The vasodilator action of nebivolol in forearm vasculature of subjects with essential hypertension

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Aims Brachial artery administration of nebivolol increases forearm blood flow in normotensive subjects through activation of the L-arginine/NO pathway. The aim of the present study was to investigate the effect of brachial artery administration of nebivolol in subjects with essential hypertension.

Methods We studied eight patients with uncomplicated essential hypertension and serum cholesterol less than 6.9 mmol l^{-1}. Antihypertensive medication was discontinued 2 weeks before the study in previously treated patients. Following cannulation of the left brachial artery, saline was infused to establish baseline blood flow, followed by increasing doses of nebivolol (88.5, 177 and 354 μg min^{-1}, each dose for 6 min), followed by saline for 12 min, followed by a 30 min infusion of L-NMMA (2 mg min^{-1}). During the final 18 min of the L-NMMA infusion, nebivolol was coinfused using the same doses as before. Forearm blood flow was measured in both arms using venous occlusion plethysmography.

Results Blood flow in the noninfused arm did not change significantly throughout the study. In the infused arm blood flow increased significantly in a dose-related manner during the first series of nebivolol infusions from 2.76 ± 0.39 ml min^{-1} 100 ml $forearm^{-1}$ during the baseline period to 4.40 ± 0.60 ml min^{-1} 100 ml $forearm^{-1}$ (mean $\pm$ s.e. mean, $n=8$, $P=0.0003$ by ANOVA). L-NMMA antagonized the vasodilator effect of nebivolol: baseline blood flow in the infused arm was 2.41 ± 0.53 ml min^{-1} 100 ml $forearm^{-1}$ and 2.94 ± 0.42 ml min^{-1} 100 ml $forearm^{-1}$ during coinfusion of the top dose of nebivolol with L-NMMA ($P=0.0006$ for an effect of L-NMMA on nebivolol response). There were no serious adverse events.

Conclusions Nebivolol causes vasodilation in the forearm vascular bed in subjects with essential hypertension. Since this response is antagonized by L-NMMA, the vasodilatation is probably caused by activation of the L-arg/NO pathway.

Keywords: essential hypertension, forearm resistance vasculature, nebivolol, N^G-monomethyl-L-arginine, nitric oxide

Introduction

Nebivolol, the most selective β_1-adrenoceptor antagonist clinically available [1], has additional haemodynamic effects attributable to systemic vasodilatation. Its vasorelaxant effect in dog coronary artery is endothelium-dependent [2]. When administered into the brachial artery of healthy normotensive human subjects in high doses, it increases forearm blood flow in contrast to atenolol which causes no change in forearm blood flow even at high doses [3]. This vasodilator effect of nebivolol does not show tachyphylaxis and is antagonized by coinfusion of L-NMMA to a similar extent as is that of an endothelium dependent vasodilator (carbachol) and more than a nonendothelium dependent vasodilator (nitroprusside). L-arginine (administered intra-arterially) restores the vasodilator effect of nebivolol when coinfused with L-NMMA. These findings suggest that the vasodilating property of nebivolol in the forearm vascular bed is mediated via the endothelial L-arginine/NO pathway [3].

Endothelial function, assessed using acetylcholine as endothelium dependent agonist, is impaired in forearm [4, 5] and coronary [6] vasculature of certain patients with essential hypertension, although this is not universal

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[7] and may represent an accelerated form of the dysfunction that occurs with advancing age [8]. Nebivolol is used therapeutically to lower arterial blood pressure and hypertensive patients could benefit from its vasodilator action, especially if this resulted from a restoration of endothelium-dependent NO biosynthesis. The object of the present investigation was therefore to determine whether nebivolol causes vasodilatation in the forearm vascular bed in patients with essential hypertension and, if so, whether this effect is antagonized by coinfusion of L-NMMA.

Methods

Otherwise healthy subjects referred to the Guy's Hypertension Clinic were screened sequentially for eligibility. Eligible subjects, none of whom had taken part in other forearm studies, were invited to take part: 11 were screened to provide eight evaluable subjects (brachial artery catheterization failed in three subjects). The protocol was approved by the Guy's Hospital Research Ethics Committee. Inclusion criteria were: men or women on antihypertensive treatment for at least 6 months, or newly diagnosed on the basis of three or more seated office readings measured by sphygmomanometry following British Hypertension Society guidelines [9] on separate visits >159 mmHg (systolic) and/or >89 mmHg (diastolic) or of a mean ambulatory diastolic pressure from 08.00 h to 20.00 h of >89 mmHg using an Accutracker II automatic device [10]. Exclusion criteria were: age <18 or >70 years, secondary hypertension (diagnosed by history, physical examination, routine biochemical investigations and special endocrinological and imaging as clinically indicated), any contraindication to β-adrenoceptor antagonists, a history of accelerated/malignant hypertension, untreated systolic blood pressure >190 mmHg, untreated diastolic blood pressure >120 mmHg, symptoms or clinical signs of cardiac or cerebrovascular disease, diabetes, clinical evidence of hepatic disease, serum total cholesterol >6.9 mmol l^{-1}, serum creatinine >135 μmol l^{-1}, clinical need for ongoing regular medication for any other chronic disease.

Following written informed consent any antihypertensive medication was discontinued for at least 2 weeks. History, physical examination and routine laboratory tests were obtained at an initial screening visit. On the study day, subjects rested supine in a temperature controlled clinical laboratory. Forearm blood flow was measured in both arms simultaneously using venous occlusion plethysmography with temperature-compensated electrically calibrated strain gauges [11, 12]. Flows were recorded for 10 s in every 15 s during the final 3 min of each infusion period, and the mean of the final five measurements used for analysis. A 27-gauge steel cannula (Cooper's Needleworks, Birmingham, UK) was inserted into the left brachial artery under local anaesthesia using <1 ml of 1% lignocaine hydrochloride (Antigen Ltd, Sligo, Ireland). Saline (NaCl 140 mmol l^{-1}, Travenol, Thetford, UK) or drugs dissolved in saline were infused at a constant rate of 1.0 ml min^{-1}. Subjects rested supine for at least 20 min before a 12-min infusion of saline and the measurement of baseline forearm blood flow. Nebivolol was infused at increasing doses (88.5, 177 and 354 μg min^{-1}) each for 6 min, and forearm blood flow measured in both arms at regular intervals during the final 3 min of each infusion period. After completion of the nebivolol infusion a further 12 min of saline was infused followed by L-NMMA (2 mg min^{-1}) for 30 min, first with saline for 12 min and then coinfused with nebivolol as used previously for 18 min. Any adverse events that occurred during the trial were noted by the investigator.

Statistical analysis

Data are summarized as means ± s.e. mean. Statistical analysis of blood flow data was by repeated measures analysis of variance. Responses to nebivolol were also calculated in terms of the increase in blood flow above the immediately preceding baseline (saline or L-NMMA). Differences were considered significant when $P<0.05$.

Results

The subject characteristics are summarized in Table 1. Figure 1 shows mean forearm blood flow in the cannulated and noncannulated arms. Blood flow in the noncannulated arm did not change significantly throughout the study, although there was a trend toward increased flow at the end of the study ($P=0.09$). When nebivolol was infused with saline there was an increase in forearm blood flow in the cannulated arm from 2.76 ± 0.39 ml min^{-1} 100 ml^{-1} forearm during the first (saline) baseline period

Table 1 Subject characteristics.

Male/Female	6/2
Previous treatment[a] (Y/N)	7/1
Age (years)	47.8 ± 3.6
Weight (kg)	79.3 ± 4.7
Serum cholesterol (mmol l^{-1})	4.83 ± 0.28
Serum creatinine (μmol l^{-1})	90.3 ± 5.2
Blood pressure[b] (mmHg)	161 ± 9/98 ± 2
Blood pressure[c] (mmHg)	148 ± 5/92 ± 2

[a]Antihypertensive drugs were: atenolol (5), enalapril (1) and doxazosin (1). [b]Measured by sphygmomanometry using an appropriate sized cuff before antihypertensive treatment, and [c]on the day of the study.

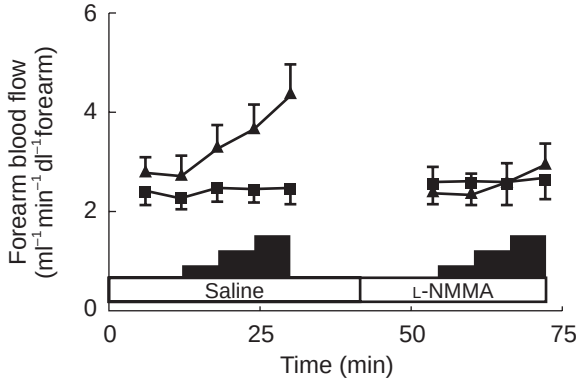

Figure 1 Forearm bloodflow responses in the infused left arm (▲) and non-infused right arm (■) to two sequential cumulative infusions of increasing doses of nebivolol (88.5, 177, and 354 $\mu g\,min^{-1}$, shown by closed bars), initially with saline coinfusion followed by coinfusion with L-NMMA (shown by open bars).

to 4.40 ± 0.60 ml min^{-1} 100 ml^{-1} forearm during the highest dose of nebivolol ($P = 0.0003$). After the recovery period and 12 min infusion of L-NMMA with saline, mean blood flow in the cannulated arm was 2.41 ± 0.53 ml min^{-1} 100 ml^{-1} forearm, compared with 2.58 ± 0.42 ml min^{-1} 100 ml^{-1} forearm in the noncannulated arm. During coinfusion of the highest dose of nebivolol with L-NMMA, mean blood flow was 2.94 ± 0.42 ml min^{-1} 100 ml $forearm^{-1}$ ($P < 0.0006$ for the comparison of infusion of L-NMMA *vs* saline with nebivolol), similar to blood flow in the noncannulated arm which was 2.98 ± 0.33 ml min^{-1} 100 ml^{-1} forearm. The highest dose of nebivolol produced an increase in blood flow above baseline of 1.64 ± 0.30 ml min^{-1} 100 ml^{-1} forearm during coinfusion of saline and 0.52 ± 0.2 ml min^{-1} 100 ml^{-1} forearm during coinfusion of L-NMMA ($P < 0.05$, 95% Confidence Intervals for the difference 0.09–2.15).

Discussion

This study demonstrates that brachial artery administration of nebivolol to subjects with essential hypertension increases forearm blood flow and that this is antagonized by L-NMMA, an inhibitor of the L-arg/NO pathway. This extends earlier findings in normotensive subjects [3]. Blood pressure on the day of study after resting supine was lower than blood pressure before antihypertensive treatment (Table 1), possibly because the time after withdrawal of antihypertensive drugs was limited to 2 weeks. The effect of L-NMMA on basal flow was similar or somewhat less marked than in previous studies, possibly because of residual vasodilatation caused by the previous infusion of nebivolol. L-NMMA had a marked effect on the response to nebivolol vasodilatation. Responses to vasodilators that are independent of the L-arg/NO pathway (e.g. verapamil, prostacyclin) are not inhibited by L-NMMA [13] so the observed inhibition of nebivolol can not be explained by a change of baseline vessel tone *per se*. The results suggest that the vasodilatation produced by intra-arterial nebivolol in essential hypertensive subjects is caused by activation of the L-arginine/NO pathway, as it is in normotensives.

The mechanism whereby nebivolol activates the L-arg/NO pathway is not known. β_2-adrenoceptor agonists cause vasodilatation in forearm resistance vasculature by an endothelium-dependent NO-mediated mechanism [13] and an endothelial NO component of α_2- and β-adrenergic vascular responses in the forearm is a target of the vascular action of insulin [14]. However, nebivolol is devoid of intrinsic sympathomimetic activity [1], ruling out direct activation of adrenoceptors as the explanation of its vasodilating action. Furthermore, both of the major stereoisomers of nebivolol cause vasodilatation in the forearm, suggesting a nonreceptor mediated mechanism [3]. Further experimental work will be needed to define the mechanism of this vasodilator action more precisely.

Release of NO in resistance vasculature could explain the vasodilator effect of nebivolol administered therapeutically to treat essential hypertension. Such vasodilatation may contribute to the favourable effects on exercise tolerance and quality of life of nebivolol in comparison with atenolol [15, 16] and explain the finding that it improves the left ventricular function of patients with hypertension [17]. NO has several potential antiatherogenic and antithrombotic effects including inhibition of monocyte and platelet adhesion, of monocyte migration, and of smooth muscle cell proliferation [18–21]. Inhibition of endogenous NO biosynthesis accelerates the development of arterial lesions in hypercholesterolaemic rabbits [22], suggesting the possibility that increasing endogenous NO biosynthesis could have a protective effect against atherosclerosis in humans with cardiovascular risk factors such as hypertension. Since most of the excess mortality and morbidity of patients with mild to moderate essential hypertension derives from the complications of atherosclerosis, a therapeutic agent that increases the endogenous production of NO in addition to causing selective β_1-adrenoceptor blockade has obvious theoretical attractions. In order to determine whether oral administration of nebivolol increases endogenous NO biosynthesis in patients with essential hypertension it will be necessary to perform further studies combining a measure of total systemic vascular resistance with biochemical measures of nitric oxide biosynthesis, such as that described recently by Forte and colleagues [23].

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