ORIGINAL ARTICLE

Effect of simvastatin and coenzyme Q10 on brain NO synthase activity in obese zucker rats

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Submitted: 2023-11-25 Accepted: 2023-12-02 Published online: 2023-12-03

Key words: **statins; coenzyme Q10; cholesterol; nitric oxide; brain cortex; cerebellum**

Act Nerv Super Rediviva 2023; **65**(3): 133–137 ANSR65323A05

Abstract

Simvastatin, 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor, is a lipophilic drug with mainly lipid-lowering effect. However, reduction of coenzyme Q10 (CoQ10) belongs among the adverse effects of simvastatin. We aimed to determine the effect of simvastatin and CoQ10 treatment on nitric oxide synthase (NOS) activity in the brain cortex and cerebellum of Zucker rats with metabolic syndrome. Twelve-week-old male obese Zucker rats were divided into the control group and groups treated with simvastatin (15 mg/kg/day), or CoQ10 (15 mg/kg/day), or with simvastatin and CoQ10 combination in the same doses. After 6 weeks, body weight and blood pressure were measured. NOS activity was determined by the formation of [3H]-L-citrulline from [3H]-L-arginine. None of the treatments was able to lower body weight or blood pressure of obese Zucker rats. Simvastatin did not significantly increase NOS activity in either the brain cortex or the cerebellum. However, CoQ10 increased total NOS activity significantly in both brain cortex and cerebellum. The combination of simvastatin and CoQ10 increased NOS activity to the level achieved after the CoQ10 treatment. In conclusion, CoQ10, an endogenous antioxidant, was able to increase NO generation in the brain with possible neuroprotective effect.

Introduction

Statins are well acknowledged drugs for their effectiveness in treating cardiometabolic diseases (Taylor *et al.* 2012, Dayar & Pechanova 2022). However, there is growing evidence that statins could also prevent neurodegenerative diseases, including Alzheimer's disease, or act as neuro-protectants in a number of neuropathological conditions (Rehakova *et al.* 2016, Kandiah & Feldman 2009; Barone *et al.* 2013). Since chronic neurodegenerative disorders have been linked to dysregulation of cholesterol homeostasis in the brain (Vance 2012), several authors have proposed using statins to treat neurodegenerative disease as an effective emerging therapy to either stop or delay the neurodegenerative process (Jick *et al.* 2000; Silva *et al.* 2013).

Statins directly inhibit the conversion of the hydroxyl-methyl-glutaryl-CoA (HMG-CoA) into L-mevalonate by inhibiting HMG-CoA reductase, which is the first step in the biosynthesis of cholesterol (Shitara & Sugiyama 2006). L-mevalovate is

the precursor of a number of different lipids such as farnesyl pyrophosphate (FPP) and geranyl pyrophosphate (GGPP) (Schonbeck & Libby 2004; Wood *et al.* 2010). Therefore, statin-induced neuroprotection in some cases has been proposed to be due to the reduction in FPP and/or GGPP levels (Cole & Vassar 2006; Li *et al.* 2012). In the central neuros system, HMG-CoA reductase is expressed with high transcription level in cortical, cholinergic and hippocampal neurons (Korade *et al.* 2007).

Moreover, statins have been shown to increase nitric oxide synthase (NOS) activity and NO generation through their impact on various signalling pathways (Gorabi *et al.* 2019). The cholesterol-dependent action of statins on NO production is reflected in the reduction of low-density lipoprotein (LDL) cholesterol and caveolin-1, a negative regulator of eNOS (Laufs 2003). Cholesterol-independent effects include eNOS upregulation (Kano *et al.* 1999, Cebova *et al.* 2018), increased eNOS phosphorylation, and inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase resulting in decreased reactive oxygen species (ROS) generation (Tong *et al.* 2018, Simko & Pechanova 2010).

Long-term statin treatment, however, may be associated with a number of adverse effects and a significant residual risk (Dayar & Pechanova 2022). One of the most important negative effects of statins is the reduction of endogenous antioxidant, coenzyme Q10 (CoQ10) synthesis. Deficiency of CoQ10 disrupts the metabolism of cellular energy and may lead to the development of myopathy and other muscular disorders. Conversely, in individuals with metabolic syndrome, CoQ10 supplementation improves glucose metabolism, triglycerides, total cholesterol, and LDL levels while reducing ROS generation (Potgieter *et al.* 2013, Zozina *et al.* 2018).

We aimed to determine the effect of simvastatin, CoQ10, and the combination of simvastatin and CoQ10 treatments on NOS activity in the brain cortex and cerebellum of Zucker rats with metabolic syndrome.

Materials and Methods

Chemicals

Most of the chemicals were obtained from Sigma-Aldrich (Saint-Louis, MO, USA); if not, the company is indicated. Simvastatin (99.7%) was isolated from the commercial drug Simvastatin-Ratiopharm 20 mg. CoQ10 was obtained from Tachyon Technology Pharm, Slovakia.

Animals and Treatment

All procedures and experimental protocols were approved by an Ethical committee of the Institute of Normal and Pathological Physiology Slovak Academy of Sciences (Ro-3601/17-221/3) according to the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purpose, Directive 2010/63/EU of the European Parliament.

Zucker rats were obtained from Charles River, USA and housed in groups of 2 animals, under a 12 h light - 12 h dark cycle, at a constant humidity (45–65%) and temperature (20–22 °C). Twelve-week-old male obese Zucker rats were divided into the control group and groups treated with simvastatin (15 mg/kg/day), or CoQ10 (15 mg/kg/day), or with simvastatin and CoQ10 combination in the same doses. Each group consists of 6 animals. Treatment was administered via gavage from the 12th week of age for 6 weeks. Daily water consumption was estimated individually for every animal and adjusted, if necessary.

Body weight and blood pressure were monitored weekly. Blood pressure was measured noninvasively, using tail-cuff plethysmography. At the end of the treatment, the animals were sacrificed, and samples of the brain cortex and cerebellum were used to determine NOS activity.

Total NOS activity determination

Total NOS activity was determined in crude homogenates of the brain cortex and cerebellum by measuring the formation of [3H]-L-citrulline from [3H]-L-arginine as previously described by Jendekova *et al.* (2006) with minor modifications (Paulis *et al.* 2009). Briefly, 50 µl of crude homogenate of the brain part (7.5 mg of wet tissue) was incubated in the presence of 50 mmol/l Tris/HCl, pH 7.4, containing 1 µmol/l [3H]-L-arginine (specific activity 5 GBq/mmol, approx. 100000 d.p.m.), 0.5 mg/ml calmodulin, 0.5 mmol/l β-NADPH, 250 µmol/l tetrahydrobiopterin, 4 µmol/l FAD, 4 µmol/l flavin mononucleotide and 1 mmol/l Ca 2+, in a total volume of 100 µl. After a 30-min incubation at 37 °C, the reaction was stopped (by adding 0.02 M Hepes containing 2 mM EDTA, 2 mM EGTA and 1 mM [3H]-L-citrulline), the samples were centrifuged, and supernatants were applied to 1-ml Dowex 50WX-8 columns (Na + form). [3H]-L-citrulline was eluted with 2 ml of water and radioactivity was determined by liquid scintillation counting. Total NO synthase activity was expressed as pkat/g of proteins.

Statistical analysis

The results are expressed as mean ± S.E.M. One-way analysis of variance and Bonferroni test were used for statistical analysis. Values were considered to differ significantly if the probability value was less than 0.05.

Results

Body weight and blood pressure

Body weight of control obese Zucker rats was 688 ± 24 g. Neither simvastatin nor CoQ10 were able to significantly reduce body weight of the animals (647 ± 16 and 667 ± 25, respectively). Similarly, combination of simv-

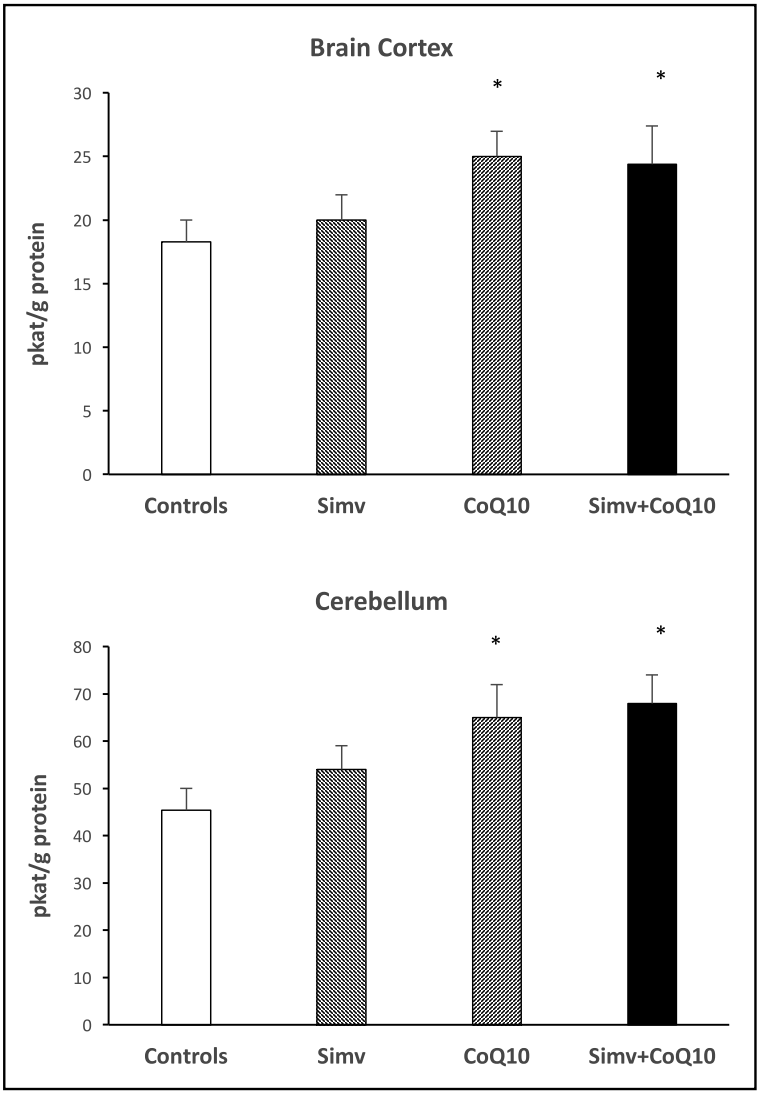

Fig. 1. Effect of simvastatin (Simv), coenzyme Q10 (CoQ10), and combination of simvastatin and CoQ10 on total nitric oxide synthase (NOS) activity in the brain cortex and cerebellum of obese Zucker rats. Data are means ± SEM from 6 animals in each group. * $p < 0.01$ compared to the control group.

astatin and CoQ10 did not decrease the body weight (641 ± 26).

At the end of experiment, blood pressure of control obese Zucker rats was 146 ± 4 mmHg. None of the treatments was able to lower blood pressure significantly (simvastatin: 139 ± 4 mmHg, CoQ10: 141 ± 5 mmHg, combination: 135 ± 7 mmHg).

Total NOS activity

Total NOS activity of control obese Zucker rats in the brain cortex was 18.3 ± 1.7 pkat/g protein and in the cerebellum 45.4 ± 4.6 pkat/g protein. Simvastatin did not significantly increase NOS activity in either the brain cortex or the cerebellum. However, CoQ10 increased total NOS activity significantly in both brain cortex and cerebellum of obese Zucker rats. The combination of simvastatin and CoQ10 increased NOS activity to the level achieved after CoQ10 treatment (Fig. 1).

Discussion

Simvastatin is a well-known inhibitor of HMG-CoA reductase, that lowers LDL, VLDL, triglycerides, and apolipoprotein B levels (Oesterle *et al.* 2017). However, its most dangerous adverse effect is myopathy, which can progress to rhabdomyolysis in extreme cases and frequently results in acute renal failure (Tomaszewski *et al.* 2011). This adverse effect has been linked in part to the decrease in CoQ10 synthesis that occurs with simvastatin treatment (Qu *et al.* 2018). Therefore, CoQ10 was administered to the rats together with simvastatin in our experimental study. Simvastatin, on the other hand, appears to have favourable lipid-independent pleiotropic effects including Akt activation, endothelial NOS upregulation, and Ser 1177 phosphorylation (Laufs 2003, Harris *et al.* 2004). Also, the antioxidant, anti-inflammatory, and neuroprotective properties of simvastatin have been well documented (Tong *et al.* 2018, Gvozdjakova *et al.* 2012). It has been demonstrated that the intake of simvastatin is associ-

ated with a decreased incidence of Alzheimer´s disease, reduced cerebrovascular accidents, or immunomodulation in the nervous system (Wolozin *et al.* 2000). The neuroprotective mechanisms of simvastatin are usually associated with increased endothelial NOS generation. Mechanisms for statin uptake into the brain include diffusion and active transport across the blood – brain barrier (BBB) depending on the acid or lactone form of statins (Wood *et al.* 2014). Hydrophobic statins like simvastatin, fluvastatin, or lovastatin can easily cross the BBB, whereas hydrophilic statins like rosuvastatin and pravastatin are thought not to cross the barrier (King *et al.* 2003, Tsuji *et al.* 1993). Therefore, simvastatin was chosen for our study.

Similarly, CoQ10 has been shown to cross the BBB. CoQ10 is a regular component of the respiratory chain in inner mitochondrial membrane. Among others, it is the only endogenous lipid antioxidant (Rauchova 2021). Its beneficial effects have been documented also in neurological diseases like Parkinson's disease, Huntington's disease, Alzheimer's disease, or cardiometabolic diseases (Rauchova 2021, Stanga *et al.* 2020).

We aimed to determine the effect of simvastatin and CoQ10 treatment on NOS activity in the brain cortex and cerebellum of Zucker rats with metabolic syndrome. However, according to our results, simvastatin did not significantly increase NOS activity in either the brain cortex or the cerebellum. Despite the fact, that simvastatin was found to be transported into cells by organic anion transporter polypeptide, which has been identified in the BBB of rats (Lee *et al.* 2001), we did not see any effect of simvastatin on NOS activity. Probably, the dose of simvastatin used in our experimental conditions was not high enough to activate NOS in the brain. On the other hand, CoQ10 increased total NOS activity significantly in both brain cortex and cerebellum. Recently, Wainwright *et al.* (2020) studied the mechanism of CoQ10 transport across the BBB, using normal and CoQ10-deficient cell culture models. They identified lipoprotein-associated uptake and efflux mechanisms regulating CoQ10 transport and documented dynamic interplay of transport receptors. Matthews *et al.* (1998) demonstrated that oral administration of CoQ10 for two months increased both brain and brain mitochondrial CoQ10 levels in male Sprague-Dawley rats. Similarly, Kwong *et al.* (2002) documented significant increase of CoQ10 in brain mitochondria in male Sprague-Dawley rats after 13 weeks of treatment. CoQ10 treatment also increased brain level in the transgenic mouse model of Huntington's disease (Smith *et al.* 2006). Thus, it seems, that in our experimental conditions, CoQ10 was able to cross BBB and activate NOS, probably by NOS upregulation and/or ROS reduction. Similarly, oral CoQ10 treatment reduced blood pressure by inducing Akt and neuronal NOS phosphorylation in the nucleus tractus solitaire of fructose-induced hypertensive rats (Chen *et al.* 2019).

In conclusion, although our results did not show increased NOS activity after simvastatin treatment, they pointed to the fact that simvastatin combined treatment with CoQ10 has many advantages. Among others, in metabolic disorders, the addition of CoQ10 to simvastatin therapy may lead to increased production of nitric oxide in the brain with various neuroprotective effects.

Acknowledgement

This work was supported by The European Regional Development Fund "Vývoj biomodelov pre zlepšenie hodnotenia účinnosti liekov a látok, ktoré majú potenciál pri liečbe COVID-19 (BIOVID-19)"—ITMS2014+:313011AVG3 and "Centrum pre biomedicínsky výskum—BIOMEDIRES—II. etapa"—ITMS2014+:313011W428.

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