Beneficial effects of nebivolol treatment on oxidative stress parameters in patients with slow coronary flow

Koroner yavaş akım olan hastalarda nebivolol tedavisinin oksidatif stres parametreleri üzerine olumlu etkileri

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Objectives: Imbalance between oxidative stress and antioxidant defense has been demonstrated in patients with slow coronary flow (SCF). The aim of this study was to investigate the effect of nebivolol treatment on oxidative stress parameters in SCF patients.

Study design: The study included 32 patients (10 females, 22 males; mean age 53.3±5.2 years) with SCF and 32 control subjects (14 females, 18 males; mean age 50.6±5.2 years) with normal coronary arteries on angiography. Coronary slow flow was determined by the TIMI frame count method. Patients with SCF received nebivolol treatment (5 mg/day) for six months. Blood samples were analyzed for malondialdehyde (MDA) and serum nitric oxide (NO) levels, and erythrocyte catalase (CAT) and erythrocyte superoxide dismutase (SOD) activities in the control group and, in SCF patients, at baseline and after six months of nebivolol treatment.

Results: The two groups were similar with respect to age, body mass index, blood pressure, heart rate, and lipid profile. Smoking was more frequent in the SCF group compared to the controls. TIMI frame counts measured from the left anterior descending, circumflex, and right coronary arteries were significantly higher in the SCF group ($p<0.0001$). Baseline MDA and NO levels, and SOD and CAT activities were significantly different between the two groups, with significantly increased MDA ($p<0.0001$), and significantly decreased SOD ($p<0.0001$), CAT ($p<0.001$), and NO ($p<0.001$) in the SCF group. After six months of nebivolol treatment, all oxidative stress parameters showed significant improvements compared to the baseline values ($p<0.0001$ for MDA, SOD, CAT, and NO) and approximated to the values of the control group.

Conclusion: Our results show that nebivolol treatment may be beneficial to improve oxidative stress parameters in patients with SCF, which is considered to be an early stage of atherosclerosis.

Key words: Adrenergic beta-antagonists; blood flow velocity; coronary circulation; lipid peroxidation; nitric oxide; oxidative stress.

Amaç: Koroner yavaş akım (KYA) olan hastalarda oksidatif stres ile antioksidan savunma arasında dengesizlik gösterilmiştir. Bu çalışmada, KYA hastalarında nebivolol tedavisinin oksidatif stres parametreleri üzerine etkisi araştırıldı.

Çalışma planı: Çalışmaya KYA saptanan 32 hasta (10 kadın, 22 erkek; ort. yaş 53.3±5.2) ve anjiyografide koroner arterleri normal bulunan 32 kontrol (14 kadın, 18 erkek; ort. yaş 50.6±5.2) alındı. Koroner yavaş akım TIMI kare sayısı yöntemiyle belirlendi. Koroner yavaş akım olan hastalara altı ay süreyle 5 mgr/gün dozunda nebivolol tedavisi uygulandı. Kontrol grubundan ve KYA hastalarından başlangıçta ve altı ay süreyle nebivolol tedavisi sonrasında alınan kan örneklerinde malondialdehit (MDA) ve serum nitrik oksit (NO) düzeyleri, eritrosit katalaz (CAT) ve eritrosit süperoksit dismutaz aktiviteleri (SOD) ölçüldü.

Bulgular: İki grup yaş, beden kütle indeksi, kan basıncı, kalp hızı ve lipit profili yönünden benzer özelliklerdeydi. Koroner yavaş akım grubunda sigara içme kontrollere göre daha yaygındı. Sol ön inen, sirkumfleks ve sağ koroner arterlerden ölçülen TIMI kare sayıları KYA grubunda anlamlı derecede fazla bulundu ($p<0.0001$). Kan incelemesinde başlangıç MDA ve NO düzeyleri ve SOD ve CAT aktiviteleri iki grup arasında anlamlı farklılık gösterdi; MDA KYA grubunda anlamlı derecede artmış bulunurken ($p<0.0001$), SOD ($p<0.0001$), CAT ($p<0.001$) ve NO ($p<0.001$) anlamlı derecede düşük değerler sergiledi. Nebivolol tedavisi sonrasında altıncı ayda oksidatif stres parametrelerinin tümünde başlangıç değerlerine göre anlamlı düzelmeler görüldü (MDA, SOD, CAT, ve NO için $p<0.0001$). Bu parametrelerin hepsi kontrol grubundaki değerlere yaklaşmıştı.

Sonuç: Bulgularımız nebivolol tedavisinin, aterosklerozun erken evresi olarak kabul edilen KYA'da oksidatif stres parametrelerinin iyileştirilmesinde yararlı olabileceğini göstermektedir.

Anahtar sözcükler: Adrenerjik beta-antagonisti; kan akım hızı; koroner dolaşım; lipit peroksidasyonu; nitrik oksit; oksidatif stres.

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Slow coronary flow (SCF) is commonly seen during routine coronary angiography and is characterized by delayed opacification of the distal vasculature in the presence of angiographically normal or near-normal coronary arteries. Tambe et al.[1] were the first to note this phenomenon in patients without an atherosclerotic lesion. Recently, capillary disorders have been suggested to be responsible for decreased coronary flow.[2] Microvascular dysfunction is associated with endothelial and vasomotor dysfunction, while occlusive coronary artery disease and myocardial ischemia are associated with impaired coronary flow.[3-6] In view of these data, SCF can be defined as a form or at least an early stage of atherosclerosis involving small coronary arteries. Several reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals may play part in the pathophysiology of atherosclerosis, stroke, and cardiovascular disease.[7] Several studies showed significant changes in plasma levels of oxidative stress parameters such as malondialdehyde (MDA), erythrocyte superoxide dismutase (SOD), and erythrocyte catalase (CAT) in patients with SCF compared to healthy individuals.[8,9] Malondialdehyde, one of the final products of lipid peroxidation, is directly associated with cell damage by plasma levels of ROS.[10,11] Superoxide dismutase and CAT are important antioxidants in the breakdown and elimination of ROS.[12] Concentration of SOD in the arterial wall is sufficiently high to suppress pathological effects including the destructive effect of peroxynitrite resulting from the reaction between superoxide anions and nitric oxide (NO).[13] Imbalance between increased oxidative stress and antioxidant defense, in particular NO inactivation induced by superoxide and other ROS may contribute to endothelial dysfunction.[14,15]

Nebivolol is a highly selective beta1-adrenoreceptor antagonist with additional vasodilatory properties for endothelium-mediated NO[16,17] and protective properties for NO bioactivity.[18-20] It is commonly used in patients with hypertension, coronary artery disease, and heart failure. In addition, it has antiproliferative effects on vascular smooth muscle cells.[20] Endothelial-dependent vasodilation has been reported to be improved in hypertensive patients receiving nebivolol treatment.[18]

In a literature search, we found no data about the effects of nebivolol on oxidative stress parameters in SCF patients with impaired endothelial dysfunction and oxidative stress. The aim of this study was to investigate the effect of nebivolol treatment on serum concentrations of NO, MDA, CAT, and SOD in SCF patients.

PATIENTS AND METHODS

Patients. The study included 32 patients (10 females, 22 males; mean age 53.3±5.2 years) with angiographically normal coronary arteries and SCF in one of the coronary arteries and 32 controls (14 females, 18 males; mean age 50.6±5.2 years) with normal coronary arteries on angiography. Patients with significant lesions, atherosclerotic heart disease, tortuous coronary arteries, coronary ectasia, muscular bridge, myocardial or valvular heart disease, left ventricular hypertrophy shown by echocardiography, uncontrolled hypertension, renal dysfunction, ligament disease, hypothyroidism, and those receiving proton pump inhibitors or antibiotics or vitamin supplements for the past eight weeks were excluded from the study.

Patients with SCF received nebivolol treatment (5 mg/day) for six months. Following the first visit, the patients were questioned about side effects of the treatment and were examined in the cardiology clinic every month. Treatment was well-tolerated by most of the patients. Nebivolol dose was reduced to 2.5 mg in two patients presenting with hypotensive symptoms. All the study subjects gave signed informed consent for participation in the study and the study protocol was approved by the ethics committee of our institution (Number: 2008/3-1, 06.03.2008).

Angiographic documentation of SCF. Coronary angiography was performed using the femoral artery approach and standard Judkins technique. Iopromide (Ultravist-370, Schering AG, Germany) was used as the contrast agent during the procedure. For quantitative measurement of coronary blood flow, the time from the first visibility of contrast agent to the endpoint of the left anterior descending (LAD) artery, circumflex (Cx) artery, or one of the arteries of the right coronary artery (RCA) was measured using a cine viewer TIMI-frame counter. The endpoints were defined as the bifurcation of the LAD and Cx and the first branch of the posterolateral artery for the RCA. TIMI frame count for each artery was obtained by subtracting the last frame from the first frame. In addition, the TIMI frame count of the LAD was divided by a factor of 1.7 to obtain corrected TIMI frame count for the LAD. The reference limits proposed by Gibson et al.[21] were used for normal TIMI frame count for each artery; hence, 36.2±2.6 for the LAD; 22.2±4.1 for the Cx; 20.4±3.0 for the RCA. Patients with a corrected TIMI frame count greater than 2 standard deviations from the normal range of a particular coronary artery were classified as having SCF, while patients with a corrected TIMI frame count of ≤2 standard deviations

from the normal were considered to have normal coronary flow.[22]

Blood analysis. Blood samples were collected from both patients and controls at baseline and at 6 months in SCF patients receiving nebivolol treatment. All blood samples were drawn from the vein in the forearm and collected into 5-ml Vacutainer tubes containing potassium ethylenediaminetetraacetate (EDTA). The samples were centrifuged at 1000 g at 4 °C for 10 minutes to separate the pellets and supernatant. The supernatant was removed cautiously. Erythrocytes were washed three times using 0.9% NaCl solution to remove residuals. The mixed solution containing erythrocytes and saline was centrifuged at 1,000 g at 4 °C for 10 minutes following each process. Hemolysates were prepared directly from washed red cells to measure biochemical parameters.

Erythrocyte catalase activity was measured using the Beutler test at 230 nm based on the rate of decomposition of hydrogen peroxide by catalase and expressed as units per gram of hemoglobin (U/gHb).[10]

Superoxide dismutase activity was measured using the Fridovich's method.[23] This method uses xanthine and xanthine oxidase to produce superoxide radicals which react with p-iodonitrotetrazolium violet to generate a red formazan measured at 505 nm. The result was expressed as units per gram of hemoglobin (U/gHb).

Lipid peroxidation of plasma samples was measured by the method of Ohkawa et al.[24] based on thiobarbituric acid-reactive MDA formation by absorption at 532 nm and was expressed as nmol/ml. In addition, hemoglobin level was measured by the cyanmethemoglobin method using a Spectronic-UV 120 spectrophotometer.

Nitric oxide concentration was measured by the enzymatic Griess assay using a reagent kit (Nitric Oxide, Colorimetric Assay, Roche). The absorbance was read at 540 nm using a spectrophotometer and expressed as U/ml.

Statistical analysis. The results were expressed in mean ± standard deviation (SD). Differences between SCF patients and controls were compared using the independent samples t-test. Pre- and post-treatment parameters of SCF patients were compared using the paired t-test. The Lilliefors test was also performed to check the normal distribution of parameters. All data were processed using the SPSS statistical software (version 11.0) and a *p* value of less than 0.05 was considered to be significant.

RESULTS

Clinical characteristics and TIMI frame counts of SCF patients and controls are summarized in Table 1. The two groups were similar with respect to age, body mass index, systolic and diastolic blood pressures, heart rate at rest, and levels of plasma glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and triglyceride. Smoking was

Table 1. Clinical and laboratory characteristics, and TIMI frame count data of the patients and controls

	Patients (n=32)			Controls (n=32)			
	n	%	Mean±SD	n	%	Mean±SD	*p*
Age (years)			53.3±5.2			50.6±5.2	0.143
Sex							0.270
Female	10	31.3		14	43.8		
Male	22	68.8		18	56.3		
Body mass index (kg/m^2)			28.6±3.9			28.2±4.8	0.787
Systolic blood pressure (mmHg)			122.2±12.6			120.9±8.5	0.644
Diastolic blood pressure (mmHg)			76.1±7.7			75.3±7.6	0.697
Heart rate (beat/min)			74.1±9.9			75.9±9.6	0.454
Smoking	19	59.4		9	28.1		**<0.001**
Plasma glucose (mg/dl)			108.6±32.2			101.8±3.4	0.241
Total cholesterol (mg/dl)			181.4±31.2			189.9±36.9	0.329
LDL cholesterol (mg/dl)			110.0±24.7			107.6±26.8	0.714
HDL cholesterol (mg/dl)			37.4±9.4			37.7±8.0	0.898
Triglyceride (mg/dl)			177.6±81.7			160.8±89.5	0.436
TIMI frame count							
Left anterior descending artery (Corrected)			37.6±9.9			19.9±1.2	**<0.0001**
Circumflex artery			30.6±11.3			21.8±1.7	**<0.0001**
Right coronary artery			26.8±11.2			19.5±1.7	**<0.0001**

Table 2. Oxidative parameters in controls and in SCF patients before and 6 months after nebivolol treatment

	Controls (n=32) (Mean±SD)	Patients with SCF (n=32) Baseline (Mean±SD)	Patients with SCF (n=32) At 6 months (Mean±SD)	p^1	p^2
Erythrocyte catalase (U/gHb)	7.3±2.6	5.3±1.9	7.0±1.8	**<0.001**	**<0.0001**
Erythrocyte superoxide dismutase (U/gHb)	1647.5±530.4	1133.0±415.3	1530.3±392.5	**<0.0001**	**<0.0001**
Malondialdehyde (nmol/ml)	2.0±0.6	3.3±1.6	2.2±0.9	**<0.0001**	**<0.0001**
Nitric oxide (U/ml)	6.1±2.9	4.1±1.6	6.1±1.5	**<0.001**	**<0.0001**

SCF: Slow coronary flow; p^1: Comparison of the two groups at baseline; p^2: Comparison between pre-and post-treatment values of patients with SCF.

more frequent in the SCF group compared to the controls and none of the patients quit smoking during the treatment. TIMI frame counts were significantly higher in the SCF group for all three coronary arteries.

Baseline MDA and NO levels, and SOD and CAT activities were significantly different between the two groups, with significantly increased MDA ($p<0.0001$), and significantly decreased SOD ($p<0.0001$), CAT ($p<0.001$), and NO ($p<0.001$) in the SCF group (Table 2).

After six months of nebivolol treatment, all oxidative stress parameters showed significant improvements compared to the baseline values ($p<0.0001$ for MDA, SOD, CAT, and NO) and approximated to the values of the control group (Table 2).

DISCUSSION

From this study, we derived two main results: *(i)* Baseline MDA levels were found to be significantly increased, and CAT, SOD, and NO activities were significantly decreased in patients with SCF compared to controls. *(ii)* Following nebivolol treatment for six months, all adverse indications of oxidative stress parameters in the SCF group showed significant improvements, with decreases in MDA levels, and increases in CAT, SOD, and NO levels. Oxidative stress indicators were similar in both the patient and control groups after nebivolol treatment.

Slow coronary flow is an angiographic finding characterized by delayed opacification of the epicardial coronary arteries, in the absence of significant obstruction, thrombus, spasm, or dissection. The incidence of SCF has been reported to be 1% in patients undergoing coronary angiography.[25] Yaymacı et al.[6] demonstrated myocardial ischemia in 85% of patients with SCF having positive scintigraphic findings. Intimal thickening, calcification through the coronary artery walls, and atheroma have been shown by intravascular ultrasound imaging in patients with SCF.[26] Mosseri et al.[3] showed histopathological changes (pathologic small coronary arteries with fibromuscular hyperplasia, hypertrophy of the media, myointimal proliferation, and endothelial degeneration) in ventricular endomyocardial biopsy specimens of six patients with large patent arteries with slow flow. Inflammation also may lead to cellular damage due to oxidation of lipids, proteins, or DNA.[9]

Malondialdehyde level is commonly used as an indicator of lipid peroxidation. Malondialdehyde inactivates membrane transporters by forming intra- and intermolecular cross links. Increased MDA level in our patients is an indicator of severe oxidative stress. On the other hand, SOD and CAT are known to be major antioxidant enzyme systems.[12] Actually, during oxidative stress, inflammatory signal-sensitive sites in promoter region may promote SOD and CAT expression which is associated with increased expression of inducible endothelial nitric oxide synthesis (iNOS).[27] Although oxidative stress is often associated with compensatory increases in SOD and CAT levels, several studies in parallel with our study have shown increased ROS production as well as decreases in SOD and CAT levels under oxidative stress. Decreased SOD and CAT levels are mostly seen in patients with high-degrees of oxidative stress and cellular damage. We also found significantly increased oxidative damage in SCF patients compared to controls.

Nebivolol, which has vasodilatory properties for NOS activation in the endothelium,[17] increases NO release and decreases endothelial cellular oxidative stress, a key factor in the mechanism of atherogenesis.[28] A direct and antioxidant interaction between nebivolol and reactive oxygen radicals has been shown in a rat study, possibly as result of a direct ROS-eliminating action.[29] Nebivolol treatment improved ROS-induced impairment of endothelium-dependent vasorelaxation.[29] Furthermore, nebivolol infusion increased forearm blood flow in normotensive subjects through blockade of NOS inhibitors and activation of the L-arginine/NO pathway.[30] Systemic oxidative stress has also been shown to be reduced in healthy controls receiving nebivolol treatment.[31]

Limitations of the study. Case-controlled, non-randomized, and open-ended design of the study with a small sample size constitutes the major limitation of our study. In addition, the follow-up of patients included only serum oxidative stress parameters. It remains unknown whether decreased oxidative stress is associated with alleviation of stress or with direct effect of nebivolol, or both.

In our study, oxidative stress parameters in patients with SCF improved significantly after six months of nebivolol treatment. Our results show that nebivolol treatment may be beneficial to improve oxidative stress parameters in patients with SCF, which is considered to be an early stage of atherosclerosis. Furthermore, nebivolol treatment can reduce the risk for cardiovascular disease in these patients.

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