressed in hypercholesterolaemic patients, and it may be improved by administration of statins, which also were shown to markedly reduce the vasoconstrictor response to angiotensin II infusion [14]. It was shown that statins could improve systemic arterial compliance in normolipidaemic patients with isolated systolic hypertension via reduction of large artery stiffness [15, 16].

By inhibiting HMG-CoA reductase, statins reduce the production of important isoprenoids, i.e. farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). The isoprenylation process was

Table II. Heart rate [min⁻¹] before and after metoprolol or 0.9% NaCl intraperitoneal administration (mean ± SE) in rats during 4-week administration of simvastatin or placebo

	Metoprolol or 0.9% NaCl injection		
	Before	After	Difference [%]
K_Na (9)	441.47 ±5.60	445.01 ±4.53	100.84 ±0.65
K_M (6)	439.29 ±14.03	374.41 ±13.32	85.27 ±1.89^t
S1_Na (6)	443.41 ±13.45	448.74 ±13.78	101.21 ±0.72
S1_M (6)	429.37 ±14.46	356.97 ±7.48	83.41 ±2.66^t
S10_Na (6)	419.40 ±10.63	418.48 ±9.93	99.81 ±0.77
S10_M (6)	481.05 ±6.60	396.63 ±8.03	82.44 ±0.75^t
S20_Na (5)	448.93 ±19.95	448.28 ±16.52	99.95 ±1.62
S20_M (7)	436.15 ±13.84	375.66 ±7.67	86.25 ±1.58^t

K_Na – control group receiving 0.9% NaCl injection, K_M – control group receiving metoprolol injection, S1_Na, S1_M – rats receiving 1 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S10_Na, S10_M – rats receiving 10 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S20_Na, S20_M – rats receiving 20 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, (n) – number of rats in group, 't' – p < 0.05 t-par

shown to influence signalling molecules, including the monomeric GTPases of the Rho and Ras families. However, isoprenylation of G protein γ subunit (Gγ) was found to be essential for membrane attachment of Gγ as well as Gβ [17, 18]. Statins, by interfering with Gγ isoprenylation, could influence membrane association and function of heterotrimeric G proteins [19-23]. The previous study demonstrated that treatment of cardiac myocytes with a statin reduced the cAMP level and induced a significant increase in β-adrenergic receptor density [11]. The above mechanisms may lead to statins' impact on the heart rate and blood pressure.

Clinical observations in the UCSD Statin Study and the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA) suggest that HMG-CoA reductase inhibitors might have some

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Table III. Blood pressure [mmHg] before and after metoprolol or 0.9% NaCl intraperitoneal administration (mean ± SE) in rats during 4-week administration of simvastatin or placebo

	Metoprolol or 0.9% NaCl injection		
	Before	After	Difference [%]
K_Na (9)			
Mean	94.01 ±4.34	93.46 ±4.43	99.38 ±0.58
Diastolic	86.96 ±4.23	84.63 ±4.09	99.08 ±0.52
Systolic	103.24 ±5.53	102.39 ±5.42	99.17 ±0.78
K_M (6)			
Mean	91.66 ±2.01	90.95 ±2.91	99.35 ±1.39
Diastolic	86.96 ±2.07	83.96 ±1.88	100.01 ±1.64
Systolic	100.35 ±3.21	99.01 ±2.41	99.82 ±1.43
S1_ Na (6)			
Mean	86.81 ±2.56	88.36 ±3.34	101.78 ±1.29
Diastolic	82.10 ±0.81	83.80 ±1.98	102.02 ±1.45
Systolic	91.96 ±4.68	93.21 ±4.93	101.33 ±1.33
S1_M (6)			
Mean	95.07 ±3.0	96.49 ±3.19	101.49 ±1.00
Diastolic	89.42 ±1.53	91.57 ±1.84	102.39 ±0.94
Systolic	101.67 ±5.17	102.17 ±5.09	100.53 ±1.23
S10_Na (6)			
Mean	88.08 ±0.86	88.34 ±0.68	100.31 ±0.54
Diastolic	85.51 ±1.03	85.89 ±0.82	100.46 ±0.43
Systolic	90.76 ±0.93	90.99 ±0.88	100.27 ±0.63
S10_M (6)			
Mean	90.55 ±1.87	90.10 ±0.99	99.54 ±0.95
Diastolic	87.67 ±1.85	87.95 ±1.41	100.35 ±0.51
Systolic	93.85 ±1.97	92.45 ±0.76	98.56 ±1.36
S20_Na (5)			
Mean	95.54 ±4.56	95.65 ±4.29	100.15 ±0.45
Diastolic	89.26 ±2.22	88.73 ±1.26	99.47 ±1.09
Systolic	101.39 ±6.83	102.49 ±7.48	101.02 ±0.74
S20_M (7)			
Mean	97.60 ±4.35	99.66 ±3.91	101.78 ±1.13
Diastolic	90.62 ±2.57	93.84 ±3.68	103.49 ±1.52
Systolic	105.24 ±6.89	105.38 ±5.01	100.48 ±1.80

K_Na – control group receiving 0.9% NaCl injection, K_M – control group receiving metoprolol injection, S1_Na, S1_M – rats receiving 1 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S10_Na, S10_M – rats receiving 10 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, S20_Na, S20_M – rats receiving 20 mg/kg simvastatin and 0.9% NaCl or metoprolol injection, (n) – number of rats in group

blood-pressure-lowering properties in addition to their effect on lipids [24, 25]. However, the CARE study showed no significant reduction of BP with statin therapy [26]. The different results in the CARE and ASCOT studies may result from the degree of LDL-C reduction achieved in the above trials.

Another point is that a time- and dose-dependent influence of statin on β1-adrenergic signalling is observed. Atorvastatin reduced total G protein α (s) subunit (Gαs) protein level concentrations dependently and time-course experiments with 1 µmol/l atorvastatin showed the first significant effect after 24 h of treatment and a slightly larger effect at 48 h [6]. Simvastatin administration in patients undergoing cardiac surgery being treated with metoprolol reversed upregulation of β1-adrenergic receptors [5]. It was connected with appeasement of the depressing metoprolol influence on heart rate and blood pressure.

Our study demonstrates no impact of simvastatin after four-week administration to rats on blood pressure and heart rate after metoprolol injection. Similar effects were observed in our studies considering a 2-week period of statin administration [7, 8]. Clinical observations of reduction in heart rate and blood pressure after 1-month concomitant administration of simvastatin and metoprolol included study patients with ischaemic heart disease or diabetes mellitus. The inter-species differences could not be excluded. The long-term concomitant administration of statin with β1-blockers and lipid disturbances could be important, considering the influence of the drug-drug interaction on heart rate and haemodynamic parameters.

In conclusion, simvastatin administration during a 4-week period in different doses did not influence heart rate or blood pressure after metoprolol injection in normocholesterolaemic and normotensive rats.

Acknowledgments

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