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Effect of nebivolol on blood oxygen transport indices and endothelial dysfunction in patients with arterial hypertension

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Summary

Endothelial dysfunction, which is characterized by impairment of nitric oxide (NO) bioavailability, plays an important role in the development of arterial hypertension. The L-arginine-NO pathway is closely related to oxygen transport to tissue. Endothelial dysfunction in patients with arterial hypertension can affect haemoglobin-oxygen affinity and tissue oxygen supply. Alterations in blood oxygen transport indices may play role in the pathogenesis of arterial hypertension. The aim of the present study was to investigate the effect of the beta-selective adrenoblocker nebilet (nebivolol) on blood oxygen transport indices and on endothelial dysfunction in patients with arterial hypertension. The study population included 52 patients with grade II and grade III arterial hypertension. The results of our studies indicate that endothelial dysfunction in hypertensive patients significantly changes blood oxygen indices. The endothelium can be involved in formation of these impairments because only NO synthesized in sufficient amounts can maintain normal blood flow and oxygen transport to tissues. Endothelial dysfunction impairs formation of different haemoglobin NO-derivatives, that influence not only on the release of NO at different sites of the vascular bed, but also on haemoglobin-oxygen affinity, and accordingly, on optimal blood oxygenation in capillaries of pulmonary circulation and its deoxygenation in capillaries of systemic circulation. Treatment of hypertensive patients with nebivolol corrects the blood oxygen transport indices, stimulates NO production and improves endothelium-dependent dilatation. Normalization of blood oxygen transport indices may regulate the activity of the L-arginine-NO pathway. Thus, nebivolol may improve efficiency of the treatment of hypertension.

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Introduction

Recent data demonstrate that the global burden of hypertension is an important and increasing health problem worldwide and that awareness and control of hypertension vary considerably (Kearney *et al.*, 2005). Nitric oxide (NO) is a molecule that has gained recognition as a crucial modulator of vascular disease. NO has a number of intracellular effects that lead to vasorelaxation, endothelial regeneration, inhibition of leucocyte chemotaxis and platelet adhesion (Luscher & Barton, 1997; Ignarro & Napoli, 2005). Endothelial dysfunction, which is characterized by impairment of NO bioavailability, plays an important role in the development of arterial hypertension (Hermann *et al.*, 2006). The main mechanisms of impairment in L-arginine-NO pathway metabolism are the absence of either the initial substrate L-arginine or cofactors, decreased expression of endothelial NO-synthase and a raised level of endogenous NO inhibitors as well as NO inactivation by free radicals (O_2^-, OH^*) (Kelm & Rath, 2001; Landmesser & Drexler, 2007). Recent research on endothelial dysfunction supports its clinical significance and has led to important insights in the pathophysiology of cardiovascular diseases and at the same time provides an important opportunity to develop new therapeutic approaches. Endothelial function represents a valuable surrogate endpoint to assess the impact of therapeutic interventions (Landmesser & Drexler, 2005).

The L-arginine-NO pathway is closely related to oxygen transport to tissue. Oxygen is known to be an important factor regulating NO-synthase activity (Cannon, 1998). NO maintains the blood flow level, thus regulates oxygen supply to tissues.

A disturbance in endothelial NO-synthase function stipulates to a great extent the loss of control over vascular tone, thus resulting in a reduction of an adequate supply of oxygen to tissue. Endothelial dysfunction in patients with arterial hypertension can affect haemoglobin-oxygen affinity and tissue oxygen supply. Alterations in blood oxygen transport indices may play role in the pathogenesis of arterial hypertension (Zinchuk *et al.*, 2004). Acting against pathological processes causing hypoxia will improve endothelial dysfunction in patients with arterial hypertension. In clinical pharmacology, some new drugs have recently been produced, which are capable of increasing NO production in the organism. One of these drugs is nebivolol, a new 'atypical' third generation beta-adrenoblocker that has pronounced vasodilatatory properties (Cockcroft, 2004). However, some NO-mediated pharmacological effects of nebivolol remain to be studied (Ignarro, 2004).

The aim of the present study was to investigate the effect of the beta-selective adrenoblocker nebilet (nebivolol) on blood oxygen transport indices and on endothelial dysfunction in patients with arterial hypertension.

Methods

Subjects

The study population included 52 patients with grade II and grade III arterial hypertension who were examined and treated in the First City Hospital of Grodno. Arterial hypertension was diagnosed in accordance to the WHO/ISH criteria approved in 1999. Grade II arterial hypertension is defined as systolic blood pressure >160 mmHg and/or diastolic blood pressure >100 mmHg. Grade III arterial hypertension is defined as systolic blood pressure >180 mmHg and/or diastolic blood pressure >110 mmHg (European Society of Hypertension, 2003). Patients with diabetes mellitus, acute infectious diseases, kidney insufficiency, with atrial fibrillation, chronic congestive heart failure and acute disturbance of cerebral circulation were excluded from the study. Causes of secondary hypertension were excluded on the basis of clinical history, physical examination and by appropriate instrumental and biochemical tests. None of the subjects had hypercholesterolemia, none of them were smokers. Table 1 summarizes the clinical characteristics of the study population.

The control group included 24 healthy volunteers (10 women and 14 men) with the mean age of 37·9 years (24–52). Normal blood pressure is defined as systolic blood pressure <130 mmHg and diastolic blood pressure <85 mmHg.

Study design

This was an open-label randomized study. Patients, who met the required criteria and gave written consent, were categorized into treatment groups using a simple randomization method. Group I included 17 patients with grade II hypertension (8 men), Group II included 12 patients with grade III hypertension (6 men). For 2 weeks patients from the I an II groups were treated with the beta-adrenoblocker – Atenolol (4-(2-Oxy-3-izopropylaminoproxy)phenyl-acetamide) with dosage of 50 mg/day, and the angiotensin-converting enzyme inhibitor – Enalapril maleate (1-[N-[S]-1-carboxy-3-phenylpropyl]-L-alanyl]-L-prolin-1′ ethylic ether) 20–40 mg/day. Group III included 11 patients with grade II hypertension (5 men) and Group IV–12 patients with grade III hypertension (5 men). For 2 weeks the patients from the III and IV groups were treated with the beta-adrenoblocker – Nebivolol (R*[S*[S*-(S*)]]]-α,α′-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-I-benzopiran- 2 metanol) with dosage of 5 mg/day, and the angiotensin-converting enzyme inhibitor – Enalapril maleate 20–40 mg/day. The patients from the above mentioned groups corresponded to the same sex, age, duration of arterial hypertension and approximate body weight.

A venous blood sample was collected in heparinized syringes. The protocols of the studies were approved by the Ethics Committee of Grodno State Medical University, and written consent was obtained from each participant. All the experimental procedures followed institutional guidelines. All the studies were performed in the morning. The subjects fast the overnight for at least 12 h before the examination.

Determination of plasma nitrite/nitrate level

The level of the end NO metabolites, nitrite/nitrate, was measured in blood plasma spectrophotometrically using the Griess method (Moshage *et al.*, 1995). The participants of the investigation have had low nitrite and nitrate diet during

Table 1 Clinical characteristics of hypertensive patients and normotensive control subjects.

	Controls	grade II hypertension	grade III hypertension
Number	24	28	24
Age (years)	37·9 ± 1·9	43·3 ± 2·1	51·4 ± 1·23
Sex (male/female)	14/10	13/15	11/13
Duration of the disease (years)	–	5·4 ± 0·8	11·2 ± 1·2
Total cholesterol (mmol l)	4·70 ± 0·41	5·12 ± 0·45	5·16 ± 0·34
Glucose (mmol l)	5·1 ± 0·6	5·3 ± 0·5	5·4 ± 0·4
Creatinine (μmol l)	72·3 ± 4·2	73·8 ± 5·6	76·2 ± 6·6
Systolic pressure (mmHg)	118·8 ± 1·22	170·54 ± 2·08	194·7 ± 3·65
Diastolic pressure (mmHg)	74·7 ± 1·17	102·3 ± 1·44	111·8 ± 3·66

the study and 3–4 days before the studies (Wang *et al.*, 1997).

Determination of endothelial function

The endothelial function was measured by strain-gauge plethysmography. The measurements were carried out in a room with a constant temperature of 22–24°C. First, the forearm blood flow was measured at rest (after the patient had rested in supine position for 20 min). Then we studied the endothelium-dependent and endothelium-independent mechanisms (Celermajer *et al.*, 1992). The endothelium-dependent dilatation of peripheral arteries was induced by reactive hyperemia using a blood pressure cuff which was placed around the arm and inflated up to 280 mmHg within 5 min. The changes in the forearm blood flow were estimated after the removal of the cuff for 5 min. After the recovery of the initial forearm blood flow, the endothelium-independent response was studied, for which the patient had to take 0·5 mg of nitroglycerine sublingually. The forearm blood flow was measured before the nitroglycerine intake and within 15 min after its intake. The percentage changes in the forearm blood flow at 90th second after the release of occlusion and the forearm blood flow at the third minute after the nitroglycerine intake were calculated taking into account the baseline measurements. The criterion for endothelial dysfunction was less than 10% increase of the forearm blood flow after reactive hyperemia.

Measurements of blood oxygen transport indices

The blood oxygen transport indices: blood pO_2, pCO_2, pH, the actual of buffer bases excess, the standard excess of buffer bases, the standard hydrocarbonate concentration and the concentration of total carbone dioxide were measured using an ABL-330 'Radiometer' microgasoanalyzer (Radiometer, Copenhagen, Denmark). The haemoglobin-oxygen affinity was determined according to the p50 index (the blood pO_2 corresponding to its 50% oxygen saturation) by the 'mixing' method in the modification of Scheid & Meyer (1978), the standard p50 was assessed under standard conditions (pH = 7·4; pCO_2 = 40 mmHg and T = 37°C), whereas the actual p50 was calculated for the real values of these factors. On the basis of the p50 values obtained, the Hill equation was used to calculate the position of the oxyhaemoglobin dissociation curve.

The above studies were carried out before and after the two-week treatment.

Statistical analysis

Differences between 2 means were compared with use of the Student's two-tailed unpaired *t*-test. Pearson's r coefficient was used to test the correlation. The results are presented as means ± SEM. Differences were considered statistically significant at a level of $P<0·05$.

Results

Our studies showed that compared to healthy subjects, patients with grade II hypertension (Groups I and III) had 34·4% ($P<0·001$) and 32·4% ($P<0·001$) decreased blood plasma nitrate/nitrite contents, whereas Group II and IV patients (grade III hypertension) showed 47·5% ($P<0·001$) and 44·4% ($P<0·001$) diminished blood plasma nitrate/nitrite contents (Table 2).

According to the plethysmography data, the grade II hypertensive patients (Groups I and III) demonstrated reduced ($P<0·001$) endothelium-dependent vasodilatation, as compared to healthy subjects (Table 2). Endothelial dysfunction was found in 23·5% of the Group I patients and in 27% of the Group III patients (less than 10% increased forearm blood flow after reactive hyperemia). In grade III hypertensive patients (Groups II and IV), the endothelium-dependent vasodilatation was

Table 2 Plasma level concentration of nitrite/nitrate (μmol l), endothelium-dependent dilatation and endothelium-independent dilatation (%) in patients with arterial hypertension before and after treatment (M ± m).

			Atenolol, enalapril maleate		Nebivolol, enalapril maleate	
Index		**Control**	**Group I II grade AH**	**Group II III grade AH**	**Group III II grade AH**	**Group IV III grade AH**
Number		24	17	12	11	12
nitrite/nitrate (μmol l)	before treatment	24·85 ± 1·13	16·29 ± 0·65[a]	13·04 ± 0·92[a]	16·80 ± 0·95[a]	13·81 ± 0·97[a]
	after treatment		18·56 ± 0·78[b]	14·62 ± 0·93	23·41 ± 1·21[b]	19·14 ± 1·20 [b]
FBF on reactive hyperemia (%)	before treatment	30·90 ± 1·71	15·90 ± 1·78[a]	9·30 ± 1·76[a,c]	16·80 ± 2·57[a]	9·70 ± 1·71[a,c]
	after treatment		18·50 ± 1·78	12·90 ± 1·92	29·01 ± 2·90[b]	18·94 ± 2·32[b]
FBF on nitro-glycerine (%)	before treatment	34·89 ± 2·01	32·3 ± 2·65	28·95 ± 2·74	34·40 ± 2·67	30·30 ± 2·43
	after treatment		32·00 ± 2·30	28·90 ± 2·66	34·10 ± 2·98	30·60 ± 2·64

[a]Significant differences from control group.
[b]Significant differences after treatment.
[c]Significant differences from II grade AH.
AH, arterial hypertension; FBF, forearm blood flow.

diminished in comparison with both healthy subjects (P<0·001) and grade II hypertension patients (P<0·05). Endothelial dysfunction was observed in 75% of the cases in Groups II and IV. Endothelium-dependent vasodilatation was maintained in all examined groups (19% showed enhanced forearm blood flow after nitroglycerine intake). The hypertensive patients demonstrated a moderate positive correlation between the nitrate/nitrite level and the value of the increase in forearm blood flow after reactive hyperemia (r = 0·48, P<0·01).

Contrary to controls, the grade II hypertensive patients showed 5·4% (P<0·05, Group I) and 5·7% (P<0·05, Group III) increases in actual p50. The standard p50 was raised by 6·8% (P<0·05, Group I) and by 6·7% (P<0·05, Group III), the pO_2 was diminished (P<0·05), and the value for pCO_2 was elevated (P<0·05) (Table 3). The reduced haemoglobin-oxygen affinity in these patients should be considered as a compensatory response to hypoxia. As compared to controls, the grade III hypertensive patients had 5·1% (P<0·05, Group II) and 5·7% (P<0·05, Group IV) actual p50 decreases. Their standard p50 values were diminished by 6·3% (P<0·01, Group II) and by 5·9% (P<0·01, Group IV), which reflects the elevation of haemoglobin-oxygen affinity (Figs 1 and 2). In this situation, the pO_2 value was decreased (P<0·01), and that of pCO_2 was increased (P<0·01) compared to healthy subjects. The pH value decreased to 7·3 ± 0·01 units (P<0·05). These variations should be considered as a decompensation of blood oxygen indices that enhances tissue hypoxia. A moderate correlation was found between the pO_2 and the blood plasma nitrite/nitrate level (r = 0·51, P<0·01) as well as between the pO_2 and the increases in forearm blood flow (r = 0·41, P<0·05). A moderate positive correlation was noted between the standard p50 and the blood plasma nitrite/nitrate level (r = 0·44, P<0·01).

Thus, the results of our studies indicate that endothelial dysfunction in hypertensive patients significantly changes blood oxygen indices.

After 2 weeks of atenolol and enalapril treatment, the blood plasma nitrite/nitrate levels in grade II hypertensive patients (Group I) were raised by 13·9% (P<0·05) (Table 2). However, the endothelium-dependent vasodilatation remained unchanged. In patients with grade III arterial hypertension (Group III), this treatment did not essentially change the nitrite/nitrate levels and endothelium-dependent vasodilatation.

The atenolol and enalapril intake by grade II hypertensive patients (Group I) normalized haemoglobin-oxygen affinity, (standard p50 was reduced by 5·2% (P<0·05)) and increased pO_2 (P<0·05) (Table 3). In grade III hypertensive patients (Group III), our treatment reduced haemoglobin-oxygen

Table 3 Blood oxygen transport indices in patients with arterial hypertension before and after treatment (M ± m).

			Atenolol, enalapril maleate		Nebivolol, enalapril maleate	
Index		**Control**	**Group I AH II grade**	**Group II AH III grade**	**Group III AH II grade**	**Group IV AH III grade**
Number		24	17	12	11	12
p50 act (mmHg)	before treatment	28·62 ± 0·36	30·17 ± 0·74[a]	27·16 ± 0·53[a]	30·26 ± 1·03[a]	26·99 ± 0·74[a]
	after treatment		28·93 ± 0·39	28·79 ± 0·42[b]	29·04 ± 0·47	29·48 ± 0·85[b]
p50 stand (mmHg)	before treatment	26·75 ± 0·25	28·58 ± 0·37[a]	25·06 ± 0·23[a]	28·54 ± 0·39[a]	25·15 ± 0·36[a]
	after treatment		26·92 ± 0·45[b]	26·73 ± 0·33[b]	26·74 ± 0·36[b]	27·25 ± 0·60[b]
pO_2 (mmHg)	before treatment	36·10 ± 0·96	31·01 ± 0·81[a]	30·09 ± 0·71[a]	31·01 ± 0·81[a]	30·36 ± 0·71[a]
	after treatment		33·65 ± 0·71[b]	31·94 ± 1·50	35·16 ± 1·36[b]	32·16 ± 0·86
MetHb (%)	before treatment	0·73 ± 0·12	0·79 ± 0·09	0·94 ± 0·22	0·61 ± 028	0·74 ± 0·20
	after treatment		0·64 ± 0·12	0·56 ± 0·21	0·74 ± 0·20	0·77 ± 0·29
pH (units)	before treatment	7·34 ± 0·007	7·32 ± 0·008	7·30 ± 0·010[a]	7·32 ± 0·007	7·30 ± 0·01[a]
	after treatment		7·33 ± 0·010	7·32 ± 0·010	7·33 ± 0·009	7·32 ± 0·01
pCO_2 (mmHg)	before treatment	49·71 ± 1·22	55·38 ± 1·53[a]	56·30 ± 1·05[a]	55·27 ± 1·68[a]	57·81 ± 1·88[a]
	after treatment		52·91 ± 1·59	52·44 ± 1·05[b]	50·75 ± 1·15[b]	51·93 ± 1·64[b]
HCO_3^- (mmol l)	before treatment	27·85 ± 0·72	28·76 ± 0·63	29·09 ± 0·65	27·76 ± 1·52	29·13 ± 0·84
	after treatment		28·87 ± 0·63	28·60 ± 1·08	26·54 ± 1·55	28·08 ± 1·07
ABE (mmol l)	before treatment	1·95 ± 0·55	1·47 ± 0·66	2·03 ± 0·56	0·25 ± 0·60	0·87 ± 0·62
	after treatment		1·64 ± 0·34	1·81 ± 0·90	0·12 ± 0·30	1·00 ± 0·79
SBE (mmol l)	before treatment	2·21 ± 0·57	2·90 ± 0·56	2·78 ± 0·63	1·04 ± 0·58	2·30 ± 0·64
	after treatment		2·12 ± 0·42	2·65 ± 0·99	0·88 ± 0·54	2·10 ± 0·89
SBC (mmol l)	before treatment	25·11 ± 0·46	24·83 ± 0·62	24·92 ± 0·56	24·80 ± 0·74	24·94 ± 0·59
	after treatment		24·41 ± 0·30	24·33 ± 1·11	24·83 ± 0·62	24·31 ± 1·12

[a]Significant differences from control group.
[b]Significant differences after treatment.
p50 act, blood pO_2 under its 50% saturation by O_2 as determined at actual pH, pCO_2 and temperature; p50 stand, p50 at 37°C, pH 7·4 and pCO_2 = 40 mmHg.
MetHb, methaemoglobin; HCO_3^-, plasma concentration of hydrocarbonates; TCO_2, concentration of total carbon dioxide; ABE, the actual excess of buffer bases; SBE, standard excess of buffer bases; SBC, standard hydrocarbonate.

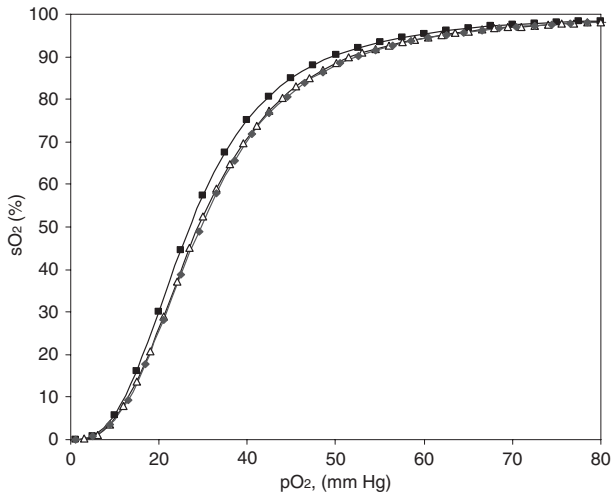

Figure 1 Actual oxyhaemoglobin dissociation curves: control (Δ), atenolol before treatment (■), atenolol after treatment (◆).

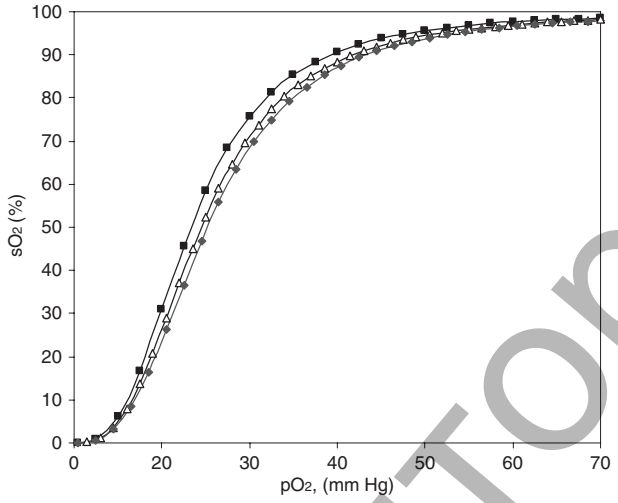

Figure 2 Actual oxyhaemoglobin dissociation curves: control (Δ), nebivolol before treatment (■), nebivolol after treatment (◆).

affinity (Fig. 1), actual p50 was elevated by 6·0% and standard p50 – by 6·1% in comparison with the initial values ($P<0·05$). However, the low values for pO_2 and high pCO_2 level and low blood pH values remained unchanged (Table 3), which indicate an imbalance between oxygen requirements and it supply to myocardium. Consequently, the atenolol and enalapril application did not completely compensate hypoxia in hypertensive patients.

Thus, the 2-week intake of atenolol and enalapril contributed to some improvement of NO synthesis, but only in grade II hypertensive patients. This treatment was, however, insufficient to correct the abnormalities found, which was confirmed by other authors (Erzen *et al.*, 2006). Higashi *et al.* (2002) also found an improvement in endothelial function after the application of angiotensin-converting enzyme inhibitor in mild hypertension and inefficiency of this treatment in severe hypertension.

Nebivolol and enalapril treatment raised nitrite/nitrate level by 39·3% ($P < 0·01$) in grade II hypertensive patients, and by 38·5% ($P < 0·01$) in grade III hypertensive patients. The endothelium-dependent vasodilatation was significantly improved in both examined groups ($P < 0·05$), especially in grade II hypertensive patients (the forearm blood flow to reactive hyperemia reach the control values). The endothelium-independent vasodilatation did not change in all the studied groups (Table 2).

The application of nebivolol in grade II hypertensive patients (Group III) improved oxygen supply, pO_2 ($P < 0·05$) was increased and pCO_2 diminished ($P < 0·05$) (Table 3). Nebivolol reduced the manifestations of hypoxia in patients of Group IV [pCO_2 was decreased ($P < 0·05$)], however, the pO_2 was not significantly changed. To a greater extent, the p50 changes depended on its initial level. In grade II hypertensive patients, the actual p50 remained unchanged and the standard p50 decreased by 6·3% ($P < 0·05$). Under the influence of nebivolol, grade III hypertensive patients showed by 9·2% increased actual p50 ($P < 0·05$) (Fig. 2) and by 8·3% increased standard p50 ($P < 0·05$), i.e. nebivolol normalized haemoglobin-oxygen affinity.

Thus, the 2 week intake of nebivolol improved endothelial function, increased NO synthesis and acted positively on blood oxygen transport indices.

Discussion

The beneficial effect of nebivolol on blood oxygen transport indices can be explained by an enhanced NO synthesis which can change haemoglobin-oxygen affinity (Zinchuk *et al.*, 2004). As a result, blood oxygen indices can affect the activity of the L-arginine-NO system. The NO formation is an oxygen-dependent process (Cannon, 1998). Oxygen is one of the essential substrates for NO synthesis and can play a limiting role for NO formation (Shaul *et al.*, 1995). Hypoxia can modify the activity of NO-synthase (Shaul *et al.*, 1993). When pO_2 is less than 30 mmHg, the enzymatic synthesis of NO decreases (Kourembanas *et al.*, 1998).

Earlier, experimental studies *in vitro* and *in vivo* showed that nebivolol had a vasodilatatory effect due to its stimulation of NO production (Cockroft *et al.*, 1995; Paranti *et al.*, 2000). Some authors show that nebivolol improved endothelium-dependent vasodilatation in healthy subjects and hypertensive patients (Cockroft *et al.*, 1995; Dawes *et al.*, 1999). However, these studies employed single intraarterial administration and disregarded the extent of hypertension severity. For clinical practice, it is important to find whether this drug would produce a similar effect after a per os intake. Some investigations demonstrated that peroral nebivolol intake improved the vasodilatatory function of the endothelium (Tzemos *et al.*, 2001; Arosio *et al.*, 2002; Ugrekhelidze *et al.*, 2006). The results of our investigation demonstrate that nebivolol improved the endothelial vasodilating function and raised the blood plasma nitrite/nitrate levels not only at early stages of the disease but also in severe hypertension.

It is known that the lowest concentration of nebivolol elevates p50 values by 4·3 + 0·8 mmHg at actual pH and CO_2. And the subsequent 2-and 3-fold increases in nebivolol concentration raised p50 value by 7·5 ± 1·1 mmHg ($P < 0·01$) and 10·6 ± 0·7 mmHg ($P < 0·01$), respectively, which demonstrates a dose-dependent effect of the drug (Zinchuk & Zinchuk, 2007). Nitric oxide can be an allosteric effector of haemoglobin, increasing or decreasing its oxygen affinity – probably, through the generation of different NO-Hb derivatives (changes in haemoglobin-oxygen affinity in experiments *in vitro* with various ratios between the NO and the haemoglobin and varying oxygen pressures) (Stepuro & Zinchuk, 2006).

Historically, red blood cells have been considered as transporters of oxygen and carbon dioxide, with the uptake of one and subsequent release of the other. With the advent of the field of NO biology, red blood cells also were thought to be scavengers of NO that could effectively suppress its bioactivity (Angelo *et al.*, 2006). Therefore, not only the interaction of haemoglobin with oxygen, but also that with NO should be taken into account. NO has much higher affinity for the deoxyhaemoglobin haeme group compared to oxygen and carbon dioxide, which suggests its competition with oxygen for the corresponding sites of partially oxygenated haemoglobin (Gladwin *et al.*, 2000).Therefore, blood flow deficiency and impaired oxygen transport to tissues are important factors controlling NO formation in the body (Zinchuk *et al.*, 2004). Haemoglobin-oxygen affinity, regulating NO level, can contribute to the equilibrium between NO and oxygen in the vascular network. On the other hand, the endothelium can be involved in formation of these impairments because only NO synthesized in sufficient amounts can maintain normal blood flow and oxygen transport to tissues. Endothelial dysfunction impairs formation of different haemoglobin NO-derivatives, that influence not only the release of NO at different sites of the vascular bed, but also on haemoglobin-oxygen affinity, and accordingly, on optimal blood oxygenation in capillaries of pulmonary circulation and its deoxygenation in capillaries of systemic circulation.

Three main NO-derivatives of haemoglobin are known. There are nitrosylhaemoglobin, nitrosohaemoglobin and methaemoglobin (Gladwin *et al.*, 2000). However, their functions are not fully established yet. NO interacts with oxyhaemoglobin to produce methaemoglobin and nitrates, and while interacting with deoxyhaemoglobin, NO forms nitrosohaemoglobin, which is desintegrated to haemoglobin and nitrite in the presence of oxygen (Kosaka, 1999; Gladwine *et al.*, 2003). About 70% of endogenous NO is metabolized to nitrates and nitrites (Kelm, 1999; Kato *et al.*, 2004). Various haemoglobin NO-derivatives are known to affect whole blood haemoglobin-oxygen affinity in various ways (Kosaka, 1999), which may be of significant importance for gaseous exchange (Zinchuk & Dorokhina, 2002). The presence of different haemoglobin compounds containing NO can affect the haemoglobin-oxygen affinity in different ways. Methaemoglobin and nitrosohaemoglobin increase the haemoglobin-oxygen affinity, whereas nitrosylhaemoglobin decreases it. The oxygen-dependent nature of the equilibrium between nitrosylhaemoglobin and nitrosohaemoglobin provides a balance between the blood flow and its requirements, i.e. an optimal balance between hypoxic vasodilatation and hyperoxic vasoconstriction. In arterioles and capillaries, erythrocytes sequestrate NO, reducing its participation in vasodilatation and forming blood oxygen transport indices (Zinchuk *et al.*, 2004).

As it is known, the vascular endothelium is heterogenous in its NO-forming function and its influences on haemoglobin-oxygen affinity. It was found that the basal level of NO production in arteries is higher than that in veins (Moncada *et al.*, 1991). Immunological studies show that more NO is synthesized in small blood vessels than in large blood vessels (Kelm & Rath, 2001). Thus, haemoglobin maintains a definite plasma pO_2 level and contributes to adequate supply of oxygen to tissues. Therefore the mechanisms of oxygen transport and blood oxygen-binding properties can regulate the activity of the L-arginine-NO pathway (Zinchuk *et al.*, 2004). The normal basal NO production by endotheliocytes plays a very important role in the mechanisms of coronary blood flow autoregulation, in the phenomenon of 'escaping' the pressing effect of angiotensine II and in the reaction of reactive hyperemia of vessels (Hermann *et al.*, 2006; Spieker *et al.*, 2006). The enhanced production of vascular NO has great adaptive significance since it does not only improve tissue perfusion, but also inhibits thrombocytes adhesion and their aggregation.

Impairment of the endothelial NO-synthase function to a great extent stipulates loss of control over the vascular tone, resulting in a decrease of adequate oxygen supply to tissues by blood flow. It has been proven that severe hypoxia provokes constriction of different vessels, including coronary arteries. This is more likely to be due to transitional inhibition of basal NO secretion rather than to production of endothelium-dependent vasoconstrictors (Mehta, 1995). Acute and chronic hypoxia in experiment disturbs NO production not only in coronary, but also in pulmonary arteries.

A specific defect of the endothelial NO-producing system, proatherogenic and vasospastic effects of endothelial dysfunction and an impaired blood oxygen function may contribute to development of cardiovascular complications related to elevated arterial pressure. Acting against the pathological processes causing the development of endothelial dysfunction and hypoxia will improve the outcome in patients with arterial hypertension. Therefore, further investigations of the mechanisms of development of the endothelial dysfunction in case of arterial hypertension and search for a possible correction of these impairments still remain to be discussed.

Thus, the results of our study indicate significant disturbances of blood oxygen transport indices in hypertensive patients with endothelial dysfunction. Treatment of hypertensive patients with nebivolol corrects the blood oxygen transport indices, stimulates NO production and improves endothelium-dependent dilatation. Normalization of blood oxygen transport indices may regulate the activity of the L-arginine-NO pathway. Thus,

nebivolol may improve efficiency of the treatment of hypertension.

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