



Influence of simvastatin at high dose and nifedipine on hemodynamic parameters in rabbits

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Abstract:

Recent findings *in vitro* have shown that statins could reduce cardiomyocyte viability. The correlation between statin cardiotoxicity, assessed *in vitro*, and cardiac efficiency, investigated *in vivo* has not been estimated so far.

The aim of the present experiment was to establish the impact of high-dose simvastatin on the hemodynamic parameters, especially on myocardium efficiency, after continuous infusion of dopamine. Moreover, hemodynamic interaction between simvastatin and nifedipine, metabolized by the same isoenzyme CYP3A4 was examined. The experiments were performed on twenty seven New Zealand white rabbits. The animals were divided into four groups receiving: 0.2% methylcellulose (MC) (control group), nifedipine, simvastatin or simvastatin + nifedipine, for 14 days (*po*). The following hemodynamic parameters were estimated: cardiac output index (COI), heart rate (HR), systolic blood pressure (SBP), mean blood pressure (MBP), diastolic blood pressure (DBP) and total peripheral resistance index (TPRI). The registration of hemodynamic parameters was performed by Doppler method (Hugo Sachs Electronic Haemodyn). Dopamine did not cause a statistically significant increase in COI in rabbits receiving simvastatin alone or concomitantly with nifedipine. Nifedipine significantly lowered COI, BP and HR in rabbits if given simultaneously with simvastatin.

In conclusion, administration of nifedipine may elicit detrimental impact on statin therapy, resulting in the worsening of cardiac performance. This may suggest another mechanism of drug-drug interaction than the one based on CYP3A4 inhibition.

Key words:

simvastatin, nifedipine, hemodynamic parameters, rabbits

Introduction

3-Hydroxy-3-methyl-glytaryl coenzyme A (HMG-CoA) reductase inhibitors (“statins”, HMGRI) have proved to be extremely useful drugs in the management of hypercholesterolemia, especially at elevated concentrations of low-density lipoprotein cholesterol (LDL-C). Recent studies showed the efficacy of HMGRI in the primary [7, 59] and secondary [51, 54, 55] prevention of ischemic heart disease. Furthermore, other studies indicated that statins due to their neuro-

protective effects might reduce the risk of acute cerebral ischemia [63].

Statins are well tolerated by most patients, but can produce a variety of muscle-related symptoms, with myopathy and rhabdomyolysis, accounting for 2 to 5% of all musculoskeletal effects [64]. The withdrawal of cerivastatin from the world market due to 100 rhabdomyolysis-related deaths [12], as well as the increased use of statins in clinical practice, have focused attention on their myotoxicity and safety profile.

The exact mechanism of statin myotoxicity still remains unclear. It is known that the risk of statin-

induced myopathy rises with plasma drug concentration [32, 43]. Thus, it may increase significantly, when HMGRIs, metabolized by the CYP3A4 isoenzyme, are prescribed concomitantly with other drugs inhibiting their metabolism *via* CYP3A4 pathway. The above-mentioned interactions were shown for macrolides, antifungal and immunosuppressive drugs, and recently for mibefradil [57] as well as diltiazem [15].

Recent findings *in vitro* have shown that statins can also damage myocardium. It has been demonstrated that HMGRIs produced a dose-dependent reduction of cardiomyocyte viability [26, 52] with oncotic and apoptotic cell death [8, 53], indicating the possibility that a part of the mortality caused by rhabdomyolysis might be due to cardiac toxicity of statins. However, the correlation between possible statin cardiotoxicity, assessed *in vitro*, and cardiac efficiency, investigated *in vivo* has not been estimated so far.

Simvastatin is a lipophilic statin, metabolized by CYP3A4 isoenzyme. Its high cytotoxicity targeting skeletal muscles has been confirmed in *in vitro* studies [14, 31, 35, 48], as well as in clinical trials indicating the highest percentage (36%) of total cases of statin-associated rhabdomyolysis (The FDA Adverse Event Reporting System, 1997–2000). Moreover, recent studies have described the induction of apoptosis in cultured rat cardiomyocytes after simvastatin administration [8].

Hyperlipidemia is the major risk factor for cardiovascular events, including atherosclerosis, and it often co-exists with hypertension. Calcium channel blockers (CCBs) possess established antihypertensive effects. They are especially indicated in the treatment of patients with coronary heart disease secondary to hypertension and hyperlipidemia, due to their antiatherosclerotic properties [3, 64, 74]. Besides, the supported clinical antiatherosclerotic advantages of combination of HMGRIs and CCBs [10, 22], include long-acting nifedipine. Moreover, nifedipine is metabolized by CYP3A4 isoenzyme of cytochrome CYP-450 [28]. This may contribute to the risk of interactions between simvastatin and other drugs, if given simultaneously (i.e. in patients with co-existing hypercholesterolemia, hypertension or ischemic heart disease).

Taking into consideration the still unresolved problem of statin cardiac side effects as well as clinically important interactions resulting from co-administration of CCBs and HMGRIs, we performed the hemodynamic estimation of cardiac function, especially its efficiency. The cardiac output under continuous infu-

sion of positive inotropic agent, dopamine was assessed as a function of myocardium efficiency. Dopamine is a catecholaminergic drug, which at 2–10 $\mu\text{g}/\text{min}/\text{kg}$ improves myocardial contractility and enhances impulse conduction *via* stimulation of both α - and β -adrenoreceptors [66]. The studies on the hemodynamic response to dopamine has been studied in cardiac diseases (i.e. myocardial ischemia, chronic heart failure) [23, 67].

The aim of the present experiments was to establish the influence of high-dose simvastatin administered alone or concomitantly with nifedipine on the hemodynamic parameters in rabbits.

Materials and Methods

Drugs: Simvastatin (Polfarmex, Poland series no. KY-SI-M20030102), nifedipine (Cordafen, Polpharma, Poland, series no.10403), dopamine (Dopaminum hydrochloricum 1%, Polfa Warszawa, Poland, series no. 01AV0203), α -chloralose (Sigma, USA, series no. 120K2505), urethane (Sigma, USA, series no. 022K1248), lidocaine (Lidocaini hydrochloricum 2%, Polfa Warszawa, Poland, series no. 14BT0403), methylcellulose (Fluka, Switzerland, series no RB 13425), Natrium chloratum 0.9% inj. (Polfa Lublin, Poland, series no 10602).

The experiments were performed on twenty seven outbred New Zealand white rabbits of both sexes and body weight of 2.5–5 kg, fed on granulated mix “LSK” with free access to water. The animals were housed in standard cages, one animal per cage. The experimental procedures were carried out in accordance with international guidelines for the care and use of laboratory animals. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments. All the procedures in these experiments were approved by the Ethics Committee of the Medical University of Łódź (Poland).

The animals were randomly allocated to four groups of rabbits:

Group I: 0.2% methylcellulose (MC), 1 ml/kg, *po* daily for 14 days (n = 9);

Group II: Nifedipine 1 mg/kg, *po* daily for 14 days (n = 6);

Group III: Simvastatin 50 mg/kg, *po* daily for 14 days (n = 6);

Group IV: Simvastatin 50 mg/kg + nifedipine 1 mg/kg, *po* daily for 14 days (n = 6).

All drugs at doses used in the previous experiments in rabbits [13, 35, 73] were administered by oral gavage, suspended in 0.2% MC.

The surgery was performed 24 h after the administration of the last drug dose. The rabbits were placed in a dorsal position on the operation table. The animals were anesthetized with α -chloralose (75 mg/kg) and urethane (500 mg/kg) [27] administered into the marginal ear vein. Anesthesia was maintained by additional bolus doses of urethane as needed. Lidocaine (5 mg/kg) [19] was used for local infiltration of the surgical sites. During the experimental procedures the thoracic cavity was opened, for further aortic flow measurement from the ascending aorta. Before thoracotomy, the trachea was intubated. Ventilator frequency was set at 30 bpm and tidal volume at 10 ml/kg.

Dopamine, dissolved in 0.9% NaCl, was infused into marginal ear vein, using a continuous infusion pump (sp100i syringe pump, WPI, England), at 10 μ g/kg/min. The infusion was started after the stabilization period.

The registration of hemodynamic parameters in rabbits was performed by using Hugo Sachs Elektronik Haemodyn (Harvard Apparatus GmbH, March, Germany).

For the measurement of systolic, mean and diastolic arterial blood pressure (SBP, MBP, DBP), a heparinized polyethylene catheter was placed into the dissected carotid artery and connected to an Isotec pressure transducer (HSE Harvard Apparatus, Germany).

After median sternotomy and pericardiotomy, an ultrasonic flow probe was placed around the ascending aorta in order to measure aortic blood flow (AF_{max} , AF_{mean} , AF_{min}). It was connected to an ultrasonic flow meter Transit Time Flowmeter TTFM Type 700 (HSE Harvard Apparatus and Transonic System Inc. USA). The AF_{mean} values were taken as an index of cardiac output. The heart rate was registered from the catheter placed in the carotid artery. After surgery the animals were killed by exsanguination while ventilation was continued.

All analog signals were amplified and recorded on a computer *via* an A/D converter (HSE Haemodyn software for Microsoft Windows 95/98/NT) and they were evaluated according to the algorithms. For further statistical analysis, output values of the hemodynamic parameters were calculated as the mean from 3-min periods.

The following derivative hemodynamic parameters were calculated: cardiac output index (COI) and total peripheral resistance index (TPRI).

– COI = CO/BW (ml/min/kg), where: CO – cardiac output (ml/min), BW – body weight (kg);

– TPRI = TPR/BW (mmHg min/ml/kg), where: TPR = MAP/CO (mmHg min/ml), MAP – mean arterial pressure (mmHg).

Statistics

The statistical analysis of hemodynamic parameters was performed using the Statistica version 5.0 Statsoft program. The statistical evaluation was performed using analysis of variance (ANOVA) and *post-hoc* comparisons were performed using Duncan test. Normal distribution of a parameter was checked by means of Kolmogorov-Smirnov test with Lillieforce correction. The homogeneity of variance was tested by Levene's 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 $p < 0.05$.

Results

Cardiac output index (Fig. 1)

In the control group, dopamine infusion caused a statistically significant increase in COI, observed from 15th min to the end of the experiment. In rabbits receiving nifedipine, a significant increase in COI was observed from 9th min to the end of the experiment. Administration of simvastatin alone or concomitantly with nifedipine did not result in the statistically significant increase in COI during continuous infusion of dopamine.

The initial values of COI observed in rabbits receiving simultaneously simvastatin and nifedipine were lower compared to simvastatin and control group. However, the initial values of COI noted in rabbits receiving simvastatin did not differ significantly from the respective values of COI noted in the control group.

The combined administration of simvastatin with nifedipine caused a marked decrease in cardiac output

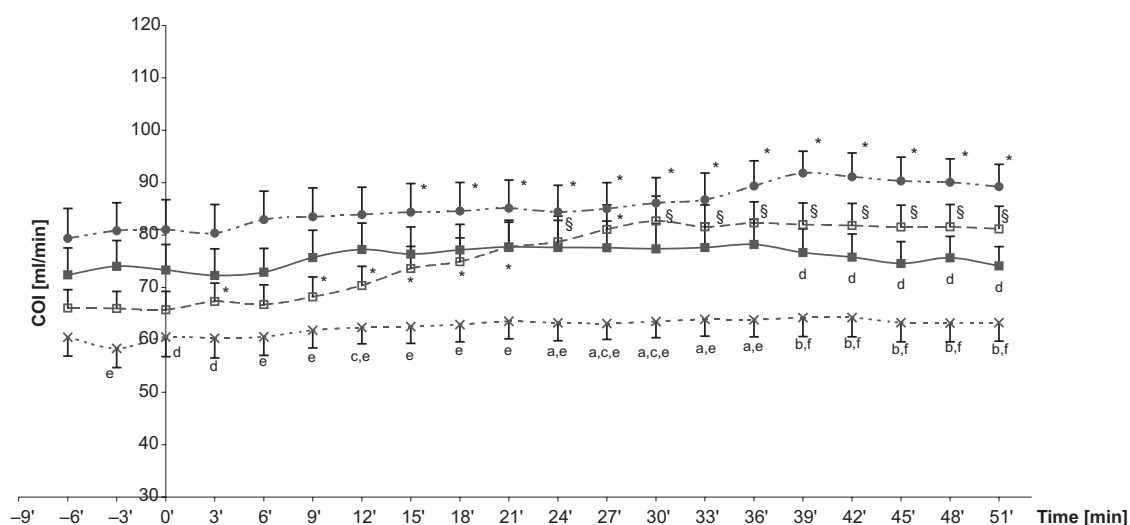


Fig. 1. The influence of nifedipine (---□---), simvastatin (—■—), nifedipine + simvastatin (---×---), in comparison to the control group (—●—), on cardiac output index (COI) in rabbits, during continuous infusion of dopamine, given simultaneously. Each value represents the mean \pm SEM. (a) $p < 0.05$, (b) $p < 0.01$ in comparison to nifedipine alone, (c) $p < 0.05$ in comparison to simvastatin alone, (d) $p < 0.05$, (e) $p < 0.01$, (f) $p < 0.001$ in comparison to the control group. * $p < 0.05$, § $p < 0.0005$ in comparison to the initial values (0'). 0' – the initiation of dopamine infusion

index, observed from 12th min of dopamine infusion as compared to simvastatin alone. Also, in rabbits receiving simvastatin with nifedipine, the reduction of COI, in comparison with nifedipine alone was observed. A statistically significant decrease in COI was noted from 24th min of dopamine infusion. The combined administration of simvastatin with nifedipine caused a significant decrease in COI as compared to

the control group, observed both before and during dopamine infusion (Fig. 1).

Blood pressure (Fig. 2, Fig. 3, Fig. 4)

Dopamine infusion did not influence systolic blood pressure values in the examined groups of rabbits. The values of SBP, observed after dopamine infusion

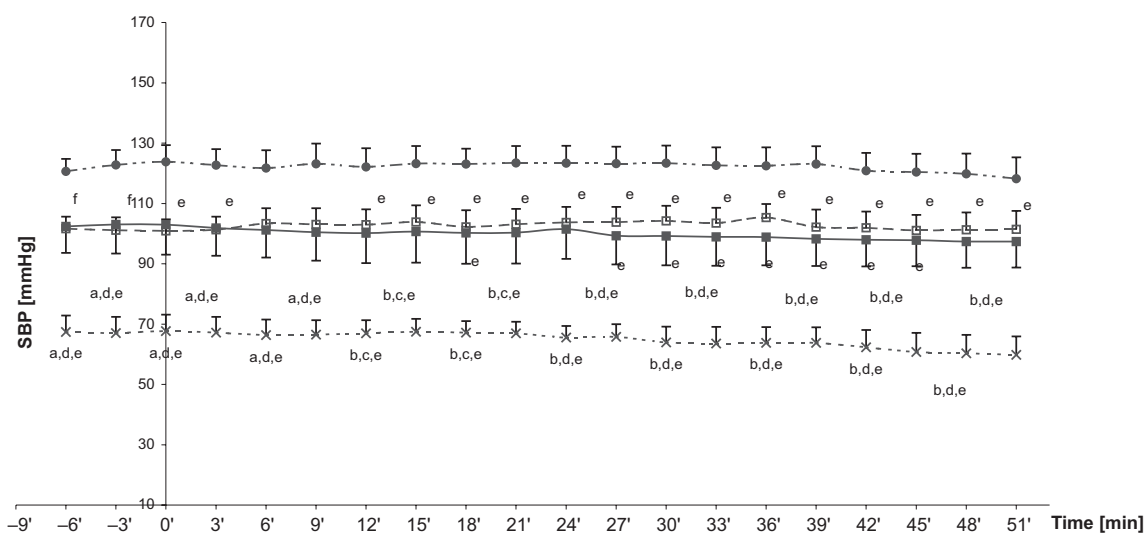


Fig. 2. The influence of nifedipine (---□---), simvastatin (—■—), nifedipine + simvastatin (---×---), in comparison to the control group (—●—), on systolic blood pressure (SBP) in rabbits, during continuous infusion of dopamine, given simultaneously. Each value represents the mean \pm SEM. (a) $p < 0.005$, (b) $p < 0.001$ in comparison to nifedipine alone, (c) $p < 0.005$, (d) $p < 0.001$ in comparison to simvastatin alone, (e) $p < 0.05$, (f) $p < 0.0001$ in comparison to the control group. 0' – the initiation of dopamine infusion

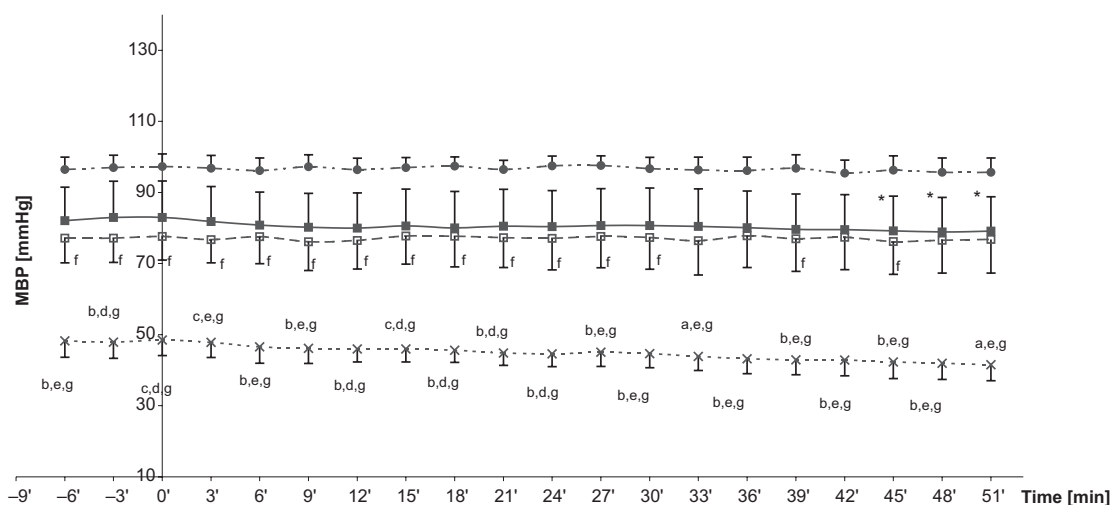


Fig. 3. The influence of nifedipine (---□---), simvastatin (---■---), nifedipine + simvastatin (---×---), in comparison to control group (---●---), on mean blood pressure (MBP) in rabbits, during continuous infusion of dopamine, given simultaneously. Each value represents the mean ± SEM. (a) $p < 0.05$, (b) $p < 0.01$, (c) $p < 0.005$ in comparison to nifedipine alone, (d) $p < 0.05$, (e) $p < 0.01$ in comparison to simvastatin alone, (f) $p < 0.05$, (g) $p < 0.0001$ in comparison to the control group, * $p < 0.05$ in comparison to the initial values (0'). 0' – the initiation of dopamine infusion

had started, did not differ significantly from the initial values.

The values of SBP in rabbits receiving simvastatin, were significantly lower as compared to the control group. Also, the values of SBP observed in rabbits receiving nifedipine alone or with simvastatin, were significantly lower as compared to the control group. The combined administration of simvastatin and nifedipine caused a marked decrease in the values of the

systolic blood pressure as compared to the simvastatin group, both before and during dopamine infusion. Also in rabbits receiving simvastatin simultaneously with nifedipine, a marked decrease in the systolic blood pressure as compared to nifedipine alone was observed (Fig. 2).

In the control group as well as in rabbits receiving nifedipine alone or simultaneously with simvastatin, dopamine did not cause any changes in MBP values.

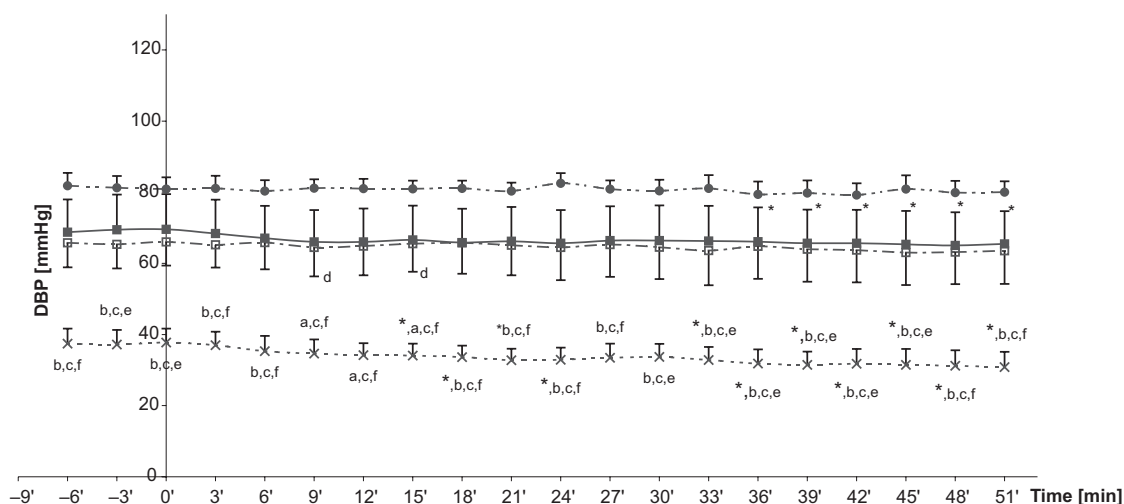


Fig. 4. The influence of nifedipine (---□---), simvastatin (---■---), nifedipine + simvastatin (---×---), in comparison to control group (---●---), on diastolic blood pressure (DBP) in rabbits, during continuous infusion of dopamine, given simultaneously. Each value represents the mean ± SEM. (a) $p < 0.01$, (b) $p < 0.005$ in comparison to nifedipine alone, (c) $p < 0.005$ in comparison to simvastatin alone, (d) $p < 0.05$, (e) $p < 0.0005$, (f) $p < 0.0001$ in comparison to the control group, * $p < 0.05$ in comparison to the initial values (0'). 0' – the initiation of dopamine infusion

No influence of dopamine infusion on the observed MBP values, except for the last 9 min of the experiment, was noted in the group receiving simvastatin alone.

The values of MBP observed in rabbits receiving nifedipine alone or with simvastatin, were significantly lower as compared to the control group both before and during dopamine infusion. The simultaneous administration of simvastatin and nifedipine caused a marked decrease in MBP values, as compared to groups receiving simvastatin alone, as well as to rabbits receiving nifedipine alone (Fig. 3).

An insignificant influence of dopamine on DBP values was observed, except for the last 18 min in rabbits receiving simvastatin alone and except for the last 39 min of the experiment in rabbits receiving simvastatin with nifedipine.

The combined administration of simvastatin with nifedipine resulted in the diastolic blood pressure reduction as compared to simvastatin or nifedipine alone and control group. A statistically significant decrease in DBP values, as compared to nifedipine alone, was noted during almost whole duration of the experiment. DBP in rabbits receiving simvastatin with nifedipine was markedly reduced as compared to simvastatin alone (Fig. 4).

Heart rate (Fig. 5)

The administration of dopamine caused a marked reduction of heart rate in groups receiving simvastatin

alone, observed from 6th min of dopamine infusion to the end of the experiment. In rabbits receiving simvastatin with nifedipine, dopamine slowed significantly rabbits' heart rate beginning from 24th min of its administration to the end of the experiment.

No statistically significant influence of dopamine on the HR values was observed in the group receiving nifedipine alone and in the control group.

The combined administration of nifedipine and simvastatin slowed HR, as compared to rabbits receiving nifedipine alone. These changes were statistically significant, beginning from 9th min of dopamine infusion. In rabbits receiving simvastatin alone statistically significant decrease in HR was observed beginning from 3rd min of dopamine infusion, as compared to the control group. The combined administration of simvastatin and nifedipine slowed HR significantly, as compared to the control group. These changes were observed both before and during dopamine infusion.

The HR values in rabbits receiving simvastatin alone were insignificantly different from the values observed in the group receiving simultaneously simvastatin and nifedipine.

Total peripheral resistance index (Fig. 6)

The infusion of dopamine caused a reduction of TPRI in the examined groups. A significant decrease in TPRI was observed after 21st min of dopamine in-

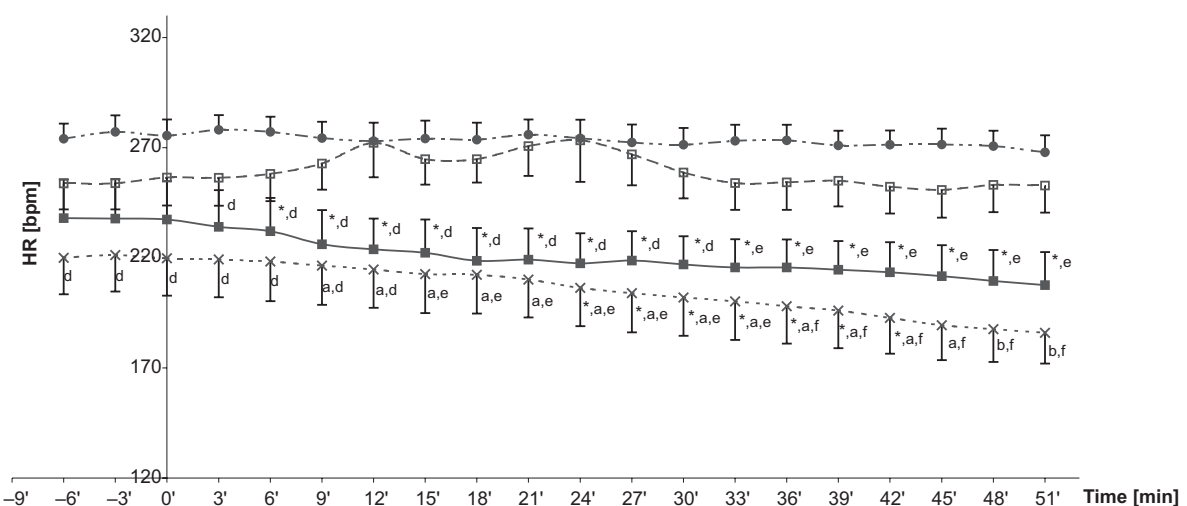


Fig. 5. The influence of nifedipine (---□---), simvastatin (—■—), nifedipine + simvastatin (—×—), in comparison to control group (—●—), on heart rate (HR) in rabbits, during continuous infusion of dopamine, given simultaneously. Each value represents the mean \pm SEM. (a) $p < 0.05$, (b) $p < 0.01$, (c) $p < 0.005$ in comparison to nifedipine alone, (d) $p < 0.05$, (e) $p < 0.005$, (f) $p < 0.0005$ in comparison to the control group* $p < 0.05$ in comparison to the initial values (0). 0' - the initiation of dopamine infusion

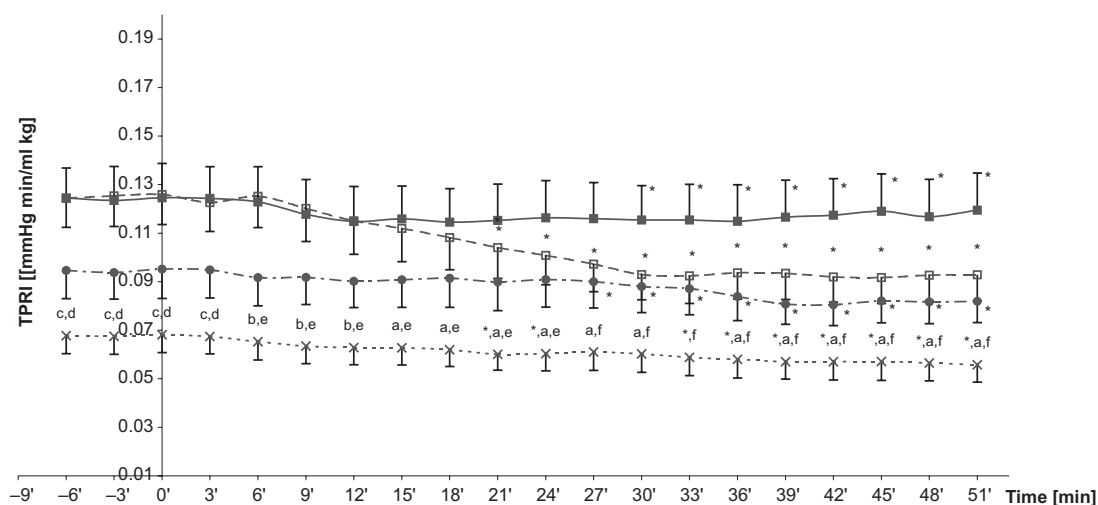


Fig. 6. The influence of nifedipine (---□---), simvastatin (—■—), nifedipine + simvastatin (---×---), in comparison to control group (—●—) on total peripheral resistance index (TPRI) in rabbits, during continuous infusion of dopamine, given simultaneously. Each value represents the mean ± SEM. (a) $p < 0.05$, (b) $p < 0.01$, (c) $p < 0.005$ in comparison to nifedipine alone, (d) $p < 0.05$, (e) $p < 0.01$, (f) $p < 0.005$ in comparison to simvastatin alone, * $p < 0.05$ in comparison to the initial values (0'). 0' – the initiation of dopamine infusion

fusion to rabbits that received nifedipine alone or with simvastatin. In group administered simvastatin alone, a marked reduction of TPRI was noted after 30th min of dopamine administration. In the control group, a significant decrease in TPRI was noted after 27th min of dopamine administration.

Simultaneous administration of simvastatin and nifedipine resulted in reduction of TPRI values as compared to simvastatin. Statistically significant changes were observed both before and after dopamine infusion, during whole experimental period.

The combined administration of simvastatin and nifedipine resulted in a reduction of TPRI values as compared to nifedipine.

Discussion

The aim of the present study was to establish the influence of high dose of simvastatin on the cardiac efficiency and to evaluate hemodynamic interaction with nifedipine. In the present study, in rabbits receiving vehicle or nifedipine, the continuous infusion of dopamine at 10 $\mu\text{g}/\text{min}/\text{kg}$, caused a significant increase in the cardiac output index. However, similar response to positive inotropic activity of dopamine was not observed after simvastatin alone or given with nifedipine (Fig. 1).

Only a few reports describing the HMGRI influence on the hemodynamic function have been found. De Lorgeril et al. [4] observed loss of myocardial reserve in patients treated with simvastatin. Studies performed on the animal ischemia-reperfusion model showed the worsened recovery of myocardial contraction [20] and enhanced myocardial stunning [56] resulting from statin therapy. On the other hand, Marz et al. [30] showed that lovastatin at a dose of 10 mg/kg significantly increased the mortality of cardiomyopathic hamsters. Also Folkers et al. [11] described the cases of five patients with dilated or ischemic cardiomyopathy that revealed increased cardiac disease after lovastatin therapy. The authors suggested that this might be associated with both CoQ10 and ATP reduction and detrimental impact of the drug on energy production in heart muscle. It is worth noting that especially diastolic LV performance is ATP-dependent, thus ATP reduction, after HMGRI administration, may suggest the relationship between statin therapy and myocardial diastolic dysfunction, with normal systolic ejection fraction [34]. Unfortunately, we were not able to examine the influence of simvastatin on systolic and diastolic LV performance, since we analyzed aortic flow as an indirect indicator of myocardium contractility.

Myocardial diastolic dysfunction may result from calcium overload [75]. Nakahara et al. [37] revealed that simvastatin and simvastatin acid elevated $[\text{Ca}^{2+}]$ in L6 rat myoblasts. The authors suggested that it

might lead to skeletal muscle cell damage and myopathy. Similarly, intracellular $[Ca^{2+}]$ increase in cardiomyocytes was reported, as well [2]. Several agents such as doxorubicin [1, 24] and halothane [25] have been reported to cause skeletal or cardiac muscle cytotoxicity, from intracellular calcium overload. Chronic $[Ca^{2+}]$ elevation may contribute to the induction of apoptosis due to activation of cellular proteases (caspases) [17]. It has been demonstrated that HMGRIs may induce cardiac toxicity *via* activation of proapoptotic factors: caspase-2 and caspase-3 [39]. Caspase-3 activation was demonstrated in myocardial ischemic injury and cardiomyopathy [39, 71]. However, the exact relationship between $[Ca^{2+}]$ elevation and caspase activation has not been elucidated.

Nifedipine significantly worsened the cardiac function if given simultaneously with simvastatin, as compared to rabbits receiving simvastatin or nifedipine alone, suggesting that nifedipine may have detrimental impact on statin therapy (Fig. 1). Nifedipine is metabolized by isoenzyme CYP3A4. However, contrary to other CCBs (diltiazem, verapamil), it does not inhibit this isoenzyme, which could result in elevation of simvastatin level. On the other hand, nifedipine, by inhibition of smooth muscle L-type calcium channels, decreases mainly intracellular calcium concentration in smooth muscle cell, having less impact on those of the heart [44, 58]. The hypothesis about detrimental impact of the elevated $[Ca^{2+}]$ on cardiomyocytes and development of diastolic dysfunction resulting from HMGRIs administration, may explain why nifedipine given simultaneously with simvastatin did not improve myocardial efficiency, as compared to simvastatin alone. However, the mechanism explaining further worsening of cardiac performance is, therefore, questionable.

In the present experiment, nifedipine caused significant reduction of MBP and SBP, as compared to control group (Fig. 2, Fig. 3). This confirms its antihypertensive properties in the examined rabbit model and results from both, the direct relaxing efficacy associated with inhibition of the smooth muscle L-type calcium channel, as well as the indirect relaxing effect related to NO release from vascular epithelium [5]. A marked reduction of SBP, MBP and DBP values (Fig. 2, Fig. 3, Fig. 4), after simvastatin and nifedipine as compared to simvastatin alone, results from vasodilatory activity of nifedipine. The significant decrease in BP values as compared to rabbits receiving nifedipine alone, may suggest the hypotensive efficacy of

HMGRIs. Only one report on the interaction between nifedipine and HMGRIs has been found. Eliot et al. [9] showed in the hyperlipidemic rat model that atorvastatin normalized the lowering effect of nifedipine on BP. It was strictly associated with hyperlipidemic state, potentiating the hypotensive effect of nifedipine by increasing its total plasma concentrations. In our experiments, normolipidemic rabbits were examined, thus a similar mechanism should be excluded. Instead, the additive effect of nifedipine and simvastatin on BP values can be hypothesized. Previous studies performed on hypertensive and (or) hyperlipidemic animal models demonstrated beneficial effects of HMGRIs on hypertension [21, 42, 68, 69], owing to its ability to increase NO production and to decrease AT1 expression [40, 70]. It seems that similar mechanisms might be responsible for the hemodynamic changes, observed in normotensive and normolipidemic rabbits, considering high doses of simvastatin used in our experiment. Moreover, it is worth noting that simvastatin administration caused statistically significant decrease in SBP values as compared to control group (Fig. 2).

Another mechanism, i.e. pharmacokinetic drug-drug interaction could also be considered. Metabolism of nifedipine may be suppressed by simvastatin, as the two drugs compete with each other for CYP3A4. Marumo et al. [29] revealed that pretreatment with simvastatin and atorvastatin markedly enhanced the hypotensive effect of diltiazem in normotensive rats. In contrast, hydrophilic pravastatin, that is not metabolized *via* CYP450 pathway, did not produce similar effect, which also confirms the impact of such drug-drug interaction. Unfortunately, due to lack of suitable equipment, in the present experiment we were not able to measure the serum concentration of nifedipine, to confirm the hypothesis of pharmacokinetic drug-drug interaction at the level of isoenzyme CYP3A4. No reports pertaining to the influence of simvastatin on nifedipine serum concentration have been found, either.

The above mechanisms of the potentiated hypotensive efficacy of nifedipine, if given with simvastatin, may explain the significant decrease in TPRI values observed in rabbits receiving nifedipine in combination with simvastatin (Fig. 6).

It is worth noting that previous findings have confirmed the functional similarity of intestinal CYP3A forms in rabbits and humans, suggesting that the rabbit is a valuable *in vivo* model for the assessment of

drug interaction occurring at the first pass of drugs ingested [38]. Moreover, studies performed on rabbits, evaluating the pharmacokinetics of other drugs metabolized in humans *via* CYP3A4 pathway (i.e. diltiazem, saquinavir) [47, 60, 72], have confirmed the usefulness of the rabbit model for such investigations.

Additionally, dopamine caused further marked reduction of TPRI in the examined groups of rabbits. Systemic and peripheral vascular resistance reduction is another hemodynamic change resulting from dopamine administration, as a consequence of D1 and D2 receptor stimulation [76].

In our study, simvastatin slowed the rabbits' heart rate significantly, as compared to the control group (Fig. 5). The latest findings revealed that HMGRIs slowed HR, *via* enhancing NO synthesis and lowering the sympathetic outflow [6, 33]. Moreover, other findings imply the statin-related reduction of AT1 receptor expression [40, 45]. The above studies were performed on the hypercholesterolemic [16, 40] or CHF model [49, 50] both characterized by significant AT1 overexpression, resulting in the enhanced vasoconstriction and sympathetic activation [18]. Our experiments were performed on normolipidemic rabbits without CHF. It is worth noting that heart rate reduction observed in CHF rabbits was dose-dependent [49]. However, similar studies relating sympathetic activity to statin therapy in healthy subjects have not been found in literature.

HR of rabbits receiving simvastatin with nifedipine was markedly decreased, as compared to the control group or nifedipine alone (Fig. 5). This seems to confirm the relationship between HR reduction and simvastatin rather than nifedipine administration, especially as no significant changes were detected after chronic administration of nifedipine alone. The latter was associated with the process of adaptation of baroreceptor reflex mechanisms to the prevailing level of blood pressure [61, 62], following the chronic nifedipine administration [41].

Interestingly enough, simvastatin administered alone or simultaneously with nifedipine slowed rabbits' HR significantly, also during continuous dopamine infusion (Fig. 5). Similarly, Patterson et al. [46], in the studies performed on hypercholesterolemic humans treated with atorvastatin, showed a gradual decrease in HR values, described as delta R-R intervals, simultaneously with increasing doses of another inotropic agent-noradrenaline, which confirms the role of HMGRIs in reflex bradycardia. Similarly to our find-

ings with dopamine infusion, no significant influence of noradrenaline on BP values has been observed, either (Fig. 2, Fig. 3, Fig. 4).

The primary aim of our experiments was to establish the influence of simvastatin at the dose provoking myopathy in the rabbit model, given alone or with nifedipine, on the cardiac output index, especially after inotropic agent administration. The changes in other hemodynamic parameters (BP, HR, TPRI) may throw some light on the complex impact of statin administration on the cardiovascular performance, with the variety of mechanisms, still remaining questionable.

The limitation of the extrapolation of our data to humans consists in the relatively high dose of simvastatin used in our experiments. However, the aim of the present study was to assess whether statins, at the dose provoking myopathy of skeletal muscles, influence myocardium efficiency, especially considering that the statin affinity for skeletal muscle in comparison to cardiac muscle has not been elucidated, yet. Thus, dose and dosage of simvastatin (50 mg/kg per day, 14 days) were established based on the previous experimental simvastatin-induced myopathy in rabbits [13, 36, 37].

The role of HMGRIs in the primary and secondary prevention of ischemic heart disease is well established. However, the mechanism of possibly the highest statin affinity for skeletal muscle in comparison to cardiac muscle has not been determined. In the performed study, high-dose simvastatin administration seems to possess negative impact on the cardiac performance. The worsening of myocardial function resulting from simultaneous nifedipine administration may suggest another mechanism of drug-drug interaction than that based on CYP3A4 inhibition. However, this issue requires further studies.

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