Nebivolol prevents remodeling in a rat myocardial infarction model: an echocardiographic study

Nebivolol sıçan miyokardiyal infarktüs modelinde ventriküler yeniden şekillenmeyi önlüyor: Ekokardiyografik bir çalışma

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ABSTRACT

Objective: Ventricular remodeling (VR) which develops after myocardial infarction (MI) plays an important role in progressive left ventricular dysfunction. We aimed to investigate the role of nebivolol treatment on VR after a MI in a rat ischemia-reperfusion model.
Methods: Rats were divided into 3 groups of 12 each: sham operated (sham-control), MI-induced (MI-control) and nebivolol treated (MI-nebivolol). Left ventricular (LV) diameters, volumes, and diastolic filling parameters were evaluated by echocardiography. On the 28th day, after recording the systemic and LV pressures and determining the plasma nitric oxide (NO) and peroxynitrite (ONOO-) levels , animals were sacrificed and heart, body and LV weights (HW, BW, LVW) were measured and infarct sizes were determined. Results were evaluated statistically by ANOVA for repeated measurements 3x3 factorial design with post-hoc Bonferroni test.
Results: After MI, while VR (an increase in LV diameters and volumes associated with a decrease in EF, FS and posterior wall thickness change (LWPc) was significant in MI-control rats ($p<0.05$ for; all comparisons) these changes were significantly less in MI-nebivolol group ($p=0.08$ and $p=0.06$ for EF and FS respectively). LV end diastolic pres-sure (LVEDP) was lower ($p<0.005$) and $\Delta\pm dp/dt$'s ($p<0.05$) were higher in MI-nebivolol group compared to MI-control animals. Although infarct sizes were similar in MI-induced groups ($p=0.79$); LVW/HW and HW/BW's were significantly greater in the MI-control group compared to sham-control ($p<0.01$ for all comparisons), these changes were not statistically significant in MI-nebivolol group. The increase in plasma NO and ONOO- levels were also prevented with nebivolol.
Conclusion: Nebivolol therapy reduced the effects of VR in rats after MI. These beneficial effects were not related to its heart rate and blood pressure reducing effects. Nitric oxide regulatory action of this compound may contribute these beneficial effects on VR developed after MI.
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Key words: Myocardial infarction, ventricular remodeling, nebivolol, echocardiography, nitric oxide, peroxynitrite, left ventricular function

ÖZET

Amaç: Miyokard infarktüsü (MI) sonrası gelişen ventriküler yeniden şekillenme (VR) ilerleyici sol ventrikül disfonksiyonunda önemli bir role sahiptir. Çalışmada sıçan iskemi-reperfüzyon modelinde MI sonrası nebivolol tedavisinin VR üzerine etkisini incelemeyi amaçladık.
Yöntemler: Sıçanlar her grupta 12 hayvan olacak şekilde 3 gruba ayrıldı; operasyonel kontrol (sham-kontrol), MI kontrol (MI-kontrol) ve nebivolol uygulanmış MI (MI-nebivolol). Sol ventrikül (LV) çapları, hacimleri ve diyastolik dolum parametreleri ekokardiyografi ile incelendi. Yirmi sekiz günlük periyodun sonunda sistemik ve LV basınçları kaydedildi, plazma nitrik oksit (NO), peroksinitrit (ONOO-) düzeyleri ölçüldü, vücut (BW), kalp (HW) ve LV (LVW) ağırlıkları ile infarkt alanları belirlendi. Istatistiksel değerlendirme tekrarlı ölçümler varyans analizi ve ikili karşılaştırmalar Bonferroni testi ile yapıldı.
Bulgular: İnfarktüs sonrası MI-kontrol grubunda belirgin VR parametreleri (LV çap ve hacminde artış, EF, FS ve arka duvar % kalınlık değişimde azalma) saptanırken (bütün karşılaştırmalar için $p<0.05$); bu değişiklikler MI-nebivolol grubunda MI-kontrol grubu ile karşılaştırıldığında kısıtlanmıştı (EF ve FS için sırası ile $p=0.08$ ve $p=0.06$). Sol ventrikül diyastol sonu basıncı (LVEDP) MI-nebivolol grubunda daha düşüktü ($p<0.005$). Yine bu grupta $\Delta\pm dp/dt$ değerleri daha yüksekti ($p<0.05$). İnfarktüs oluşturulmuş gruplarda infarkt alanları benzer olmasına rağmen ($p=0.79$); LVW/HW

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ve HW/BW değerleri MI-kontrol grubunda sham-kontrol grubuna göre daha yüksekti (bütün karşılaştırmalar için p<0.01). Bu değerler MI-nebivolol grubunda istatistiksel olarak anlamlı değildi. Plazma NO ve ONOO- düzeylerindeki artış da MI-nebivolol grubunda önlenmişti.
Sonuç: Nebivolol tedavisi sıçanlarda MI sonrası gelişen VR'i azaltmıştır. Bu etki nebivololün kalp hızı ve kan basıncı düşürücü etkilerine bağlanamamıştır. Molekülün NO düzenleyici etkisi MI sonrası gelişen VR'de nebivololün yaralı etkilerinden sorumlu olabilir.
(Anadolu Kardiyol Derg 2010; 10: 18-27)
Anahtar kelimeler: Miyokard infarktüsü, ventriküler yeniden şekillenme, nebivolol, ekokardiyografi, nitrik oksit, peroksi nitrit, sol ventrikül fonksiyonu

Introduction

Coronary artery disease is the major cause of cardiovascular mortality worldwide. It is known that acute coronary syndromes are the initial presentation of coronary artery disease in most patients and nearly half of subjects with an acute coronary syndrome have acute myocardial infarction (MI) (1).

In patients with MI, the size of infarction and the function of the remote myocardium are the most important factors affecting the short and long-term prognosis (2). The basic mechanism of post-MI left ventricular (LV) dysfunction is the progressive remodeling (VR) initiated by the neuro-hormonal activation which induces an extension of infarct area in the early phase and, if not controlled, structural changes and dysfunction of remote myocardium in the late phase (3). Current pharmacological treatment to control the neurohormonal activation with angiotensin converting enzyme (ACE) inhibitors and beta-blockers has been shown to decrease mortality and morbidity by reducing remodeling (4, 5). However, differently from ACE inhibitors, the cardioprotective activity of beta-blockers has been commonly attributed to their antiarrhythmic and energy-sparing properties. Recently a preventive effect of carvedilol on remodeling has also been demonstrated (6). Nebivolol is a selective beta1-blocker with a nitric oxide (NO) mediated vasodilating activity (7, 8). Favorable effects of nebivolol on ventricular function in patients with chronic heart failure have been reported (9, 10). However, the use of nebivolol on VR after MI has not been evaluated yet.

In this study, we aimed to investigate the effects of early use of low dose nebivolol on remodeling in an experimental MI model in rats.

Methods

Study design and dose determination

Study design: Prospective controlled animal laboratory study

Animal groups: Twelve weeks old Sprague-Dawley rats with a mean weight of 250-300 g were divided in 3 groups of 12 each; sham operated control (sham-control), MI induced control (MI-control) and MI induced, nebivolol treated (MI-nebivolol).

Animals were housed in a temperature-controlled animal facility with a 12-hour light-dark cycle, with tap water and rodent chow ad libitum and handled in accordance with the 'Guide for the Care and the Use of Laboratory Animals' published by the US National Institutes of Health. All experimental procedures were approved by the local 'Institutional Animal Ethic Committee'.

Administration of drug and dose regimen: Nebivolol was administrated within the 10 min of reperfusion at dose of 0.1mg/kg i.v and continued orally at dose of 0.5 mg/kg, by gastric gavage once daily for 28 days. The i.v. dose of nebivolol was selected as the minimum beta-blocker dose (absence of significant effect on blood pressure and heart rate), by a preliminary dose-response study, (using 0.1, 0.5, and 1mg/kg of nebivolol iv) according to Sacco et al. (11) (Fig. 1). The oral dose was selected according to Sanchez et al. (12).

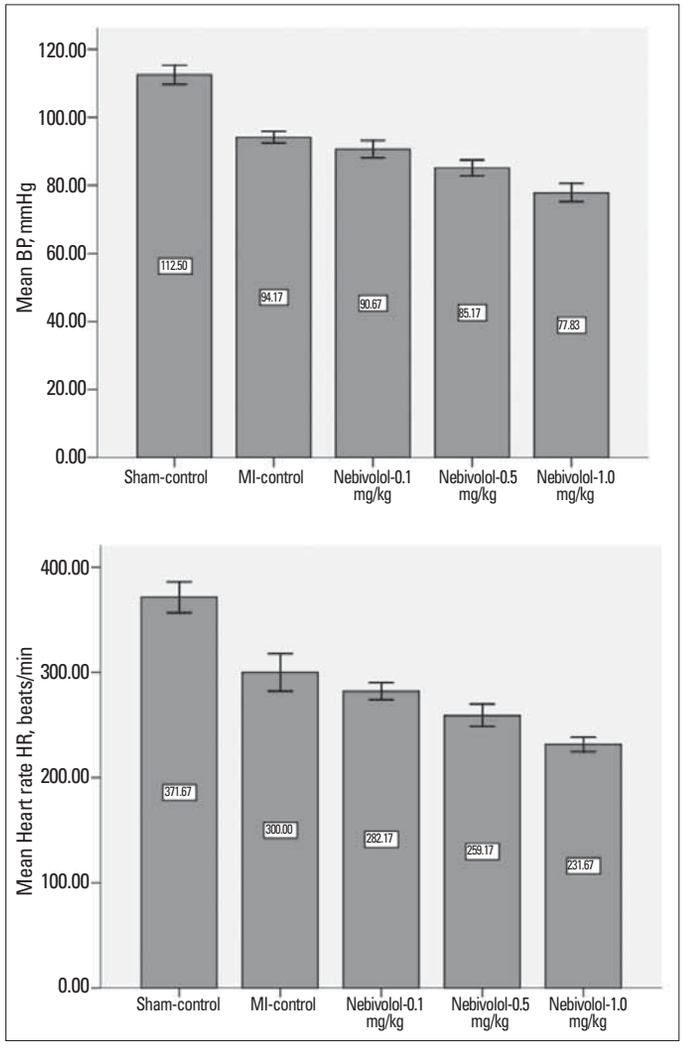

Figure 1. Blood pressure and heart rates recorded during 60 min in the preliminary dose-response study

Though blood pressure and heart rates were decreased with the increasing nebivolol dose, these changes were only statistically significant in 1.0 mg/kg treated group (p=0.012 and p=0.002, respectively)
BP - blood pressure, HR - heart rate, MI - myocardial infarction

Induction of myocardial infarction

Myocardial infarction was induced by the ligation of the left anterior descending coronary artery (LAD) as described previously (13). After an anesthesia with ketamine and xylazine combination, rats were intubated through a tracheotomy and ventilated with a volume-cycled small-animal ventilator (TOPO ventilator, Kent Scientific, USA). An anterior thoracotomy was performed. The heart was rapidly exteriorized and LAD was ligated about 2 mm from its origin with 6-0 prolene suture, which was released after 30 min. Myocardial infarction was confirmed by regional cyanosis, ST-elevation on electrocardiogram (ECG), and elevation of serum creatine kinase myocardial band (CK-MB) and troponin T levels. After the release of the ligature, reperfusion was confirmed by myocardial blush over the risk area. Positive end-expiratory pressure was applied to fully inflate the lungs and then the chest was closed in layers. The sham-control rats underwent the same procedure except ligation. All surgical procedures were done under aseptic conditions.

Echocardiographic assessment

All the animals underwent echocardiography under anesthesia just before the operation (baseline) and on 2, 7, 14, 21 and 28 days after MI. Animals were lightly anesthetized with ketamine and xylazine combination. Transthoracic echocardiography was performed as described previously (14). Two-dimensionally (2D)-guided M-mode echocardiography and pulse-wave Doppler echocardiography were performed using an echocardiographic system equipped with a 10 MHz sector probe at 6MHz (Vivid 7, General Electric, Horten Norway). For M-mode recordings, the parasternal short-axis view was used to image the heart in 2D at the level of the papillary muscle. LV volumes were calculated via 2D measurements by a formula.

LV volumes were calculated from apical two chamber view by using "area-length" method. All the measurements and calculations were done in accordance with the American Society of Echocardiography (15). The following M-mode measurements were done: LV internal dimensions at both diastole and systole (LVIDd and LVIDs respectively), LV posterior wall dimensions at diastole and systole (LVPWd and LVPWs respectively), interventricular septal dimensions at both diastole and systole (IVSd and IVSs, respectively). From these measurements; end diastolic and end systolic volumes, (EDV and ESV), fractional shortening (FS), ejection fraction (EF) of the LV, stroke volume (SV), cardiac output (CO) were derived (16-18). Left ventricular posterior wall thickness change (LVPWc) was calculated using the following formula (18):

LVPWC (%) = [(LVPWs - LVPWd) / LVPWd] x 100

The following Doppler measurements were taken using the apical four-chamber view: early diastolic filling peak velocity (E wave), late diastolic peak velocity (A wave), E/A ratio and E deceleration time.

Hemodynamic measurements

Twenty-eight days after drug therapy, hemodynamic measurements were performed according to the method described by Pfeffer et al. (19). In brief, after weighing the animals, the right carotid artery was dissected and a heparinized saline-filled polyethylene-tubing catheter (PE-50) was inserted in to the artery. Catheter was connected to the pressure transducer (MLT 0699, PowerLab, ADI Instruments,UK) and pressures recorded on a physiological recorder (10T Hardware System, PowerLab, ADI Instruments, UK). After the record of the ascending aortic pressure, the catheter was advanced to LV and LV systolic and end-diastolic pressure (LVSP, LVEDP) and maximum rise and fall of LV pressures (+dp/dt and -dp/dt respectively) were recorded.

Perfusion-fixation and infarct size determination

After the hemodynamic measurement, the catheter was pulled back to the aorta and the heart was arrested in diastole with IV KCl injection (3cc, 10% solution). Thorax was opened quickly and the right atrium was cut for the drainage of the blood and fixative. The heart was perfused-fixed with 10% phosphate-buffered formalin at a pressure of 7.5 cm H2O for one hour. After the fixation, the heart was excised quickly and weighted. Then the left ventricle was dissected, weighted, dehydrated and embedded in paraffin.

Ten µm thin sections were serially cut from apex to base at 1-mm intervals, deparaffinized and stained with hematoxylin-eosin (HE) and Masson's trichrome (MS). The specimens were evaluated under optic microscope. MI size was average of all slices and expressed as:

MI size (%) = total infarct length / [(epicardial+endocardial length) /2] x 100

Plasma nitric oxide and peroxynitrite measurements

At the end of the 28-day period, plasma NO and peroxynitrite (ONOO-) levels were measured in all experimental groups. Measurements were done in accordance with the manufacturer's instructions. While NO was measured as nitrite/nitrate (NOx) concentrations which are stable metabolites of NO by spectrophotometer (Nitric Oxide Colorimetric Assay, Roche, Germany). ONOO- was determined as nitrotyrosine the fingerprint of ONOO- by enzyme-linked immunoassay (ELISA) (HyCuit biotechnology, USA)

Statistical analysis

Statistical analyses were performed using SPSS for Windows software version 15.09 (Chicago, IL, USA). All the variables are expressed as mean±SD. Data for hemodynamic, histological and biochemical measurements were analyzed by ANOVA with post-hoc Bonferroni test. Data for echocardiographic assessment were submitted to a 3x3 factorial design ANOVA post-hoc Bonferroni test. A value of $p<0.05$ in a two-tailed distribution was considered statistically significant. Correlation coefficients were calculated by Pearson correlation analysis.

Results

A total of 36 rats were subjected to the MI or sham operation. Mortality was followed up for 28 days after surgery. Except 3

perioperative death, within the first 24 hours after surgery (2 MI-control and 1 MI-nebivolol), no more death was seen in that period.

Echocardiography

Echocardiographic assessments of LV geometry and function and a representative M-mode echocardiogram are shown in Table 1 and Figure 2, respectively.

Basal M-mode echocardiography parameters were similar in all groups. When compared to sham-control rats, MI-control animals exhibited significant LV structural changes of VR as thickening of the remote non-infarcted myocardial wall, increase in LVIDd and LVIDs, immediately after MI ($p<0.05$ and $p<0.0001$ respectively). These changes were statistically significant as early as 2 days post MI and remained elevated during the 28 day-period ($p<0.01$ and $p<0.001$ for LVIDd and LVIDs respectively) (Table 1; Fig. 3).

Functional abnormalities in-MI-control rats were in accordance with the structural changes. EF and FS were significantly decreased immediately after MI (2^{nd} day) ($p<0.05$ for both comparisons). This trend continued to 28^{th} day when compared to sham-control group ($p<0.001$ for both comparisons) (Table 2; Fig. 4).

Doppler recordings of mitral inflow showed profound and progressive alterations in LV diastolic filling characteristics, manifested by increased early filling velocity (E) ($p<0.05$) and decreased atrial filling velocity (A) ($p<0.001$) in MI-control rats, in comparison with sham-control animals. Whereas MI-nebivolol group was characterized by an opposite pattern of E and A wave velocity with significantly lower E/A ratio (Table 3, Fig. 5).

LV structure and functions were maintained in MI-nebivolol group both in acute (on day 2 after MI) and sub-acute (on day 28 after MI) period of MI. In contrast to MI-control, LV dimensions and functional parameters (EF, FS) were not significantly changed in MI-nebivolol group during the study period ($p=0.08$ and $p=0.06$ for EF and FS respectively). In the MI-nebivolol group LVIDs, LVIDd and LVPWd were significantly smaller than those of MI-control group ($p<0.005$ for all comparisons). EF and FS of MI-nebivolol group were also significantly higher than those of MI-control group throughout the time-course ($p<0.001$ for both comparisons) (Table 1-2, Fig. 3-4). In the MI-nebivolol group, the mitral inflow patterns were also maintained after MI (Fig. 5).

Hemodynamic assessments

Hemodynamic parameters measured in the three groups of rats are shown in Table 4. Compared with the sham-control group, heart rate (HR) and mean blood pressure (MBP) were both significantly decreased in MI-control and MI-nebivolol groups ($p<0.005$ for both comparisons). MI-induced rats developed increased left ventricle end-diastolic pressure

Table 1. Left ventricular M-mode echocardiography measurements

Groups/Variables	IVSDd, cm	IVSDs, cm	LVIDd, cm	LVIDs, cm	LVPWd, cm	LVPWs, cm	LVPWc, %
Sham-control							
baseline	0.13±0.05	0.28±0.02	0.65±0.03	0.34±0.05	0.12±0.02	0.20±0.06	68.50±6.92
2^{nd} day	0.15±0.02	0.25±0.04	0.67±0.09	0.39±0.06	0.15±0.02	0.22±0.03	65.00±6.99
28^{th} day	0.13±0.03	0.24±0.03	0.68±0.04	0.37±0.03	0.13±0.02	0.22±0.04	65.67±4.27
MI-control							
baseline	0.18±0.08	0.27±0.03	0.64±0.06	0.33±0.09	0.15±0.02	0.22±0.05	67.00±5.89
2^{nd} day	0.16±0.03	0.23±0.02	0.67±0.07	0.47±0.05*$^{\delta}$	0.17±0.03	0.20±0.04	49.17±7.08*$^{\delta}$
28^{th} day	0.15±0.04	0.21±0.06$^{\delta}$	0.74±0.10*$^{\delta}$	0.57±0.04*$^{\delta}$	0.24±0.08*$^{\delta}$	0.31±0.07*$^{\delta}$	43.17±3.51*$^{\delta}$
MI-nebivolol							
baseline	0.15±0.02	0.26±0.06	0.64±0.03	0.36±0.05	0.16±0.04	0.24±0.07	66.50±5.79
2^{nd} day	0.13±0.04	0.24±0.04	0.65±0.06	0.33±0.05$^{\psi}$	0.12±0.02	0.23±0.03	64.50±6.09$^{\psi}$
28^{th} day	0.12±0.07	0.26±0.04$^{\psi}$	0.69±0.02$^{\psi}$	0.32±0.05*$^{\psi}$	0.14±0.09$^{\psi}$	0.22±0.04$^{\psi}$	65.00±5.29$^{\psi}$
F	3.869	3.763	74.642	43.880	48.307	88.818	2481.615
p	0.05	0.05	0.04	<0.001	<0.001	<0.001	<0.001

Data are presented as mean±SD

ANOVA for repeated measurements 3x3 factorial design

post hoc Bonferroni test:

*$p<0.05$ compared to sham-control group at same point in time

$^{\psi}p<0.05$ compared to MI-control group at same point in time

$^{\delta}p<0.05$ compared to baseline

IVSd- interventricular septal thickness at diastole, IVSs- interventricular septal thickness at systole, LVIDd- left ventricular internal dimension at diastole, LVIDs- left ventricular internal dimension at sys-tole, LVPWc- left ventricular posterior wall change LVPWd- left ventricular posterior wall thickness at diastole, LVPWs- left ventricular posterior wall thickness at systole, MI- myocardial infarction

Table 2. Left ventricular volumes, contractility and hemodynamic parameters calculated from M-mode echocardiographic measurements

Groups/Variables	EDV, ml	ESV, ml	EF, %	SV, ml	CO,ml/min	FS, %
Sham-control						
baseline	0.65±0.04	0.18±0.04	71.83±6.20	0.42±0.19	167±38	34.82±5.30
2nd day	0.70±0.04	0.21±0.04	70.17±6.20	0.45±0.19	169±38	36.83±5.35
28th day	0.66±0.05	0.21±0.03	69.50±6.20	0.46±0.13	171±27	32.83±6.33
MI-control						
baseline	0.68±0.07	0.22±0.04	70.66±6.20	0.42±0.15	172±42	34.92±5.30
2nd day	0.78±0.06*	0.26±0.07*	58.17±7.90*$^{\delta}$	0.47±0.18	153±42*$^{\delta}$	25.61±5.71*$^{\delta}$
28th day	0.81±0.04*	0.28±0.08*	56.00±6.79*$^{\delta}$	0.30±0.18*	127±34*$^{\delta}$	12.67±6.23*$^{\delta}$
MI-nebivolol						
baseline	0.68±0.05	0.22±0.02	70.83±6.20	0.45±0.19	176±27	37.24±4.32
2nd day	0.74±0.12	0.24±0.10	66.17±3.98*$^{\psi}$	0.43±0.03	157±30*$^{\psi}$	32.19±2.22$^{\psi}$
28th day	0.77±0.06*	0.28±0.08*	65.60±2.79*$^{\psi}$	0.44±0.08	155±34*$^{\psi}$	25.33±2.07$^{\psi}$
F	695.66	34.29	3137.469	115.69	18152.19	21808.57
p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Data are presented as mean±SD
ANOVA for repeated measurements 3x3 factorial design
post hoc Bonferroni test:
*p<0.05 compared to sham-control group at same point in time
$^{\psi}$p<0.05 compared to MI-control group at same point in time
$^{\delta}$p<0.05 compared to baseline
CO- cardiac output, EDV- end-diastolic volume, EF- ejection fraction, ESV- end-systolic volume, FS- fractional shortening, MI- myocardial infarction, SV- stroke volume

Table 3. Left ventricular diastolic flow patterns measured by pulsed-Doppler echocardiography

Groups/Variables	E, cm/s	A, cm/s	E/A ratio
Sham-control			
baseline	69±14	39±9	1.68±0.70
2nd day	65±14	34±6	1.74±0.70
28th day	66±12	38±10	1.72±0.50
MI-control			
baseline	62±14	32±9	1.58±0.50
2nd day	72±8*$^{\delta}$	23±16*	3.2±0.6*$^{\delta}$
28th day	88±14*$^{\delta}$	11±8*$^{\delta}$	6.57±0.50*$^{\delta}$
MI-nebivolol			
baseline	64±14	35±9	1.62±0.70
2nd day	68±10	31±8$^{\psi}$	2.26±0.80$^{\psi}$
28th day	73±12$^{\psi}$	28±5$^{\psi}$	2.38±0.80$^{\psi\delta}$
F	6355.77	1170.11	1467.03
p	<0.001	<0.001	<0.001

Data are presented as mean± SD
ANOVA for repeated measurements 3x3 factorial design
post hoc Bonferroni test:
*p<0.05 compared to sham-control group at same point in time
$^{\psi}$p<0.05 compared to MI-control group at same point in time
$^{\delta}$p<0.05 compared to baseline
A- atrial filling velocity, E- left ventricular early filling velocity, MI- myocardial infarction

(LVEDP) and reduced Δ±dp/dt (p<0.05). These changes were significantly less common in MI-nebivolol group compared with those in the MI-control group (p<0.05 for Δ±dp/dt and p<0.005 for LVEDP) (Table 4).

Histological assessments

Body weights and postmortem cardiac chamber weights for three groups are shown in Table 5. Whereas body weights (BW) were similar, heart and LV weights (HW and LVW, respectively), LVW/HW and HW/BW ratios were significantly greater in the MI-control group compared to sham-control rats (p<0.01 for all comparisons). These changes were not significant in MI-nebivolol group. There were no significant differences in the MI sizes among MI-control and MI-nebivolol groups (p=0.79).

Biologic assessments

Plasma NO and ONOO- levels are shown in Table 6. As seen in the table compared to sham-control rats, both plasma NO and ONOO- levels were high in the MI-control group (p<0.005 for both comparisons). However most dramatic increase was seen in the plasma ONOO- levels. In the MI-nebivolol group, plasma NO levels were similar with sham-control animals (p=0.067). Compared to sham-control animals though plasma ONOO- levels were statistically higher in the MI-nebivolol group (p<0.05); these levels were lower than in MI-control rats (34.2±3.0 and 191.7±3.8 for MI-nebivolol and MI-control groups respectively, p<0.005). Plasma ONOO- levels were also correlated with the

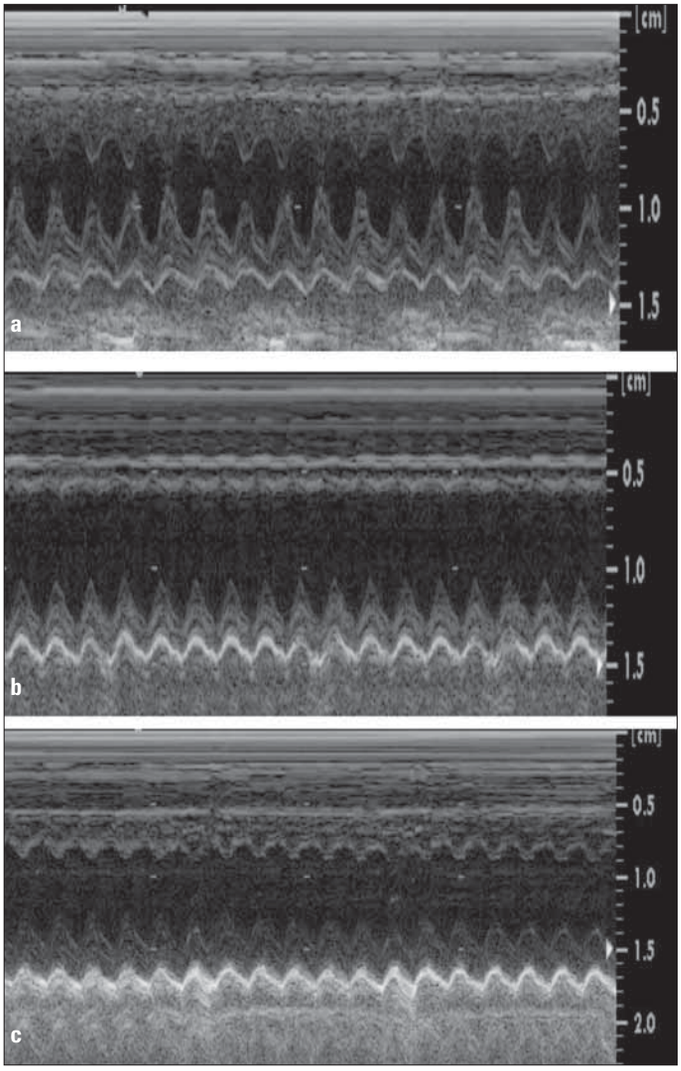

Figure 2. M-mode echocardiograms of representative left ventricular wall motion abnormalities

Sham-control with characterized normal left ventricular wall motions (a), MI induced demonstrating severe hypokinesia of septum (b), and nebivolol treated rat showing mild to moderate hypokinesia of septum (c) on 28th day

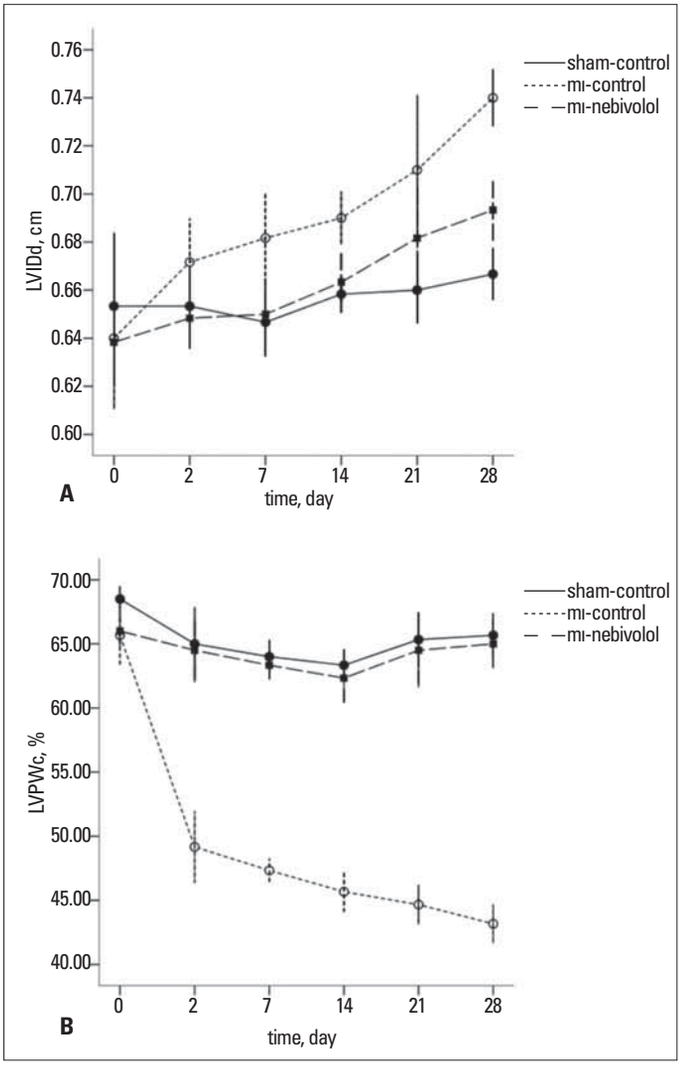

Figure 3. Left ventricular structural changes measured by M-mode echocardiography

Compared to sham-control rats, MI-control animals exhibited significant left ventricular structural changes. While LVIDd was increased as early as 2 days post MI, LVPWc was decreased. These changes were remained elevated during the 28 day-period. In the MI-nebivolol group, LVIDd and LVPWc were similar with the sham-control rats throughout the study period

LVIDd- left ventricular internal dimension at diastole, , LVPWc- left ventricular posterior wall change, MI- myocardial infarction

MI-sizes also (correlation coefficients are 0.908 and 0.929 respectively) (Fig. 6).

Discussion

In this study, we found that, when compared to sham-control rats; MI-control animals exhibited significant LV structural changes of VR (characterized by increase in LVIDd and LVIDs) immediately after MI (significant as early as 2 days post MI) and this trend continued during 28-day period. Functional abnormalities in-MI-control rats were in accordance with the structural changes (characterized by decrease in EF, FS and LVPWc). These changes were significantly less in MI-nebivolol group. Although, heart rate (HR) and mean blood pressure (MBP) levels were decreased in MI-induced rats; LVEDP was lower and $\Delta\pm dp/dt$'s were higher in MI-nebivolol group. Infarct sizes were similar in MI-induced groups however, LVW/HW and HW/BW's were significantly greater in the MI-control group compared to sham-control. These changes were not statistically significant in MI-nebivolol group. The increase in plasma NO and ONOO- levels were also prevented with nebivolol.

Despite all improvements in the treatment strategies, MI still continues to be a serious health problem with high risk of mortality and morbidity. MI and subsequent VR, causes structural and functional changes in jeopardized and remote healthy myocardium (20). Beta-blockers, used in the treatment of both post-MI and heart failure, show their useful effect by controlling the neuro-hormonal activation (6, 21, 22). Recent studies with carvedilol have also showed that, these useful effects were not only mediated by their adrenergic receptor blocker activity, but also by other mechanisms such as antioxidant activity (23, 24). Nebivolol is a third generation beta-blocker with NO mediated

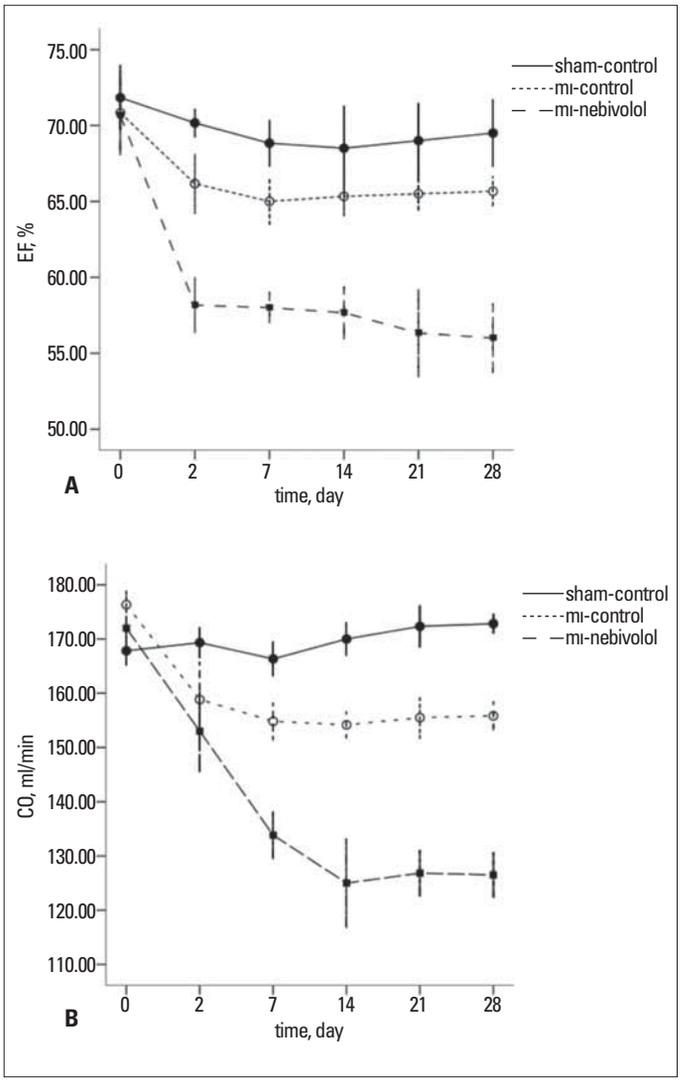

Figure 4. Left ventricular functions evaluated by echocardiography

Left ventricular functional changes are in accordance with the structural changes. Although EF (A) and CO (B) were decreased significantly in the MI-control group; these changes were prevented in the MI-nebivolol group

CO - cardiac output, EF - ejection fraction, MI - myocardial infarction

vasodilating activity and antioxidant effects (25, 26). Although there are studies investigating the effect of nebivolol in chronic heart failure models (27-30), its effects on VR in MI model has not been investigated yet. This experimental trial is the first one, which studied the effects of nebivolol on VR after MI.

Differently from previous animal studies with beta-blockers (23, 24), in our study nebivolol was administered IV at low dose in the acute phase of MI and then continued orally. This is in agreement with the therapeutic strategy of MI, as suggested by the results of large clinical trials and by the international guidelines for the treatment of MI (28-32). The parenteral dose of nebivolol selected in our study was 0.1 mg/kg, in order to assess the effect of drug on VR at the lowest beta-blocking activity with a minimum hemodynamic effect and to avoid impairment of coronary perfusion due to systemic hypotension. As a matter of fact, MBP and HR were not different between MI-control and MI-nebivolol groups in our study.

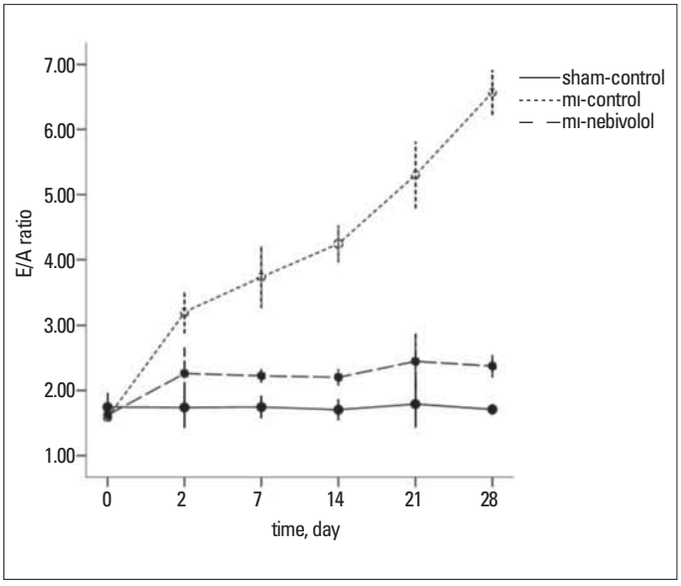

Figure 5. Left ventricle mitral inflow parameters

Increased E/A ratio resulting from increased early filling velocity (E) and decreased atrial filling velocity (A) was seen in the MI-control group over the study period. The change in the E/A ratio was similar between sham-control and MI-nebivolol groups

A - atrial filling velocity, E - early filling velocity, MI - myocardial infarction

Table 4. Hemodynamic parameters 28 days after MI

Variables	Sham-control	MI-control	MI-nebivolol
HR, bpm	331±21	283±24*	287±30*
MAP, mmHg	110.1±6.7	98.8±8.3*	92.3±8.1*
LVSP, mmHg	133.0±8	123.6±6.8*	118.5±8.4*
LVEDP, mmHg	2.3±0.3	31.8±3.3*	12.1±4.8*ψ
+dp/dt, mmHg/s	6600±352	3915±584*	4838±351*ψ
-dp/dt, mmHg/s	5058±618	2853±265*	3350±322*ψ

Data are presented as mean± SD

One-way ANOVA test, post hoc Bonferroni test:

*p<0.05 compared to sham-control group at same point in time

ψp<0.05 compared to MI-control group at same point in time

+dp/dt- maximum rise rate of left ventricular pressure, -dp/dt- maximum fall rate of left ventricular pressure, HR- heart rate, LVEDP- left ventricular end-diastolic pressure, LVSP- left ventricular systolic pressure, MAP- mean arterial pressure, MI- myocardial infarction

Table 5. Infarct sizes, body and heart weights 28 days after MI

Variables	Sham-control	MI-control	MI-nebivolol
LVW, mg	728±62	988±68*	732±36ψ
HW, mg	929 ± 23	1110± 52*	950±20ψ
BW, g	323±9	325±10	318±11
LVW/HW	0.75±0.03	0.92±0.04*	0.83±0.03ψ
HW/BW	2.95±0.09	3.44±0.08*	2.98±0.05ψ
Infarct size, %	-	44.88±4.9	45.22±3.5

Data are presented as mean± SD

One-way ANOVA test, post hoc Bonferroni test:

*p<0.05 compared to sham-control group at same point in time

ψp<0.05 compared to MI-control group at same point in time

BW- body weight, HW- heart weight, LVW- left ventricle weight, MI- myocardial infarction

Table 6. Plasma NO and ONOO levels 28 days after MI

Variables	Sham-control	MI- control	MI-nebivolol
NO, µmol/l	29.4±2.3	41.7±4.3*	28.4±4.1$^{\psi}$
ONOO, nmol/l	19.9±4.5	191.7±8.9*	34.2±3.0*$^{\psi}$

Data are presented as mean± SD
one-way ANOVA test, post hoc Bonferroni test:
*p<0.05 compared to sham-control group at same point in time
$^{\psi}$p<0.05 compared to MI-control group at same point in time
MI - myocardial infarction, NO - nitric oxide, ONOO - peroxynitrite

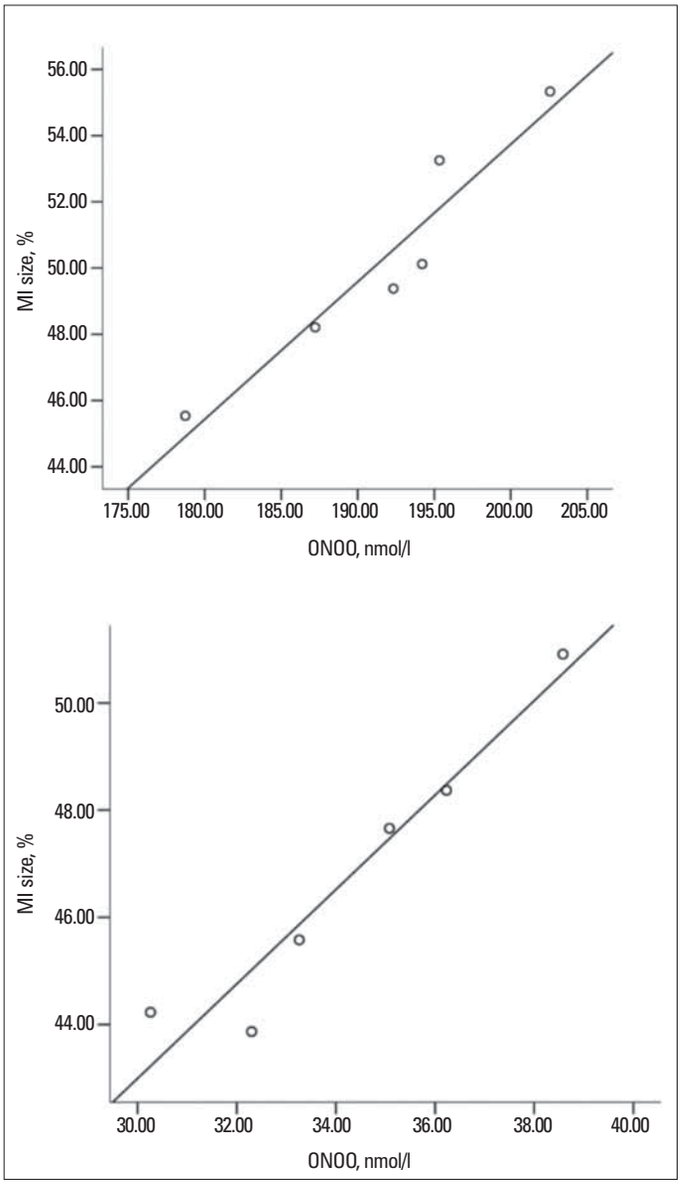

Figure 6. Correlation curves of plasma ONOO- levels with MI sizes
Plasma ONOO- levels were correlated with the MI sizes both (a) MI-control (b) MI-nebivolol groups (correlation coefficients are 0.908 and 0.929 respectively)
MI- myocardial infarction, ONOO – peroxynitrite

Our results showed that; although MI-control rats developed progressive VR (characterized by progressive LV dilation, associated with increased EDV, LVEDP and decreased FS, SV, CO and EF) both in early and sub-acute period of MI, there was a significant limitation on VR (shown by the less increase in LVID and EDV together with slight decrease in EF, CO, FS and SV) in the MI-nebivolol group. Compared to MI-control animals, a less deterioration was also observed in diastolic functions shown by insignificant change in LVEDP and dp/dt resulting from maintenance of systolic performance.

In our study, one of the important indicators of VR in the MI-control group was the development of marked hypertrophy in the healthy myocardium. Despite the hypertrophy in the healthy myocardium, developed due to compensatory mechanism, contractility did not increase in the same proportion (decrease in posterior wall thickening (LVPWc). Similar findings were reported in other post MI pre-clinic studies as well (29, 30). Investigators explain the reason for insufficient contraction of the healthy myocardium in various ways. One of them is the "insufficient hypertrophy" of the healthy myocardium. Compensation response to tissue loss in MI does not occur as rapid as the development of MI. In our study, the increase in the LVPWc reached to a significant level on the 21st day and this finding also supports this explanation. Healthy myocardium could not increase its thickness parallel to increased wall tension (afterload-mismatch).

In the MI-control group, we determined an increase in E wave velocity, a decrease in A wave velocity and as a result a significant increase in E/A ratio. This kind of changes in diastolic mitral flow are described as restrictive type LV diastolic dysfunction which is typical in the terminal heart failure (17). Restrictive type ventricular diastolic dysfunction reflects the significant increase in left atrial pressure. In our study, the left atrial pressure was not measured directly. Nevertheless, LVEDP indirectly reflecting left atrial pressure increased markedly in MI-control group. A similar type of diastolic dysfunction was shown to develop in MI and heart failure studies with rats (31, 34).

Recent studies reported that some of beta-blockers (metoprolol, propranolol, carvedilol) prevent VR (23, 24,32). However, attenuation of VR with propranolol and metoprolol was attributed to their effects on neuro-hormonal activation (33, 34), cardioprotective effects of carvedilol might be related to additional pharmacological properties such as the antioxidant and anti-neutrophil effects (23, 24, 35). In comparison with carvedilol and metoprolol (36), in our study nebivolol prevented VR to a greater extent. However, though nebivolol appears to be more effective, there is no comparative study in the same model.

In this study, nebivolol significantly decreased the negative structural and functional findings of VR in acute (on the 2nd day of MI) and sub-acute (on the 28th day of MI) periods after MI. Nebivolol limited the decrease in LV systolic function by preventing the increase in LV volume and LVEDP and preserved the EF and SV. Nebivolol showed these beneficial effects even in low dose, which has not hemodynamic effect related to beta-blockade. We also demonstrated that the increase in the plasma ONOO- and NO levels were prevented by nebivolol treatment. It is known that excessive amount of NO inhibits myocyte contractility (37, 38), induces myocardial cell loss through

apoptosis (39). It is also known that the toxic effects of high levels of NO have been reported to be mediated by the formation of ONOO- (40). Inconsistent with this knowledge, in this study increases in NO production and ONOO- levels were associated with decreased myocardial function characterized with gradually decreased FS during the sub-acute period of MI in the MI-control rats, however this decrease was prevented in the MI-nebivolol group. Moreover, we found positive correlation between infarct sizes and plasma ONOO- levels.

Study limitations

The most important limitation of our study was the sample size. We would perform our experiments in a larger population to increase the statistical power of the study.

Conclusion

Nebivolol treatment prevented VR in a rat MI model. No statistically significant relation was determined between these effects of nebivolol and its heart rate and blood pressure reducing effects. NO-mediated effects of nebivolol, may probably be involved in the favorable hemodynamic profile and prevention of VR. Further studies are needed for evaluating the NO-mediated effects of nebivolol in MI models. The results of our study have a promising clinical interest in the therapy of MI that represents the final pathway to heart failure.

Conflict of interest: None declared

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