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Antioxidant Effects of Statins in Patients with Atherosclerotic Cerebrovascular Disease

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Background and Purpose Oxidative stress is involved in the pathophysiological mechanisms of stroke (e.g., atherosclerosis) and brain injury after ischemic stroke. Statins, which inhibit 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, have both pleiotropic and low-density lipoprotein (LDL)-lowering properties. Recent trials have shown that high-dose statins reduce the risk of cerebrovascular events. However, there is a paucity of data regarding the changes in the oxidative stress markers in patients with atherosclerotic stroke after statin use. This study evaluated changes in oxidative stress markers after short-term use of a high-dose statin in patients with atherosclerotic stroke.

Methods Rosuvastatin was administered at a dose of 20 mg/day to 99 patients who had suffered an atherosclerotic stroke and no prior statin use. Blood samples were collected before and 1 month after dosing, and the serum levels of four oxidative stress markers−malondialdehyde (MDA), oxidized LDL (oxLDL), protein carbonyl content (PCO), and 8-hydroxy-2'-deoxyguanosine (8-OHdG)−were evaluated to determine the oxidation of MDA and lipids, proteins, and DNA, respectively, at both of those time points.

Results The baseline levels and the degrees of reduction after statin use differed among the oxidative stress markers measured. MDA and PCO levels were associated with infarct volumes on diffusion-weighted imaging (r=0.551, p<0.05, and r=0.444, p=0.05, respectively). Statin use decreased MDA and oxLDL levels (both p<0.05) but not the PCO or 8-OHdG level. While the reduction in MDA levels after statin use was not associated with changes in cholesterol, that in oxLDL levels was proportional to the reductions in cholesterol (r=0.479, p<0.01), LDL (r=0.459, p<0.01), and apolipoprotein B (r=0.444, p<0.05).

Conclusions The impact of individual oxidative stress markers differs with time after ischemic stroke, suggesting that different oxidative markers reflect different aspects of oxidative stress. In addition, short-term use of a statin exerts antioxidant effects against lipid peroxidation via lipid-lowering-dependent and -independent mechanisms, but not against protein or DNA oxidation in atherosclerotic stroke patients. **J Clin Neurol 2014;10(2):140-147**

Key Words atherosclerosis, ischemic stroke, statin, oxidative stress, cholesterol.

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Introduction

Oxidative stress is involved in the pathophysiological mechanisms of stroke (e.g., atherosclerosis) and brain injury after ischemic stroke (e.g., reperfusion injury).[1] Antioxidant levels and activities have been found to decrease after stroke as a consequence of increased oxidative stress, and thereafter increase gradually over time.[2] It has also been reported that low plasma antioxidant activity is associated with high lesion volume and severe neurological impairment in stroke.[3] Moreover,

the brain is at risk for oxidative damage because it has high oxidative damage potential but a low antioxidant capacity.[4]

Statins, which inhibit HMG-CoA reductase, have both pleiotropic and low-density lipoprotein (LDL)-lowering properties.[5] Two randomized trials−Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) and Justification for the Use of Statin in Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER)−showed that medication with high-dose statins reduces the risk of cerebrovascular events.[6,7] Based on the results of the SPARCL trial, the American Heart Association/American Stroke Association recently recommended the use of statins in patients who had suffered ischemic stroke or transient ischemic attack.[8] In addition, the prior use of statins has been shown to reduce the severity of ischemic stroke and to be associated with better clinical stroke outcome.[9,10] However, the precise mechanisms underlying the effects of statins in this context remain unclear. The improved clinical outcomes observed with statin use may be due to facilitated recanalization, collateral perfusion enhancement, or a direct neuroprotective effect.[11] Statins have also been shown to exert plaque-stabilizing effects, with evidence of the occurrence of atherosclerotic plaque regression and reverse remodeling in medicated patients.[12]

There is a paucity of data regarding the effects of statins on oxidative stress in patients with atherosclerotic stroke. Under the assumption that the short-term use of statins exerts various antioxidant effects in patients with atherosclerotic stroke, a comprehensive study of the antioxidant effects of a statin was conducted using a variety of markers of oxidative stress, from lipids to DNA.

Methods

Patient selection and blood collection

Stroke patients with presumed atherosclerotic stroke and no use of statins prior to the onset of stroke were enrolled prospectively. All patients underwent electrocardiography, echocardiography, and brain magnetic resonance imaging (3.0 tesla; Achieva, Philips Medical Systems, Best, The Netherlands) including diffusion-weighted imaging (DWI) and magnetic resonance angiography of the cervical and intracranial vessels. Briefly, the semiautomatic threshold approach was used to measure the lesion volume on DWI using Medical Image Processing, Analysis and Visualization (MIPAV, version 6.0.1; National Institutes of Health, Bethesda, MD, USA). The categories for the stroke mechanisms were assigned based on a modified Trial of Org 10172 in Acute Stroke Treatment classification system.[13]

In addition to patients with acute ischemic stroke (within 7 days of symptom onset), chronic atherosclerotic stroke patients (after 3 months of symptom onset) were included in this study to preclude the effects of stroke at the level of oxidative stress markers. All human samples were used in accordance with procedures approved by the local institutional review boards. All patients provided written informed consent to participate in the study.

Rosuvastatin was administered at a dose of 20 mg/day after work-ups for stroke and blood sampling. Blood samples were collected using ethylenediaminetetraacetic acid-plasma collection tubes before and 1 month after treatment. Plasma was separated by centrifugation at 3000 rpm and 4ºC for 15 min, and then stored at -70°C until analysis.

Laboratory assays

All blood samples were subjected to comprehensive biochemical assays. Serum concentrations of lipids, high-sensitivity C-reactive protein (hs-CRP), apolipoprotein B (ApoB), and lipoprotein lipase (LPL) were measured by immunoturbidimetry.

Changes in the oxidative stress in the peripheral blood were determined using molecular targets of reactive oxygen species (ROS), which comprise proteins, lipids, and DNA. Four oxidative stress markers were evaluated, covering the oxidation of lipids [oxidized LDL (oxLDL) and malondialdehyde (MDA)], proteins [protein carbonyl content (PCO)], and DNA [8-hydroxy-2'-deoxyguanosine (8-OHdG)]. Plasma MDA, oxLDL, and 8-OHdG levels were determined using commercially available enzyme-linked immunosorbent assay kits according to the manufacturers' protocols (Cell Biolabs, San Diego, CA, USA; Mercodia, Uppsala, Sweden; and Cayman Chemical, Ann Arbor, MI, USA). The level of PCO, a marker of protein oxidation, was determined by 2,4-dinitrophenylhydrazine spectrophotometry, as described previously.[14]

Statistical analysis

The Statistical Package for the Social Sciences (SPSS) 17.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis, and the level of statistical significance was set at $p<0.05$. Except where indicated otherwise, data are expressed as mean±SD values. Statistical analysis consisted of the independent *t*-test for continuous variables, and the chi-squared or Fisher's exact test for categorical variables. In addition, a paired Student's *t*-test was used to compare values between baseline and treatment samples. Pearson's correlation coefficient was used to assess associations between measured parameters.

Results

Baseline characteristics of the patients

The patterns of oxidative stress markers were measured in the plasma from 99 patients who had suffered ischemic stroke (71

in the acute stroke group and 28 in the chronic stroke group) with intracranial (n=73) or extracranial (n=26) atherosclerotic stenosis. Rosuvastatin (20 mg/day) was typically administered for at least 1 month (39.1±15.1 days), starting at 5.4±3.7 days after stroke onset in the acute stroke group and 161.1±97.0 days in the chronic stroke group.

The patients' baseline clinical characteristics and laboratory findings are given in Table 1. Age, gender, and risk-factor profiles did not differ significantly between the groups, with the exception of current smoking, which was more prevalent in the acute stroke group (p=0.01). Furthermore, the initial lipid profile and ApoB levels did not differ significantly between the two groups.

Effects of rosuvastatin on lipid profile and inflammation

Table 2 lists the changes in the lipid profile and inflammatory markers before and after statin treatment. In both stroke groups, the statin dramatically reduced the serum levels of total cholesterol (mean changes from baseline: 34% in acute stroke and 33% in chronic stroke; p<0.01), triglycerides (27% and 22%, p<0.01 and <0.05, respectively), LDL-cholesterol (50% and 51%, p<0.01 for both), and ApoB (51% and 46%, p<0.01 for both). High-density lipoprotein levels significantly increased after treatment in both the acute (9%, p<0.05) and chronic (7%, p<0.05) stroke groups.

Rosuvastatin significantly reduced the LPL levels in both the acute (64%, p<0.01) and chronic (48%, p<0.01) stroke groups.

Table 1. Baseline characteristics of the patients

Variable	Acute stroke (n=71)	Chronic stroke (n=28)	p
Age (years)	66.8±10.7	66.1±11.1	0.76
Male gender, n (%)	46 (64.8)	12 (42.9)	0.05
Risk factors, n (%)			
Hypertension	39 (54.9)	15 (53.6)	0.90
Diabetes	21 (29.6)	4 (14.3)	0.30
Current smoking	25 (35.2)	1 (3.6)	<0.01
Laboratory findings			
Fasting glucose	124.8±41.5	115.2±26.4	0.28
Total cholesterol (mg/dL)	191.2±39.4	188.1±48.3	0.75
Triglyceride (mg/dL)	164.6±84.9	133.0±66.1	0.09
HDL-cholesterol (mg/dL)	43.9±12.4	46.1±8.6	0.41
LDL-cholesterol (mg/dL)	120.1±37.2	121.9±43.3	0.84
ApoB (μg/mL)	95.0±30.9	108.3±39.1	0.21
Inflammatory biomarkers			
hs-CRP (mg/dL), mean (range)	0.09 (0.05–0.23)	0.07 (0.03–0.15)	0.24
LPL (mg/dL)	52.1±15.1	79.9±27.8	0.03

Except where indicated otherwise, data are mean±SD values.
ApoB: apolipoprotein B, HDL: high-density lipoprotein, hs-CRP: high-sensitivity C-reactive protein, LDL: low-density lipoprotein, LPL: lipoprotein lipase, N/A: not assessed.

Table 2. Changes in lipid profiles and inflammatory markers following statin use

Marker	Acute stroke			Chronic stroke		
	Pre-Tx	Post-Tx	p	Pre-Tx	Post-Tx	p
Lipid profile (mg/dL)						
Total cholesterol	191.2±39.4	127.1±25.4	<0.01	188.1±48.3	125.7±27.6	<0.01
Triglycerides	164.6±84.9	120.8±46.3	<0.01	133.0±66.1	103.5±29.5	0.02
HDL-cholesterol	43.9±12.4	43.9±12.5	0.04	46.1±8.6	49.5±13.4	0.02
LDL-cholesterol	120.1±37.2	59.6±20.2	<0.01	121.9±43.3	60.1±17.4	<0.01
ApoB (μg/mL)	95.0±30.9	47.0±14.1	<0.01	108.3±39.1	58.9±10.8	<0.01
Inflammatory markers						
hs-CRP (mg/dL), mean (range)	0.09 (0.05–0.23)	0.07 (0.04–0.14)	0.05	0.07 (0.03–0.15)	0.05 (0.03–0.15)	0.61
LPL (ng/mL)	52.1±15.1	18.7±7.9	<0.01	79.9±27.8	41.3±19.1	<0.01

Except where indicated otherwise, data are mean±SD values.
ApoB: apolipoprotein B, HDL: high-density lipoprotein, hs-CRP: high-sensitivity C-reactive protein, LDL: low-density lipoprotein, LPL: lipoprotein lipase, Post-Tx: posttherapy, Pre-Tx: before therapy.

Although there was a trend toward a reduction in serum hs-CRP levels with statin use in the acute stroke group, it did not reach statistical significance.

Levels of oxidative stress markers in stroke patients

The increases in the serum levels of MDA and PCO were greater in the acute stroke patients than in the chronic stroke patients, while levels of oxLDL and 8-OHdG did not differ significantly between the two groups.

Serum MDA was significantly correlated with the initial DWI lesion volume in the acute stroke group (r=0.551, p<0.05) (Fig. 1A). Serum PCO also increased with increasing DWI lesion volume in the acute stroke patients (r=0.444, p=0.05) (Fig. 1C). Conversely, oxLDL and 8-OHdG levels were not correlated with DWI lesion volume in the acute stroke group (Fig. 1B and D).

Effects of rosuvastatin on oxidative stress

The degree of reduction in oxidative stress markers after statin treatment differed according to the specific marker (Fig. 2). Specifically, levels of MDA decreased in the acute stroke patients (p<0.05) but not in the chronic stroke patients (p=0.49) (Fig. 2A), whereas the levels of oxLDL were decreased in both of the patient groups (p<0.01 for both) (Fig. 2B). Rosuvastatin did not significantly affect PCO levels in either the acute or chronic stroke group (p=0.97 and 0.08, respectively) (Fig. 2C). Plasma levels of 8-OHdG were significantly increased after statin treatment (p<0.01) in the acute stroke group, but tended to increase in the chronic stroke group, although that change was not statistically significant (p=0.89) (Fig. 2D).

The mechanisms underlying the effects of rosuvastatin on oxidative stress markers were explored by assessing the correlations between changes in lipid and oxidative stress markers from the respective pretreatment levels to the posttreatment levels (Fig. 3). The posttreatment reduction of MDA levels was not associated with the observed changes in cholesterol levels

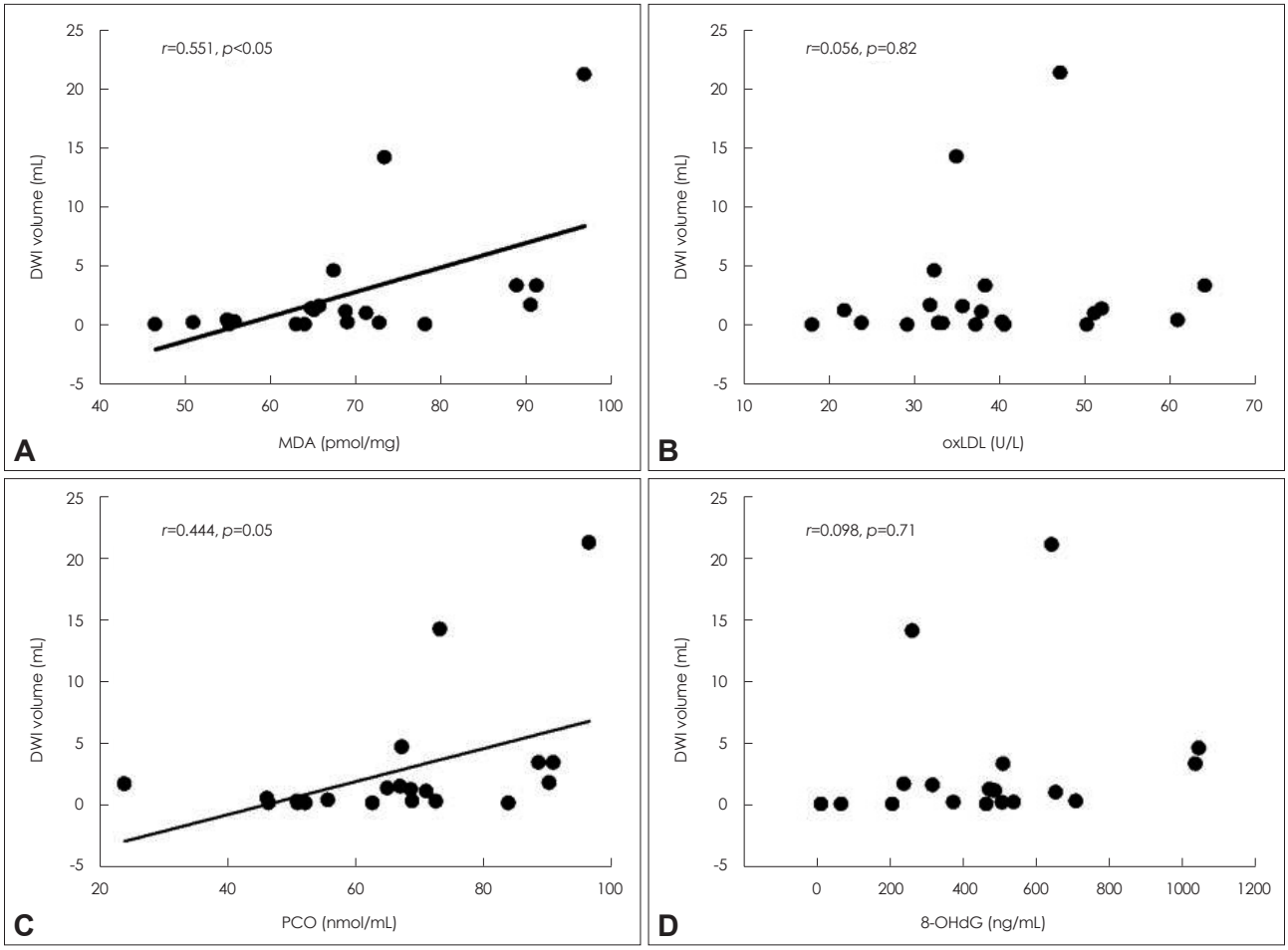

Fig. 1. Correlations between oxidative stress markers and lesion volume on initial DWI in acute stroke patients. Individual values and the linear regression line are displayed. DWI: diffusion-weighted imaging, MDA: malondialdehyde, oxLDL: oxidized low-density lipoprotein, PCO: protein carbonyl content, 8-OHdG: 8-hydroxy-2'-deoxyguanosine.

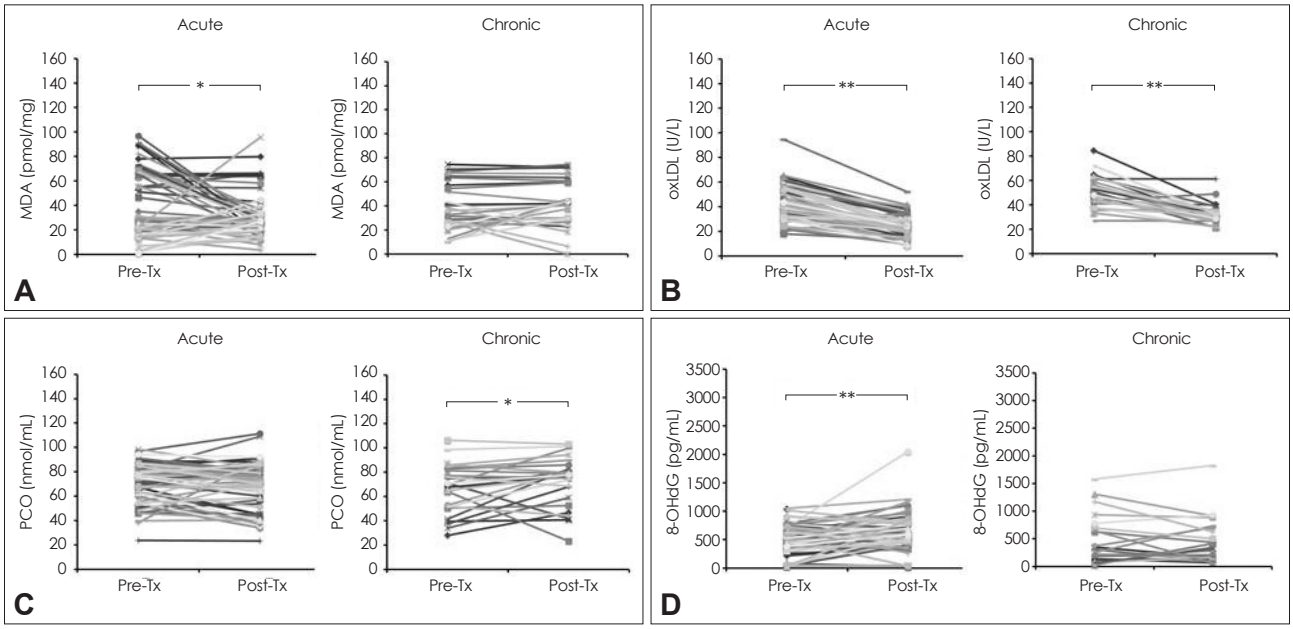

Fig. 2. The changes in oxidative stress markers before and after statin use. A: Rosuvastatin significantly reduced serum MDA levels in the acute stroke group but not in the chronic stroke group. B: There was a marked reduction in serum oxLDL level in both the acute and chronic stroke groups. C: There was no significant change in PCO in either stroke group after statin use. D: Surprisingly, 8-OHdG levels increased more in the acute stroke group than in the chronic stroke group after statin use. *p<0.05, **p<0.01. MDA: malondialdehyde, oxLDL: oxidized low-density lipoprotein, PCO: protein carbonyl content, Post-Tx: posttherapy, Pre-Tx: before therapy, 8-OHdG: 8-hydroxy-2'-deoxyguanosine.

(Fig. 3C and D). Conversely, significant positive correlations were observed between the changes in oxLDL levels and those in total cholesterol (r=0.479, p<0.01), LDL (r=0.459, p<0.01), and ApoB (r=0.444, p<0.05), suggesting a lipid-lowering-dependent reduction in serum oxLDL (Fig. 3A and B). Rosuvastatin had no effect on serum PCO and 8-OHdG levels (data not shown).

Discussion

The major findings of this study were as follows: 1) the levels of circulating oxidative stress markers appear to reflect different aspects of oxidative stress, 2) short-term use of a high-dose statin exerted antioxidant effects against lipid peroxidation but not protein oxidation or DNA damage, and 3) the antioxidant effects of statin against lipid peroxidation occurred via lipid-lowering-dependent and -independent mechanisms.

Reactive oxygen species might play critical roles in brain damage after ischemia and reperfusion by destroying the balance of the redox potential in cells and triggering protein oxidation, lipid peroxidation, and DNA damage.[15-17] ROS are also involved in the pathogenesis of atherosclerosis. Thus, measurement of oxidative stress markers in peripheral blood provides a useful tool for exploring the pathophysiological mechanisms and assessing the effects of antioxidants in stroke patients. However, the findings regarding the circulating oxidative stress markers and the effects of statin on these markers in stroke patients are conflicting. The possible reasons for this are threefold. First, stroke is a heterogeneous condition compared to coronary heart disease. Previous studies have encompassed all major stroke etiological subtypes, including cardioembolic, lacunar, and even hemorrhagic stroke. Second, and more importantly, different oxidative stress markers may be involved in different clinical settings in patients with ischemic stroke (Table 3). Few studies have evaluated the kinship between serum oxidative stress markers and the phase of ischemic stroke (i.e., acute and chronic) using a comprehensive approach.[18] Finally, various types and doses of statin have been studied; the lipid-lowering and non-lipid-lowering (pleiotropic) effects may differ among individual statins, which would explain the differing results between statin trials. Thus, in the present study the antioxidant effects of a high-dose statin were tested using various markers of oxidative stress in atherosclerotic stroke patients.

The results of this study show that the baseline levels and the degrees of reduction observed after statin use differed among the oxidative stress markers measured. Interestingly, there was no correlation between the baseline levels of the four oxidative stress markers and the changes after statin treatment (data not shown). MDA levels were associated with the index of stroke severity (initial DWI lesion volume), whereas oxLDL was not. These findings suggest that these two oxidative stress markers reflect different aspects of oxidative stress. Several studies have demonstrated that acute stroke patients possess high serum lev-

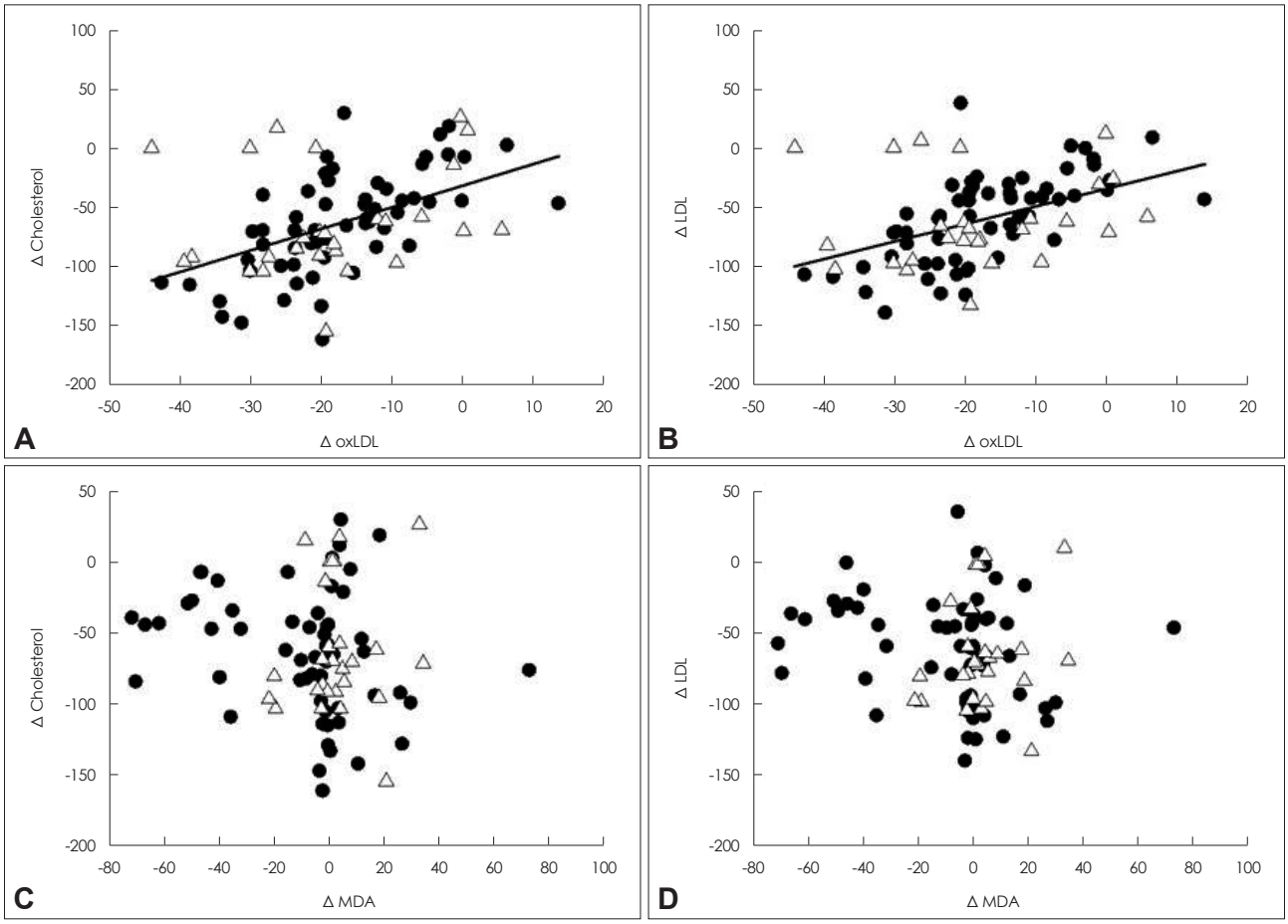

Fig. 3. Correlation between changes in the levels of lipids and markers of oxidative stress. Black circle and white triangle signify acute and chronic stroke patients, respectively. Individual values and linear regression lines are displayed. LDL: low-density lipoprotein, MDA: malondialdehyde, oxLDL: oxidized low-density lipoprotein.

Table 3. Changes in oxidative stress markers of ischemic cerebrovascular disease; a literature review

Marker	Clinical setting		
	Subclinical (atherosclerosis)	Acute stroke	Chronic stroke
MDA		Increased[18-20,30,35,36]	Increased[18,21]
			No change[36]
oxLDL	Increased[22,37,38]	Increased[24]	Increased[21]
	Reduced with statin[38]		
PCO		Increased[18]	No change[18]
		No change[30]	
8-OHdG		Increased[15,* 31,* 32*]	

*Animal model of transient middle cerebral artery occlusion.
MDA: malondialdehyde, oxLDL: oxidized low-density lipoprotein, PCO: protein carbonyl content, 8-OHdG: 8-hydroxy-2'-deoxyguanosine.

els of MDA,[19,20] but few studies have addressed the level of MDA in the chronic stage of stroke, and the obtained results have been controversial.[18,21] On the contrary, oxLDL is reportedly increased in patients with atherosclerosis and is associated with the progression and severity of that condition.[22,23] Nevertheless, few studies have focused on the levels of oxLDL in acute stroke patients.[24]

The present findings also show that statin exerts an antioxidant action against lipid peroxidation via both lipid-lowering-dependent (by reducing oxLDL) and -independent (by reducing MDA) mechanisms. Several studies have indicated that therapy with statins could reduce lipoprotein oxidation and ameliorate free-radical injury. In general, plasma levels of oxLDL are significantly correlated with the total levels of LDL. As expected, statin therapy reduced the circulating oxLDL, an effect that was dependent upon the degree of LDL reduction.[25,26] There was also no significant relationship between the changes in MDA and cholesterol levels. There have been conflicting

results regarding the concordant decrease of MDA and the decrease in cholesterol levels. Specifically, one study showed that with statin therapy MDA levels reduced in parallel with reductions in cholesterol levels,[27] whereas in another study there was no significant decrease in MDA levels after statin use, despite a significant reduction in cholesterol levels.[28] MDA is a highly reactive, three-carbon dialdehyde that is produced as a byproduct of polyunsaturated fatty acid peroxidation.[29] It can thus be assumed that the MDA-lowering mechanism of statin is a byproduct of peroxidation.

The present study found that markers of protein oxidation but not of DNA damage were changed in patients with ischemic stroke. The most widely studied marker of protein oxidation is PCO. High levels of protein carbonyls have been reported in several neurological disorders.[16,18,30] Plasma levels of 8-OHdG, which has been employed as a marker of oxidative DNA damage, were found to be increased in an animal model of ischemic stroke;[15,31,32] however, there have been no studies of the plasma level of 8-OHdG in stroke patients.

Protein carbonyl content and 8-OHdG have been investigated in only a small number of studies. Several preclinical studies demonstrated that statin exerts antioxidant effects by preventing protein oxidation or DNA damage due to scavenging hydroxyl radicals.[33] However, in the present study, statin therapy did not reduce either protein oxidation or oxidative DNA damage in the plasma of stroke patients. Several reports have suggested that proteins and lipids interact during the oxidation process. The presence of protein carbonyl is not necessarily an indication of direct oxidation of amino acid residues in proteins, and may occur via the Michael addition reaction with products of lipid peroxidation.[29] In addition, lipid-dependent carbonylation is inhibited by antioxidants, while lipid-independent protein carbonylation is antioxidant-insensitive.[34] Based on the data obtained in the present study, it appears that statin therapy does not reduce protein carbonylation by lipid-independent direct oxidation. Further studies are needed to establish the antioxidant effects against protein oxidation and DNA damage after long-term statin use.

The present study was subject to some limitations. First, a relatively small cohort from a single center was studied. That being said, the cohort comprised a relatively homogeneous group of patients, all of whom had been diagnosed with atherosclerotic stroke after an extensive work-up for stroke mechanisms, for which the current guidelines recommend the use of a statin.[8] Second, these results represent the short-term effects of statin therapy, and long-term follow-up data are needed. However, stroke recurrence and neuronal damage as a result of oxidative stress occur most frequently soon after an index stroke, suggesting the importance of short-term antioxidant therapy. Finally, a high dose of rosuvastatin was used in the present study because it is well known that intensive statin therapy may further reduce the risk of stroke compared to less-intensive therapy. However, the antioxidant effects may differ among the various statins, and the pleiotropic effects of rosuvastatin are less well known.

In conclusion, the results of this study suggest the need for a comprehensive approach in the study of oxidative stress markers. Short-term use of a high-dose statin has not only lipid-lowering and LDL-dependent antioxidant effects, but also exerts antioxidant effects against lipid peroxidation in an acute setting (e.g., reperfusion injury or acute ischemic injury) via LDL-independent mechanisms. Further studies are needed to establish the long-term antioxidant effects of statins in patients with atherosclerotic stroke.

Conflicts of Interest

The authors have no financial conflicts of interest.

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