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Małgorzata Kobusiak-Prokopowicz, Beata Jołda-Mydłowska, Andrzej Mysiak

Effect of Simvastatin on Endothelial Function Parameters in Patients with Hypertension and Ischemic Heart Disease

Wpływ simwastatyny na wskaźniki funkcji śródbłonka u chorych na nadciśnienie tętnicze i chorobę niedokrwienną serca

Department of Cardiology, Wroclaw Medical University, Poland

Abstract

Background. Dysfunctions in the vascular endothelium precede the development of atherosclerosis in patients with arterial hypertension. Prevention of endothelial damage may potentially delay the appearance of atherosclerotic plaques in blood vessels.
Objectives. The objective of the study was to evaluate changes in endothelial function based on serum nitric oxide (NO) level and serum von Willebrand factor activity during an 8-week simvastatin treatment.
Material and Methods. The study population consisted of 31 patients aged 40–80 years (mean: 60.8 ± 11.9 years), 24 females and 7 males, with disturbances in lipid metabolism, primary arterial hypertension, and ischemic heart disease, not previously treated with hypolipidemic agents. The patients were divided into two groups: group A (16 patients with hyperlipidemia and mild/moderate arterial hypertension) and group B (15 patients with hyperlipidemia and mild/moderate arterial hypertension and second class ischemic heart disease). The control group comprised 10 healthy age-matched individuals. Blood samples were taken for measurements of serum NO and lipids and plasma vWf. Simvastatin at a dose of 40 mg/day was then administered orally. The biochemical measurements were also performed after 4 and 8 weeks of simvastatin treatment.
Results. Serum NO concentration in the control group was significantly higher than in all the patients during visits 0 (baseline) and 1 ($p < 0.05$). NO concentration was significantly lower in group A than in the control group during visits 0 and 1. In group B it was significantly lower in all three visits. Moreover, a significantly lower serum NO level was found during visit 2 in group B than in group A. Plasma von Willebrand factor level in the controls was not significantly different from that of the patients during all visits. At baseline, patient serum NO concentration correlated with total cholesterol level, cholesterol LDL, cholesterol HDL, and triglycerides. During visit 2 it correlated with HDL cholesterol level and von Willebrand factor level.
Conclusions. Simvastatin treatment significantly improves some parameters of endothelial function in patients with dyslipidemia and arterial hypertension (**Adv Clin Exp Med 2010, 19, 2, 195–201**).

Key words: simvastatin, endothelial function, NO, von Willebrand factor, ischemic heart disease.

Streszczenie

Wprowadzenie. Dysfunkcja śródbłonka naczyniowego wyprzedza rozwój zmian miażdżycowych u chorych na nadciśnienie tętnicze. Zapobieganie uszkodzeniu śródbłonka na tak wczesnym etapie może opóźniać pojawianie się zmian miażdżycowych.
Cel pracy. Ocena zmian czynności śródbłonka na podstawie stężenia tlenku azotu (NO) w surowicy i aktywności czynnika von Willebranda w osoczu podczas 8 tygodni leczenia simwastatyną.
Materiał i metody. Badaniem objęto 31 chorych w wieku 40–80 lat, w tym 24 kobiety i 7 mężczyzn z zaburzeniami gospodarki lipidowej, pierwotnym nadciśnieniem tętniczym i chorobą niedokrwienną serca, nieleczonych wcześniej lekami hipolipemizujacymi. Chorych podzielono na 2 grupy: grupę A stanowiło 16 chorych na hiperlipidemię i nadciśnienie tętnicze; grupę B stanowiło 15 chorych na hiperlipidemię, nadciśnienie tętnicze oraz chorobę niedokrwienną serca, u których jednorazowo pobrano próbki krwi w warunkach spoczynkowych. Stężenie NO i poziom czynnika von Willebranda oceniano przed rozpoczęciem leczenia, po 4 tygodniach leczenia (obserwacja 1) i 8 tygodniach leczenia (obserwacja 2). Dobowa dawka simwastatyny wynosiła 40 mg.

Wyniki. Stężenie NO w surowicy w grupie kontrolnej było istotnie większe w porównaniu z wszystkimi badanymi chorymi w obserwacji 0 (wyjściowej) i obserwacji 1 ($p < 0.05$). Stężenie NO było istotnie mniejsze u chorych z grupy A w porównaniu z osobami z grupy kontrolnej w obserwacjach 0 i 1 ($p < 0.05$), u chorych z grupy B natomiast było istotnie mniejsze we wszystkich trzech obserwacjach ($p < 0.5$). Stężenie czynnika von Willebranda w osoczu u osób zdrowych nie różniło się istotnie w porównaniu z chorymi z grup A i B (pNS). W obserwacji 0 u wszystkich chorych stężenie NO w surowicy korelowało ze stężeniem cholesterolu całkowitego (beta –0.410), cholesterolu LDL (beta –0.386), cholesterolu HDL (beta 0.441) i trójglicerydów (beta –0.736), a w obserwacji 2 ze stężeniem cholesterolu HDL (beta 0.678) i stężeniem czynnika von Willebranda (beta 0.542).
Wnioski. Leczenie simwastatyną istotnie poprawia niektóre wskaźniki funkcji śródbłonka u chorych z zaburzeniami lipidowymi i nadciśnieniem tętniczym (**Adv Clin Exp Med 2010, 19, 2, 195–201**).

Słowa kluczowe: simwastatyna, czynność śródbłonka, NO, czynnik von Willebranda, choroba niedokrwienna serca.

Disorders of the vascular endothelium precede the development of atherosclerotic lesions in hypertensive patients. Prevention of endothelial damage in the early phase could delay the occurrence of these lesions. Repair processes leading to recovery of lost properties of the endothelium could slow the progression of atherosclerosis.

Statins, which are 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors, are being more commonly used. This is justified by their hypolipidemic action, which correlated with decreases in cardiovascular morbidity and mortality [1, 2]. The so-called "pleiotropic" action of statins on the organism comprises their effects on inflammatory processes, oxidative stress, blood coagulation, and endothelial function [3]. Experimental and clinical studies relate to the dynamics of this "pleiotropic" action of statins, its relationship with their hypolipidemic action, and its significance in improving survival. Experimental studies from the end of the 1990s indicated a possible effect of statins on endothelial function unrelated to the changes in serum lipid profile. In the clinic, simultaneous evaluation of changes in serum lipid profile and selected factors reflecting improvement or worsening of endothelial function is mandatory [4].

One of the most active vasodilatative substances produced by endothelial cells is nitric oxide (NO). Its effect is due to relaxation of the smooth muscle of vessels. Among NO's other known biological effects is a protective influence on the vascular wall which consists in preventing lipid oxygenation and limiting free oxygen radical functioning. Moreover, the antiatheromatous effect of NO is related to its inhibition of contraction and proliferation of smooth muscles, inhibition of endothelin production, monocyte adhesion, and platelet adhesion and aggregation. It should be emphasized that NO has synergistic action with prostacyclin, particularly with regard to platelet aggregation inhibition. NO also has an impact on the myocardium. Previous *in vitro* studies indicated both positive and negative inotropic effects of NO [5, 6]. It has been suggested that the inotropic action may be related to serum NO level; low NO levels would have inotropic-positive action and high levels inotropic-negative [7].

Von Willebrand factor, regarded as a good marker of endothelial function, is a procoagulant in coagulation activation [8, 9]. Increased von Willebrand factor activity has been found in many cardiovascular disorders. In prospective studies this was related to worse prognosis, higher incidence of myocardial infarction and stroke, and higher risk of mortality [10, 11]. In a study by Whincup et al., patients with the highest activity of von Willebrand factor had higher relative risk of developing ischemic heart disease than those from the lower tertile [12]. This was also confirmed in a meta-analysis of the five most comprehensive studies on the relationship between von Willebrand factor activity and severity of ischemic heart disease and in an additional prospective study, comprising 1524 cases of ischemic heart disease compared with 19,830 subjects of a control group. The study indicated that the relative risk of ischemic heart disease was significantly increased in the highest tertile [12].

The objective of the present study was to evaluate changes in endothelial function based on serum nitric oxide (NO) level and serum von Willebrand factor activity during an 8-week simvastatin treatment.

Material and Methods

The study population consisted of 31 patients aged 40–80 years (mean: 60.8 ± 11.9 years), with 24 women aged 40–80 years (mean: 59.2 ± 11.1 years) and 7 men aged 46–80 years (mean: 67.0 ± 13.9 years) with disturbances in lipid metabolism, primary arterial hypertension, and ischemic heart disease, not treated with hypolipidemic agents in their medical history. Ischemic heart disease was diagnosed based on typical history, positive electro-

cardiographic stress test, and coronary angiography. The study was accepted by the appropriate bioethics committee. All the subjects gave written informed consent before entering the study according to the recommendations and approval of the bioethics committee acting in accordance with the rules of Good Clinical Practice and the Declaration of Helsinki.

Patients were divided into two groups: group A, 16 patients with hyperlipidemia and mild/moderate arterial hypertension (according to the European Society of Cardiology [ESC] classification), aged 40–73 years; group B, 15 patients with hyperlipidemia and mild/moderate arterial hypertension (according to the ESC classification) and class 2 ischemic heart diseases according to the Canadian Cardiovascular Society (CCS), aged 46–80 years.

Patients treated previously with statins or fibrates and those with uncontrolled arterial hypertension, poorly controlled diabetes, heart failure, liver failure, renal failure, systemic connective tissue disorders, cancer, acute or chronic inflammatory diseases, or coagulation disorders were excluded from the study. Treatment of arterial hypertension and ischemic heart disease was according to the standards of the European Society of Cardiology and remained unchanged. If the concomitant treatment of arterial hypertension and/or ischemic heart disease required a significant change, the patient was excluded from the study.

The control group consisted of 10 healthy sex- and age-matched volunteers. In this group, one blood specimen was taken at rest.

Concentration of NO and activity of von Willebrand factor, lipid parameters, and liver function markers (GOT, GPT levels) were evaluated before the initiation of the study treatment (baseline, visit 0) and after 4 (visit 1) and 8 (visit 2) weeks of the study treatment. A dose of 40 mg of simvastatin (Zocor, MSD) was given once daily, in the evening. During the observation the patients were not administered any medication possibly affecting lipid metabolism.

Plasma von Willebrand factor's activity was measured using an ELISA method (Asserachrom vWF:Ag kit). Blood was taken from the basilic vein into test tubes with 3.2% sodium citrate. The blood was centrifuged for 15 minutes at 2500 rpm. The obtained plasma was then stored at –70°C. Intra- and inter-series variability was below 5%. Nitric oxide level was determined using a spectrophotometric method (R&D Systems Total Nitric Oxide Assay kit, catalogue no. DE 1600). After blood taking from the basilica vein, it was centrifuged over 10 minutes at 1000 rpm. The obtained plasma was then stored at –70°C. In determining NO level, the transformation to nitrite (III) and nitrate (V) with nitrate reductase was used. The total transformed nitrogen (III) was than detected as the product of the Griess reaction with a spectrophotometric method. Intra- and inter-series variability was below 5%.

Statistical analysis was performed using Statistica 5.0 PL software on the basis of mean values of the determined variables and their standard deviations. The Shapiro-Wilk's W test was used to confirm a normal distribution of data. When this criterion was fulfilled, further analysis was carried out using Student's t test for paired and unpaired samples. In the samples in which the above criterion was not fulfilled, the differences were investigated using the non-parametric Mann-Whitney U test. To evaluate the statistical significance of the qualitative changes, the highest reliability χ^2 test with Yates' modification and the exact Fisher test were used. Correlation between the investigated variables was assessed by Pearson's linear correlation coefficient (Pearson's r) as well a non-parametric correlation coefficient (Spearman's R). Differences were considered significant at $p < 0.05$.

Results

The results are presented in Tables 1–3. The serum NO concentration in the control group was 19.6 ± 6.1 ng/ml. It was significantly higher than in the patient group during visit 0 (baseline) and visit 1 ($p < 0.05$) (Table 1). NO concentration was significantly lower in group A than in the control group during visits 0 and 1 ($p < 0.05$) (Table 2). In group B it was significantly lower in all three visits ($p < 0.5$) (Table 3). Moreover, in group B a significantly lower serum NO level was found during visit 2 compared with group A ($p < 0.05$). Plasma von Willebrand factor level in the control group was 98.15 ± 34.7%. It was not significantly different from the patient group during visits 0, 1, and 2. In all the patient groups a significant decrease in total cholesterol and LDL cholesterol was observed starting with 4 weeks of treatment. Total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride levels in groups A and B were not statistically different between visits 1 and 2.

Correlations

At baseline, serum NO concentration in all patients correlated with total cholesterol level (beta = –0.410), cholesterol LDL (beta = –0.386), cholesterol HDL (beta = 0.441), and triglycerides (beta = –0.736). During visit 2 it correlated with

Table 1. Nitric oxide (NO) concentration, von Willebrand factor activity, and lipid levels in all the patients with arterial hypertension and ischemic heart disease at baseline (visit 0), after 4 weeks (visit 1), and after 8 weeks (visit 2)

Tabela 1. Stężenie tlenku azotu (NO), aktywność czynnika von Willebranda i stężenie lipidów u pacjentów z nadciśnieniem tętniczym i chorobą wieńcową na początku (wizyta 0), po 4 tygodniach (wizyta 1) i po 8 tygodniach (wizyta 2)

Parameter (Wskaźnik)		Control group (Grupa kontrolna) n = 10	Patient group (Grupa badana)			*p* Statistical difference between visits (Różnica istotna statystycznie między wizytami)		
			visit 0 (wizyta 0) n = 31	visit 1 (wizyta 1) n = 31	visit 2 (wizyta 2) n = 31	0 – 2	0 – 1	1 – 2
von Willebrand factor (Czynnik von Willebranda) %	*X* *SD*	98.15 34.7	115.98 34.73	93.45 39.40	114.08 41.97	ns.	0.01	ns.
Nitric oxide – NO (Tlenek azotu) ng/mL	*X* *SD*	19.6 6.1	13.10 6.30	13.55 7.16	15.37 9.23	ns.	ns.	ns.
Total cholesterol (Cholesterol całkowity) mg/dL	*X* *SD*	216.16 24.84	252.17 43.71	179.73 30.76	166.85 32.66	0.0001	0.0001	ns.
LDL cholesterol mg/dL	*X* *SD*	128.06 22.41	170.81 32.75	94.77 22.25	86.38 29.06	0.0001	0.0001	ns.
HDL cholesterol mg/dL	*X* *SD*	52.95 12.62	52.39 12.76	54.89 11.49	51.30 10.64	ns.	ns.	ns.
Triglycerides mg/dL	*X* *SD*	149.2 60.78	136.13 71.29	121.55 33.13	143.42 68.66	ns.	ns.	ns.

Table 2. Nitric oxide (NO) concentration, von Willebrand factor activity, and lipid levels in the patients of group A

Tabela 2. Stężenie tlenku azotu (NO), aktywność czynnika von Willebranda i stężenie lipidów u pacjentów z grupy A

Parameter (Wskaźnik)		Group A (Grupa A)			*p* Statistical difference between visits (Różnica istotna statystycznie między wizytami)		
		visit 0 (wizyta 0) n = 16	visit 1 (wizyta 1) n = 16	visit 2 (wizyta 2) n = 16	0 – 2	0 – 1	1 – 2
von Willebrand factor (Czynnik von Willebranda) %	*X* *SD*	110.84 42.07	79.75 27.05	122.67 54.50	ns.	ns.	ns.
Nitric oxide – NO (Tlenek azotu) ng/mL	*X* *SD*	13.47 7.43	15.50 9.77	20.26 12.12	0.05	ns.	ns.
Total cholesterol (Cholesterol całkowity) mg/dL	*X* *SD*	260.00 45.16	181.40 34.02	156.40 58.74	0.0001	0.0001	ns.
LDL cholesterol mg/dL	*X* *SD*	174.66 31.24	90.66 15.41	91.25 35.07	0.0001	0.0001	ns.
HDL cholesterol mg/dL	*X* *SD*	50.56 11.14	54.60 10.90	50.87 8.33	ns.	ns.	ns.
Triglycerides mg/dL	*X* *SD*	143.75 87.81	124.11 37.40	134.44 78.00	ns.	ns.	ns.

Table 3. Nitric oxide (NO) concentration, von Willebrand factor activity, and lipid levels in the patients of group B

Tabela 3. Stężenie tlenku azotu (NO), aktywność czynnika von Willebranda i stężenie lipidów u opacjentów z grupy B

Parameter (Wskaźnik)		Group B (Grupa B)			*p* Statistical difference between visits (Różnica istotna statystycznie między wizytami)		
		visit 0 (wizyta 0) n = 15	visit 1 (wizyta 1) n = 15	visit 2 (wizyta 2) n = 15	0 – 2	0 – 1	1 – 2
von Willebrand factor (Czynnik von Willebranda) %	*X* *SD*	122.41 22.77	108.53 46.43	106.91 31.82	ns.	ns.	ns.
Nitric oxide – NO (Tlenek azotu) ng/mL	*X* *SD*	12.71 5.05	11.59 1.93	11.88 4.92	ns.	ns.	ns.
Total cholesterol (Cholesterol całkowity) mg/dL	*X* *SD*	242.53 41.54	177.88 28.62	159.40 19.78	0.0001	0.0001	ns.
LDL cholesterol mg/dL	*X* *SD*	166.00 35.33	98.88 27.88	78.60 16.10	0.0001	0.0001	ns.
HDL cholesterol mg/dL	*X* *SD*	54.83 14.81	55.22 27.88	52.00 14.74	ns.	ns.	ns.
Triglycerides mg/dL	*X* *SD*	126.76 51.39	119.00 30.32	159.60 51.39	ns.	ns.	ns.

HDL cholesterol level (beta = 0.678) and von Willebrand factor activity level (beta = 0.542). NO serum level in group A during visits 0 and 2 negatively correlated with triglyceride level (r = = –0.825, $p < 0.01$ and r = –0.972, $p < 0.02$, respectively). In group B the serum NO level negatively correlated during visit 1 with total cholesterol and LDL cholesterol levels (r = –0.346, $p < 0.04$ and r = –0.404, $p < 0.04$, respectively) and during visit 2 with triglyceride level (r = –0.885, $p < 0.04$). Von Willebrand factor level at baseline in all subjects correlated positively with age (r = 0.426, $p <$ < 0.03). Multiple regression analysis showed that von Willebrand factor level at baseline was related only to age (beta = 0.426) and during visit 2 to serum NO level (beta = 0.536).

Discussion

The pleiotropic action of statins, not directly related to its hypolipidemic action, is due to the effect on endothelial function and release of inflammatory factors. A distinct pleiotropic effect is observed some hours after the initiation of treatment [13]. Marketou et al. evaluated changes in peroxides, interleukin-6, TNF, and sICAM-1 levels two hours after simvastatin or atorvastatin administration. After this short period, significant changes in subjects with lipid disturbances were observed, indicating a beneficial effect of statins, expressed as limitation of oxidative stress, decreased release of proinflammatory cytokines, and endothelial activation. These effects increased with time. Normalization of endothelial function parameters was observed during one to three weeks after initiation of treatment [14].

Pathophysiological disturbances characteristic of endothelial function do not always have a direct effect on the severity of atherosclerosis and the clinical condition of patients. Chung et al. reported significant changes in endothelial function parameters, including von Willebrand factor level, and angiogenesis and thrombogenesis indicators in patients with ischemic heart disease. However, no correlation with severity of atheromatous lesions was found [10]. Yildirir et al. examined other endothelial parameters, including E-selectin. Serum E-selectin level revealed correlation with the severity of atheromatous lesions, but only in a group of patients with unstable angina [15].

The production of peroxide anions by the endothelium inactivates nitric oxide (NO). Reports of improved endothelial function after administration of statins suggest that statins increase serum NO level due to the inhibition of NO degradation in the presence of free radicals [16]. The observations of the present study indicated that there is a negative relationship between total cholesterol, LDL cholesterol, and triglyceride levels and serum NO concentration. This indicates the coexistence of endothelial dysfunction and lipid disturbances.

The positive correlation between NO level and HDL cholesterol level indicates protective action of this lipid fraction on NO. This positive correlation was sustained over the entire observation period. This indicates that the administration of simvastatin makes it possible to maintain a beneficial relationship between HDL cholesterol and NO.

The present study found that the use of simvastatin in a group of patients with lipid disturbances and arterial hypertension is associated with increased serum NO levels. The aforementioned studies on the effect of statins indicate that these agents restore endothelial function and that this is not related to their hypolipidemic action. It should be considered why during the statin treatment the NO level increased only in the group of patients with arterial hypertension but not in the group of patients with arterial hypertension and ischemic heart disease. Possibly, the presence of more advanced atheromatous lesions, arising from endothelial dysfunction, makes it impossible to show the effect of simvastatin on the restoration of endothelium. It seems that for severe atheromatous lesions in vessels, long-term observations of the effect of statin treatment are warranted.

The participation of von Willebrand factor in the formation of thrombi, documented in acute myocardial infarction, justifies studies on this subject also in stable ischemic heart disease as a new therapeutic target [17]. In the present study, simvastatin normalized the lipidogram but there was no relationship between the observed dynamic changes in lipid levels and von Willebrand factor level. In subsequent visits the positive correlation was maintained between plasma von Willebrand factor level and serum NO level, indicating feedback. In this feedback mechanism an increase in the dysfunction parameter, i.e. von Willebrand factor, releases a reactive increase in the opposite factor, i.e. NO improving endothelial function.

Limitations of the Study

The number of patients investigated defines the study as a pilot study. Further observation on the relationship between lipid profile and NO would require a larger study population. Lack of correlation between simvastatin treatment and von Willebrand factor activity requires more study. The authors of the closed Edinburgh Artery Study with 12 years of follow-up looked for any effect of hemostatic mechanisms and inflammation on the development of atherosclerotic changes. They found a higher impact of inflammatory factors on the progression of atherosclerosis compared with hypercoagulabity [18].

The authors concluded that simvastatin treatment significantly improves some parameters of endothelial function in patients with dyslipidemia and arterial hypertension.

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Address for correspondence:

Małgorzata Kobusiak-Prokopowicz
Department of Cardiology
Wroclaw Medical University
Pasteura 4
50-367 Wrocław
Poland
Tel: +48 71 784 26 12
E-mail: kobusiak@poczta.fm

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