Research Article

Effect of Surgical Treatment on Lipid Peroxidation Parameters and Antioxidant Status in the Serum of Patients with Peripheral Arterial Disease

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The various risk factors for peripheral arterial disease (PAD) are almost identical to those for atherosclerosis and include abnormal levels of lipids or lipoproteins. Lipid peroxidation parameters and total antioxidant capacity in the serum of male patients with PAD before surgery as well as 3–5 days and 7–10 days after surgery were measured. We also compared these parameters with those in a group of patients receiving simvastatin therapy. Concentrations of lipid hydroperoxides (LOOHs) and malondialdehyde, the total antioxidant capacity (assessed by ferric reducing antioxidant power assay), concentration of thiol (-SH) groups, and ceruloplasmin activity were determined spectrophotometrically in PAD patients treated surgically (Group I) or pharmacologically (Group II). The patients before surgical treatment had significantly higher concentrations of malondialdehyde but lower ceruloplasmin activity than those observed in Group II, treated with simvastatin. No significant differences before surgery in ferric reducing antioxidant power or thiol concentrations were found between the two groups. However, in Group I, both ferric reducing antioxidant power and thiol group concentrations decreased 3–5 days postoperatively, and ceruloplasmin activity increased 7–10 days after surgical treatment. The presented results demonstrate diverse oxidative stress responses to surgical treatment and confirm the beneficial effects of statin therapy in PAD.

1. Introduction

Peripheral arterial disease (PAD) commonly results from progressive narrowing of arteries in the lower extremities due to atherosclerosis [1]. It has become evident that PAD is an important predictor of substantial coronary and cerebral vascular risk [2, 3]. The most common symptomatic manifestation of mild to moderate atherosclerotic PAD is intermittent claudication [4], observed in ~5% of individuals older than 60 years [5, 6]. Several studies, using the ankle-brachial index, have revealed that the presence of PAD, even when being asymptomatic and in patients with no history of other cardiovascular diseases, is a marker of greatly increased cardiovascular morbidity and mortality [7, 8]. Oxidative stress, through the extra generation of reactive oxygen species (ROS), is also involved in the development and progression of PAD [9].

Moreover, risk factors associated with PAD [10], similar to those for coronary heart disease [11], include high blood concentrations of total cholesterol and LDL-cholesterol. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, called statins, are well-known

lipid-lowering drugs. Statins not only reduce lipid levels, but they also have important pleiotropic effects, improving endothelial function, reducing oxidative stress, affecting the lipid metabolism enzyme activities, and so forth [12–14]. Three main reasons have been suggested for initiating statin therapy for patients with PAD of the lower limbs [15]: (1) prevention of coronary heart disease; (2) treatment of lower extremity peripheral arterial disease; and (3) perioperative treatment of patients with peripheral arterial disease [16].

Although several trials have shown that treatment with statins is associated with a reduction in coronary heart disease events [17], such as stroke [18, 19] and mortality [20–23], most of these trials did not specifically address the treatment of patients with PAD. The aim of the present study, therefore, was to evaluate lipid peroxidation parameters and total antioxidant capacity in the serum of patients with PAD before surgery as well as 3–5 days and 7–10 days after surgery. We also compared these parameters with those in a group of patients receiving simvastatin therapy.

2. Material and Methods

2.1. Experimental Subjects. The study group consisted of PAD patients, treated either surgically (Group I, $n = 35$ males, aged 61 ± 8 years) or pharmacologically (Group II, $n = 18$ males, aged 62 ± 7 years) in the Department of General and Vascular Surgery at the Poznan University of Medical Sciences. Patients included in Group I were admitted for surgical treatment (implantation of an aortobifemoral bypass graft). They were diagnosed by measurement of the ankle brachial index (ABI) and arteriography of the lower limbs, and were clinically stable without any accompanying diseases. Subjects with diabetes were excluded. The patients treated surgically had shown critical ischemia (ABI less than 0.90, rest pain, ulcer or necrosis of the lower limbs, and ankle pressure $\leq$ 50 mmHg). Blood samples were collected without anticoagulant, in the fasting state, before surgery as well as 3–5 days and 7–10 days after surgical treatment. Group II had been receiving statin therapy, with 20 mg of simvastatin daily, for at least 3 months. The study protocol was approved by the Ethics Committee of Poznan University of Medical Sciences, and the subjects were fully informed and gave their written consent.

2.2. Reagents and Apparatus. All the reagents were of analytical grade of purity (Sigma-Aldrich, St. Louis, MO, USA). Spectrophotometric measurements were carried out on SP-8001 UV/Visible Spectrophotometer (Metertech Inc., Taiwan).

2.3. Procedures. The subjects' lipid profile (total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides), glucose, uric acid, apolipoproteins (Apo A-I, Apo B) and fibrinogen concentrations in serum were measured by standard laboratory procedures, using the Roche Cobas 6000 analyzer system, Dade Behring BN II system, and the Sysmex CA-500 Series system. The total antioxidant potential (capacity) of serum was determined by ferric reducing antioxidant power (FRAP) assay according to the Benzie and Strain method [24], based on the reduction of ferric ion Fe(III)

to ferrous ion Fe(II) at a low pH. This method measures the change in absorbance at 593 nm owing to the formation of a blue-colored Fe(II)-tripyridyltriazine complex from the colorless oxidized Fe(III) form, by the action of electron-donating antioxidants.

Lipid peroxidation was measured by estimation of both lipid hydroperoxides (LOOHs) and malondialdehyde (MDA) concentrations by the Sodergren method [25] and Ohkawa et al. method [26], respectively. The concentration of thiol (-SH) groups was determined according to the Hu method [27]. The oxidase activity of ceruloplasminin serum was determined spectrophotometrically according to the Schosinsky method [28]. Ceruloplasmin, in the presence of *o*-dianisidine hydrochloride in an acidic solution (pH 5.0), forms a stable yellow-brownish product. Absorbance was measured at 540 nm and ceruloplasmin activity was expressed in U/L (μmol of oxidized substrate in 1 mL of serum in 1 min).

2.4. Statistical Analysis. The means and standard errors of the mean were calculated. Statistical analysis was performed using Instant GraphPad software. The paired Student's *t*-test (to compare the values before and after surgery), the two-tailed unpaired *t*-test (to compare Group I and Group II), and the *F*-test were used for statistical analysis of the data.

The mean changes (Δ) in lipid peroxidation parameters and antioxidant capacity, that is, 3–5 days (Δ_1) and 7–10 days (Δ_2) after surgery, in relation to the values obtained before surgery, were calculated. Pearson's correlation coefficients r_1 and r_2 were computed for the results found before surgery and the changes observed during treatment (Δ_1, Δ_2). Statistical significance was accepted at the $P < 0.05$ level.

3. Results

As shown in Table 1, generally, the clinical parameters of the two groups of patients included in this study did not differ significantly. However, the concentrations of total cholesterol and LDL cholesterol were found to be significantly higher in Group I before surgery than those in Group II on simvastatin therapy.

Group I patients before surgery had enhanced oxidative stress, as demonstrated by elevated serum levels of MDA, in comparison to those treated pharmacologically. We did not observe any significant decrease in LOOH and MDA concentrations in Group I patients after surgery (Figures 1 and 2).

The total antioxidant capacity (expressed as FRAP) and -SH group concentrations in the serum of patients on statin therapy and in those before surgery were similar (Figures 3 and 4). However, a decrease was observed in FRAP level 3–5 days after surgery, and 7–10 days after surgery, it was significantly lower in comparison to the values before surgery and on simvastatin therapy. Moreover, thiol group concentrations 3–5 days postoperatively were lower than those before surgery and 7–10 days after surgery and were lower than those in patients treated with simvastatin (Figure 4).

A significant difference in the oxidase activity of ceruloplasmin between Group II and Group I before surgery was observed. Its oxidase activity in serum was higher in Group II

Table 1: Clinical characteristics of Group I patients (before surgery) and Group II patients (treated pharmacologically with simvastatin).

Parameter	Group I before surgery	Group II treated with simvastatin	P value
Total cholesterol [mmol/L]	5.9 ± 0.9	5.1 ± 1.1	<0.05
HDL cholesterol [mmol/L]	1.2 ± 0.3	1.3 ± 0.4	ns
LDL cholesterol [mmol/L]	4.2 ± 1.5	3.0 ± 1.1	<0.05
Triacylglycerols [mmol/L]	1.8 ± 1.0	1.6 ± 0.7	ns
Glucose [mmol/L]	6.6 ± 1.9	5.6 ± 0.8	ns
Uric acid [mg/dL]	4.9 ± 1.2	5.4 ± 1.4	ns
Fibrinogen [g/L]	4.4 ± 1.8	4.1 ± 1.1	ns
Apo A-I [g/L]	1.4 ± 0.3	1.5 ± 0.2	ns
Apo B [g/L]	1.0 ± 0.2	1.1 ± 0.2	ns
Apo B/A-I	0.8 ± 0.2	0.7 ± 0.2	ns
C-reactive protein [mg/L]	12.3 ± 13.7	7.5 ± 11.9	ns
Body mass index	25.5 ± 4.3	23.3 ± 3.6	ns
Age [years]	61 ± 8	62 ± 7	ns

HDL: high-density lipoprotein; LDL: low-density lipoprotein; Apo A-I: apolipoprotein A-I; and Apo B: apolipoprotein B.
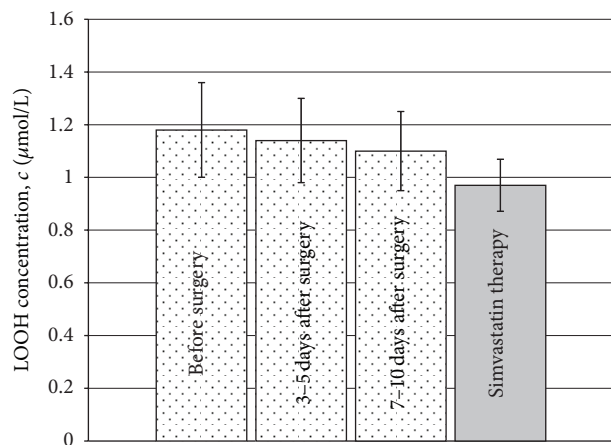

Figure 1: Concentrations of lipid hydroperoxides (LOOHs) in Group I patients before surgery, in the postoperative phase, and on simvastatin therapy.
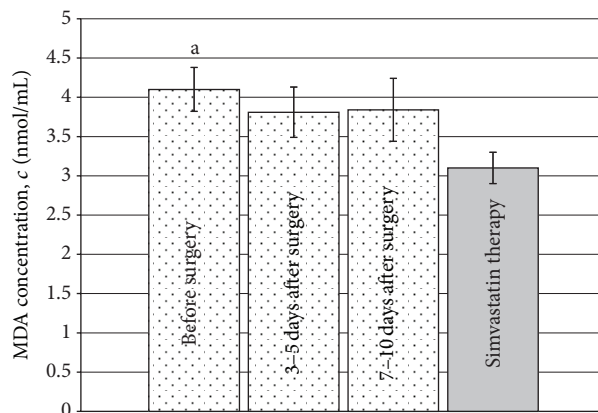

Figure 2: Malondialdehyde (MDA) concentrations in Group I patients before surgery, in the postoperative phase, and on simvastatin therapy. a—versus simvastatin therapy; $P < 0.05$.

and 7–10 days after surgery in Group I, in comparison to the values before and 3–5 days after surgery (Figure 5).

In addition, all the studied parameters (LOOH, MDA, FRAP, -SH groups, and ceruloplasmin) in Group I patients before surgery were significantly correlated with mean changes at 3–5 days (Δ_1) and/or 7–10 days (Δ_2) after surgery (Table 2).

4. Discussion

Oxidized LDL is a biochemical marker of oxidative damage and has atherogenic effects, including the stimulation of foam cell formation and activation of the inflammatory process in the vascular wall [29]. The findings of the present study are broadly consistent with the hypothesis that lipid peroxidation may be one mechanism through which several risk factors may promote PAD.

MDA and LOOH are suitable markers for evaluating oxidative stress. In our study, we observed an increased MDA

Table 2: Pearson's correlation coefficients r_1 and r_2 for the analyzed parameters before surgery versus 3–5 days (Δ_1) and 7–10 days (Δ_2) after surgery, respectively.

Parameter	r_1 (Δ_1)	r_2 (Δ_2)	P value
MDA	-0.575	-0.516	<0.05
LOOH	-0.671	-0.778	<0.05
FRAP	-0.555	-0.637	<0.05
Thiol groups	-0.664	-0.599	<0.05
Ceruloplasmin	n.d.	-0.682	<0.05

MDA: malondialdehyde; LOOHs: lipid hydroperoxides; FRAP: ferric reducing antioxidant power; and n.d.: no difference.

level in the patients before surgery, which suggests that they were in a state of high oxidative stress. At neutral pH, MDA exists as a low reactive enolate anion, but it is still toxic and may damage many biologically important molecules. Determination of the lipid peroxidation product MDA in PAD is important because, as was shown earlier, MDA damages
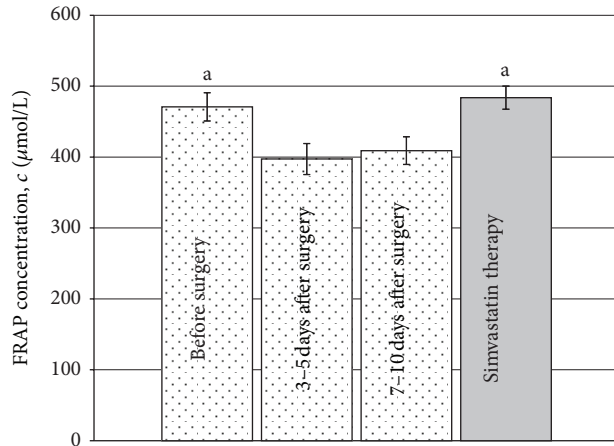
FIGURE 3: Ferric reducing antioxidant power (FRAP) in Group I patients before surgery, in the postoperative phase, and on simvastatin therapy. a—versus 3–5 days and 7–10 days after surgery; $P < 0.05$.
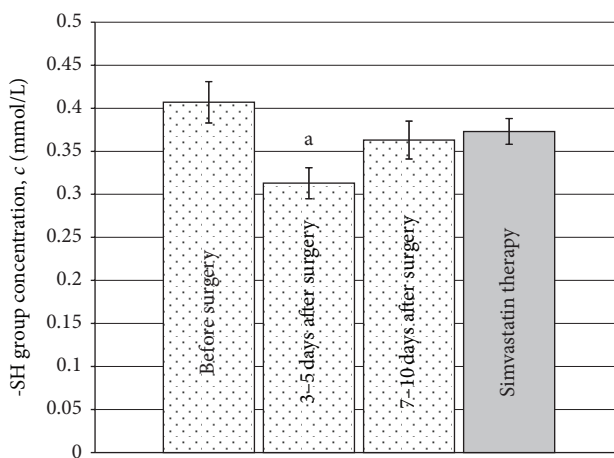

FIGURE 4: Thiol (-SH) group concentrations in Group I patients before surgery, in the postoperative phase, and on simvastatin therapy. a—versus before surgery, 7–10 days after surgery, and simvastatin therapy; $P < 0.05$.

collagen by forming transverse intramolecular bonds that cause rigidity of blood vessels [30]. In addition, modification of native LDL by MDA causes the accumulation of cholesteryl esters within the cell in which the atherosclerotic reaction takes place [31].

Several trials have shown that statin therapy relieves symptoms and improves function in patients with lower extremity PAD [32, 33]. MDA concentrations in the serum of the patients treated with simvastatin were lower than those in Group I patients before surgery. Since PAD patients are usually undertreated with regard to the use of lipid-lowering agents, compared to patients with coronary heart disease, our results provide strong support for the efficacy of statin therapy in PAD.
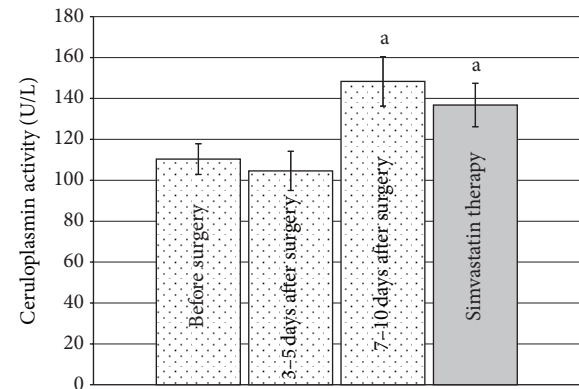

FIGURE 5: Ceruloplasmin activity in Group I patients before surgery, in the postoperative phase, and on simvastatin therapy. a—versus before surgery and 3–5 days after surgery; $P < 0.05$.

Human cells and tissues contain many antioxidant enzyme systems and nonenzymatic antioxidants, which protect against the action of toxic free radicals [34]. The FRAP assay used here is a useful indicator of the body's antioxidant status, which determines the level of non-enzymatic antioxidants, such as uric acid, ascorbic acid, α-tocopherol, and some protein-containing -SH groups.

An increase in FRAP level in the patients before surgical treatment is consistent with similar results obtained by Gawron et al. [35] in a group of patients with coronary heart disease and by Lantos et al. [36] in patients with hypertension. An increased antioxidant capacity of serum seems to be response to an increase in the concentration of lipid peroxidation products.

The reduction in FRAP level observed in our Group I patients 3–5 and 7–10 days after surgery can be partly explained by a decrease in their serum antioxidant status and an increase in serum oxidant levels following surgery, as indicated by the negative correlations found (Table 2). In their study, Girona et al. [37] analyzed the antioxidant effect of simvastatin both in vitro and in vivo. They showed that simvastatin therapy increases the resistance of both LDL and HDL to oxidation. In addition, simvastatin inhibits the ability of activated macrophages to oxidize LDL in vitro in a dose-dependent manner [38]. A similar effect has been shown for other statins, such as atorvastatin [39], which reduces LDL levels and HDL oxidation and protects paraoxonase—an enzyme bound to HDL.

With regard to FRAP in our study, simvastatin therapy (20 mg daily) did not influence the total antioxidant potential. However, Shin et al. [40] found a significant increase in antioxidant ability in their group of hypercholesterolemic patients treated with 40 mg simvastatin daily. They suggested the possibility of a dose-dependent effect of simvastatin on plasma antioxidant status. This could be the reason why we did not detect any effect of simvastatin on FRAP in our study.

Glutathione and other proteins, such as albumin, are an important part of serum antioxidant activity against free radicals. Wayner et al. [41] believe that protein participation makes up to 50% of the antioxidant status. The thiol groups

react with peroxide radicals in the first stage of the oxidation reaction and also act in the lag phase, which is an accepted marker of serum oxidizability. In our group of patients with PAD, the decrease in thiol group concentrations, observed 3–5 days after surgery, followed by an increase 7–10 days after surgery, may be explained by oxidative stress intensification in reperfusion and by the actions of -SH groups, leading to their reduction. Furthermore, a decrease in both lag phase and albumin level has also been reported in the serum of patients with aneurysmal or arterial occlusive disease [42].

Some studies have confirmed that ceruloplasmin is an independent risk factor for cardiovascular disease [43, 44]. Elevated serum ceruloplasmin activity has been reported in patients with atherosclerosis obliterans [45]. Authors of that study suggest that increased ceruloplasmin activity is related to the acute phase response to inflammation and necrosis in atherosclerosis obliterans and results from increased ROS production, mostly of superoxide radicals. Ceruloplasmin contains four free thiol groups [46]. Therefore, the preferential oxidative destruction of thiol groups can explain the greater susceptibility of ceruloplasmin to oxidative stress.

Recent data show that statins may reduce oxidative stress [47] by decreasing the generation of ROS, thereby synergizing with the biological effects of antioxidants [48]. The results of our study show that ceruloplasmin activity was influenced by both simvastatin therapy and surgical treatment, but only 7–10 days postoperatively. In our opinion, simvastatin may be implicated in antioxidant properties of ceruloplasmin, as reflected by the increase in its activity in men on simvastatin therapy. Several in vitro studies have indicated that ceruloplasmin is a potent catalyst of LDL oxidation and that has been attributed to the presence of a reduced copper atom on its surface. ROS dissociate copper from ceruloplasmin and therefore can markedly alter its structure and function, which could explain the initial lack of differences in its activity 3–5 days after surgery.

In view of the escalating prevalence of PAD in our aging population, further studies are needed to clarify precisely not only the mechanisms involved but the relative importance of the lipid-related and nonlipid-related effects of statins as well.

5. Conclusions

Results of this study show diverse oxidative state responses to surgical treatment and confirm the beneficial effects of statin therapy in PAD.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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