Pharmacological Reports 67 (2015) 907–913

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original research article

Effects of simvastatin on malondialdehyde level and esterase activity in plasma and tissue of normolipidemic rats

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ARTICLE INFO

Article history:
Received 3 November 2014
Received in revised form 28 January 2015
Accepted 13 February 2015
Available online 26 February 2015

Keywords:
Simvastatin
Paraoxonase 1
Butyrylcholinesterase
Malondialdehyde

ABSTRACT

Background: We investigated the possible non-lipid effects of simvastatin (SIMV) on paraoxonase 1 (PON1) and butyrylcholinesterase (BuChE) activity, as well as on malondialdehyde (MDA) levels in normolipidemic rats.
Methods: Two experimental groups of Wistar rats (10 mg/kg/day of SIMV) and two control groups (saline) underwent a 21-day treatment period (TP). On the 22nd day one experimental and one control group of rats were sacrificed. Remaining groups of animals were sacrificied on the 32nd day of the study (10-day after-treatment period (AT)). Blood samples and slices of liver, heart, kidney, and brain tissue were obtained for the measurement of PON1 and BuChE activity and levels of MDA. Data were analyzed by means of *t*-test for independent samples. p values ≤ 0.05 were considered as statistically significant.
Results: SIMV caused a significant decrease of serum and liver PON1 activity (18–24%, $p \leq 0.05$) and MDA concentrations in the plasma, heart, liver, kidney, and brain (9–40%, $p \leq 0.05$), while plasma and liver BuChE activity increased by 29% ($p \leq 0.05$) and 18%, respectively. All effects of SIMV were largely diminished following AT. The exception was MDA, which remained significantly decreased in plasma and all tissues analyzed.
Conclusion: SIMV significantly decreased PON1 activity and MDA levels and increased BuChE activity. We suggest that the decrease of MDA levels is a beneficial therapeutic effect of SIMV, for example in cardiovascular disorders, while the increase of BuChE activity, especially in brain, may be a potential adverse effect in patients with Alzheimer disease.
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Introduction

Statins represent drugs of first choice for treatment of hypercholesterolemia since they inhibit 3-hydroxy-3-methylglutarylcoenzyme A (HMG-CoA) reductase, a rate-limiting enzyme in mevalonate and cholesterol biosynthesis [1]. Hypercholesterolemia leads to a pronounced increase in reactive oxygen species (ROS) like free oxygen radicals, oxygen ions, and hydrogen peroxides (H_2O_2) [1]. Large amounts of H_2O_2 stimulate the peroxidation of LDL-cholesterol, which causes a significant amount of malondialdehyde (MDA) formation. Therefore MDA is very often used as an index of oxidative status [2]. Individuals with high exposure to oxidative stress, such as those with hypertension [3], have an increased formation of oxidized LDL-cholesterol that is accumulated in the arterial walls and causes endothelial dysfunction. In this situation the endogenous antioxidant defensive effects of superoxid dismutase, catalase, and glutathione peroxidase are very important [3].

Experiments *in vitro* and *in vivo* have shown that statins contribute to the protection of organism against oxidative stress

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http://dx.doi.org/10.1016/j.pharep.2015.02.005
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through their anti-inflammatory and anti-thrombotic actions, and through the scavenging of superoxide anions and hydroxyl radicals [1,4–6]. All these effects are well-known cholesterol-independent or "pleiotropic" effects of statins. It is also suggested that antioxidative effects of statins depend on patients' cholesterol levels. Thus, in hypercholesterolemic patients atorvastatin and pravastatin were capable of protecting LDL-cholesterol from oxidation *in vivo* only in early treatment phase [5] while fluvastatin treatment did not improve oxidative stress or inflammation in patients with arterial hypertension and normal cholesterol levels [6].

Statins may also modulate the activity of two serum esterases, paraoxonase 1 (PON1) [7] and butyrylcholinesterase (BuChE) [8], with unknown physiological function at present. Both enzymes are synthesized in the liver and hypothetically are involved in metabolism of lipids. Paraoxonase 1 (PON1, aryldialkilphosphatase, EC 3.1.8.1, PON1) is secreted into the plasma where it is associated with HDL-cholesterol and apolipoprotein (apo) AI. PON1 is included in the protection of the organism against oxidative stress [7] and it has been suggested that possible antioxidant actions of PON1 are hydrolysis of oxidized lipids formed on LDL- and HDL-cholesterol and protection of HDL-cholesterol from peroxidation [9]. The evidence about the effect of statins on PON1 activity remains equivocal [7,10].

The enzyme BuChE (EC 3.1.1.8, pseudocholinesterase, serum cholinesterase) is secreted in the blood after its synthesis in the liver [11]. A known special target in the organism is cleavage of choline esters like acetylcholine (ACh) and butyrylthiocholine. The hypothesis that LDL-cholesterol is formed from very low-density lipoproteins (VLDL-cholesterol) in the presence of BuChE is supported by the fact that increased BuChE activity is associated with an abnormal lipid metabolism in humans [11]. It has been shown that BuChE activity is increased in patients with hypercholesterolemia, hypertension, obesity, and diabetes, where this increased enzyme activity correlates positively with serum levels of LDL-cholesterol and triacylglycerols, and inversely with HDL-C [11,12].

The presence of BuChE has also been detected in various tissues [11], specially in neurons and glial cells of the human brain [13] where it hydrolyses ACh, but less efficiently than acetylcholinesterase (AChE) [13]. It is known that the most striking neurochemical disturbance in Alzheimer disease (AD) is a deficiency of ACh [14,15]. Since both cholinesterases are involved in cholinergic transmission in the brain, cholinesterase inhibitors are used for treatment of AD [14,15]. According to the results *in vitro* and *in vivo*, some statins can cause either the inhibition of both enzymes or have no effect on AChE and BuChE activity [16,17].

Since the results of human and animal studies about the effects of statins on BuChE and PON1 activity and oxidative stress vary, our aims were to investigate the effects of multiple administration of lipophilic simvastatin (SIMV) on BuChE and PON1 activities and MDA level in the serum and different tissues of normolipidemic rats, as well as to observe the levels of enzyme activity and MDA 10 days after discontinuation of the treatment.

Material and methods

Test substances

SIMV (CAS-79902-63-9) (Statex® 20) was obtained from Pliva, Croatia. It was suspended in saline and administered daily (9.00–10.00 a.m.) into the stomach by oral gavage. The daily dose was 10 mg/kg body weight during 21 day.

Treatment of animals

Male Wistar rats (Department of Pharmacology, School of Medicine, University of Zagreb) weighing 250–350 g were used in this study. Animals were maintained under controlled laboratory conditions. Standard diet in pellet form was available *ad libitum*. Handling and treatment of the animals were conducted according to international guidelines regulating the use of laboratory animals. The experiments had been approved by the local ethics committee.

Study design

The study was divided into a 21-day treatment period (TP) and a 10-day after-treatment period (AT). Two experimental groups of Wistar rats were on SIMV treatment (10 mg/kg/day) and two control groups on saline. In each group there were 7–8 animals. SIMV and saline were given for a 21-day TP. After an overnight fast of 12 h, one experimental and one control group of rats were sacrificed under diethyl ether anesthesia on the 22nd day of the study. The remaining groups of animals which received no SIMV or saline for the 10-day ATP were sacrificied after a 12-h overnight fast on the 32nd day of the study. Blood samples for measuring serum PON1 and plasma BuChE activity, plasma lipids, and plasma MDA levels in all groups of rats were obtained by cardiac puncture. Plasma lipids were measured 2–4 h after sampling. All other serum and plasma samples were frozen at $-20\,^{\circ}$C immediately after sampling, until further processing. Heart, liver, kidney, and brain tissues for determining both PON1 and BuChE activity and MDA level were frozen at $-70\,^{\circ}$C immediately after sacrifice of the animals, until further processing. Before collecting, the liver had been washed out of blood with saline *in situ via* the vena cava superior. The brain was also rinsed with saline.

Measurement of PON1 activity in serum and liver

Serum PON1 activity was measured using synthetic diethyl-p-nitrophenyl phosphate (paraoxon, o,o-diethyl-p-nitrophenylphosphate; Sigma Chemical Co., London, UK) and $CaCl_2$ (1 mM in 0.1 M TRIS buffer pH 7.4) as moderator. The activity toward paraoxon was determined by measuring the initial rate of substrate hydrolysis to p-nitrophenol. Slices of liver tissue (0.2 g) were homogenized in four volumes of saline and centrifuged at $3500 \times g$ for 15 min. In brief, the reaction for the hydrolysis of paraoxon contained 200 μL of 0.1 M TRIS buffer (pH 7.4)-$CaCl_2$ and 800 μL of 1 mM paraoxon solution, to which 100 μL undiluted serum or 100 μL of supernatant (for measurement of liver PON1 activity) was added to start the reaction. The increase in absorbance at 405 nm was monitored for 3 min [18].

Measurement of BuChE activity in plasma and liver

Plasma and liver BuChE activity was measured by spectrophotometric method of Ellman et al. [19], using butyrylthiocholine (0.9 mM) (Sigma ChemCo, St. Louis, USA) as the substrate. Slices of liver tissue (0.2 g) were homogenized in four volumes of saline and centrifuged at $3500 \times g$ for 15 min. The reaction for the hydrolysis of butyrythiocholine in plasma and liver contained 1 mL of mixture of 3 mL 0.1 M phosphate buffer and 100 μL 0.38 mM 5.5-dithio-bis(2-nitrobenzoic acid) (DTNB), 100 μL of butyrylthiocholine and 50 μL of plasma or 50 μL of supernatant. The reaction for the measurement of liver BuChE activity was repeated after the addition of 50 μL of etopropazine hydrochloride, which is a specific BuChE inhibitor. The increase in absorbance at 412 nm and a temperature of 25 °C was monitored for 3 min.

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