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Impact of nebivolol on levels of serum nitric oxide, plasma von Willebrand factor and exercise stress testing parameters in hypertensive and ischemic heart disease patients

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Abstract

Background: *The dysfunction of vascular endothelium precedes the development of atherosclerosis in patients with arterial hypertension. Nebivolol is a very specific beta-blocker, which can be characterized by a strong endothelial vasodilatative effect. The aim of the study was the assessment of changes in concentrations of serum nitric oxide (NO), plasma von Willebrand factor (vWf) and selected parameters of electrocardiographic exercise tests after 4-week nebivolol treatment.*

Methods: *Twenty-one patients were included in the study, aged from 34 to 82 years with primary arterial hypertension or primary arterial hypertension and ischemic heart disease. Blood samples were taken for measurements of serum NO and plasma vWf. Electrocardiographic stress tests were also performed. Subsequently, nebivolol was administered for four weeks and the aforementioned measurements were repeated.*

Results: *A significant increase in serum NO concentration was found in all the investigated patients after nebivolol treatment. A prolongation of exercise time, increase in metabolic equivalent and decrease in double product were also noted in patients after nebivolol treatment.*

Conclusions: *Nebivolol treatment improves parameters of electrocardiographic exercise test in patients with arterial hypertension. The improvement of the parameters of the exercise test was not observed in those patients who showed no significant increase in serum NO concentration following nebivolol treatment.* (Cardiol J 2008; 15: 162–168)

Key words: nebivolol, nitric oxide, von Willebrand factor, arterial hypertension, ischemic heart disease

Introduction

The dysfunction of vascular endothelium precedes the development of atherosclerosis in patients with arterial hypertension. The prevention of endothelial damage may potentially delay the appearance of atherosclerotic plaques in blood vessels. It can be assumed that reparative processes, which allow vascular endothelium to regain its lost functional integrity, should slow down the progression of atherosclerotic processes.

Nebivolol is a beta-blocker with very special qualities [1]. It has the highest cardiac beta-receptor selectivity among all beta-blockers and a strong

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endothelial nitric oxide-mediated vasodilatative effect. It has been confirmed that endothelial function-dependent vasodilation becomes ameliorated in hypertensive patients receiving nebivolol treatment. Tzemos et al. [2] demonstrated the additional vasoprotective effects of nebivolol as compared with atenolol. Nebivolol increased both stimulated and basal endothelial nitric oxide release, whereas, for the same degree of blood pressure control, atenolol had no effect on nitric oxide (NO) bioactivity. The vasodilatory response to acetylcholine was significantly increased with nebivolol, but not with atenolol. Concomitantly, the endothelium-dependent vasoconstrictive response to N(G)-monomethyl-L--arginine (L-NMMA) was significantly reduced, which again was not demonstrated with atenolol [2].

Brehm et al. [3] also demonstrated that nebivolol inhibits the proliferation of human coronary smooth muscle cells induced by growth factors, whereas classical beta-blockers do not affect cell growth. During incubation of endothelial cells (HaECs) with nebivolol, NO formation of HaECs increased, while endothelin-1 transcription and secretion were suppressed. The previous nebivolol investigations demonstrated both vasodilating properties of nebivolol and significant mortality reduction in patients treated with this unique beta-blocker [4].

The plasma von Willebrand factor, regarded as a very good indicator of endothelial dysfunction, contributes to the activation of the coagulation cascade [5, 6]. An increased plasma concentration of von Willebrand factor was found in patients suffering from various cardiovascular diseases. The prospective studies demonstrated that the increased plasma concentration of this coagulation factor was strongly related to poor prognosis in cardiovascular patients, an increased number of major vascular events (myocardial infarction, brain stroke) and high mortality rate [7].

The aim of the study was the assessment of the dynamics of changes in concentrations of serum nitric oxide, plasma von Willebrand factor and the selected parameters of electrocardiographic treadmill tests after one month of nebivolol treatment in hypertensive patients with chronic ischemic heart disease.

Methods

Twenty-one patients aged from 34 to 82 years (mean age 59.3 ± 12.2 years; 15 females and 6 males) with primary arterial hypertension or primary arterial hypertension and ischemic heart disease, untreated before with beta-blockers, entered the study. The diagnosis of ischemic heart disease was confirmed in the investigated patients after considering the typical clinical symptoms, the presence of myocardial ischemia during electrocardiographic stress test and the outcome of coronary arteriography. The patients affected with renal or hepatic failure, systemic connective tissue diseases, cancer, or acute or chronic inflammatory diseases were excluded from the study.

The investigated patients were divided into two groups:

— group 1 — 11 patients (mean age 56.6 ± 10.9 years) with arterial hypertension of moderate degree according to the European Society of Cardiology (ESC) classification;
— group 2 — 10 patients (mean age 62.3 ± 10.6 years) with arterial hypertension of moderate degree according to ESC classification and ischemic heart disease in stage II according to Canadian Cardiac Society (CCS).

Depending on changes in serum NO concentration after nebivolol treatment, two subgroups of patients were established:

— group 3 — 11 patients (mean age 64 ± 13.0 years) — increase in serum NO concentration was observed;
— group 4 — 10 patients (mean age 62.6 ± 9.7 years) — increase in serum NO concentration was not observed.

Blood samples were taken in the morning hours from the decubital vein in all the study patients for measurements of concentrations of serum NO, plasma von Willebrand factor and blood lipids. Subsequently, echocardiographic interrogations and electrocardiographic (ECG) stress tests were also performed. Afterwards, nebivolol in a dose of 5 mg per day (Nebilet, Berlin-Chemie Company) was orally administered to all the examined patients according to the 4-week follow-up protocol. The afore-mentioned biochemical measurements and ECG stress tests were performed again after the 4-week nebivolol treatment. All patients underwent electrocardiographic stress tests according to Bruce protocol (Burdick T 600 computerized system combined with ECG apparatus, Sicard 460 S — Siemens company). The following criteria of exercise test termination were accepted for the investigation protocol: 1) examined patient reached age-limited increase in MET (metabolic equivalent) and/ /or submaximal heart rate; 2) presence of myocardial ischemia in ECG tracings; 3) acute anginal chest pain; 4) wishes of a patient — symptoms of fatigue. Afterwards, the following ECG stress test parameters were

Table 1. Concentration of serum nitric oxide, plasma von Willebrand factor, blood lipids and left ventricular ejection fraction in all investigated patients with arterial hypertension and ischemic heart disease.

Parameter	Before nebivolol treatment	After nebivolol treatment	Statistical significance
von Willebrand factor (%)	105.5 ± 21.9	124.4 ± 48.3	NS
Nitric oxide [ng/ml]	12.3 ± 4.7	15.4 ± 5.9	< 0.05
Total cholesterol [mg%]	216.6 ± 36.7	193.7 ± 49.9	NS
Cholesterol LDL [mg%]	129.3 ± 29.7	120.9 ± 52.6	NS
Cholesterol HDL [mg%]	58.1 ± 19.4	54.7 ± 15.9	NS
Triglycerides [mg%]	115.1 ± 50.0	103.0 ± 23.8	NS
Left ventricular ejection fraction (%)	71.0 ± 5.8	64.3 ± 20.9	NS

NS — non-significant

evaluated: time of exercise, MET, degree of ST depression and double product (heart rate multiplied by systolic blood pressure during peak exercise).

Echocardiographic interrogation was performed in every patient using Wingmed LG apparatus with a 3.5 MHz transducer. The left ventricular ejection fraction was determined using biplane Simpson's rule.

The concentration of plasma von Willebrand factor was measured with ELISA immunoassay using Asserachrom vWf:Ag kit. The blood samples were placed into test tubes with a 3.2% solution of sodium citrate and then centrifuged for 15 min at 2500 revolutions per minute (rpm). Afterwards, the obtained plasma was stored at –70°C. The intra- and interassay variability was below 5%.

The serum NO concentration was measured with spectrophotometry using R&D Systems' Total Nitric Oxide Assay kit, catalogue number DE 1600. The blood samples were centrifuged for 10 min at 1000 rpm. Afterwards, the obtained plasma was stored at –70°C. The measurements of plasma NO concentration were made by using transformation of NO into nitrate (III) and nitrate (V) catalyzed by nitrate reductase. The total transformed nitrate (III) was then detected spectrophotometrically using the Griess reaction. The intra- and interassay variability was below 5%.

The study was approved by the local ethical committee and all patients gave their informed consent.

Statistical anaylysis

Statistical analysis was performed using STATISTICA 5.0. Means and standard deviations were calculated for the selected parameters. Normal distribution of data was tested by the Shapiro-Wilk test. The non-parametric U-Mann-Whitney and Kruskal-Wallis tests were applied when the normal distribution of data was not confirmed. If the normal distribution of data was confirmed, further analysis was performed by means of Student's t-test for paired and unpaired samples. The highest reliability χ^2 test with Yates' correction and Fisher's exact test were used when the statistical significance of quantitative changes in the examined parameters was tested. The correlations between investigated parameters were assessed using Pearson's linear coefficient and the non-parametric Spearman's coefficient. Statistical significance was considered at $p < 0.05$

Results

Significantly lower serum NO concentrations were found in all the examined patients, compared with healthy volunteers before the start of the investigation (12.3 ± 4.7 μM *vs*. 19.6 ± 6.1 μM; $p < 0.02$). The von Willebrand factor levels in the study group were not significantly different from those of the control group (105.5 ± 21.9% *vs*. 98.15 ± ± 34.7%).

A significant increase in serum NO concentrations was found in all the investigated patients after nebivolol treatment. However, serum NO levels were still much lower than those of the control group (Table 1). Significant changes in von Willebrand factor levels were not observed during the investigation period.

A significant increase in serum NO concentration was found after nebivolol treatment in patients from groups 1 and 2. However, no significant changes in von Willebrand factor concentrations were observed (Table 2). A very high increase in serum NO concentration was observed in group 2. After 4-week nebivolol treatment, the serum NO levels were comparable to those of the control group. The NO and von

Table 2. Concentration of serum nitric oxide, plasma von Willebrand factor versus exercise stress test parameters in patients with arterial hypertension (group 1) and ischemic heart disease (group 2).

Investigated parameter	Before nebivolol treatment	After nebivolol treatment	Statistical significance
Group 1			
von Willebrand factor (%)	105.9 ± 25.9	124.8 ± 53.4	NS
Nitric oxide [ng/ml]	10.9 ± 2.2	14.8 ± 6.5	< 0.05
Exercise time [min]	8.5 ± 1.7*	9.9 ± 1.1**	< 0.05
Peak workload [MET]	8.4 ± 2.1*	9.7 ± 1.5*	< 0.01
ST depression [mm]	3.5 ± 2.5	2.5 ± 2.0*	NS
Double product [mm Hg × beats/min]	26815 ± 4737**	24284 ± 4156**	< 0.05
Group 2			
von Willebrand factor (%)	105.2 ± 18.6	123.9 ± 45.8	NS
Nitric oxide [ng/ml]	13.8 ± 6.1	16.3 ± 5.7	< 0.05
Exercise time [min]	5.5 ± 3.9	6.4 ± 2.5	NS
Peak workload [MET]	5.3 ± 3.6	5.8 ± 3.4	NS
ST depression [mm]	4.5 ± 2.6	4.0 ± 3.4	NS
Double product [mm Hg × beats/min]	20606 ± 4398	18800 ± 4331	< 0.05

*Statistically significant differences between analogous parameters in patients from groups 1 and 2 ($p < 0.05$); **statistically significant differences between analogous parameters in patients from groups 1 and 2 ($p < 0.01$); NS — non-significant

Willebrand factor levels at the beginning of the investigation were similar to those found after 4-week nebivolol treatment in groups 1 and 2.

The prolongation of exercise time, increase in MET and decrease in double product were observed after nebivolol treatment in group 1. The significantly longer exercise time, higher increase in MET and higher double product were observed both before and after nebivolol treatment in group 1, as compared with group 2. The ST- segment depression after nebivolol treatment in groups 1 and 2 was smaller than that recorded before the onset of the investigation. Unfortunately, the observed differences were not statistically significant. However, the ST- segment depression at peak exercise in group 1 was respectably smaller than that recorded in group 2.

The significant increase in serum NO concentrations after nebivolol treatment was accompanied by a significant decrease in double product in groups 2 and 3 (Tables 2 and 3). Neither of the investigated biochemical or electrocardiographic exercise test parameters were changed significantly in group 4.

A significant negative correlation between baseline serum NO concentration and double product calculated at peak exercise before the onset of nebivolol treatment was found in all the examined patients ($R = 0.426$; $p < 0.03$). A positive correlation between plasma von Willebrand factor concentrations and total cholesterol levels both before and after nebivolol treatment was observed in the study group ($R = 0.714$; $p < 0.01$ and $R = 0.848$; $p < 0.03$). A positive correlation between serum NO concentrations and exercise time was found in group 1 after nebivolol treatment ($R = 0.921$; $p < 0.02$). A significant positive correlation between serum NO concentrations after nebivolol treatment and double product calculated at peak exercise before nebivolol treatment was also observed ($R = 0.895$; $p < 0.01$).

Significant fluctuations in blood concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol or triglycerides were not found. The echocardiographic interrogation did not show any significant change of left ventricular ejection fraction (LVEF).

Discussion

The treatment of arterial hypertension and ischemic heart disease with beta-blockers has been a gold standard for many years. Intensive research on novel beta-blockers with more and more unique biochemical properties has contributed to the development of three generations of these drugs. The novel beta-blockers can be distinguished by various degrees of cardiac selectivity and vasodilating properties. Nebivolol seems to be a promising tool in the complex treatment of arterial hypertension, ischemic heart disease and the prevention of atherosclerosis, owing to it having specific vasodilating properties and the highest cardiac selectivity among

Table 3. Concentration of serum nitric oxide, plasma von Willebrand factor versus exercise stress test parameters in patients with increase in serum nitric oxide concentration (group 3) and patients without increase in serum nitric oxide concentration (group 4).

Investigated parameter	Before nebivolol treatment	After nebivolol treatment	Statistical significance
Group 3			
von Willebrand factor (%)	106.5 ± 24.4	128.9 ± 53.8	NS
Nitric oxide [ng/ml]	12.3 ± 4.6	16.1 ± 6.2**	< 0.05
Exercise time [min]	7.7 ± 2.7	8.6 ± 2.4	NS
Peak workload [MET]	7.5 ± 2.9	8.5 ± 2.4	NS
ST depression [mm]	4.1 ± 2.6	3.5 ± 2.7	NS
Double product [mm Hg × beats/min]	25999 ± 4510	21917 ± 2428	< 0.001
Group 4			
von Willebrand factor (%)	101.2 ± 19.3	100.1 ± 12.8	NS
Nitric oxide [ng/ml]	11.8 ± 5.1	11.5 ± 5.0	NS
Exercise time [min]	9.0 ± 3.6	9.9 ± 3.2	NS
Peak workload [MET]	6.7 ± 3.2	7.2 ± 3.0	NS
ST depression [mm]	2.3 ± 2.0	2.0 ± 1.8	NS
Double product [mm Hg × beats/min]	20138 ± 3984	18997 ± 3012	NS

*Statistically significant differences between analogous parameters in patients from groups 1 and 2 ($p < 0.05$); **statistically significant differences between analogous parameters in patients from groups 1 and 2 ($p < 0.01$); NS — non-significant

beta-blockers [8, 9]. The antiatherosclerotic effect of nebivolol is closely associated with NO-mediated inhibition of LDL cholesterol-oxidation, constriction and proliferation of smooth muscle cells, adhesion and aggregation of platelets, adhesion of monocytes and favourable hemodynamic profile despite an impaired endothelial function [10]. The neutral effect on carbohydrate economy and the renin-angiotensin-aldosterone system is another clinically important property of nebivolol [11, 12]. On the other hand, NO demonstrates an immediate effect on heart function, which is not fully understood. The previous in-vitro investigations showed both an inotropic positive and inotropic negative effect of NO on the heart muscle [13, 14]. It was even postulated that the inotropic effect of NO was dependent on serum NO concentration, i.e. at lower serum concentrations, NO exerted an inotropic positive effect, and at higher serum levels, NO might have an inotropic negative effect on the heart muscle [15]. There is also a lack of investigations on humans to elucidate fully the issue of inotropic effects of NO on the heart. Rassaf et al. [16] demonstrated in their recent investigation on healthy volunteers that the baseline serum NO concentration determined its beneficial effect on heart function, manifested by the maintenance of optimal stroke volume index.

Regarding the short follow-up of our study, we decided to treat the selected parameters of electrocardiographic stress test as part of clinical observation. It must be stressed that ST-segment depression without concomitant anginal pain during exercise tests in hypertensive patients with normal coronary arteriogram results from dysfunction of coronary microcirculation [17]. It is highly probable that the significant differences between groups 1 and 2 in the degree of ST-segment depression during exercise tests are associated with amelioration of coronary microcirculation after nebivolol treatment only in patients with arterial hypertension. Galderisi et al. [18] demonstrated amelioration of coronary flow reserve after 4-week nebivolol treatment in hypertensive patients without ischemic heart disease. Erdogan et al. [19] obtained similar results in patients with idiopathic dilated cardiomyopathy. This can be related to a decrease in double product just after 4-week nebivolol treatment, as was demonstrated by our study.

It is very interesting that an increase in serum NO concentration was observed after nebivolol treatment in patients with increased double product before the treatment protocol was started. Moreover, the positive correlation between the double product, as calculated at peak exercise before nebivolol protocol and serum NO concentration after nebivolol treatment, was found in these patients. These observations may give an incentive to further investigations aimed at selecting patients who will actually derive benefit from nebivolol treatment.

It should be noted that an increase in systolic blood pressure and heart rate at peak exercise, i.e. an increase in double product, was found in hypertensive patients with normal coronary arteries. Consequently, it is obvious that the vasodilating power of NO and beneficial effect of nebivolol will be potentiated in this group of patients. On the other hand, a significant increase in serum NO concentration, concomitant with a decrease in double product after nebivolol treatment, was observed in patients with ischemic heart disease (group 2), whereas a positive correlation between double product and serum NO concentration, as measured before and after nebivolol administration, was confirmed in this group of investigated patients. Hence, it can be assumed that the presence of atherosclerotic foci in coronary arteries does not exclude the meaningful effect of nebivolol on the vessel wall.

The previous investigations demonstrate that the afore-mentioned pathophysiological changes do not necessarily go together with the clinical status of patients with ischemic heart disease. Chung et al. [7] found significant changes in parameters of endothelial function, including von Willebrand factor, angiogenesis and thrombogenesis factors (VEGF, sFlt-1, TF), in patients with ischemic heart disease. However, these parameters did not correlate with the severity of atherosclerosis in coronary arteries. Yildirir et al. [20] investigated other endothelial parameters and demonstrated that E-selectin levels correlated with the severity of atherosclerosis. However, statistical significance was achieved only in patients with unstable angina pectoris.

The documented contribution of von Willebrand factor to thrombus formation in acute myocardial infarction also justifies further research on this issue in patients with stable angina pectoris [21]. The positive correlation between plasma concentration of von Willebrand factor and total cholesterol level, i.e. the fundamental risk factor for progression of atherosclerosis, was confirmed by our study. The Edinburgh Artery Study was one of the first studies to search for relationships between plasma concentration of hemostatic and inflammatory markers and severity of atherosclerosis. The 12-year follow-up demonstrated that inflammatory factors are stronger risk factors for progression of atherosclerosis than the state of hypercoagulability [22]. These observations combined with our study results demonstrate that von Willebrand factor is a generally accepted marker of vessel wall damage. Nevertheless, the current stage of knowledge does not allow connection to be made between fluctuations in plasma concentration of von Willebrand factor and clinical predictors of progression of ischemic heart disease.

The investigation included quite a small group of patients; therefore, it is more of a pilot study than a thorough research study. Nonetheless, it gives an incentive to more in-depth analysis and investigations on the effect of beta-blockers on the clinical course of coronary artery disease.

Conclusions

We concluded that 4-week nebivolol treatment ameliorates heart function as manifested by a decrease in double product, concomitant with an increase in serum NO concentration. Nebivolol treatment improves the parameters of electrocardiographic exercise tests in patients with arterial hypertension. No such improvement was observed in patients without a significant increase in serum NO concentration following nebivolol treatment.

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