

Effects of atorvastatin and simvastatin on oxidative stress in diet-induced hyperhomocysteinemia in *Wistar albino* rats: a comparative study

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Received: 29 March 2017 / Accepted: 9 June 2017 / Published online: 15 June 2017
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Abstract Considering the well-known antioxidant properties of statins, it seems important to assess their impact on major markers of oxidative stress (superoxide anion radical, nitric oxide, and index of lipid peroxidation) to compare the antioxidative potentials of atorvastatin and simvastatin during the different degrees of hyperhomocysteinemia (HHcy) in rats. This study was conducted on adult male Wistar albino rats ($n = 90$; 4 weeks old; 100 ± 15 g body mass) in which HHcy was achieved by dietary manipulation. For 4 weeks, the animals were fed with one of the following diets: standard rodent chow, diet enriched in methionine with no deficiency in B vitamins (folic acid, B6, and B12), or diet enriched in methionine and deficient in B vitamins (folic acid, B6, and B12). At the same time, animals were treated with atorvastatin at doses of 3 mg/kg/day i.p. or simvastatin at doses of 5 mg/kg/day i.p. Levels of superoxide anion radical and TBARS were significantly decreased by administration of simvastatin in normal and high-homocysteine (Hcy) groups ($p < 0.05$). At 4 weeks after feeding with purified diets, the

concentrations of the GSH, CAT, and SOD antioxidants were significantly affected among all groups ($p < 0.05$). Our results indicated that statin therapy had variable effects on the redox status in hyperhomocysteinemic rats, and simvastatin demonstrated stronger antioxidant effects than did atorvastatin.

Keywords Hyperhomocysteinemia · Oxidative stress · Statins · Rat

Abbreviations

Hcy	Homocysteine
NOS	Nitric oxide synthase
–SH	Sulfhydryl group
CVD	Cerebrovascular disease
ROS	Reactive oxygen species
ONOO–	Peroxynitrite
NO	Nitric oxide
GSH	Reduced glutathione
CAT	Catalase
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
NO ₂	Nitrites
O ₂	Superoxide anion radical
EDTA	Ethylenediaminetetraacetic acid
HRPO	Horseradish peroxidase
TRIS	2-Amino-2-hydroxymethyl- propane-1,3-diol buffer

Introduction

The pathophysiological mechanism of cardiovascular disease includes both functional and structural impairment [1, 2]. Endothelial dysfunction, coordination disorder of the

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relaxation and contraction of corporal smooth muscles via the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway and RhoA/Rho-kinase (ROCK) pathway, autonomic neuropathy, and apoptosis in corporal smooth muscles simultaneously occurred. Therefore, to maintain the endothelial function, it is important to reduce risk factors, such as oxidative stress, HHcy, and dyslipidaemia [3, 4].

Excess prevalence of HHcy, an independent risk factor of cardiovascular diseases (CVD), has been observed in patients with dyslipidaemia [5].

HHcy may promote atherosclerotic plaque development and instability [6]. Hcy dose-dependently induced the apoptosis of the cells via increasing NADPH (nicotinamide adenine dinucleotide phosphate), oxidase (Nox) 1 and Nox2, which are oxidative stress inducing enzymes, suggesting that cell apoptosis by oxidative stress may be one of the underlying mechanisms of Hcy-induced vascular fragility [7–9].

Conversely, statins inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which acts as a rate-limiting enzyme for endogenous cholesterol synthesis and is considered a key enzyme in the mevalonate pathway. Statins are widely used to prevent cardiovascular events [10]. Statins improve the prognosis in patients with coronary heart diseases by decreasing the incidence of vascular events and ameliorating oxidative stress by regulating oxidant and antioxidant enzymes. Many studies have reported the cholesterol-lowering effects of statins, which exert pleiotropic effects, including cardioprotection [11, 12]. Clinical studies have also shown that statins can reduce the risk of heart attack, stroke, and death in patients with heart disease, such as coronary artery disease and cardiovascular disease [13, 14]. The efficacy of statins is related to the ‘common soil’ hypothesis, which proposes oxidative stress and inflammation as the primary pathophysiological processes in the disease group of diabetes and endothelial dysfunction [15].

Nevertheless, other studies have reported conflicting results, which indicated that statin therapy does not decrease the incidence of cardiovascular events, and it could be a risk for CVD, especially in the presence of one risk factor. One of the potential factors contributing to this discrepancy is the variability in the pleiotropic effects of different statins. Chemical properties vary among different statins, influencing the solubility and drug distribution, and the bioavailability and pleiotropic effects [16].

Therefore, we hypothesized that different statins might different prevent Hcy-induced oxidative stress and apoptosis. To date, however, there are few comparative studies investigating the effects of atorvastatin and simvastatin on the redox status in the presence of intermediate or severe HHcy [17].

Moreover, considering the well-known antioxidant properties of statins, it seems important to assess their impact on major markers of oxidative stress (superoxide anion radical, nitric oxide, and index of lipid peroxidation) to compare the antioxidative potentials of atorvastatin and simvastatin based on the degree of HHcy in rats.

Materials and methods

Ethical approval

All research procedures were performed in accordance with European Directive for welfare of laboratory animals No: 2010/63/EU and principles of good laboratory practice (GLP). The protocol of the current study was approved by the ethics committee for experimental animal well-being of the Faculty of Medical Sciences of the University of Kragujevac, Serbia (No: 01-11794). The investigators understand the ethical principles under which the journal operates, and that their work complies with the animal ethics checklist.

Animals and dietary manipulation

The animals were acclimatized for two weeks in the animal house of Faculty of Medical Sciences in Kragujevac before dietary manipulation. Two rats were housed per wire floored cage in an air-conditioned room (22 ± 2 °C) with a 12-h light/dark cycle and had free access to standard or special laboratory chow diet and water ad libitum.

The study was conducted on adult male *Wistar albino* rats ($n = 90$; 4 weeks old; 100 ± 15 g body mass) in which HHcy was achieved by dietary manipulation [18].

Age-matched littermates were selected for each experimental group. For 4 weeks, the animals were fed one of the following diets (*Mucedola SRL, Milan, Italy*): standard rodent chow, diet enriched in methionine with no deficiency in B vitamins (folic acid, B₆ and B₁₂), or diet enriched in methionine and deficient in B vitamins (folic acid, B₆ and B₁₂) (Table 1). At the same time, animals were exposed to pharmacology treatment with atorvastatin at a dose of 3 mg/kg/day i.p or simvastatin at a dose of 5 mg/kg/day i.p at the same time every day, according to equivalent therapeutic doses of these statins (10 mg atorvastatin = 20 mg simvastatin). Animals were divided into the following nine [9] different groups as follows:

- (1) Animals fed a standard rodent chow without statins (S);
- (2) Animals fed a diet enriched in methionine with no deficient in B vitamins (folic acid, B₆, and B₁₂) without statins (M);

Table 1 Composition of diets

Food composition	Standard food	Diet enriched in methionine	Diet enriched in methionine and deficient in B vitamins
The main ingredients			
Methionine (g/kg)	3.8	7.7	7.7
Vitamin B ₆ (mg/kg)	70.0	70.0	0.01
Vitamin B ₉ (mg/kg)	2.0	2.0	0.08
Vitamin B ₁₂ (mg/kg)	0.03	0.03	0.01
Protein (Casein, %)	20	18.5	18.5
Other ingredients			
Lipids (%)	7.5	7.5	7.5
Carbohydrates (%)	2	2	2
Vitamin A (IU/kg)	4000	4000	4000
Vitamin D ₃ (IU/kg)	1000	1000	1000
Vitamin E (mg/kg)	25	25	25
Na (Sodium, %/kg)	0.15–0.25	0.15–0.25	0.15–0.25
Fe (Ferric citrate, mg/kg)	40.2	40.2	40.2
P (Phosphorus, %/kg)	min. 0.5	min. 0.5	min. 0.5
Mn (Manganese carbonate, mg/kg)	10.5	10.5	10.5
Zn (Zinc carbonate, mg/kg)	33.5	33.5	33.5
Cu (Copper carbonate, mg/kg)	6.2	6.2	6.2
I (Potassium iodide, mg/kg)	0.21	0.21	0.21
Se (Sodium selenite, mg/kg)	0.15	0.15	0.15
Mo (Ammonium molybdate, mg/kg)	0.15	0.15	0.15
t-butylhydroquinone (mg/kg)	14	14	14
Ash (%)	10	4	4

- (3) Animals fed with diet enriched in methionine and deficient in B vitamins (folic acid, B₆, and B₁₂) without statins (MD);
- (4) Animals fed a standard rodent chow with atorvastatin at a dose of 3 mg/kg/day i.p. (S + Atorva);
- (5) Animals fed a standard rodent chow with administration of simvastatin at a dose of 5 mg/kg/day i.p. (S + Simva);
- (6) Animals fed a diet enriched in methionine with no deficiency in B vitamins (folic acid, B₆, and B₁₂) with atorvastatin at a dose of 3 mg/kg/day i.p. (M + Atorva);
- (7) Animals fed a diet enriched in methionine with no deficient in B vitamins (folic acid, B₆, and B₁₂) with administration of simvastatin at a dose of 5 mg/kg/day i.p. (M + Simva);
- (8) Animals fed a diet enriched in methionine and deficient in B vitamins (folic acid, B₆, and B₁₂) with administration of atorvastatin at a dose of 3 mg/kg/day i.p. (MD + Atorva);
- (9) Animals fed a diet enriched in methionine and deficient in B vitamins (folic acid, B₆, and B₁₂) with administration of simvastatin at a dose of 5 mg/kg/day i.p. (MD + Simva).

Biochemical assay in blood

After week-dietary manipulation, animals were sacrificed, and rat blood samples were collected by exsanguination. In serum samples, we determined the concentration of Hcy and lipids, such as the total cholesterol (tChol), high-density lipoprotein (HDL) and triglycerides (Try). In plasma samples, we measured the concentration of pro-oxidative markers, such as superoxide anion radical (O₂⁻), nitric oxide (NO⁻), and index of lipid peroxidation measured as TBARS (TBARS). In lysate, we determined the activity of nonenzymatic antioxidant, such as reduced glutathione (GSH), and activity of the enzymatic defence system, by evaluating of catalase (CAT) and superoxide dismutase (SOD) concentrations.

Determination of homocysteine (Hcy)

Total serum Hcy concentrations were measured with a high-performance liquid chromatography (HPLC) procedure with reverse phase separation and fluorescence detection, as previously described [19]. The total serum concentrations were determined using commercial kits

from SIEMENS, at automatic analyser CENTAUR XP SIEMENS, with a sensitivity assay range $0.5\text{--}65\ \mu\text{mol/L}$.

Determination of lipid profile

Prior to blood collection to measure plasma parameters, animals were fasted for 12 h to minimize the interference of food intake in the lipid profile results. Blood was collected during sacrifice of animals into micro-tubes. Total cholesterol (tChol), high-density lipoprotein (HDL), and triglycerides (Try) were determined using commercial kits from SIEMENS Diagnostica, an automatic analyser Dimension R \times L Max.

Determination of superoxide anion radical (O_2^-)

The concentration of the superoxide anion radical (O_2^-) was measured after the reaction of nitro blue tetrazolium in TRIS buffer with plasma at 530 nm. Distilled water solution served as a blank [20].

An indirect method for monitoring nitric oxide (NO) by determining nitrate (NO_3^-) and nitrite (NO_2^-)

The nitrate (NO_3^-) and nitrite (NO_2^-) levels are measured and NO levels calculated as previously described [21]. A total of 0.5 mL of plasma was precipitated with 200 μl of 30% sulfosalicylic acid, vortexed for 30 min, and centrifuged at $3000\times g$. Equal volumes of the supernatant and Griess reagent, containing 1% sulphanilamide in 5% phosphoric acid/0.1% naphthalene ethylenediamine dihydrochloride, were added and incubated for 10 min in the dark and measured at 543 nm [21].

TBARS determination (index of lipid peroxidation)

The degree of lipid peroxidation in the plasma was estimated by measuring the TBARS using 1% thiobarbituric acid in 0.05 NaOH, incubated with plasma at 100 $^\circ\text{C}$ for 15 min, and measured at 530 nm. Distilled water served as a blank [22].

Determination of reduced glutathione (GSH)

The level of reduced glutathione (GSH) was determined based on GSH oxidation with 5,5'- dithio-bis-6,2-nitrobenzoic acid using the method reported by Beutler [23].

Determination of catalase (CAT)

CAT activity was determined according to Aebi. Lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove haemoglobin; then 50 μl of CAT buffer, 100 μl of ample, and 1 mL of

10 mM H_2O_2 were added to the samples. Detection was performed at 360 nm [24].

Determination of superoxide dismutase (SOD)

SOD activity was determined by the epinephrine method of Beutler. One hundred μl lysate and 1 mL of carbonate buffer were mixed; then 100 μl of epinephrine was added. Detection was performed at 470 nm [25].

Reagents and diets

All reagents and substances were purchased from Sigma-Aldrich (*Sigma-Aldrich Chemie GmbH, Germany*): Atorvastatin calcium salt trihydrate $\geq 98\%$ ($\text{C}_{33}\text{H}_{34}\text{FN}_2\text{O}_5 \times 0.5\ \text{Ca} \times 1.5\cdot\text{H}_2\text{O}$) MW: 604.69, (product number: PZ0001-25MG) and Simvastatin $\geq 97\%$ ($\text{C}_{25}\text{H}_{38}\text{O}_5$), MW: 418.35 (product number: S6196-25MG). All diets for animals were purchased from *Mucedola Corporation (Milan, Italy)*.

Statistical analysis

Data are presented as the mean \pm SD. The effects of dietary methionine and folate intake on oxidative parameters and antioxidant status between groups were analysed by one-way ANOVA (Scheffé's F test) and the nonparametric analogue test of ANOVA (Kruskall–Wallis test). Differences were considered to be significant at $p < 0.05$.

Results

Homocysteine concentration

In MD and M groups, the average serum concentrations of Hcy were 63.89 ± 0.1 and $22.43 \pm 0.3\ \mu\text{mol/L}$, while it was $8.11 \pm 0.1\ \mu\text{mol/L}$ in the control group. In the S + Atorva and S + Simva groups, the average serum concentrations of Hcy were less than $15\ \mu\text{mol/L}$; in the M + Atorva and M + Simva groups, they were in range from 15 to $30\ \mu\text{mol/L}$, and in the MD + Atorva and MD + Simva groups, they were above $30\ \mu\text{mol/L}$. According to these results, all groups were divided into normal (S, S + Atorva, and S + Simva), high (M, M + Atorva, and M + Simva), and very high (MD, MD + Atorva, and MD + Simva) homocysteine groups (Table 2).

Lipid profile

The values of tChol, HDL, and Try were significantly decreased after the SIM administration in the Meth-purified

Table 2 Levels of homocysteine in all groups

Groups	Total Homocysteine in serum ($\mu\text{mol/L}$)		
	S group	M group	MD group
	8.11 \pm 0.1	22.43 \pm 0.3	63.89 \pm 0.1
Groups	S + Atorva group	M + Atorva	MD + Atorva
	11.57 \pm 3.27	16.10 \pm 4.32	59.41 \pm 7.12
Groups	S + SIMVA group	M + Simva	MD + Simva
	11.41 \pm 1.92	30.22 \pm 5.71	58.51 \pm 4.49

Data are presented as the mean value \pm standard deviation (M \pm SD)

groups ($p < 0.05$). The values of tChol and Try were significantly decreased after the ATO administration in the Meth-B deficient groups, while the levels of HDL were not. Interestingly, blood concentrations of Chol, HDL, and Try were not significantly affected by atorvastatin or simvastatin in the groups fed a standard diet ($p > 0.05$) (Table 3).

Concentration of pro-oxidant markers

The levels of superoxide anion radical were significantly decreased by the administration of simvastatin in the normal and high-homocysteine groups ($p < 0.05$); however, in group with Hcy levels above 30 $\mu\text{mol/L}$, the level of O_2^- was not significantly changed ($p > 0.05$) (Fig. 1). Simvastatin significantly decreased the level of O_2^- in the M groups compared to atorvastatin, while the atorvastatin decreased the level of this pro-oxidative marker in MD groups more than simvastatin without reaching statistical significance. Compared to the control groups, SIM and atorvastatin significantly decreased O_2^- in the hyperhomocysteinemic groups (Fig. 1).

Table 3 Lipid profiles of all groups

Lipids	CHOL (mmol/L)	HDL (mmol/L)	TG (mmol/L)
Group			
Standard	1.32 \pm 0.02	1.63 \pm 0.01	0.67 \pm 0.02
Meth purified	2.87 \pm 0.03	2.23 \pm 0.04	1.23 \pm 0.01
Meth-B deficient	1.92 \pm 0.05	1.36 \pm 0.02	1.12 \pm 0.04
ATO + Standard	1.66 \pm 0.01	1.56 \pm 0.01	0.98 \pm 0.02
ATO + Meth purified	2.65 \pm 0.06	2.43 \pm 0.02	0.84 \pm 0.03
ATO + Meth-B deficient	1.34 \pm 0.01 [#]	1.23 \pm 0.02	0.34 \pm 0.002 [#]
SIM + Standard	1.58 \pm 0.01	1.21 \pm 0.003	0.87 \pm 0.01
SIM + Meth purified	2.23 \pm 0.05*	1.83 \pm 0.04*	0.44 \pm 0.001*
SIM + Meth-B deficient	1.85 \pm 0.02	1.63 \pm 0.03	0.90 \pm 0.02

Data are presented as the mean value \pm standard deviation (M \pm SD). Statistical significance is presented as follows: asterisk represents significant differences between the signed parameter in all Meth-purified groups ($*p < 0.05$) and hash represents significant differences between signed parameters in all Meth-B deficient groups ($\#p < 0.05$)

The level of NO (nitrites+nitrates) was not significantly changed in the groups with hyperhomocysteinemia compared to control groups ($p < 0.05$) (Fig. 2). Compared to control groups (S, M, and MD groups), both statins had similar effects on the NO levels after 30 days of administration (Fig. 2).

The index of lipid peroxidation was significantly altered (Fig. 3). Compared to the control groups without drug administration, atorvastatin increased the levels of TBARS in the S and M groups, while the levels of this marker were decreased in the MD groups. Conversely, simvastatin administration decreased the levels of TBARS in the M+SIM group compared to all other groups and compared to control conditions (Fig. 3). Simvastatin, compared to atorvastatin, significantly decreased the levels of TBARS in the M group of animals fed with meth-purified food.

Concentration of antioxidant markers

At 4 weeks after feeding with purified diets, the blood concentrations of the GSH antioxidant in blood were significantly affected among all groups (Fig. 4). Interestingly, at a higher concentration of Hcy in blood, simvastatin induced increasing levels of GSH; in normal conditions (Hcy $< 15 \mu\text{mol/L}$), simvastatin reduced the levels of GSH compared to the Atorva-groups. Additionally, compared to groups without drug administration, simvastatin and atorvastatin alone affected this parameters in the same manner, simvastatin had higher potency (Fig. 4).

Furthermore, simvastatin administration significantly increased the CAT values among all groups that were given this statin compared to atorvastatin ($p < 0.05$) (Fig. 5). Separately, simvastatin induced increased CAT levels compared to control groups without drug administration, as well as atorvastatin. Additionally, by comparing their

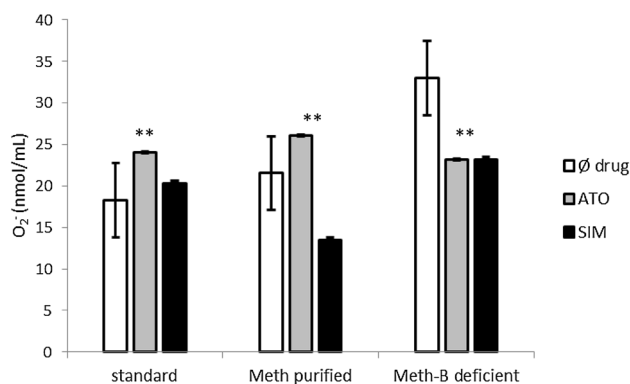


Fig. 1 Values of superoxide anion radical (O_2^-) levels (nmol/mL) in all groups of rats (mean + SD). Statistical significance was considered for a p value less than 0.05 ($*p < 0.05$)

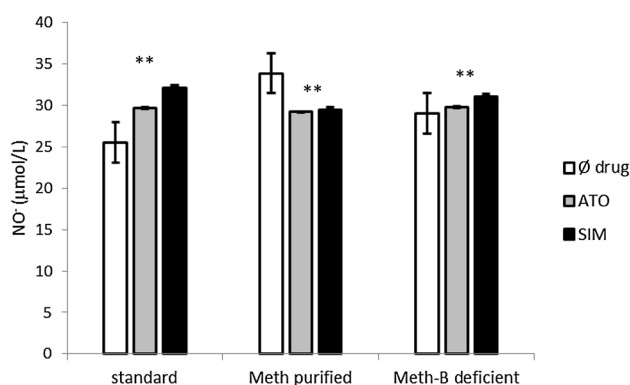


Fig. 2 Values of nitric oxide (NO^-) levels ($\mu\text{mol/L}$) in all groups of rats (mean + SD). Statistical significance was considered at p values less than 0.05 ($*p < 0.05$)

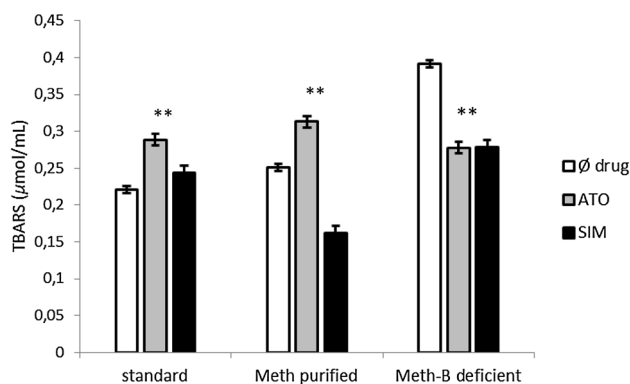


Fig. 3 Values of index of lipid peroxidation (TBARS) levels ($\mu\text{mol/mL}$) in all groups of rats (mean + SD). Statistical significance was considered at p values less than 0.05 ($*p < 0.05$)

separate effects, simvastatin exerts stronger antioxidant activity (Fig. 5).

Finally, atorvastatin and simvastatin had similar actions on the levels of superoxide dismutase in groups with high-homocysteine concentrations. However, in extreme conditions, such as very high or very low Hcy concentrations,

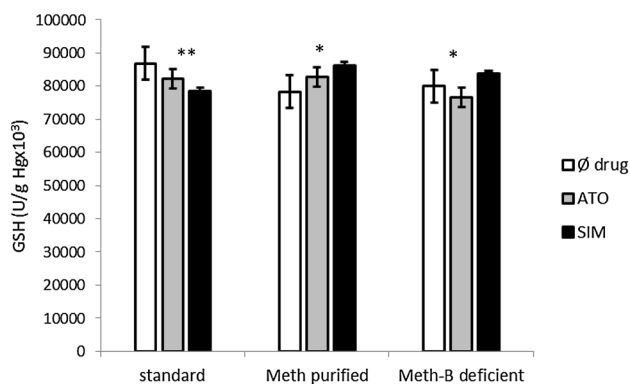


Fig. 4 Values of reduced glutathione (GSH) levels (nmol/mL/RBC) in all groups of rats (mean + SD). Statistical significance was considered at p values less than 0.05 ($*p < 0.05$)

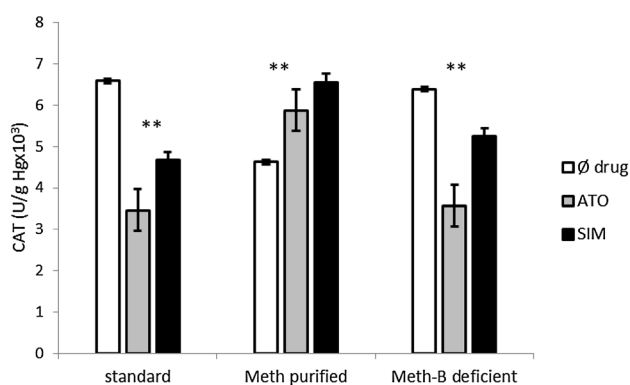


Fig. 5 Values of catalase (CAT) levels ($\text{U/gHg} \times 10^3$) in all groups of rats (mean + SD). Statistical significance was considered at p values less than 0.05 ($*p < 0.05$)

simvastatin significantly increased the SOD levels compared to atorvastatin ($p < 0.05$) (Fig. 6). Compared to groups without drug treatment, simvastatin alone induced increased levels of SOD, but there was no difference in relation to the Hcy levels. However, compared to groups without drug treatment, atorvastatin alone was increased during the normal and very high Hcy levels, while it was decreased compared to simvastatin for the normal and very high Hcy groups (Fig. 6).

Discussion

Several clinical and epidemiological studies have shown that HHcy was independently associated with vascular disorders, and it has recently been considered a co-morbid risk factor for cardiovascular disease [26–30]. Nevertheless, the oxidant potential of HHcy in the heart is still unclear.

In the present study, we used a rat model of diet-induced HHcy with the high intake of methionine to mimic, as

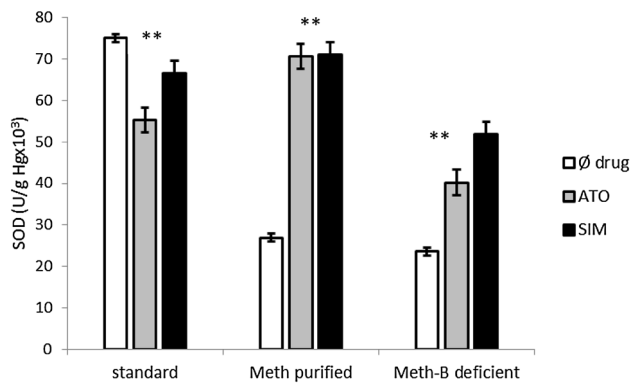


Fig. 6 Values of superoxide dismutase (SOD) levels ($\text{U/gHg} \times 10^3$) in all groups of rats (mean + SD). Statistical significance was considered at p values less than 0.05 ($*p < 0.05$)

much as possible, the clinical condition of HHcy. This type of experimental design updated the current knowledge combining elevated Hcy levels with and without deficiency vitamin B₁₂, B₆ and folic acid. At the termination of treatment, we diagnosed mild and moderate HHcy by biochemical and pathological evaluation, confirming the reliability of the model.

Prior studies have reported that elevated serum levels of Hcy have various mechanisms of influence on the cardiovascular system, such as a role in endothelial dysfunction, proliferation, inflammation, and oxidative processes [31, 32].

Hcy can be viewed as a first risk factor for atherosclerosis that is thought to exert its effects through a mechanism involving oxidative damage [39], inducing endothelial dysfunction and leading to atherosclerosis and other cardiovascular diseases. Clinical *in vivo* studies have confirmed the occurrence of endothelial dysfunction in HHcy [17]. The basis of the Hcy-induced oxidative stress hypothesis relies on the chemical auto-oxidation of Hcy. In our study, it can be clearly observed that HHcy can be associated with increased release of O_2^- and TBARS (Figs. 1, 3). Well, our results show that both control groups with different levels of HHcy had higher values O_2^- and TBARS than control group fed with standard diet (Figs. 1, 3).

Therefore, understanding the mechanisms by which HHcy affects the signalling pathways linked with oxidative damage could form the basis for developing new treatment strategies. It is known that statins prolong the lifespan of lower organisms [33] and reduce the total human mortality, even in people who have normal lipid levels, and statins are the first-line drugs in the primary and secondary prevention of cardiovascular diseases [30, 31].

Due to pleiotropic effects of statins, which are not associated with lipid-lowering effects, we examined the

effects of widely used statins, atorvastatin, and simvastatin, on the redox status of rats with normal and high levels of Hcy and normal levels of lipoproteins in the blood.

First, we tested the well-known observation that the statins are a class of drugs used for decades to treat hypercholesterolaemia. During the experimental period, groups of animals that fed a methionine-purified diet with high levels of B vitamins had significantly higher growth and body mass indexes compared to the other groups. In agreement, the blood of these animals had higher levels of tChol and Try (Table 3), and atorvastatin and simvastatin had similar effects on the lipid profiles in the same state of HHcy. Our results agree with previous results [40, 41], which suggested that equivalent doses of atorvastatin and simvastatin have equivalent lipid-lowering effects.

In the second part of our study, we evaluated the effects of atorva/simvastatin on pro-oxidant markers in intermediate and severe HHcy compared to physiological conditions.

Simvastatin exerts more potential effects in reducing the O_2^- level at lower Hcy levels compared to atorvastatin (Fig. 1). Hcy is readily oxidized when added to plasma, principally because of auto-oxidation, leading to the formation of Hcy, homocysteine-mixed disulphides, and homocysteine thiolactone. During oxidation of the sulfhydryl group, superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2) are generated, and these oxygen-derived molecules are believed to account for the endothelial cytotoxicity of homocyst(e)ine. In the same conditions of oxidation Hcy in plasma, simvastatin strongly decreased the level of superoxide anion radical. Additionally, TBARS were changed in the same manner by simvastatin (Fig. 3). By contrast, nitric oxide (measured in form of nitrites), as a potent vasodilator, was not altered by long-term simvastatin or atorvastatin at different Hcy levels (Fig. 2). Compared with control groups without drug administration, we can confirm the dominant effects of simvastatin (Figs. 1, 2, 3). Our findings indicate that statins exhibit differential effects in preventing oxidative stress when administered at equivalent lipid-lowering doses.

Crespo et al. compared the effects of daily administration over a four-week period at low doses (10 mg/kg per day) of atorvastatin (AV), simvastatin (SV), and pravastatin (PV) on cardiac performance and oxidative stress in diabetic rats. These researchers determined that statins decrease the malondialdehyde (MDA) content without affecting the eNOS and iNOS protein levels [34], which correlates with our results. A clinical study also suggested that atorvastatin had slight effects on inflammation and oxidative stress, while rosuvastatin had the best anti-inflammatory effect. Simvastatin again achieved the best antioxidant effects [35]. Previous reports noted that all three statins decreased the media thickness, perivascular fibrosis, and both MDA and 4-HAE

in the aortas of diabetic rats and observed that the haemodynamic benefits were cholesterol-independent. These benefits appear to be secondary to the improved endothelial function and to the reduced vascular tone and remodelling that result from decreased oxidative stress. However, both literature and our data indicate no change in the nitrite dynamics after statin treatment.

In every previous study and in ours, nitrite was not altered with any statin treatment compared to the control conditions [13–16]. This result can be a positive, considering that a decrease in the NO bioavailability is related to impaired endothelial function. Also, pro-oxidant effects of HHcy are less evident under drug treatment (Figs. 1–3). It seems that statins failed to diminish pro-oxidant effects of hyperhomocysteinemia via still unknown mechanisms.

Although anti-inflammatory and antioxidant properties of statins are well-known, the antioxidant properties of atorvastatin and simvastatin during HHcy have not been thoroughly elucidated. To assess one potential mechanism through which statins interfere with ROS during HHcy, we measured the activity of antioxidant enzyme system. After 4-wk feeding rats with purified diets, concentrations of the antioxidant GSH in blood were significantly affected among all groups. In the Hcy-control groups without drug administration, all antioxidants were significantly reduced before statin treatments, and there was stronger antioxidant activity with simvastatin (Figs. 4, 5, 6). Interestingly, at a higher concentration of Hcy in blood, simvastatin induced increasing levels of GSH, but in normal conditions (Hcy < 15 $\mu\text{mol/L}$), simvastatin reduced the levels of GSH compared to the Atorva-groups (Fig. 4). CAT values were less increased. Finally, atorvastatin and simvastatin had similar effects on the levels of superoxide dismutase in groups with high-homocysteine concentrations; however, in extreme conditions, such as very high or very low Hcy concentrations, simvastatin significantly increased the levels of SOD compared to atorvastatin (Figs. 5, 6). Simvastatin generally exerts more antioxidant properties than atorvastatin, especially with severe HHcy, and only the groups treated with atorvastatin exhibited decreased GSH and CAT activity compared to the simvastatin groups.

Few clinical studies suggest that atorvastatin and pravastatin, but not simvastatin, exhibit anti-inflammatory and antioxidant activity in different diseases, i.e., endotoxin-induced acute lung injury [36] and acute nephropathy [33], but the duration of pretreatment and comorbidity can also affect each drug's antioxidant properties.

However, our data are not consistent with the results of Wang et al., who observed that atorvastatin and rosuvastatin had similar effectiveness against contrast media-induced oxidative stress, while simvastatin was less effective. These researchers showed that atorvastatin was the most effective against NO system dysfunction and cell

apoptosis, while rosuvastatin was most effective against inflammation [37]. Li and co-workers concluded that atorvastatin reduces oxidative stress more effectively than simvastatin [38]. The diversity of experimental models could be a reason for the inconsistent results because these authors examined the effects of widely prescribed statins in the absence of a strong generator of ROS, HHcy. HHcy is likely an important interfering co-factor in lowering pro-oxidants with any potential antioxidants, as a producer of reactive oxygen species.

One of the potential explanations for the more potent impact of simvastatin on reducing of oxidative stress by elevating of GSH and CAT activity compared to atorvastatin is the difference in their pharmacological properties, which may result in different antioxidant activities [27–32]. During metabolism, simvastatin is given as lactone prodrug and converted to the active beta-hydroxy form, simvastatin hydroxy acid (SVA), which is the most potent competitive inhibitor of HMG-CoA reductase. Conversely, atorvastatin metabolism primarily occurs through cytochrome P4503A4 hydroxylation to form active ortho- and parahydroxylated metabolites, as well as various beta-oxidation metabolites which are responsible for 70% of systemic HMG-CoA reductase activity [39].

Furthermore, it is speculated that the strong antioxidant effects of simvastatin are related to its ability to inhibit the isoprenoid compounds produced by the mevalonate pathway and their ability to inhibit the activation of nicotinamide adenine dinucleotide phosphate [30]. Additionally, reduction in leukocytes by simvastatin could directly reduce redox markers. Other antioxidant mechanisms of atorvastatin and simvastatin are inhibition of platelet Nox275, reducing the circulating concentration of the catalytic subunit of Nox247; increasing adiponectin, which inhibits Nox activation; increasing catalase and SOD by phosphorylation of Akt41 and by S-nitrosylation27; and increasing of the activity of PON1, which is responsible for the antioxidant properties of HDL cholesterol [26–37].

These various mechanisms of action in statins can be different because of differences between pharmacokinetic characteristics of atorvastatin and simvastatin, such as hydrophobicity, bioavailability, metabolism in the liver, and half-life associated with the dose and duration of drug use. These features should be considered.

Conclusions

To the best of our knowledge, few studies have compared the effects of atorvastatin and simvastatin on markers of oxidative stress in rats with HHcy. Our results have indicated that statin therapy resulted in variable effects on the redox status in hyperhomocysteinemic rats, and simvastatin

demonstrated stronger antioxidant effects than atorvastatin, independent of its effects on the lipid profile and dependent on the homocysteine concentration. Sufficiently powered prospective clinical and intervention studies are needed to investigate whether simvastatin lowers oxidative stress more effectively than other statins.

Funding This work was supported by the Ministry of Science and Technical Development of the Republic of Serbia (Grant No. 175043) and the Faculty of Medical Sciences, University of Kragujevac (Junior Project No. 09/2011).

Compliance with ethical standards

Conflict of interest No conflicts of interest, financial, or otherwise, are declared by the authors.

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