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Effect of long term, non cholesterol lowering dose of fluvastatin treatment on oxidative stress in brain and peripheral tissues of streptozotocin-diabetic rats

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ABSTRACT

One of the main goals of treatment of diabetes mellitus is to prevent its complications. Oxidative stress is universal in diabetes, being ultimately involved with the development complications. As a result of hyperglycemia, reactive oxygen/nitrogen species are produced in various tissues that leads to tissue damage with lipid peroxidation and protein oxidation, along with disruption in cellular homeostasis and accumulation of damaged molecules. Hence, supplementation with antioxidant compounds may offer some protection against diabetic complications. The pleiotropic effects of statins, including antioxidant and anti-inflammatory properties, represent an area of great interest in prevention and therapy of cardiovascular and neurological disorders. Using biomarkers of oxidative stress, in this study we examined the effect of non cholesterol lowering dose, long term fluvastatin treatment on oxidative stress in streptozotocin-diabetic rats. Experiments were conducted in 24 Wistar adult male rats. Diabetic and non-diabetic rats were treated orally for 6 months with fluvastatin (2 mg/kg/day, p.o) starting one week after streptozotocin injection (55 mg/kg, i.p.), (preventive study). In brain, heart, liver, pancreas and kidney homogenates malondialdehyde, lipid hydroperoxide, protein carbonyl content, advanced oxidation protein products, 3-nitrotyrosine levels and superoxide dismutase, catalase activities were measured. Hyperglycemia and dyslipidemia in diabetic groups remained unchanged after fluvastatin treatment. The drug act as antioxidant in the tissues. Hence, antioxidant property of fluvastatin, independent of cholesterol lowering effect, may play a role in prevention of diabetic complications. Clinical relevance of this effect of fluvastatin seems worthy of further studies.

1. Introduction

Over the past decade, there has been substantial interest in oxidative stress and its potential role in development of diabetic complications. Diabetes mellitus, in left untreated, initiate degenerative processes in tissues because of excess oxidative stress. In diabetes and its complications, oxidative stress result from an overproduction of reactive oxygen/nitrogen species generated by glucose autoxidation, mitochondria dysfunction, polyol pathway and protein glycation, and from decreased antioxidant defenses (Valko et al., 2007; Negre-Salvayre et al., 2009). One of the main goals of treatment of diabetes is to prevent its complications and there is accumulating evidence that supplementation antioxidant compounds may offer some protection against diabetic complications (Maritim et al., 2003; Da Ros et al., 2004; Ceriello 2006; Juranek et al., 2010; Cumaoglu et al., 2010; Sivitz and Yörek, 2010).

Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, are widely used in clinical practice for their cholesterol lowering effect and in reducing morbidity and mortality from cardiovascular disease. In recent years, it becomes clear that all the clinical benefits of statins therapy can not be explained solely by their lipid lowering properties because a variety of experimental data revealed that these drugs have direct antioxidant/anti-inflammatory effects that were unrelated to the lipid lowering effects (Rikitake et al., 2001; Stoll et al., 2004; Zhou and Liao, 2010). These beneficial cholesterol-independent or pleiotropic effects of statins likely contribute to their clinical efficacy in treating cardiovascular disease as well as other chronic conditions associated with increased oxidative stress. Many of these pleiotropic effects are mediated through inhibition of isoprenoid synthesis with subsequent inhibition of isoprenoid-mediated activation of small GTP-binding proteins, such as Rho family members, Rac1 and RhoA. Rac1 binds to and leads to activation of the NADPH oxidase system and subsequent generation of reactive oxygen species in many cells (Zhou and Liao, 2010). Fluvastatin is one of the frequently prescribed statins worldwide and has a structure similar to alpha-tocopherol, a natural antioxidant and, its major metabolites have also a direct scavenging activity on

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hydroxyl radicals and superoxide anions (Suzumura et al., 1999a,b; Nakashima et al., 2001a,b; Guan et al., 2004). The direct inhibitory effect on LDL oxidation has also been reported (Suzumura et al., 1999a,b; Rikitake et al., 2001; Nakashima et al., 2001b; Kaneko et al., 2003). Thus, the antioxidative effect of fluvastatin causes by not only inhibition of NADPH oxidase activity, but also the scavenging action of reactive oxygen species. Although a large number of groups have investigated the antihiperlipidemic effect of fluvastatin, its effect on oxidative stress have been examined very little in diabetic tissues. Considering the importance of oxidative stress in the pathophysiology of diabetic complications, in this study, using markers of oxidative stress, we investigated the effects of non cholesterol lowering dose, long term fluvastatin treatment on lipid peroxidation, protein oxidation and antioxidant defense enzymes and low molecular weight antioxidant molecules in brain, heart, liver, pancreas and kidney tissues from streptozotocin-diabetic rats.

2. Materials and methods

2.1. Animals and treatment

Diabetes was induced in male Wistar rats weighing 250–300 g by single dose i.p. injection of 55 mg/kg of streptozotocin (n: 12). Streptozotocin freshly dissolved in 0.1 M sodium citrate buffer (pH 4.5). Control rats (n:12) were only received vehicle. Blood samples were taken from the tail vein, glucose levels were determined using Accu-Chek Go© glucometer (Roche Diagnostics, Germany) and the rats with blood glucose levels >250 mg/dl at one week after streptozotocin injection were considered diabetic. Then, fluvastatin was administered to the diabetic (n:6) and control (n:6) rats by gavage at a dose of 2 mg/kg body weight daily for 6 months (preventive study). The dose of fluvastatin choosen to not affect cholesterol levels, based on previously published studies (Rikitake et al., 2001; Sumi et al., 2001; Mitani et al., 2003). Fluvastatin sodium (Novartis Pharma, Basel, Switzerland) dissolved in water. The animals were supplied from Ankara University, Faculty of Pharmacy, Animal Care Unit, were kept in temperature controlled facilities on 12-h light/dark cycle, standart rat chow and top water were provided *ad libidum* throught the study. The animal use protocol was approved by Ankara University Animal Care Ethic Committee. The principles of laboratory animals care (NIH publication No. 85-23, revised 1985) were observed.

2.2. Blood and tissue analyses

At the end of the treatment period, blood glucose levels were determined using glucometer, then the rats were anaesthetised with ketamine (100 mg/kg), blood samples were collected from left ventricule by cardiac puncture and plasma samples were obtained. Cholesterol and triglyceride concentrations in plasma were determined using analyser (Roche Diagnostic). Brain, heart, liver, pancreas and kidney were removed, washed with ice-cold physiological saline solution, dried on filter paper and stored in −70 °C. Oxidative stress parameters were quantified as follows:

Malondialdehyde, an end product of unsaturated fatty acid peroxidation, can react with thiobarbituric acid to form a colored complex called thiobarbituric acid reactive substances. Thiobarbituric acid reactivity was assayed by the method of Uchiyama and Mihara (1978). Butanol phase was measured with microplate reader at 532 nm. The results were expressed with tetramethoxypropane standart curve within the range from 0 to 20 nmol.

Lipid hydroperoxide levels were determined according to the method of ferrous oxidation with xylenol orange (Zadeh-Nourooz et al., 1994). Homogenates were transferred into microcentrifuge tubes. 10 mM triphenylphosphine was added to vials to remove hydroperoxides. Methanol alone was added to the remaining vials. The lipid hydroperoxide content in the homogenates were determined as a function of the absorbance difference of samples with and without elimination of lipid hydroperoxides by triphenylphosphine. FOX2 reagent was added into samples. After incubation at room temperature for 30 min, the samples were centrifuged and supernatant was carefully placed into well plate. A Bio-Tek ELX800 absorbance microplate reader (Bio-Tek Instruments Inc., USA) was used to measure the absorbance at a wavelength 560 nm. Concentration of lipid hydroperoxide was calculated by using the extinction coefficient of blue-purple complex, $1.5\times10^{4}\ M^{-1}\ cm^{-1}$.

Protein carbonyl content was measured in homogenates according to the method of Levine et al. (1990). Protein carbonyl groups react with 2,4-dinitrophenylhydrazine to generate chromophoric dinitrophenylhydrazones. 800 µl volume of 2,4-dinitrophenylhydrazine was added to the sample tube and 800 µl of 2.5 M HCl to the blank tube. After 1 h incubation, 1 ml tricloroacetic acid was added for precipitation of proteins. Both tubes were centrifuged again to remove debris and placed in the well plate. A plate reader was used to measure the absorbance at a wavelength between 340 and 370 nm. Concentration of protein carbonyl was calculated by using the extinction coefficient of 2,4-dinitrophenylhydrazine, $22\times10^{4-1}cm^{-1}$.

3-nitrotyrosine levels were measured by a commercially available ELISA kit (HyCult Biotechnology, Uden, NL). Tissue samples were homogenized (1:10, w/v) in ice-cold phosphate buffer (pH: 7.4) complete protease inhibitor cocktail using a glass teflon homogenizer.

Advanced oxidation protein products with characteristic absorbance were based on spectrophotometric detection according to Witko-Sarsat et al. (1996). Tissue homogenates diluted 1:10 with phosphate buffered saline, and only phosphate buffered saline as blank were applied on a microtiter plate. 10 µl of 1.16 M Kl and 20 µl of acetic acid were added, and absorbance at 340 was measured immediately. Concentration of advanced oxidation protein products were calculated by using the extinction coefficent of 261 $mM^{-1}\ cm^{-1}$.

Glutathione levels in homogenates were measured by a commercially available assay kit (Cayman, Ann Arbor, MI, USA). This kit utilises an enzymatic recycling method based on the reaction between glutathione and 5.5′-dithiobis-2-nitrobenzoic acid that produces a yellow colored compound 5-thio-2-nitrobenzoic acid. The rate of 5-thio-2-nitrobenzoic acid production is directly proportional to the concentration of glutathione in the sample. Measurement of the absorbance of 5-thio-2-nitrobenzoic acid at 412 nm provides an accurate estimation of glutathione in the sample. Due to the presence of glutathione reductase, which reduces the disulphide dimer of glutathione to glutathione, in the reaction buffer, both glutathione and disulphide dimer of glutathione are measured and the assay reflects total glutathione present in the sample.

Superoxide dismutase activity was assayed using nitro blue tetrazolium by a previously reported method of Sun et al. (1988). The samples were subjected to ethanol–chloroform (5:3) extraction before the enzyme activity assay. The nitro blue tetrazolium was reduced to blue formazan by O_2^-, which has a strong absorbance at 560 nm. The calculated superoxide dismutase activity was expressed as U/mg of protein in the tissue.

Catalase activity in homogenates was assayed following the procedure of Aebi (1984). The molar extinction coefficient of 43.6 M/cm was used to determine catalase activity. One unit of activity is equal to the moles of H_2O_2 degraded/min per mg protein. Total protein concentration in the homogenates were determined by the Bradford (1976) method using bovine serum albumin as a standard.

2.3. Statistical analyses

The reported datas are means of measurements with their standart error of means (S.E.M) values. ANOVA test was used for comparisons of the groups. Student-Newman-Keul's test was used as the post hoc analyses. A values of $P<0.05$ was set as the limit of statistical significance.

Table 1
General charactheristics of rat groups.

	Untreated control	Fluvastatin treated control	Untreated diabetic	Fluvastatin treated diabetic
Body weight (g)	352 ± 20	342 ± 12	240 ± 25*	250 ± 28*
Blood glucose (mg/dl)	122 ± 10	129 ± 13	543 ± 32*	555 ± 27*
Total cholesterol (mg/dl)	48 ± 5	51 ± 3	65 ± 3*	60 ± 3*
Triglyceride (mg/dl)	55 ± 4	51 ± 3	80 ± 6*	81 ± 7*

Values are mean ± S.E.M, $n = 6$ for each group. *$p < 0.05$ *vs.* untreated controls.

3. Results

3.1. Basic parameters

Significant weight reduction, elevated blood glucose, triglyceride and total cholesterol levels were observed in rats following period of diabetes. Fluvastatin treatment did not affect the general parameters in both control and diabetic rats (Table 1).

3.2. Oxidative/nitrosative stress markers

Oxidative/nitrosative stress markers measured in all group are given in Table 2.

Thiobarbituric acid reactive substances and lipid hydroperoxide levels were significantly higher and glutathione levels were significantly lower in diabetic brain tissue, when compared with those of control group. Fluvastatin treatment prevented the alterations on thiobarbituric acid reactive substances and glutathione levels in diabetic brain tissue homogenates, but no significant differences were detected in the other measured parameters.

When compared with control group, thiobarbituric acid reactive substances, lipid hydroperoxide, protein carbonyl content, 3-nitrotyrosine levels and catalase activity were significantly increased and glutathione levels were significantly decreased in heart homogenates of diabetic rats. Fluvastatin treatment did not affect catalase activity and glutathione levels, but significantly attenuated thiobarbituric acid reactive substances, lipid hydroperoxide, protein carbonyl content, 3-nitrotyrosine levels after treatment of diabetic animals.

In diabetic liver homogenates, superoxide dismutase, thiobarbituric acid reactive substances, protein carbonyl content, advanced oxidation protein products, 3-nitrotyrosine levels were significantly higher when compared with those of control group. Fluvastatin treatment was only normalized superoxide dismutase activity.

In diabetic pancreas homogenates we found increased superoxide dismutase and catalase activities, thiobarbituric acid reactive substances, protein carbonyl content and 3-nitrotyrosine levels. Fluvastatin treatment did not change superoxide dismutase activity, but

Table 2
Effects of fluvastatin treatment on oxidative stress and antioxidant defense system in tissues of rat groups.

		Untreated control	Fluvastatin treated control	Untreated diabetic	Fluvastatin treated diabetic
Brain	TBARS	2.79 ± 0.27	3.55 ± 0.45	4.98 ± 0.81*	3.25 ± 0.22#
	LHP	5.39 ± 0.93	4.79 ± 0.20	8.96 ± 1.21*	6.17 ± 1.60#
	PCC	8.41 ± 0.69	10.74 ± 0.75	10.09 ± 0.93	9.14 ± 0.60
	AOPPs	1.68 ± 0.17	1.92 ± 0.17	1.78 ± 0.10	1.79 ± 0.11
	3-NT	51.04 ± 4.73	53.31 ± 5.62	51.16 ± 2.26	46.44 ± 1.77
	SOD	0.14 ± 0.02	0.17 ± 0.01	0.16 ± 0.006	0.15 ± 0.01
	CAT	2.85 ± 0.23	2.91 ± 0.18	2.74 ± 0.15	3.01 ± 0.22
	GSH	8.45 ± 0.27	7.17 ± 0.51	6.17 ± 0.31*	8.44 ± 0.27#
Heart	TBARS	1.80 ± 0.20	2.50 ± 0.42	3.10 ± 0.35*	2.12 ± 0.18#
	LHP	9.32 ± 0.33	9.04 ± 0.58	15.71 ± 1.23*	9.73 ± 0.80#
	PCC	12.48 ± 1.31	14.42 ± 2.73	19.75 ± 1.98*	16.12 ± 1.20*
	AOPPs	3.78 ± 0.27	3.73 ± 0.31	4.41 ± 0.33	4.20 ± 0.37
	3-NT	82.13 ± 2.81	73.56 ± 6.05	96.66 ± 4.21*	60.98 ± 3.28*, #
	SOD	0.075 ± 0.01	0.077 ± 0.011	0.089 ± 0.005	0.089 ± 0.005
	CAT	24.58 ± 2.80	36.23 ± 1.95	40.45 ± 2.38*	39.93 ± 1.36*, #
	GSH	18.77 ± 1.30	16.26 ± 0.95	14.00 ± 1.01*	16.13 ± 0.80
Liver	TBARS	0.85 ± 0.03	0.88 ± 0.11	1.29 ± 0.05*	1.08 ± 0.19
	LHP	4.39 ± 0.37	5.31 ± 0.96	4.89 ± 0.16	4.61 ± 0.4
	PCC	10.01 ± 0.80	9.80 ± 1.08	14.21 ± 1.50*	9.05 ± 1.33
	AOPPs	2.25 ± 0.24	2.50 ± 0.23	3.55 ± 0.29*	3.23 ± 0.37
	3-NT	74.45 ± 4.04	85.05 ± 3.90	100.35 ± 5.16*	78.49 ± 5.20#
	SOD	0.13 ± 0.01	0.18 ± 0.005	0.16 ± 0.009*	0.15 ± 0.003#
	CAT	1093 ± 70	1216 ± 44	1251 ± 60	1196 ± 64
	GSH	24.90 ± 2.06	24.19 ± 0.98	22.08 ± 1.97	25.01 ± 1.86
Pancreas	TBARS	0.48 ± 0.029	0.38 ± 0.039	0.70 ± 0.083*	0.47 ± 0.033#
	LHP	4.71 ± 1.66	3.61 ± 0.72	5.80 ± 0.62	5.80 ± 0.62
	PCC	23.07 ± 3.34	31.18 ± 6.19	35.90 ± 2.67*	29.93 ± 1.41#
	AOPPs	2.12 ± 0.21	2.62 ± 0.69	2.48 ± 0.23	2.53 ± 0.51
	3-NT	83.38 ± 8.99	101.73 ± 8.46*	121.21 ± 11.95*	74.13 ± 4.13#
	SOD	0.098 ± 0.022	0.152 ± 0.020	0.178 ± 0.020*	0.181 ± 0.021*
	CAT	312.87 ± 24.66	306.64 ± 13.73	436.57 ± 28.34*	339.62 ± 12.11#
	GSH	10.19 ± 1.01	9.57 ± 0.77	9.19 ± 0.69	11.62 ± 0.42
Kidney	TBARS	1.65 ± 0.22	1.99 ± 0.40	2.25 ± 0.19*	1.71 ± 0.19#
	LHP	9.40 ± 0.18	12.06 ± 2.47	11.10 ± 1.48	8.38 ± 0.78
	PCC	10.25 ± 0.60	12.45 ± 0.79	14.72 ± 0.43*	11.47 ± 1.10#
	AOPPs	2.65 ± 0.24	2.65 ± 0.16	3.47 ± 0.23	2.96 ± 0.34
	3-NT	139.24 ± 2.13	146.31 ± 7.08	162.17 ± 5.94	140.44 ± 4.96
	SOD	0.106 ± 0.006	0.114 ± 0.009	0.144 ± 0.036	0.130 ± 0.087
	CAT	917.28 ± 64.27	952.48 ± 92.84	792.67 ± 42.81	888.06 ± 24.26
	GSH	19.73 ± 0.46	19.77 ± 1.92	16.28 ± 1.33*	16.36 ± 0.62

Thiobarbituric Acid Reactive Substance (TBARS: nmol/mg protein); Lipid Hydroperoxide (LHP: nmol/mg protein); Protein Carbonyl Content (PCC: nmol/mg protein); Advanced Oxidation Protein Products (AOPP:, nmol/mg protein); 3-Nitrotyrosine(3-NT: pmol/mg protein); Superoxide Dismutase activity (SOD: IU/mg protein); Catalase activity (CAT: IU/mg protein); Glutathione levels (GSH: nmol/mg protein).
$n = 6$ for each group. Values are mean ± S.E.M.
* $p < 0.05$ vs. untreated controls, # $p < 0.05$ vs. untreated diabetic.

significantly prevented the alterations on thiobarbituric acid reactive substances, protein carbonyl content, 3-nitrotyrosine levels and catalase activity in diabetic group. Interestingly, 3-nitrotyrosine levels were increased significantly in pancreas homogenates from control rats after the treatment.

Finally, in diabetic kidney tissue homogenates, we found significantly increased 3-nitrotyrosine, protein carbonyl content and thiobarbituric acid reactive substances, and significantly decreased glutathione levels when compared with those of control group. Treatment with fluvastatin prevented augmentations on thiobarbituric acid reactive substances, protein carbonyl content and 3-nitrotyrosine levels. We determined diversified antioxidant enzyme activities in diabetic kidney tissue homogenates, but the differences did not reach to statistical significance.

4. Discussion

Streptozotocin-diabetic rat model is a prototype of type 1 diabetes mellitus with an intense oxidative stress due to hyperglycemia (Kakkar et al., 1995, 1998). Fluvastatin dosage used in the present study did not significantly affect the cholesterol levels, as expected, allowing us to investigate *in vivo* direct antioxidant effect of the drug. Thus, our results indicate that the lipid lowering effect did not contribute to the antioxidant effect of fluvastatin in the tissues. Chronic hyperglycemia promotes endogen free radical generation and deplete antioxidant defense systems. Free radicals play an important role as endogenous initiators and promoters of lipid and protein oxidation that contribute to diabetes and its complications. Thus, uncontrolled diabetes leads to diabetic complications including cardiovascular disease, nephropathy, neuropathy and retinopathy (West, 2000; Ceriello, 2006). Antioxidant effects of fluvastatin, independent of its hypolipidemic effect, have already been demonstrated in several hypercholesterolemic rabbits (Rikitake et al., 2001; Sumi et al., 2001; Yamaguchi et al., 2002; Mitani et al., 2003), reactive oxygen species-generated rat model (Bandoh et al., 2003), myocardial infarction rat model (Zhou et al., 2008), hyperhomosysteinemic rat (Morita et al., 2005), oxygen-induced retinophatic mice (Bartoli et al., 2009), and recently, in streptozotocin-diabetic rat model (Matsuki et al., 2010). The molecular basis of antioxidant/anti-inflammatory effects of statins relate to their ability block the production and/or activity of reactive oxygen species. Statins have been shown to block the isoprenylation (geranylgeranylation) and activation of members of the Rho family, such as RhoA and Rac1. Rac1 regulates NADPH oxidase, which is a major source of reactive oxygen species in cells. Studies have shown that statins attenuate oxidative stress through inhibition of Rac1 (Stoll et al., 2004; Davignon, 2004). It has been reported that treatment of 5 mg/kg/day (noncholesterol lowering dose) fluvastatin decreases protein kinase C activator-dependent reactive oxygen species generation in rat peritoneal neutrophils and this effect was reversed by the combined administration with mevolanate. This indicates that fluvastatin inhibits reactive oxygen species generation via the inhibition of isoprenylation (geranylgeranylation) and the HMG-CoA reductase and the downstream mevalonate pathway even at non cholesterol lowering doses (Bandoh et al., 2003). Importantly, fluvastatin can inhibit angiotensin II-induced vascular reactive oxygen species production and inflammation at 3 mg/kg/day in atherosclerotic mouse model. Further, low dose fluvastatin actes synergistically with an angiotensin AT_1 receptor blocker on the inhibitions (Li et al., 2004). On the other hand, there are several reports that fluvastatin supresses lipid peroxidation by scavenging reactive oxygen species *in vivo* and *in vitro* (Bandoh et al., 2003). Thus, the antioxidative effect of fluvastatin is not only by inhibition of reactive oxygen species generation, but also by the scavenging action of the radicals. Fluvastatin has chemical structure similar to alpha-tocopherol (Nakashima et al., 2001a,b), its metabolites also have scavenging activity against reactive oxygen species (Suzumura et al., 1999a,b). Statins can also up-regulate antioxidant enzymes but the studies are few in number and not entirely consistent (Davignon, 2004; Stoll et al., 2004).

Fluvastatin, is synthetic, relatively hydrophilic, compared with the semisynthetic inhibitors. Brain perfusion studies in rats indicated that small but measurable brain uptake occurres (Guillot et al., 1993). Thus, the drug has been studied little for it's central effects. For ex., at 4 mg/kg/day dosage causes significant remodeling of the basilar artery in hypertensive rats (Ledingham and Laverty, 2002), at 7.5 mg/kg dosage alters psychomotor performance in rats (Baytan et al., 2006). Cerebral infarction and hemorrhage are more common in diabetic patients, and the central complications of hyperglycemia include the potentiation of neuronal damage. It has been reported that superoxide dismutase, but not catalase activity, was reduced in the brain from 4-week streptozotocin-diabetic rat (Nazaroglu et al., 2009). In our previous study, superoxide dismutase activity of brain was unchanged by the long term diabetes and the lipid peroxidation was increased (Ulusu et al., 2003). The antioxidant enzyme activity is tissue dependent and varies from tissue to tissue and that the duration and severity of diabetes are major contributing factors for the alterations. Although largely unknown, the alterations is also related to the level of reactive oxygen species and/or location of the enzymes (Gumieniczek et al., 2002; Ulusu et al., 2003). In the present study we did not detect any significant changes on catalase and superoxide dismutase activities in diabetic brain tissue. But thiobarbituric acid reactive substances and lipid hydroperoxide levels were significantly higher and glutathione levels were lower in diabetic group compared to control group. Fluvastatin treatment prevented these alterations. In addition to the vascular beneficial pleiotropic effects of statins (improving endothelial function, attenuating vascular remodeling, stabilizing atherosclerotic plaques), there are increasing data to suggest that these agents have neuroprotective effects with antioxidant and anti-inflammatory properties. Studies are going in this area for the potential role of statins in treating stroke and neurological diseases (Reiss and Wirkowski, 2007; Zhou and Liao, 2010).

Thiobarbituric acid reactive substances, lipid hydroperoxide, protein carbonyl content, 3-nitrotyrosine levels and catalase activity were significantly higher and glutathione levels were lower in heart of diabetic rats. Fluvastatin treatment did not affect glutathione levels, but significantly attenuated the other measured parameters. In a recent study, fluvastatin, at non cholesterol reducing doses (5 and 10 mg/kg) has been found to be effective on the protection of heart against isoproterenol-induced myocardial infarction through maintaining endogenous antioxidant enzyme activities (Zhou et al., 2008). Obata et al. (2009) tested the effect of 5 mg/kg/day fluvastatin treatment in rat and they found that fluvastatin scavenges hydroxil radical and block the LDL oxidation in heart perfusion study. Cardiac hypertrophy is mediated, in part, by myocardial oxidative stress, and Rac is an important mediator of this pathology (Zhou and Liao, 2010). Thus, Rac1 activity and reactive oxygen species generation can be attenuated by fluvastatin. In a recent study, 10 mg/kg/day of fluvastatin treatment for 2 weeks significantly reduced the myocardial levels of NADPH oxidase subunit $p22^{phox}$ mRNA expression in streptozotocin-diabetic rat (Matsuki et al., 2010). Hence, fluvastatin can prevent diabetic cardiomyopathy by its antioxidative property.

In diabetic kidney, fluvastatin treatment prevented the alterations on thiobarbituric acid reactive substances, protein carbonyl content and 3-nitrotyrosine levels. Antioxidant enzyme activities did not change significantly. Previously, in rat kidney damage model, podocyte damage and macrophage infiltration were found to be reduced by six week-fluvastatin treatment at 5 mg/kg/day dosage (McKenney, 2003). Fluvastatin also prevents the oxidative DNA damage in kidney and liver of streptozotocin-diabetic mice (Imaeda et al., 2002). Peripheral artery disease and end-stage renal disease are an important diabetic complications and fluvastatin seems to be effective in this tissue. In

clinical trials renoprotective effects of statins are uncertain because of relatively sparse data (Strippoli et al., 2008).

Pancreatic damage is also important point in the development of diabetes and its complications. Fluvastatin treatment prevented the alterations in pancreas of diabetic group, except on superoxide dismutase activity. Elevation in thiobarbituric acid reactive substances in the oxidative damage model of hamster pancreas was also suppressed by low dose fluvastatin administration (Kaneko et al., 2003). Thus, fluvastatin appears to be effective in preventing oxidative stress and damage in diabetic pancreas. Interestingly, we found that 3-nitrotyrosine levels were increased in pancreas of fluvastatin treated control rats. This unexpected augmentation seems to be specific for this tissue and the reason needs to be clarified. Experimental streptozotocin-model is well-established model, however, it should be taken into account that this insulinopenic model is associated with extremly high blood glucose levels, which are not countered in humans. Interestingly, in a recent meta-analysis it was reported that statin therapy was associated with a small, but significant, increased risk of diabetes. The mechanisms are not understood. However, the risk might be affected by the amount of drug-induced cholesterol reduction and/or high doses (Sattar et al., 2010). On the contrary, a few clinical studies have indicated that use of statins can delay the progression of diabetes. Though the mechanism underlying this phenomenon is still elusive, but elevated serum cholesterol can impair insulin secretion and increase the rate of apoptosis in beta cells (Qian et al., 2010). In our intensive oxidative stress model chronic fluvastatin treatment, at non cholesterol reducing dosage, did not affect the blood glucose levels.

In diabetic liver homogenates, fluvastatin treatment only normalized superoxide dismutase activity. In rat liver microsomes, it has been shown that fluvastatin scavenges reactive oxygen species and inhibits lipid peroxidation (Yamamoto et al., 1998).

The clinical dosage of fluvastatin for human adults is usually 20–30 mg/day, which corresponds to a dose per g body weight of 0.33–0.50 mg/kg, given to a patient with an average body weight of 60 kg (Kaneko et al., 2003). Thus, the dosage of fluvastatin used in our study is closer, but not comparable (higher) to usual dosage used clinically. The peculiarities specific to lipid metabolism in rodents make these drugs to use high dosage, but, experiments on rodents is suitable for examining the extra-lipid effect of statins. For ex., Rikitake et al. (2001) reported that fluvastatin at the dose of 2 mg/kg/day did not reduce plasma lipids in their study, although 12.5–50 mg/kg/day dosage has been shown to effectively reduce plasma lipids in hyperlipidemic rabbits (Kurokawa et al., 1995).

5. Conclusions

The antioxidative pleiotropic action of fluvastatin might contribute to its beneficial effects, by slowing organ damage in diabetes. But, the differences between rodent and human lipoprotein metabolism make a direct translation of our findings, with the dose of fluvastatin used in this study, to humans impossible. Therefore, further studies are necessary to prove whether our results can be translated to humans with diabetes. If so, addition of early, non cholesterol lowering dose of fluvastatin, irrespective of the plasma cholesterol levels, in conventional antidiabetic regime may prevent diabetic complications. Further, fluvastatin deserves special attention which oxidative stress play a major role in the ethiology of a wide variety of chronic diseases including central nervous system, and comparable dose is must be nonlipid lowering dose.

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