Concomitant administration of simvastatin with ivabradine in contrast to metoprolol intensifies slowing of heart rate in normo- and hypercholesterolemic rats

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Abstract

Introduction: β-Blockers play a significant role in therapeutic heart rate (HR) management and angina control. In patients who are unable to tolerate β-blockers ivabradine could be particularly useful. The aim of the study was to establish whether concomitant administration of simvastatin with ivabradine or metoprolol had any effect on rat HR and blood pressure (BP).

Material and methods: The experiments were performed in hyper- and normocholesterolemic outbred Wistar rats. Animals were divided into 2 groups: receiving during 4 weeks normal diet (normocholesterolemic rats) or diet with 5% cholesterol and 2.5% cholic acid (hypercholesterolemic rats). Then rats received placebo (0.1% methylcellulose), 2) metoprolol 30 mg/kg bw; 3) ivabradine 10 mg/kg bw; 4) simvastatin 10 mg/kg bw; 5) simvastatin 10 mg/kg bw + metoprolol 30 mg/kg bw; 6) simvastatin 10 mg/kg bw + ivabradine 10 mg/kg bw. Drugs were given during a 4-week period. HR and BP measure were provided by an Isotec pressure transducer connected to a direct current bridge amplifier. For the further lipid profile examination, 0.25 ml of blood samples were taken.

Results: After administration of ivabradine with simvastatin to normocholesterolemic and hypercholesterolemic rats the mean HR was significantly reduced as compared to rats receiving simvastatin (312.0 ±30.2 min^{-1} vs. 430.7 ±27.8 min^{-1}, $p < 0.05$); (329.8 ±24.2 min^{-1} vs. 420.5 ±9.2 min^{-1}, $p < 0.05$) or ivabradine alone (312.0 ±30.2 min^{-1} vs. 350.2 ±16.0 min^{-1}, $p < 0.05$); (329.8 ±24.2 min^{-1} vs. 363.0 ±21.7 min^{-1}, $p < 0.05$).

Conclusions: Concomitant administration of simvastatin with ivabradine intensified slowing of HR, although it did not influence BP in normo-and hypercholesterolemic rats. Statin-induced intensification of HR deceleration after metoprolol administration was not observed.

Key words: simvastatin, ivabradine, metoprolol, heart rate, rats.

Introduction

β-Adrenergic blocking drugs turned out to reduce both mortality and recurrent myocardial infarction in patients with a previous myocardial infarction [1]. Anti-anginal and anti-ischemic therapy with β-adrenergic blocking drugs in some patients with coronary artery disease might be complicated by contraindications and drug side effects [2-5]. Alternative pharmacotherapy by ivabradine – the novel selective for the I_f current lowering heart rate agent – seems to have beneficial effects in patients with

stable coronary artery disease (CAD) [1, 6]. It reduces heart rate in the sino-atrial node and does not affect blood pressure, myocardial contractility, intracardiac conduction or ventricular repolarization [7]. In ischemic heart disease (IHD) patients the position of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) inhibitors in primary and secondary prevention of cardiovascular events is well established [8, 9]. Their beneficial activity depends on limiting cholesterol synthesis as well as cholesterol independent "pleiotropic" effects [10]. The possible drug-drug interactions involving statins might limit the safety of concomitant therapy with potent CYP3A4 inhibitors [11-13]. Also it was established that simvastatin increased the area under plasma concentration time curve (AUC) ratio of other agents metabolized via the CYP3A4 pathway [14]. Moreover, HMG-CoA inhibitors turned out to interact with other drugs, probably by desensitization of β-adrenergic signaling resulting from reduced isoprenylation of G-proteins [15]. Therefore in the present study we have examined whether concomitant administration of simvastatin and ivabradine or metoprolol to rats had any effect 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) – 3/ŁB405/2008. The experiments were performed in 120, Wistar rats, outbred males, 200-260 g body weight (bw). A several day adaptation period was scheduled prior to the beginning of the experiment. After the adaptation period, animals were divided into 2 groups: receiving normal diet – granulated MIX "LSK" (normocholesterolemic rats) or normal diet with 5% cholesterol and 2.5% cholic acid (hypercholesterolemic rats). After a four-week period each group was divided into 6 subgroups receiving intragastrically (i.g.) during 4 weeks: 1) placebo (0.1% methylcellulose); 2) metoprolol 30 mg/kg bw; 3) ivabradine 10 mg/kg bw; 4) simvastatin 10 mg/kg bw; 5) simvastatin 10 mg/ kg bw + metoprolol 30 mg/kg bw; 6) simvastatin 10 mg/kg bw + ivabradine 10 mg/kg bw.

All rats were provided with free access to food and water throughout the study. After 4 weeks of treatment, heart rate and hemodynamic 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 the further surgical procedures, anesthesia was initiated by an intraperitoneal (*i.p.*) dose of pentobarbital sodium at 60 mg/kg bw. The anesthesia was maintained with intraperitoneal bolus injections of pentobarbital sodium at 10 mg/kg bw as needed. To measure the 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) for 20 min after the hemodynamic parameter stabilization period. For the further lipid profile examination, 0.25 ml of blood samples were taken. Surgical procedures, and heart rate and blood pressure recording were performed as described previously [16, 17].

Statistical analysis

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

Results

Metoprolol administration to normocholesterolemic rats heart rate resulted in significant dece leration in comparison with control group (390.8 ±20.5 min^{-1} vs. 429.8 ±19.5 min^{-1}, $p < 0.05$). Similar results were observed in hypercholesterolemic group, as well (384.3 ±21.9 min^{-1} vs. 418.0 ±29.0 min^{-1}, $p < 0.05$). Statistically significant reduction of heart rate after ivabradine administration to normocholesterolemic rats (350.2 min^{-1} ±16.0 vs. 429.8 ±19.5 min^{-1}), and hypercholesterolemic rats (363.1 ±21.7 min^{-1} vs. 418.0 ±29.1 min^{-1}) as compared to control groups were noted, as well. Insignificant changes of heart rate were noted in normocholesterolemic (430.7 ±27.8 min^{-1} vs. 429.8 ±19.5 min^{-1}) and hypercholesterolemic rats (420.5 ±9.2 min^{-1} vs. 418,0 ±29.0 min^{-1}) receiving simvastatin at 10 mg/kg bw alone as compared to control groups. The mean heart rate after concomitant administration of metoprolol and simvastatin to normocholesterolemic rats statistically decreased as compared to the control group (370.5 ±15.5 min^{-1} vs. 429.8 ±19.5 min^{-1}, $p < 0.05$) and as compared to the group receiving simvastatin alone (370.5 ±15.5 min^{-1} vs. 430.7 ±27.7 min^{-1}, $p < 0.05$). Similar observations were made in hypercholesterolemic rats. Significant slowing of heart rate after concomitant administration of simvastatin with metoprolol as compared to control rats (386.5 ±22 min^{-1} vs. 418.0 ±29.0 min^{-1}, $p < 0.05$) and as compared to rats receiving sim-

vastatin alone (386.5 ±22 min^{-1} vs. 420.5 ±9.2 min^{-1}, $p < 0.05$) were observed, as well. The concomitant administration of simvastatin with metoprolol to normocholesterolemic and hypercholesterolemic rats insignificantly influenced the mean heart rate as compared to rats receiving metoprolol alone (Table I, Figures 1, 2). After administration of ivabradine with simvastatin to normocholesterolemic and hypercholesterolemic rats the mean heart rate was significantly reduced as compared to the control group (312.0 ±30.2 min^{-1} vs. 429.8 ±19.5 min^{-1}, $p < 0.05$); (329.8 ±24.2 min^{-1} vs. 418.0 ±29.0 min^{-1}, $p < 0.05$) and as compared to rats receiving simvastatin (312.0 ±30.2 min^{-1} vs. 430.7 ±27.8 min^{-1}, $p < 0.05$); (329.8 ±24.2 min^{-1} vs. 420.5 ±9.2 min^{-1}, $p < 0.05$) or ivabradine alone (312.0 ±30.2 min^{-1} vs. 350.2 ±16.0 min^{-1}, $p < 0.05$); (329.8 ±24.2 min^{-1} vs. 363.0 ±21.7 min^{-1}, $p < 0.05$) (Table I, Figures 1, 2). Metoprolol, ivabradine, and simvastatin given alone or in combination (i.e. simvastatin with metoprolol; simvastatin with ivabradine) insignificantly influenced the mean, systolic and diastolic blood pressure in normocholesterolemic and hypercholesterolemic rats (Table II).

Table I. Values of the mean heart rate [beats/min] in rats fed normo- and hypercholesterolemic diet measured after 4-wk administration of drugs

K	429.81 ±19.50
K_H	418.03 ±29.05
S	430.66 ±27.76
S_H	420.51 ±9.18
IW	350.18 ±16.02*
IW_H	363.05 ±21.68*
M	390.76 ±20.54*
M_H	384.28 ±21.87*
SIW	312.02 ±30.25*,a,b
SIWH	329.83 ±24.16*,a,b
SM	370.54 ±15.55*,a
SM_H	386.50 ±22.20*,a

*K – normocholesterolemic control group, K_H – hypercholesterolemic control group, IW – normocholesterolemic group receiving ivabradine, IW_H – hypercholesterolemic group receiving ivabradine, S – normocholesterolemic group receiving simvastatin, S_H – hypercholesterolemic group receiving simvastatin, SIW – normocholesterolemic group receiving simvastatin with ivabradine, SIW_H – hypercholesterolemic group receiving simvastatin with ivabradine, *p < 0.05 as compared to control group, ap < 0.05 as compared to rats receiving simvastatin alone, bp < 0.05 as compared to rats receiving ivabradine alone*

Discussion

Heart rate reduction is a major pharmacological effect of ivabradine. The probability of side effects such as symptomatic bradycardia during ivabradine therapy is very low – approximately 0.2%. However, these effects might be potentiated by concomitant administration of several inhibitors or substrates of cytochrome P450 isoenzyme CYP3A4 [14]. As it was shown in previous studies, statins in humans are substrates of the CYP3A4 isoenzyme and they can competitively inhibit liver microsomes [18-20]. Although simvastatin metabolism in rats is different to humans, similar interaction between simvastatin and ivabradine is very likely.

In this pathway ivabradine plasma concentration might result in potentiating the slowing of heart rate [14, 21]. Our observations of heart rate in rats after concomitant administration of simvastatin and ivabradine to normo- and hypercholesterolemic rats might be the result of the drug–drug metabolism interaction. In previous studies it was reported that HMG-CoA reductase inhibitors might decrease the inhibitory effect on the platelet aggregation of clopidogrel (mainly metabolized by CYP3A4), and they might decrease the clearance of midazolam (CYP3A4 substrate) [13, 16]. Hypercholesterolemic diet in our study did not seem to have an influence on the possible interaction after combined administration of simvastatin and ivabradine. Similarly to our previous observations, no impact of simvastatin during its combined admin-

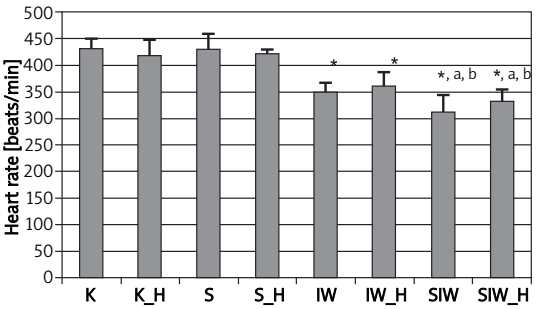

Figure 1. Values of the mean heart rate in rats fed normo- and hypercholesterolemic diet measured after 4-wk administration of drugs
**p< 0.05 as compared to control group, ap < 0.05 as compared to rats received simvastatin alone, bp < 0.05 as compared to rats receiving ivabradine alone*

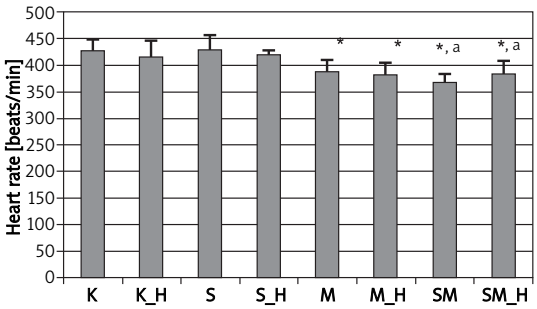

Figure 2. Baseline mean heart rate in rats fed normo- and hypercholesterolemic diet measured after 4-wk administration of drugs
**p < 0.05 as compared to control group, ap < 0.05 as compared to rats receiving simvastatin alone*

Table II. Summary statistics (mean ± SD) for blood pressure [mm Hg] in normo- and hypercholesterolemic rats

	Diastolic	Mean	Systolic
K	84.11 ±6.96	93.38 ±5.51	104.88 ±6.31
K_H	86.32 ±3.82	91.74 ±3.65	105.61 ±4.30
S	85.99 ±2.41	91.38 ±3.92	105.08 ±4.12
S_H	82.83 ±3.75	91.54 ±2.61	104.50 ±3.08
IW	85.97 ±2.25	93.47 ±3.27	105.23 ± 4.80
IW_H	81.39 ±3.66	92.26 ±2.01	106.71 ±2.52
M	83.89 ±4.22	92.68 ±2.70	105.18 ±3.03
M_H	86.86 ±3.80	94.30 ±4.43	105.89 ± 3.32
SIW	83.56 ±3.57	92.93 ±2.61	107.16 ±5.15
SIW_H	81.83 ±2.92	92.51 ±2.51	107.43 ±3.26
SM	80.68 ±3.14	89.35 ±2.27	106.49 ±5.72
SM_H	86.22 ±2.34	92.90 ±2.75	106.94 ±4.10

istration with metoprolol to normo- and hypercholesterolemic rats on the heart rate, as compared to rats receiving ivabradine alone, was observed [17, 22]. Conversely, others showed that during concomitant therapy with simvastatin and metoprolol in preoperative cardiac surgery patients (bypass grafting), heart rate was higher if metoprolol was taken alone [23]. The authors concluded that a possible explanation for the above interaction might be the influence of statin on the β-adrenergic signaling via reducing isoprenylation of G-proteins. They suggested that statin therapy seemed to counter-regulate the up-regulation of β-adrenoceptor density [23]. Also Mühlhäuser *et al.* in a study on neonatal rat cardiac myocytes treated with atorvastatin showed that as a result of reduced geranylgeranylation of G-protein, γ subunit (Gγ) statins might desensitize cardiac myocytes to β-adrenergic stimulation [15]. Statins, by inhibiting HMG-CoA reductase, reduce the production of important isoprenoids such as farnesyl pyrophosphate (FPP) and geranylgeranyl pyrophosphate (GGPP). Isoprenylation is involved in the functioning of numerous signaling molecules, including the monomeric GTPases of the Rho and Ras families. Isoprenylation of Gγ has been found to be essential for membrane attachment of Gγ, as well as Gβ [24]. Statins, by interfering with Gγ isoprenylation, might influence the membrane association and heterotrimeric G-proteins activity [23, 25, 26]. We suggest that the different effect on heart rate after concomitant administration of metoprolol with statin as compared to results from combined statin and ivabradine therapy could depend on blood concentration of HMG-CoA reductase inhibitors or on duration of experiments. Although concomitant administration of simvastatin with ivabradine seems to intensify slowing of heart rate, no impact on systolic, diastolic or mean blood pressure was observed. These observations are comparable with the previous studies [7]. The results of previous large clinical trials have not definitely proved the anti-hypertensive activity of statins. The authors of the UCSD Statin Study and the Anglo-Scandinavian Cardiac Outcomes Trial-Lipid Lowering Arm (ASCOT-LLA) suggest that HMG-CoA reductase inhibitors may have some blood-pressure-lowering properties in addition to their effect on lipids [27, 28]. However, in the CARE study no significant reduction of blood pressure with statin therapy was observed [29]. The anti-hypertensive activity of HMG-CoA inhibitors might be the result of their influence on endothelial function, and decrease of oxidative stress and inflammation [10, 30]. The possible mechanisms of statin BP-lowering effects might result from e.g. increased endothelial production of NO with up-regulation of endothelial NO synthase expression [31, 32], decreased level of vasoconstrictor endothelin-1 [33], reduced production of reactive oxygen species (e.g. superoxide anion and hydroxyl radicals) [34] or down-regulation of the angiotensin II – type 1 receptor with reduced vasoconstrictor response to angiotensin II infusion [35]. Moreover, statins could improve systemic arterial compliance by reducing large artery stiffness and blood pressure in normolipidemic patients with isolated systolic hypertension [36, 37]. Although *in vitro* studies suggested a possible lowering effect of statins on blood pressure, *in vivo* observations have provided inconsistent results.

In conclusion, heart rate is an important predictor of all-cause and cardiovascular mortality in both subjects with and those without left ventricular dysfunction. Concomitant administration of simvastatin, well established in primary and secondary prevention of cardiovascular events, with ivabradine, a novel, alternative drug for patients with stable coronary artery disease with left ventricular systolic dysfunction, intensifies slowing of heart rate. No impact on blood pressure in normo- and hypercholesterolemic rats was observed, however. A similar simvastatin-induced intensification of heart rate deceleration during metoprolol therapy was not observed. However, no results of interaction between simvastatin and metoprolol in blood pressure changes were observed. We suggest that the possible explanation for such interaction between statins and ivabradine might be linked to a metabolic pathway. Further studies are required to confirm such interaction considering its clinical importance and safety with the possibility of changes in dosage regimen in patients with cardiovascular diseases receiving statin with ivabradine.

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