Nebivolol therapy improves endothelial function and increases exercise tolerance in patients with cardiac syndrome X

Kardiyak sendrom X'li hastalarda nebivolol tedavisi ile endotel fonksiyonu ve egzersiz toleransı düzelmektedir

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Abstract

Objective: We sought to determine whether nebivolol affects coronary endothelial function and exercise induced ischemia in patients with cardiac syndrome X (CSX).

Methods: The study protocol undertaken was based on a single-blind randomized controlled prospective study. After a 2-week washout period, 38 patients with cardiac syndrome X were randomized to receive either nebivolol 5 mg daily (n=19) or metoprolol 50 mg daily (n=19) in a single-blind design for 12 weeks. The control group under study was consisted of 16 age- and gender-matched subjects with negative treadmill exercise tests. Plasma endothelial nitric oxide (NOx), L-arginine, and asymmetric dimethylarginine (ADMA) were measured in all patients at baseline and after 12 weeks of treatment. Statistical differences among groups were tested by one-way analysis of variance and unpaired samples t test for parametric; Kruskal-Wallis and Mann-Whitney U tests for non-parametric variables, respectively. A paired – samples t test was used to compare continuous variables before and after drug therapy.

Results: At baseline, plasma level of NOx, L-arginine, and L-arginine/ADMA ratio were lower ($p<0.001$ for all) in patients with CSX than in the control patients. Whereas, the plasma ADMA levels were increased in the patient group ($p<0.001$). After 12 weeks of drug therapy, the patients taking nebivolol had increased levels of plasma NOx , plasma L-arginine, the L-arginine/ADMA ratio and decreased levels of plasma ADMA compared to those of the patients taking metoprolol ($p<0.001$). In addition, exercise duration to 1-mm ST depression and total exercise duration significantly increased after treatment in the nebivolol group compared to the metoprolol group ($p<0.01$). In the nebivolol group, Canadian Cardiovascular Society (CCS) angina classification improved by one or more categories in 12 (70%) patients, whereas it deteriorated or remained in the same category in 5 (30%) patients. Meanwhile, in the metoprolol group, the CCS angina classification improved by one or more categories in 7 (41%), whereas it deteriorated or remained in the same category in 10 (59%) patients.

Conclusion: Circulating endothelial function parameters (plasma ADMA, L-arginine, NOx levels) were impaired in patients with CSX. Nebivolol treatment was associated with better improvements in both circulating endothelial function and exercise stress test parameters than metoprolol. We believe that further studies are needed to evaluate the effects of nebivolol treatment on long-term clinical outcomes in patients with CSX. *(Anadolu Kardiyol Derg 2009; 9: 371-9)*

Key words: Cardiac syndrome X, nebivolol, endothelial function, asymmetric dimethylarginine, nitric oxide

Özet

Amaç: Çalışmamızda, kardiyak sendrom X'li (KSX) hastalarda nebivolol tedavisinin endotel fonksiyonu ve egzersizin indüklediği iskemi üzerine etkisini araştırdık.

Yöntemler: Çalışma tek kör, randomize kontrollü ve prospektif olarak dizayn edildi. İki haftalık ilaçsız periyod sonrasında kardiyak sendrom X'li 38 hasta nebivolol 5 mg/gün (n=19) veya metoprolol 50 mg/gün (n=19) gruplarına tek kör olarak randomize edilerek 12 haftalık tedavi sonrası tekrar değerlendirildi. Kontrol grubu olarak efor testi negatif olan yaş ve cinsiyet uyumlu 16 hasta alındı. Tüm hastalarda tedavi öncesi ve 12 haftalık tedavi sonrası plazma endotelyal nitrik oksit (NOx), L-arjinin ve asimetrik dimetilarjinin (ADMA) düzeyleri ölçüldü. Gruplar arası çoklu karşılaştırmalar parametrik değişkenler için tek yönlü ANOVA ve eşleştirilmemiş örneklem t testleri, parametrik olmayan değişkenler için ise Kruskal–Wallis ve Mann Whitney U testleri ile yapıldı. İlaç tedavisi öncesi ve sonrası değişkenlerin karşılaştırılması eşleştirilmiş örneklem t testi ile yapıldı.

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Bulgular: Kardiyak sendrom X'li hastaları 16 kişiden oluşan sağlıklı kontrol grubu ile karşılaştırıldığında, kontrol grubuna kıyasla KSX'li hastalarda plazma NOx, L-arjinin ve L-arjinin/ADMA düzeyi azalmış, plazma ADMA düzeyi ise artmış olarak bulundu. On iki hafta süren tedavi sonrası metoprolol grubuna kıyasla nebivolol grubunda plazma NOx, L-arjinin ve L-arjinin /ADMA düzeyi artmış, ADMA düzeyi ise azalmış olarak saptandı ($p<0.001$). Ayrıca nebivolol grubunda toplam egzersiz süresi ve egzersiz sırasında 1 mm ST depresyonu gelişme zamanı uzadı ($p<0.01$). Kanada Kardiyovasküler Derneği (CCS) angina sınıflamasına göre nebivolol grubundaki hastaların %70'de angina skoru en az bir kategori düzelirken %30 hastada aynı kaldı veya kötüleşti. Metoprolol grubunda ise hastaların %41'nin angina skoru düzelirken %59 hastanınki ise aynı kaldı veya kötüleşti.

Sonuç: Kardiyak sendrom X'li hastalarda kanda endotel fonksiyonunu gösteren belirteçlerin (plazma NOx, L-arjinin, ADMA) bozulduğunu göstermiş olduk. Nebivolol tedavisi bu belirteçleri düzeltmekte ve egzersiz süresini uzatmaktadır. Yapılacak çalışmalarla KSX'in tedavisinde nebivolol'ün uzun süreli sonuçları incelenmelidir. *(Anadolu Kardiyol Derg 2009; 9: 371-9)*

Anahtar kelimeler: Kardiyak sendrom X, nebivolol, endotelyal fonksiyon, asimetrik dimetilarjinin, nitrik oksit

Introduction

The cause of chest pain in patients with angina in spite of normal coronary arteries is a common clinical challenge. Cardiac syndrome X (CSX) is a clinical entity in which patients have angina-like pain on effort, ST segment depression on an exercise stress test and normal coronary arteries at angiography in the absence of any other cardiac diseases or systemic diseases (e.g. hypertension, diabetes) known to influence vascular function (1). The real cause of the syndrome has not been established, but it is most frequently associated with impaired endothelial-dependent arterial vasodilatation, decreased nitric oxide production, and increased sensitivity to sympathetic stimulation (2). Recent evidence has suggested that endothelial nitric oxide (NOx) bioavailability and inhibition of nitric oxide (NO) synthesis by endogenous inhibitors of NO synthase (NOS) may be casually involved in the progression of coronary microvascular dysfunction in a significant portion of patients with syndrome X (3, 4).

Moreover, recent studies have revealed that increased plasma level of asymmetric dimethylarginine (ADMA) -which is known to inhibit NO synthesis by competing with L-arginine, for the active site of endothelial NOS- contribute to the impairment of NOx bioavailability in patients with CSX (5).

Beta blockers have been regarded as a therapeutic option in patients with CSX. Convincing data to the efficacy of these agents are sparse or lacking, and when available, not very encouraging. These circumstances may arise from the fact that atenolol or metoprolol have been preferred as beta- blocker selection. However, these agents have been shown to decrease anginal symptoms, delay or eliminate ischemic electrocardiogram changes on an exercise treadmill, and improve diastolic filling parameters in a CSX population with positive treadmill stress electrocardiograms (6). There are no influential data on the effect of these agents on endothelial dysfunction. Nebivolol is a highly selective beta1-adrenergic receptor antagonist that also possesses vasodilator properties that are attributed to endothelium-derived NO (7-11). Several previous studies have reported that nebivolol causes vasodilatation in the human forearm vascular bed and that this effect can be blocked by inhibitors of NOS (10).

Accordingly, the present study has two hypothesis, the first, nebivolol has beneficial effect on systemic endothelial function by decreasing plasma levels of ADMA in a well-defined group of patients with CSX; and the second, nebivolol therapy is associated with better improvement on exercise-induced ischemia in patients with CSX in comparison to metoprolol therapy.

Methods

Patient population

The study population consisted of 38 prospectively-enrolled consecutive patients with diagnosis of CSX. The diagnosis of CSX was based on the presence of a typical exercise-induced angina pectoris, transient ischemic ST-segment depression (>1 mm) during exercise, and angiographically normal coronary arteries in the absence of coronary artery spasm (determined by hyperventilation maneuver). Patients who had a history of established hypertension, significant arrhythmia, left bundle branch block, left ventricular hypertrophy (assessed by 2-dimensional echocardiography), valvular heart disease, mitral valve prolapse, myocardial infarction, unstable angina, cardiomyopathy, congestive heart failure, diabetes mellitus or collagen vascular disease were excluded. In all patients, the extra-cardiac causes of chest pain including musculo-skeletal and esophageal causes were ruled out.

The study was approved by the local ethics committee, and all patient-subjects gave informed written consent.

Study protocol

The study protocol undertaken was based on a single-blinded, randomized, and metoprolol-controlled design. Some patients received antianginal treatment prior to the study. The medications known to influence the endothelial function/dysfunction were withdrawn at least two weeks before the study. Only sublingual nitrates were allowed for the relief of chest pain during the pharmacological wash-out period. After a 2-week washout period, patients with CSX who fulfilled the criteria were randomized to receive either nebivolol (5 mg daily) or metoprolol succinate (50 mg daily) for 12 weeks. During the whole study period, all cardiovascular medication apart from sublingual nitroglycerin for anginal attacks and aspirin 100 mg/day was discontinued.

Patients were evaluated at each four-week follow-up visit by means of physical examination, blood pressure reading, drug compliance, and electrocardiography (ECG). Patients were also interrogated to ischemic symptoms or adverse effects during

this period. The frequency of angina pectoris with or without exercise was recorded according to the Canadian Cardiovascular Society (CCS) angina classification (12).

Exercise testing

All patients underwent a standard exercise stress test using the modified Bruce protocol on two occasions: before randomization and at the end of 12 weeks. Blood pressure, heart rate and 12-lead ECGs were recorded at rest, at one-minute intervals during exercise, at peak exercise, and for at least 8 minutes in the recovery phase. The ECG and ST-segment depression were continuously displayed and measured automatically by a computer-assisted system (Marquette-Case treadmill system, General Electric, Milwaukee, USA) in all 12 leads. Only leads I, II, III aVL, aVF, and V2 to V6 were used for analysis. The subjects were exercised until one of the end-points was reached: age-specific target heart rate or the development of symptoms necessitating termination of the test. Patients were encouraged to perform their maximum effort and the symptoms developed during exercise test were recorded. The test was considered positive for ischemia when more than 1 mm of down-sloping or horizontal ST depression at 60 to 80 ms after the J point occurred. Total exercise duration, rate-pressure-product (heart rate X systolic blood pressure mmHg/min), time to maximum ST-segment depression, and time to 1 mm ST-segment depression during exercise testing were compared after treatment with nebivolol 5 mg or metoprolol succinate 50 mg for 12 weeks.

Cardiac catheterization

Coronary angiograms were performed with a femoral approach using the Judkins technique without the use of nitroglycerin, adenosine or a calcium channel blocker. Angiograms were recorded on a DICOM digital media (Dicom-viewes; MedCom GmbH; Darmstadt; Germany) at 25 frame/s and were reviewed by two experienced angiographers who had no knowledge of the patients' clinical information. Coronary angiograms were judged with regard to smooth appearance, luminal wall irregularities, epicardial local or diffuse caliber reduction and stenosis. Coronary arteries were classified as normal on the basis of visual assessment of the absence of any luminal irregularities. To exclude the possibility of coronary artery vasospasm, during coronary arteriography all patients underwent a hyperventilation test, which was performed by asking the patients to breathe quickly and deeply for 5 min.

Blood investigations

From each subject, a fasting blood sample (approx. 20 cm^3) was obtained from the antecubital fossa vein in the morning hours before randomization and at the end of the 12^{th} week. These samples were immediately centrifuged at 2,000 rpm for 10 minutes with plasma, then being transferred into aliquots for storage at -80°C. Analysis for lipid profile (β-quantification) and fasting glucose was undertaken on fresh samples. Levels of total cholesterol (TC), high-density lipoprotein -cholesterol (HDL-C), and triglycerides (TG) in serum were measured using an Abbott Aeroset autoanalyzer with original kits. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald equation. Serum homocysteine (Hcy) level was measured by high-performance liquid chromatography with fluorescence detection using Chromsystems kits (Germany). High sensitive-C reactive protein (Hs-CRP), lipoprotein a (Lp-a), apolipoprotein A (Apo-A) and apolipoprotein B (Apo-B) levels were determined using nephelometric method with Beckman Image Immunochemistry system (Beckman Instruments, Fullerton, CA, USA).

Determination of plasma nitricoxide (NOx) levels: NOx levels in plasma were determined spectrophotometrically, based on the reduction of $NO_3^{\cdot -}$ to $NO_2^{\cdot -}$ by $VaCl_3$. Nitric oxide level was measured by the Griess reaction. Sodium nitrite and nitrate solutions (1, 10, 50, 100µM) were used as standards. Serum samples were deproteinized prior to assay. Samples were added to 96% cold ethanol (1/2 v/v) and then vortexed for 5 min. After incubation for 30 min. at +4°C, the mixture was centrifuged at 14.000 rpm for 5 min and the supernatants were used for the Griess reaction (13) .

Measurements of plasma levels of L-arginine, and ADMA: Measurements of ADMA and arginine were accomplished by HPLC, using the method described by Chen et al (14). In brief, to 1 ml plasma, 20 mg of 5-sulfosalisilic acid (5-SSA) was added and the mixture was left in an ice-bath for 10 min. The precipitated protein was removed by centrifugation at 2000 g for 10 min. Ten microliters of the supernatant, which was filtered through a 0.2 µm filter was mixed with 100 µl of derivatization reagent (prepared by dissolving 10 mg o-phtaldialdehyde in 0.5 ml of methanol, and 2 ml of 0.4 M borate buffer (pH 10.0) and 30 µl of 2-mercaptoethanol were added) and then injected into the chromatographic system. Separation of ADMA was achieved with a 150x4 mm I.D. Nova-pak C18 column with a particle size of 5 µm (Waters, Millipore, Milford, MA, USA) using 50 mM sodium acetate (pH 6.8), methanol and tetrahydrofurane as mobile phase (A, 82:17:1; B, 22:77:1) at a flow-rate of 1.0 ml/min. The areas of peaks detected by fluorescent detector (Ex: 338 nm; Em: 425nm) were used for quantification. The variability of the method was less than 7%, and the detection limit of the assay was 0.1 µM.

Statistical analysis

The SPSS statistical software package (SPSS, version 10.0 for windows; SPSS Inc., Chicago, Illinois, USA) was used to perform all statistical calculations.

Continuous variables were given as mean±SD; categorical variables were defined as percentage. Data were tested for normal distribution using the Kolmogorov-Smirnov test. Statistical differences among groups were tested by one-way analysis of variance (ANOVA) and Kruskal-Wallis tests for parametric and non-parametric variables, respectively. When a significant difference between three groups were observed by using one-way ANOVA test and Kruskal-Wallis tests, independent samples t test and Mann-Whitney U test were used for determination of difference between 2 groups. A paired-samples

T test was used to compare continuous variables before and after drug therapy. Differences were considered statistically significant at P<0.05.

Results

Patient characteristics

Of the 38 patients initially randomized to receive either nebivolol (n=19) or metoprolol (n=19), only 34 completed the study. Four patients were withdrawn from the study for several different reasons: three patients (two in the metoprolol group and one in the nebivolol group) were withdrawn because of lack of compliance at the second month of therapy, and one taking nebivolol was withdrawn because of a severe symptomatic bradycardia development. There were no significant difference in basic characteristics between the 34 patients with syndrome X and the 16 control subjects at baseline. As shown in Table 1, significant differences between metoprolol, nebivolol and control groups were found for aspirin and statin usage, which were more prevalent in CSX patients than control group. The pretreatment biochemical parameters were similar among all groups. (Table 1). There were no significant changes in these parameters after 12-week treatment both with nebivolol and metoprolol succinate (Table 2).

The affect of nebivolol on exercise performance

Exercise testing results are presented in Table 3. Hemodynamic parameters at different stages were similar between the two patient groups before treatment. For both groups, resting, at 1-mm ST-segment depression, and at peak

Table 1. Clinical characteristics

Variables	Nebivolol group	Metoprolol group	Control group	p*	F**	Chi-Square***
Number of patients	17	17	16			
Age, years	47.2±7.3	49.5±7.3	46.1±5.6	ns	0.292	
Gender, female/male	9/8	9/8	8/8	ns		
Body mass index, kg/m^2	25±3	26±4	25±3	ns	0.495	
Family history of CAD, n (%)	6 (35)	5 (29)	6 (35)	ns		
Cigarette smoking, n (%)	4 (23)	4 (23)	5 (31)	ns		
Diastolic BP, mmHg	73±3	74±4	72±4	ns	0.313	
Systolic BP, mmHg	114±9	112±8	114±7	ns	0.412	
Waist / Hip ratio, cm	0.84±0.11	0.86±0.12	0.86±0.10	ns	0.389	
Postmenopausal, n (%)	4 (23)	5 (29)	5 (29)	ns		
HRT use, n (%)	2 (12)	2 (12)	2 (12)	ns		
Aspirin use, n (%)	8 (47)	6 (35)	-	<0.01		
Statin use, n (%)	6 (35)	5 (29)	-	0.02		
Use of beta-blockers, n	3	3	-	0.15		
Nitrate use, n	2	1	-	0.32		
CCB use, n	1	2	-	0.32		
Total Cholesterol, mg/dl	201±38	203±22	198±25	ns	0.512	
Triglycerides, mg/dl	159±106	160±83	162±65	ns	0.292	
LDL Cholesterol, mg/dl	116±29	125±20	122±23	ns	0.678	
HDL Cholesterol, mg/dl	50±11	51±14	49±12	ns	0.197	
Hs-CRP, mg/dl	0.54±0.12	0.46±0.15	0.33±0.11	ns	1.822	
Apo A, mg/dl	140±26	136±20	133±21	ns	1.232	
Apo B, mg/dl	99±26	101±16	104±15	ns	0.879	
Lipoprotein a, mg/dl	19.2±2.2/17.4 (9.9-64.4)♦	23.2±2.5/16.2(9.9-85.2)♦	22.1±2.6/11.9 (8.2-81.3)♦	ns		0.756
Homocysteine, mg/l	10.7±2.0/11.2 (5-35.6)♦	11.1±2.2/10.9 (6.5-33.2)♦	9.5±2.2/9.8 (6.2-42.3)♦	ns		0.094

♦Data are expressed as mean±SD, median (minimum-maximum) values, and percentages/proportions

*p values for Chi-square test, Kruskal–Wallis test, Mann-Whitney U-test, or one-way ANOVA test followed by unpaired t-test

**F values for one-way ANOVA test

***Chi-square values for Kruskal-Wallis test

Apo A - apolipoprotein A, Apo B - apolipoprotein B, BP - blood pressure, CAD - coronary arter disease, CCB - calcium channel blockers, Hs-CRP - high sensitive C-reactive protein, HDL - high-density lipoprotein, HRT - hormone replacement therapy, LDL - low-density lipoprotein, ns - not significant

Table 2. Results of biochemistry analyses before and after nebivolol or metoprolol for 12 weeks

Variables	Nebivolol (n=17)	Metoprolol (n=17)
Total Cholesterol, mg/dl		
Before	201±38	203±22
After	191±40	181±23
p*	0.39	0.19
Triglycerides, mg/dl		
Before	159±106	160±83
After	155±82	134±56
p*	0.70	0.65
LDL Cholesterol, mg/dl		
Before	116±29	125±20
After	110±26	106±25
p*	0.64	0.42
HDL Cholesterol, mg/dl		
Before	50±11	51±14
After	49±13	45±6
p*	0.37	0.09
Apo A, mg/dl		
Before	140±26	136±20
After	147±27	136±23
p*	0.88	0.98
Apo B, mg/dl		
Before	99±26	101±16
After	96±23	96±16
p*	0.64	0.23
Lipoprotein a, mg/dl		
Before	19.2±2.2	23.2±2.5
After	20±1.9	25.7±2.1
p*	0.98	0.88
Hs-CRP, mg/dl		
Before	0.54±0.12	0.46±0.15
After	0.45±0.11	0.41±0.14
p*	0.62	0.46
Homocysteine, mg/l		
Before	10.7±2.0	11.1±2.2
After	11.3±3	11±4.9
p*	0.32	0.92

Data are presented as mean±SD
*Paired – samples T test
Apo A - apolipoprotein A, Apo B - apolipoprotein B, Hs-CRP - h -igh sensitive C-reactive protein, HDL - high-density lipoprotein, LDL - low-density lipoprotein

exercise after treatment heart rates were significantly lower than pretreatment levels ($p<0.05$) but there were no difference between the two treatment groups. At the end of the 12 weeks both exercise duration to 1-mm ST depression ($p<0.01$) and total exercise duration increased significantly after treatment ($p < 0.01$) in patients who received nebivolol. Although such parameters were slightly increased in metoprolol recipients, this difference did not reach statistical significance. In addition, RPP ($p<0.05$) and the maximum ST-segment depression at peak exercise ($p<0.01$) were found significantly decreased in the patients taking nebivolol (Table 4).

The affect of nebivolol on angina score

In the nebivolol group, CCS angina classification improved by one or more categories in 12 (70%) patients, whereas it deteriorated or remained in the same category in 5 (30%) patients. Meanwhile, in the metoprolol group, the CCS angina classification improved by one or more categories in 7 (41%), whereas it deteriorated or remained in the same category in 10 (59 %) patients. Moreover, complete disappearance of chest pain was noted in five patients within the nebivolol treatment group, the relief of chest pain was seen in two patients within the metoprolol treatment group.

The affect of nebivolol on endothelial function

Before drug treatment, the plasma level of NOx (53.45±12.2 vs 68.66±8.1 μM, $p<0.001$), L-arginine (22.51±3.29 vs 28.20±2.43 μM, $p<0.001$), and L-arginine/ADMA ratio (14.58±5.25 vs 26.27±11.15, $p<0.001$) were lower in the patient's group than they were in the control group, whereas plasma ADMA levels (1.92±0.88 vs 1.29±0.2 μM, $p < 0.001$) were increased in the patient's group (Fig. 1). Plasma NOx, ADMA, L-arginine, and the L-arginine/ADMA ratio were comparable between the two treatment groups before treatment (Fig. 2).

After 12-weeks of drug therapy, the patients taking nebivolol had increased levels of plasma NOx, plasma L-arginine, the L-arginine/ADMA ratio and decreased levels of plasma ADMA compared to those of the patients taking metoprolol (Fig. 2).

Discussion

The cardinal findings of this study indicate that: (1) compared with control subjects, plasma ADMA levels were increased, whereas plasma levels of NOx, L-arginine and L-arginine/ADMA ratio were reduced in CSX patients, suggesting that systemic endothelial function is impaired in patients with CSX; (2) compared to metoprolol, nebivolol therapy was associated with longer exercise duration, lesser exercise-induced myocardial ischemia and lesser angina attacks; (3) nebivolol was also associated with better influence on plasma endothelial function parameters compared to metoprolol, namely more prominent decrease in plasma ADMA levels and more prominent increase in plasma L-arginine, NOx levels and L-arginine/ADMA ratio. These results suggested that nebivolol treatment may improve NO bioavailability and systemic endothelial function by both reducing plasma ADMA and increasing L-arginine levels contributing to the improvement of coronary microvascular function and exercise-induced myocardial ischemia in patients with CSX.

The pathophysiology of CSX has not been clearly identified yet. The most influential explanation includes generalized endothelial dysfunction, inflammation, and increased pain

Table 3. Results of treadmill exercise test before and 12 weeks after nebivolol or metoprolol treatment

Variables	Nebivolol (n=17)		Metoprolol (n=17)	
	before	after	before	after
Exercise treadmill test baseline				
Heart rate, beats/min	75±13	69±11	77±12	71±12
SBP, mm Hg	138±22	133±20	135±19	130±19
DBP, mm Hg	77±11	75±10	78±9	75±10
At 1-mm STD				
Heart rate, beats/min	139±27	126±25*	138±26	125±25***
SBP, mm Hg	168±19	166±20	165±18	168±18
DBP, mm Hg	78±12	80±11	83±13	81±12
1-mm STD ET, s	380±144	431±122**	384±155	392±129
RPP, beat X mmHg/min	23352±4254	20916±4113*	22770±4288	21000±4167
At peak exercise				
Heart rate, beats/min	148±25	135±23	151±22	139±23***
SBP, mm Hg	169±22	170±21	164±19	165±22
DBP, mm Hg	81±13	82±11	80±13	78±14
Total ET, s	524±155	577±161**	532±149	540±153
RPP, beat X mmhg/min	25012±4312	22950±4277*	24764±4201	22935±4121
Maximum ST-segment depression, mm	2.2±0.11	1.3±0.10**	2.1±0.12	1.8±0.11

Data are presented as mean±SD
Paired – samples t test:
*p < 0.05 versus before nebivolol,
** p< 0.01 versus before nebivolol
*** p < 0.05 versus before metoprolol
DBP - diastolic blood pressure, ET - exercise time, SBP - systolic blood pressure, STD - ST-segment depression, RPP - rate-pressure product

Table 4. The changes of endothelial function parameters before and after 12-week treatment with nebivolol and metoprolol in patients with syndrome X

Variables	Nebivolol (n=17)		Metoprolol (n=17)	
	before	after	before	after
Plasma NOx levels, μM	51.6±14.4	70.2±19.5*	55.3±19.6	57.7±15.3
Plasma ADMA levels, μM	1.93±0.89	1.15±0.51*	1.89±0.7	1.84±0.65
Plasma L-arginine levels, μM	22.75±4.0	24.36±5.5*	22.28±3.5	22.65±3.7
L-arginine/ADMA ratio	12.99±7.3	22.82±12.9*	16.17±10.8	17.75±10.0

Data are presented as mean±SD
Paired – samples t test: * p<0.001
ADMA - asymmetric dimethylarginine, NOx - endothelial nitric oxide

sensitivity. Endothelial dysfunction is a plausible mechanism elucidating anginal chest pain by decreased coronary flow response to endothelium mediated vasodilator stimuli by decreasing NO bioavailability. There is also evidence of a basal decrease in NO levels and a decreased ratio of the vasodilator NO to the vasoconstrictor endothelin-1 in these patients (15).

For the time being, the responsible pathogenetic mechanism remains uncertain; in addition no exact therapy of CSX is found. Nitrates, beta-blockers, calcium channel blockers, and tricyclics are conventionally used in the treatment of CSX but none of these agents were studied extensively in terms of efficacy. This is in part because older data suggested that although symptomatic improvement with various treatment strategies might have been limited, the prognosis in regard to cardiac events or increased mortality was benign. When considering endothelial dysfunction as a cause of anginal pain in patients

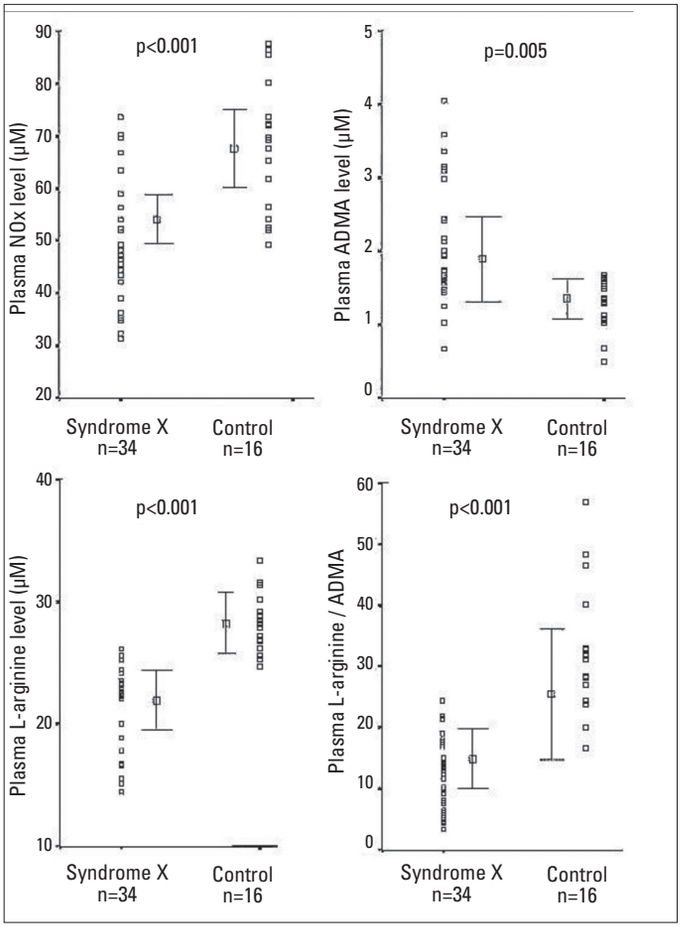

Figure 1. Plasma NOx, ADMA, L-arginine levels and L-arginine / ADMA ratio in 16 control subjects and 34 patients with cardiac syndrome X before treatment

ADMA - asymmetric dimethylarginine, NOx - endothelial nitric oxide

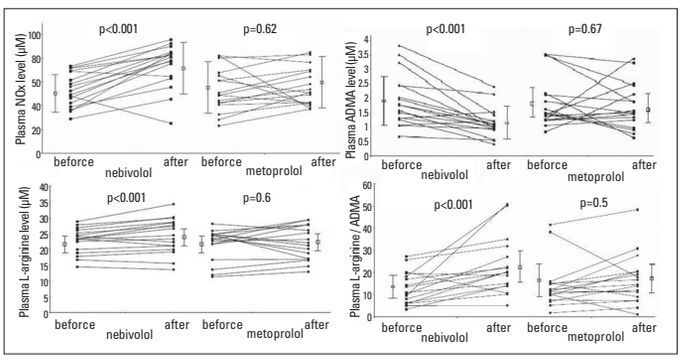

Figure 2. The changes of plasma NOx levels, ADMA levels, L-arginine levels, and plasma L-arginine/ ADMA ratio after 12-week treatment with nebivolol and metoprolol in patients with syndrome X

ADMA - asymmetric dimethylarginine, NOx - endothelial nitric oxide

with CSX, therapies that improve endothelial function such as statins (16) and angiotensin- converting enzyme inhibitors (5) are being proposed recently. A small, but well-designed and placebo controlled study of 40 patients with CSX showed improvement in endothelial function, exercise duration, and time to 1 mm ST depression on treadmill testing in patients treated with pravastatin, regardless of cholesterol level (16). Chen et al. (5) have found that coronary flow reserve and exercise duration were improved in a CSX population after 8 weeks of enalapril therapy. In addition, after receiving oral L-arginine (2 g three times per day), significant improvement in angina class, systolic blood pressure, and quality of life was shown in a hypertensive group with microvascular dysfunction (17).

Nebivolol is a newly developed, highly selective β_1-blocker, which has been shown to cause vasodilatation, due to effects on the L-arginine/ NOx pathway (9-11). The vasodilator action of nebivolol appears to be endothelium-dependent and attributed to endothelium-derived NOx (10, 11, 18, 19). Unlike other traditional β-blockers, nebivolol causes a prompt decrease in systemic vascular resistance and reduction in systemic blood pressure (7, 20). Several lines of experimental evidence suggested that endothelium-derived NO played a crucial role in nebivolol induced vasodilatation. In several studies nebivolol produced endothelium dependent relaxation that was eliminated by endothelial denudation or NO inhibitors (21, 22). In one clinical study, it was found that nebivolol increased the forearm blood flow in normotensive and hypertensive subjects, and this action was antagonized by NG monomethyl- L-arginine (L-NMMA, an NO inhibitor) (23). Moreover, nebivolol has been shown to increase the basal and stimulated release of endothelial NO in patients with essential hypertension (24).

In addition in a previous study it was shown that conventional β-blocker therapy with metoprolol was associated with increased serum ADMA levels whereas nebivolol had no effect (25). Derangement of the L-arginine-nitric oxide pathway by ADMA has been implicated as a possible contributing factor to endothelial dysfunction (26). Elevation of ADMA has been observed in plasma from patients with various risk factors of atherosclerosis (27), hypertension (28), diabetes (29) and insulin resistance (30). On the other hand, in contrast to L-arginine as the substrate for NO synthase, ADMA may also uncouple the electron transfer between NO synthase and L-arginine, increase oxidative stress, and further impair the availability of endothelium-derived NO (26). This evidence suggests that elevated concentrations of ADMA may be associated with increased oxidative stress that may decrease the bioavailability of NO and further contribute to the development of endothelial dysfunction. Accordingly, in the present study, the patients with CSX have increased plasma ADMA level, and decreased plasma levels of NOx and L-arginine compared to those of control subjects.

The affect of metformin was evaluated in nondiabetic patients diagnosed with syndrome X. After 8 week treatment significant improvements in both maximal ST-segment depression and chest pain incidence occurred in the metformin recipients but such parameters were unaltered in placebo recipients (31). In a recent study nebivolol prolonged the time to 1 mm ST depression in stage 1 hypertensive patients with CSX (32). Similarly in our study, exercise duration to 1-mm ST depression and total exercise duration significantly increased after treatment in the nebivolol group compared to the metoprolol group.

In brief, nebivolol has proved favorable effects on endothelial function by increasing NOx bioavailability. Endothelial dysfunction is blamed to take a major role in the CSX pathophysiology. Improvement in endothelial function may accordingly improves symptoms and exercise capacity in patients with CSX.

Limitations of the study

One of the most important limitations was the single-blind protocol of the study. Although the person who evaluated the exercise-test and measured the biochemical parameters had no information about the characteristics of the study participants. The other major limitation was that the endothelial function was examined by measuring the plasma levels of plasma ADMA, L-arginine, NOx and were not corroborated by an independent method, such as coronary flow reserve or flow mediated dilatation (FMD) of brachial artery. However, these circulating markers were found to be concordant with coronary flow reserve and FMD values (33, 34).

In addition, plasma levels of NOx were evaluated instead of measuring NO level. Because of circulating NOx levels are not an expression of NO pathway activation only. They are also derived from the catabolism of NO and its breakdown by oxidative stress. We assumed that NOx levels correlate with plasma levels of NO, although this measurement was somewhat indirect. Another limitation was the diet of the study participants were not considered though the plasma NOx levels may be influenced from diet status.

Although ergonovine test is superior to the hyperventilation test, we preferred to use the hyperventilation test instead of the ergonovine injection to rule out the coronary spasm in patients with CSX, because of the possibility of persistent and severe, painful spasm with ergonovine.

One of the major limitations of our study was the relatively small patient population; however, our population was quite homogenous with a limited number of cardiovascular risk factors. Lastly, the patients under nebivolol treatment were not compared to placebo group due to difficulty in obtining plascebo preparates. Thus, we have preferred the metoprolol for the control group as it has not effect on endothelial function.

Conclusion

We have found that the circulating endothelial function parameters (plasma ADMA, L-arginine, NOx levels) were impaired in patients with CSX. Endothelial function p and exercise stress test parameters improved with nebivolol treatment when compared to metoprolol treatment. We believe that further studies with larger study population and with longer follow up are needed to evaluate the effects of nebivolol treatment on long-term clinical outcomes in patients with CSX.

Conflict of interest: none declared.

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