Simvastatin Decreased Coenzyme Q in the Left Ventricle and Skeletal Muscle but not in the Brain and Liver in L-NAME-Induced Hypertension

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Running title: Simvastatin decreased myocardial CoQ level in NO-deficient hypertension

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Summary

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (statins) have been proven to effectively reduce cholesterol level and morbidity and mortality in patients with coronary heart disease and also in primary prevention in patients with dyslipoproteinemia. Statins inhibit synthesis of mevalonate, a precursor of both cholesterol and coenzyme Q (CoQ). Inhibited biosynthesis of CoQ may be involved in some undesirable actions of statins. We investigated the effect of simvastatin on tissue CoQ concentrations in the rat model of NO-deficient hypertension induced by chronic L-NAME administration. Male Wistar rats were treated daily for 6 weeks with L-NAME (40 mg/kg) or with simvastatin (10 mg/kg), another group received simultaneously L-NAME and simvastatin in corresponding doses. Coenzyme Q$_9$ and Q$_{10}$ concentrations were analyzed by high performance liquid chromatography. L-NAME and simvastatin alone had no effect on CoQ concentrations. However, simultaneous application of L-NAME and simvastatin significantly decreased concentrations of both CoQ homologues in the left ventricle and slightly decreased CoQ$_9$ concentration in the skeletal muscle. No effect was observed on CoQ level in the liver and brain. We conclude that administration of simvastatin in the condition of NO-deficiency reduced the level of CoQ in the heart and skeletal muscle what may participate on adverse effect of statins under certain clinical conditions.

Key words: Simvastatin; NO-deficient hypertension; tissue coenzyme Q; L-NAME
Introduction

Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase - statins have been proven to effectively reduce cholesterol level and morbidity and mortality in patients with coronary heart disease and also in primary prevention in patients with dyslipoproteinemia without evident ischemic heart disease. Effects of statin therapy beyond their lipid lowering potential are called pleiotropic effects involving improvement of endothelial function, antioxidant and antiinflammatory properties, immunosuppressive activity and effect against pathological remodelling of the heart and vessels (Endres 2006, Harst et al. 2006, Simko 2007).

Pleiotropic effect, however, need not be only beneficial, but also neutral and even undesirable. Statins inhibit synthesis of mevalonate, a precursor of both cholesterol and coenzyme Q (CoQ). Indeed, inhibited biosynthesis of CoQ may be involved in some undesirable action of statins (Harst et al. 2006), such as liver injury, muscular weakness and even rhabdomyolysis (Bliznakov 2002). Coenzyme Q is an essential part of mitochondrial respiratory chain responsible for ATP production and statins could affect mitochondrial function and worsen the skeletal and cardiac muscle function (Mitchell 1991, De Pinieux et al. 1996, Crane 2001). A number of studies confirmed increased risk of coenzyme Q$_{10}$ deficiency in patients with heart failure and improvement the patient’s symptoms with CoQ$_{10}$ supplementation (Folkers 1993, Morisco et al. 1993, Gottlieb et al. 2000, Tousoulis et al. 2007). Moreover, coenzyme Q besides its bioenergetic function in mitochondria is acting as scavenger of free radicals in membranes and its deficit in tissues can contribute to the decreased antioxidant protection.
and worsened function of affected organs. Previously we found depletion of CoQ<sub>10</sub> in plasma and endomyocardial biopsies related to rejection episodes in transplanted heart patients (Kucharská et al. 1998), decreased mitochondrial concentrations of CoQ and increased lipoproteinperoxidation in the heart and liver of the rats with streptozotocin-induced diabetes (Kucharská et al. 2000), and reduced concentrations of both CoQ homologues in brain mitochondria of spontaneously hypertensive rats (Kucharská et al. 2005).

The aim of this study was to show the effect of simvastatin on tissue coenzyme Q concentrations in condition of NO-deficient hypertension induced by L-NAME administration.

**Material and Methods**

Male Wistar rats 15 weeks old were divided into four groups, each consisting of 8 rats: 1/ Control animals (C) 2/ L-NAME (L) was given in the dose 40 mg/kg/day in tap water for 6 weeks 3/ Simvastatin was given in the dose 10 mg/kg/day in tap water for 6 weeks (S) 4/ L-NAME (NG-nitro-L-arginine methyl ester) and simvastatin were administered simultaneously as in the groups 3 and 4 (LS). The animals were fed a standard pellet diet ad libitum. All experiments were carried out according to guidelines for the care and use of laboratory animals. The systolic blood pressure was measured by non-invasive tail-cuff plethysmography (Hugo Sachs Elektronik KG, Freiburg, Germany) in the sixth week. At the end of experiment, animals were sacrificed by decapitation, tissues samples 30-100 mg from myocardial left ventricle (LV), skeletal muscle, brain and liver were weighed and frozen until analyses. Concentrations of oxidized
forms of coenzyme Q₉ (CoQ₉) and Q₁₀ (CoQ₁₀) were determined by isocratic high-performance liquid chromatography (HPLC) according to Lang et al. (1986) with some modifications as follows. Tissues were homogenized in water with addition of t-butylhydroxytoluene (BHT) and sodium dodecylsulphate (SDS) and extracted twice by mixture of hexane/ethanol (5/2, v/v). All steps of sample preparation were carried out in the dark. Collected organic layers were evaporated under nitrogen, the residue was taken up in ethanol and injected on the column SGX C18 7μm (Tessek Ltd, Czech Republic). The mobile phase consisted of methanol/acetonitrile/ethanol (6/2/2, v/v/v). Concentrations of compounds were detected spectrophotometrically at 275 nm using external standards (Sigma, Germany). Data were collected and processed using CSW32 chromatographic station (DataApex Ltd, Czech Republic). The results were evaluated using Student’s t-test for unpaired data, values of p<0.05 were considered statistically significant.

Results

After six weeks of L-NAME administration systolic blood pressure (SBP) increased significantly in comparison with controls (182.4 ± 0.31 vs 123.8 ± 2.06 mm Hg, p<0.001). In the L-NAME + simvastatin (LS) group SBP decreased to 162.2 ± 1.46 mm Hg but was higher than in controls (p<0.001) (Fig. 1). L-NAME and simvastatin had no significant effects on coenzyme Q₉ and Q₁₀ concentration in the left ventricle, skeletal muscle, liver and brain tissues in comparison with control rats (Fig. 2, 3, 4, 5). However, the simultaneous application of L-NAME and simvastatin significantly decreased concentrations of
CoQ\textsubscript{9} and CoQ\textsubscript{10} in the left ventricle of myocardium when compared to controls (126.0 ± 11.7 vs 204.0 ± 18.6 nmol/g ww and 11.2 ± 1.73 vs 17.5 ± 1.88 nmol/g ww, respectively) (Fig. 2). In the skeletal muscle, CoQ\textsubscript{9} concentration decreased marginally significant in LS group 19.1 ± 1.47 nmol/g ww compared to controls (26.7 ± 3.12 nmol/g ww, p<0.069) (Fig. 3). The concentrations of CoQ were not changed in the liver and brain tissues in LS group (Fig. 4 and 5).

**Discussion**

We investigated the effect of simvastatin on coenzyme Q concentrations in different tissues in condition of NO-deficient hypertension induced by L-NAME administration. L-NAME and simvastatin alone had no effect on CoQ concentrations. However, simultaneous application of L-NAME and simvastatin significantly decreased concentrations of both CoQ homologues in the left ventricle and slightly decreased CoQ\textsubscript{9} concentration in the skeletal muscle. No effect was observed on CoQ levels in the kidney and brain.

A number of studies documented the effect of statins on coenzyme Q concentrations in animals. Lovastatin treatment during 4 weeks (400 mg/kg) decreased CoQ\textsubscript{9} and CoQ\textsubscript{10} concentrations in the blood, heart and liver of rats, and supplementation with CoQ\textsubscript{10} completely reversed these effects (Willis *et al.* 1990). Nakahara *et al.* (1998) reported decreased skeletal muscle ubiquinone content in rabbits treated with simvastatin (50 mg/kg) and pravastatin (100 mg/kg) for 4 weeks. However, mitochondrial activities were not affected.
Similar results were achieved with cerivastatin in rats. It was observed the elevation of plasma level of creatine kinase (a clinical marker of myopathy), histologically proved damaging of myofibrills and slightly decreased CoQ₉ in the skeletal muscle, yet without any changes in mitochondria (Schaefer et al. 2004). The dose 50 mg/kg of simvastatin used by Fukami et al. (1993) in rabbits did not affect skeletal muscle ubiquinone content, but severe lesions in skeletal muscles associated with abnormal elevation of creatine kinase and lactate dehydrogenase activities occurred. These authors found significantly reduced ubiquinone content in the liver and cardiac muscle. Others observed decreased content of ATP and creatine phosphate in the heart muscle of the rats treated with simvastatin for 30 days (Pisarenko et al. 2001) or myopathy and worsening of myocardial function in rabbits, when simvastatin was administered for 14 days (50 mg/kg) (Jasinska et al. 2006).

Although a number of studies demonstrated a significant decline in plasma coenzyme Q₁₀ level in patients treated with statins (Folkers et al. 1990, Watts et al. 1993, De Pinieux et al. 1996, Rundek et al. 2004), only few studies investigated effect of statins on tissue coenzyme Q in humans and with contradictive findings. Laaksonen et al. (1996) found no significant changes in muscle high-energy phosphates and CoQ₁₀ concentrations in muscle biopsies of hypercholesterolemic patients treated with simvastatin (20 mg/day) for 6 months and Lamperti et al. (2005) found only a mild-decrease in muscle CoQ₁₀ concentration in patients with statin-related myopathy. On the other hand, high-dose of simvastatin (80 mg/day) administered for 8 weeks decreased CoQ₁₀ concentration and respiratory chain enzyme activities in human skeletal muscle biopsies (Päivä et al. 2005). Moreover authors of these studies suggested that
serum and muscle ubiquinone are differently regulated and that serum ubiquinone levels can not be used as a marker for tissue ubiquinone level. In addition, lactic acidosis and higher blood lactate/pyruvate ration observed in patients treated with statins indicate an interference of statins with the mitochondrial respiratory chain (De Pinieux et al. 1996, Goli et al. 2002), leading potentially to energy depletion and oxidative stress, damaging muscles and other tissues (Parker et al. 2003, Al-Ruzzeh et al. 2004).

In our experiment, the sole use of simvastatin did not induce a significant change in CoQ concentration in the left ventricle, skeletal muscle, brain or kidney. It is generally known that statin induced myopathy is dose dependent. The dose of simvastatin was lower in our experiment (10 mg/kg/day), compared to 50 mg/kg/day used by Nakahara et al. (1998) or Fukami et al. (1993) in rabbits, what migh have determined the different effects of simvastatin on skeletal muscle observed in these experiments.

On the other hand, the simultaneous treatment with simvastatin and L-NAME decreased CoQ$_9$ and CoQ$_{10}$ in the LV in this experiment. Previously we have shown that in L-NAME-induced hypertension simvastatin partly prevented the decrease of nitric oxide synthase (NOS) activity in the brain and kidney induced by L-NAME, however, not in the LV (Simko et al. 2004). It may be supposed that decreased NOS activity in the LV with presumed reduction of NO synthesis and attenuated vasodilatative ability of coronary arteries may increase the oxidative stress in the LV. The free radicals production may even be supported by decreased CoQ concentration, having antioxidant and free radicals scavenging properties. Enhanced oxidative stress may have participated on the
development of the hypertrophy and fibrosis of the LV observed in our previous work despite chronic simvastatin treatment (Simko et al. 2004).

We conclude that administration of simvastatin in the condition of NO-deficiency reduced the level of coenzyme Q in the heart and skeletal muscle what may participate on adverse effect of statins under certain clinical condition.

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Figure captions

Figure 1  Effect of L-NAME, simvastatin and L-NAME + simvastatin on systolic blood pressure after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M., **p<0.001.

Figure 2  Effect of L-NAME, simvastatin and L-NAME + simvastatin on coenzyme Q_9 and Q_{10} concentrations in the myocardial left ventricle after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M., *p<0.05.

Figure 3  Effect of L-NAME, simvastatin and L-NAME + simvastatin on coenzyme Q_9 and Q_{10} concentrations in the skeletal muscle after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M., m.s. - marginally significant.

Figure 4  Effect of L-NAME, simvastatin and L-NAME + simvastatin on coenzyme Q_9 and Q_{10} concentrations in the liver after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M.

Figure 5  Effect of L-NAME, simvastatin and L-NAME + simvastatin on coenzyme Q_9 and Q_{10} concentrations in the brain after 6-week treatment. C - control, L - 40 mg/kg/day L-NAME, S - 10 mg/kg/day simvastatin, LS - L-NAME + simvastatin in corresponding doses. Data are means ± S.E.M.
Figure 1

Systolic blood pressure

mm Hg

Control  L-NAME  Simvastatin  L-NAME+Simvastatin

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Figure 2

Left ventricle

CoQ<sub>9</sub>  
CoQ<sub>10</sub>

Control  L-NAME  Simvastatin  L-NAME+Simvastatin
Figure 3

Skeletal muscle

![Graph showing CoQ<sub>9</sub> and CoQ<sub>10</sub> levels in skeletal muscle samples treated with different conditions: Control, L-NAME, Simvastatin, and L-NAME+Simvastatin. The x-axis represents the conditions, and the y-axis represents the levels in mmol/g ww. The graph includes error bars.]
Figure 4

Liver

![Graph showing liver CoQ9 and CoQ10 levels with different treatments: Control, L-NAME, Simvastatin, and L-NAME+Simvastatin.](image)
Figure 5

Brain

CoQ₉

CoQ₁₀

nmol/g ww

C  L  S  LS  C  L  S  LS

Control  L-NAME  Simvastatin  L-NAME+Simvastatin