

The Effect of Different β -Blockers on Vascular Graft Nitric Oxide Levels: Comparison of Nebivolol Versus Metoprolol

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WHAT THIS PAPER ADDS

The aim of this study was to investigate the effects of the vasodilating β -blocker nebivolol and the cardioselective β -blocker metoprolol on nitric oxide levels at vascular graft endothelium and vasa vasorum compared to controls in patients undergoing coronary artery bypass graft surgery. According to our results, we think that nebivolol may be safer and preferable in order to diminish graft spasm in patients undergoing coronary artery bypass graft surgery due to the nitric oxide-mediated vasodilating effect.

Objectives: The aim of this study was to investigate the effects of the vasodilating β -blocker nebivolol and the cardioselective β -blocker metoprolol on nitric oxide (NO) levels at vascular graft endothelium and vasa vasorum compared to controls in patients undergoing coronary artery bypass graft surgery.

Methods: This was a prospective study. Fifty-five patients were divided into three groups: nebivolol group (group N, $n = 23$), metoprolol group (group M, $n = 16$), and control group (group A, $n = 16$). Group N received nebivolol 5 mg once daily, and group M received metoprolol 50 mg once daily for 15 days in the preoperative period. Control patients did not use β -blocker therapy. Tissue samples of both left internal mammary artery (LIMA) and saphenous vein grafts were investigated for NO activity using immunohistochemical methods.

Results: Demographic characteristics and risk factors were similar between groups. We observed the highest NO activity in group N in both endothelial and vasa vasorum samples of LIMA and saphenous veins. NO activity of metoprolol group was similar to controls.

Conclusions: According to our results, we think that nebivolol may be safer and preferable in order to diminish graft spasm in patients undergoing coronary artery bypass graft surgery due to the NO-mediated vasodilating effect.

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Article history: Received 3 July 2013, Accepted 2 November 2013, Available online 10 November 2013

Keywords: Beta blocker, Coronary artery bypass graft surgery, Metoprolol, Nebivolol, Nitric oxide

INTRODUCTION

Ischemic heart disease occurs as a result of mismatch between myocardial oxygen demand and vascular supply. Coronary artery bypass graft (CABG) surgery is a widely utilized treatment of ischemic heart disease depending on the clinical condition. However, graft spasm, especially in arterial conduits, and graft stenosis still creates a major problem in patients undergoing CABG surgery.¹ Graft spasm can present with angina, acute hemodynamic collapse, ST segment elevation, or ventricular fibrillation, or a combination of these additional factors. Insufficient blood flow through arterial grafts may also cause a "hypoperfusion syndrome" manifested

by left ventricular failure, increasing pulmonary wedge pressure, and by cardiac arrest. According to the literature, its incidence varies between 0.8 and 1.3%.² However, the incidence and mortality rates may be underestimated because it is mainly the surviving cases that are reported, and an exact diagnosis can only be achieved with coronary angiography.²

β 1-adrenergic receptor blockers have become a hallmark in the management of patients after acute myocardial infarction.³ In contrast to conventional selective β 1 adrenergic receptor antagonists such as metoprolol-succinate, the selective β 1 adrenergic receptor blocker nebivolol has been shown to possess additional actions—stimulating endothelial cell nitric oxide (NO) production, in particular, which is thought to be mediated by β 3-receptor activation and by interaction with the estrogen receptor.³ The release of the endothelium-derived relaxing factor NO has been suggested to mediate vasodilatory properties of nebivolol because nebivolol-induced vasodilation is almost completely blocked by inhibitors of NO synthase.⁴

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<http://dx.doi.org/10.1016/j.ejvs.2013.11.003>

Vasodilation effects based on endothelial NO liberation following treatment with nebivolol have been demonstrated in peripheral macrovessels and in human coronary microvessels.⁵ In addition, nebivolol has been shown to cause an increase in stroke volume, associated with a reduction in vascular resistance, resulting in a maintained cardiac output despite reduced heart rate.⁶ These different effects of NO, resulting in increasing blood flow and considerable advantages in terms of the hemodynamic profile on cardiac output, may also contribute to graft spasm after CABG surgery. However, this activity seems not to be a class effect of β -blockers. Yet, no clinical data on CABG patients are available, but a rabbit model of carotid venous bypass grafting indicates that nebivolol might decrease the development of intimal hyperplasia.⁷

The aim of this study was to investigate the effects of the vasodilating β -blocker nebivolol and the cardioselective β -blocker metoprolol on NO levels at vascular graft endothelium and vasa vasorum compared with controls in patients undergoing CABG surgery.

MATERIALS AND METHODS

This study was approved by the local institutional review board. Written informed consent was obtained from all participants. Fifty-five consecutive patients who underwent CABG surgery between January and March 2007 were randomly divided into three groups by simple randomization constructed with tables of random numbers: nebivolol group (group N, $n = 23$), metoprolol group (group M, $n = 16$), and control group (group A, $n = 16$). Group N received nebivolol 5 mg once daily, and group M received metoprolol 50 mg once daily for 15 days in the preoperative period. Control patients did not use β -blocker therapy. Tissue samples of both left internal mammary artery (LIMA) and saphenous vein grafts were investigated for NO activity using immunohistochemical methods.

Patient selection

Patients were evaluated by physical examination, laboratory tests, transthoracic echocardiography, electrocardiography, and coronary angiography. Hypertension was defined as a mean systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, and/or use of antihypertensive medications. Hyperlipidemia was defined as total cholesterol level >240 mg/dL (6.2 mmol/L), the current use of lipid-lowering treatment, or both. Patients with New York Heart Association (NYHA) class IV heart failure, left ventricular ejection fraction $\leq 30\%$, previous CABG surgery, and patients older than 75 years, requiring emergency surgery and inotropic support were excluded. Patients having additional cardiac (valvular or congenital) and systemic (chronic obstructive pulmonary disease, and renal and hepatic failure) pathologies were also excluded.

Surgery

Anesthetic technique was standard for all patients. General anesthesia and intratracheal intubation were done. Median

sternotomy was performed under general anesthesia. LIMA was prepared in suitable patients. Saphenous veins were readied in patients requiring more than one graft. Vascular samples of 1 cm length were taken from LIMA and saphenous vein grafts prior to systemic heparin administration during either conventional or off-pump CABG surgery. Venous or arterial grafts other than LIMA and saphenous veins were not used. Following sampling surgical procedure was completed as usual. Surgical technique (off-pump or conventional) were not taken into consideration.

Immunohistochemistry

Sampled vessels were preserved in 10% formalin solution at room temperature. Afterwards samples of vascular wall and vasa vasorum were evaluated for NO activity. Immunohistochemical staining was performed using the goat ImmunoCruz Staining System (sc-2053; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Primary antibody was inducible nitric oxide synthase epitope-specific rabbit antibody (0.5 mL for 200 ng/mL). Samples were evaluated using light microscopes after preparation. NO activity was scored according to histopathological findings.

Immunohistochemical expression was quantified and scored by assessing a proportion of percentage as an intensity score according to Allred's scoring protocol.⁸ The intensity of the immunostaining in the arteries was graded semiquantitatively from 0 to 2, with 0 corresponding to the absence of staining, 1 corresponding to the intermediate staining, and 2 representing strong staining. The grading was performed by a single investigator, who was unaware of the clinical and hemodynamic data.

Statistical analysis

Data were analyzed using SPSS version 13.0 for Windows (SPSS, Chicago, IL, USA). A normal distribution of the quantitative data was checked using the Kolmogorov–Smirnov test.

Table 1. Demographic and clinical characteristics of patients.

	Group A ^a	Group M ^b	Group N ^c	<i>p</i>
Gender (M/F)	9/7	12/4	10/13	.14
Age (y)	64 \pm 8.93	63.2 \pm 9.13	60.1 \pm 1.7	.63
Hypertension (mmHg)	6/16 (37.5%)	7/16 (43.8%)	14/23 (60.8%)	.31
Diabetes mellitus	9/16 (56.2%)	6/16 (37.5%)	7/23 (69.6%)	.26
Hyperlipidemia	7/16 (43.8%)	10/16 (62.5%)	13/23 (56.5%)	.55
Number of coronary grafts ($n = 1$)	2	0	7	.09
Number of coronary grafts ($n > 1$)	14	16	16	.43

Note. M = male; F = female.

^a Control group.

^b Metoprolol group.

^c Nebivolol group.

Table 2. Nitric oxide (NO) activity in endothelium and vasa vasorum of left internal mammary artery (LIMA) grafts.

Groups	Grade	Group A ^a	Group M ^b	Group N ^c	<i>p</i>
Endothelial NO activity of LIMA grafts	0	16 (100%)	16 (100%)	11 (55.0%)	1.00 (A vs. M)
	1	0 (0%)	0 (0%)	2 (10.0%)	.002 (A vs. N)
	2	0 (0%)	0 (0%)	7 (35.0%)	.002 (M vs. N)
Vasa vasorum NO activity of LIMA grafts	0	12 (75.0%)	14 (87.5%)	2 (10.0%)	.81(A vs. M)
	1	2 (12.5%)	2 (12.5%)	6 (30.0%)	.001(A vs. N)
	2	2 (12.5%)	0 (0%)	12 (60.0%)	.001(M vs. N)

^a Control group.

^b Metoprolol group.

^c Nebivolol group.

Parametric tests were applied to data of normal distribution, and non-parametric tests were applied to data of questionably normal distribution. The distribution of categorical variables in both groups was compared using Pearson's chi-square test, continuity correction, and Fisher's exact tests. Data are expressed as mean \pm SD or percentages, as appropriate. Statistical significance was assumed to be $p < .05$.

RESULTS

Demographic characteristics of patients

Fifty-five patients (31 men, 24 women) were included in the study. Demographic characteristics are presented in Table 1. There were no statistically significant differences between three groups regarding age, gender, and presence of cardiovascular risk factors or number of coronary artery anastomosis.

Immunohistochemical findings

We evaluated 45 saphenous vein and 52 LIMA graft samples during the study.

Endothelial NO activity of LIMA grafts. NO activity was absent at LIMA endothelium at all patients from control and metoprolol groups, and 55% of patients from nebivolol group. However, 10% had grade 1 and 35% had grade 2 NO

activity in group N (Table 2). Nebivolol group had the highest activity among groups ($p = .002$) (Fig. 1).

Vasa vasorum NO activity of LIMA grafts. Similarly, NO activity was highest in nebivolol group, which was statistically more significant than groups M and A ($p = .001$). NO activity was comparable in groups M and A (Fig. 1).

Endothelial NO activity of saphenous vein grafts. Patients receiving nebivolol treatment had the highest NO activity (grades 1 and 2), which was statistically significant compared with both groups M and A ($p = .014$). NO activity was similar between groups M and A (Fig. 1; Table 3).

Vasa vasorum NO activity of saphenous vein grafts. Even though groups M and A had slightly increased NO activity, overall activity was significantly higher in group N compared with the other two groups ($p = .002$) (Fig. 1; Table 3).

DISCUSSION

We investigated the effects of the vasodilating β -blocker nebivolol and the cardioselective β -blocker metoprolol on NO levels at vascular graft compared to controls in patients undergoing CABG surgery. We demonstrated that patients receiving nebivolol 5 mg once daily showed higher NO activity at both endothelium and vasa vasorum of LIMA than

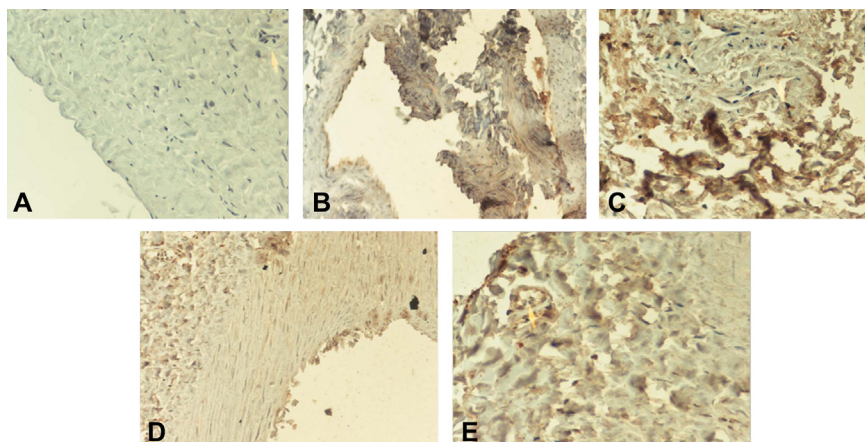


Figure 1. Immunohistochemical findings among groups. (A) Grade 0 nitric oxide (NO) activity at endothelium and vasa vasorum of left internal mammary artery (LIMA) (20 \times magnification, immunohistochemistry). (B) Grade 2 NO activity at endothelium of LIMA (20 \times magnification, immunohistochemistry). (C) Grade 1 NO activity at vasa vasorum of LIMA (20 \times magnification, immunohistochemistry). (D) Grade 2 NO activity at endothelium of saphenous vein (20 \times magnification, immunohistochemistry). (E) Grade 2 NO activity at vasa vasorum of saphenous vein (20 \times magnification, immunohistochemistry).

Table 3. Nitric oxide (NO) activity at endothelium and vasa vasorum of saphenous grafts.

Groups	Grade	Group A ^a	Group M ^b	Group N ^c	<i>p</i>
Endothelial NO activity of saphenous vein grafts	0	15 (93.8%)	12 (100%)	10 (58.8%)	.50 (A vs. M)
	1	0 (0%)	0 (0%)	6 (35.3%)	.001 (A vs. N)
	2	1 (6.3%)	0 (0%)	1 (5.9%)	.001 (M vs. N)
Vasa vasorum NO activity of saphenous vein grafts	0	10 (62.5%)	8 (66.7%)	1 (5.9%)	.50 (A vs. M)
	1	4 (25.0%)	3 (25.0%)	6 (35.3%)	.002 (A vs. N)
	2	2 (12.5%)	1 (8.3%)	10 (58.8%)	.002 (M vs. N)

^a Control group.

^b Metoprolol group.

^c Nebivolol group.

patients on metoprolol. We hypothesize that this effect may be beneficial for improving graft patency and survival.

The endothelium modulates the tone of the underlying vascular smooth muscle cells by producing relaxing factors, mainly NO and prostacyclin, and constricting factors, such as endothelin. Endothelial dysfunction is a common feature of cardiovascular diseases. Therefore, it seems highly desirable to target therapeutic strategies towards the improvement (i.e., repair) of endothelial function.⁹

Metoprolol and nebivolol have a similar potency in binding to β 1- and β 2-adrenergic receptors, but nebivolol, in particular, is suggested to exert NO-releasing effects, probably via reduction of reactive oxygen species, whereas metoprolol is lacking this pleiotropic effect on vascular function.¹⁰ The use of third-generation β -blockers with additional vasodilatory properties, in the treatment of hypertension, heart failure and ischemic syndromes is increasing.¹⁰ In contrast with most of the β -adrenoreceptor blockers, nebivolol has vasodilatory properties that are dependent on the presence of the endothelium and are associated with activation of endothelial nitric oxide synthase.¹¹ There is evidence that nebivolol, in addition to its β 1-adrenoreceptor blocking effects, can stimulate endothelial NO production, which has been suggested to be mediated, at least in part, by a β 3-agonistic effect.^{12,13} Furthermore, nebivolol has been suggested to exert antioxidant effects that have been attributed, at least in part, to prevention of nicotinamide adenine dinucleotide phosphate oxidase activation in response to hyperlipidemia or angiotensin II.^{5,14}

There is evidence that β 3-adrenoreceptor stimulation may be involved in the release of endothelial NO induced by nebivolol.¹⁵ Conversely, there are reports in the literature on the activation of estrogen receptors and the implications of this in the vascular effects of nebivolol, especially with respect to the anti-atherosclerotic property of nebivolol, which inhibits smooth muscle growth.¹⁶ Broeders et al.³ showed that in vivo metabolites of nebivolol increased NO activity and endothelial calcium concentrations in vitro vascular segments. Several other studies also proved that nebivolol induced vasodilation, which could be inhibited by *N*-monomethyl-L-arginine.^{3,11} Ignarro et al.¹⁷ showed that the mechanism of nebivolol-induced vasodilation is through NO and the cyclic guanosine monophosphate receptor.

Goldstein et al.¹⁸ compared the effect of nebivolol versus atenolol in patients undergoing CABG surgery and found that atenolol increased both systemic vascular resistance and cardiac index, whereas nebivolol increased cardiac index while lowering systemic vascular resistance. Our data suggest that nebivolol increased NO activity in vascular graft endothelium. Moreover, the highest NO grades were observed in group N. Interestingly, NO activity was absent in LIMA endothelium in all patients in group M and 55% of patients from group N. This may indicate that regional differences may exist regarding β -adrenoreceptor signaling in endothelial cells.

A strong limitation of the present study has to be expressed. β -Blockers have been compared concerning hard end-points, and it was recently shown that carvedilol was superior to both metoprolol and nebivolol in reducing mortality in heart failure and patients with myocardial infarction.¹⁹ Another limitation is the absence of data on blood pressures (both before and after the initiation of treatment with the two β -blockers) and low-density lipoprotein cholesterol levels in the different groups.

In conclusion, we think that the documented NO activity in patients younger than 75 years with NYHA I–III heart failure, left ventricular ejection fraction >30%, and no history of additional cardiac and systemic disease undergoing CABG surgery is very important because higher NO activity in graft endothelium and vasa vasorum may reduce ischemia, graft spasm, and, perhaps, graft stenosis. As β -blocker agents are widely used in patients undergoing CABG surgery, nebivolol may be safer and preferable in order to diminish graft spasm in patients undergoing CABG surgery due to the NO-mediated vasodilating effect.

ACKNOWLEDGMENTS

None.

CONFLICT OF INTEREST

None.

FUNDING

None.

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