Nebivolol decreases systemic oxidative stress in healthy volunteers

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Aims Nebivolol is a selective, vasodilatory β_1-adrenergic receptor antagonist which has been suggested to possess additional antioxidative properties. The aim of the present study was to assess the actions of nebivolol in antihypertensive doses on systemic oxidative stress in healthy volunteers, reflected by 24 h urinary excretion of 8-iso-$PGF_{2\alpha}$.

Methods In a double-blind, cross-over study, 12 healthy volunteers received 5 mg nebivolol once daily or placebo for a total of 7 days, separated by a wash out period of 2 weeks. After each treatment period 24 h urinary excretion of 8-iso-$PGF_{2\alpha}$ was determined by gas chromatography-tandem mass spectrometry.

Results After the 7 day treatment period nebivolol decreased significantly urinary excretion of 8-iso-$PGF_{2\alpha}$ by 24% from 55.3 ± 5.1 pmol $mmol^{-1}$ creatinine during the placebo period to 42.3 ± 4.7 pmol $mmol^{-1}$ creatinine (mean $\pm$ s.e. mean, $P = 0.01$), a mean decrease of 13 pmol $mmol^{-1}$ creatinine (95% CI: -22.8; -3.1).

Conclusions Our data show for the first time that nebivolol decreases systemic oxidative stress in young healthy volunteers.

Keywords: β-adrenoceptor antagonists, oxidative stress, healthy volunteers

Introduction

Nebivolol, a vasodilating and highly selective β_1-adrenoceptor antagonist, has been associated with additional antioxidative effects [1–3]. After hydroxyl radical (OH) induced injury in right ventricular rabbit trabeculae, nebivolol in contrast to propranolol completely abolished blunting of the force-frequency relationship which can normally be observed during the OH injury phase. Hydroxyl radical induced systolic and diastolic dysfunction could be prevented almost completely by nebivolol [3].

Isoprostanes are prostaglandin (PG)-like compounds which are produced by nonenzymatic free radical-catalysed peroxidation of arachidonic acid [4]. Formation of 8-iso-$PGF_{2\alpha}$, one of the most abundant isoprostanes formed *in vivo* [5], was found to be increased in animals in which oxidative stress had been experimentally induced as well as in certain cardiovascular disease associated syndromes, e.g. coronary reperfusion with thrombolytic drugs, clamp release in patients undergoing coronary artery bypass grafting [6], hypercholesterolaemia [7] and atherosclerosis [8].

Whether nebivolol decreases oxidative stress after oral doses commonly used in antihypertensive therapy has not been investigated in man. Therefore the present trial was conducted to determine the effect of nebivolol on systemic oxidative stress by measuring urinary excretion of 8-iso-$PGF_{2\alpha}$ in healthy volunteers.

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Methods

Subjects

Twelve healthy, non obese volunteers (six male, six female), age (mean $\pm$ s.d.) 25.1 ± 2.5 years were included in this study. All had normal clinical history and examination, 12-lead electrocardiogram, haematological and biochemical screen. Only extensive metabolizers of nebivolol were included into the study [9]. None of the volunteers was receiving drugs which might alter free radical status, and dietary habits were constant during the study. Informed consent was obtained to the study protocol previously approved by the local Ethics

Committee, and the investigation was conducted in accordance with the Declaration of Helsinki.

Study design

The subjects received in a randomized, double-blind, cross-over design either nebivolol (one tablet, 5 mg daily) or placebo for 7 days. The study periods were separated by a washout phase of 2 weeks. On day 7 of each medication phase, the subjects collected 24 h urine for analysis of 8-iso-$PGF_{2\alpha}$ and creatinine.

Quantification of urinary 8-iso-$PGF_{2\alpha}$

Urinary 8-iso-$PGF_{2\alpha}$ was determined by GC-tandem MS analysis as described previously [10]. Briefly, a 5 ml aliquot of urine was spiked with 5 ng of internal standard [2H 4]-8-iso-$PGF_{2\alpha}$ (Cayman, Ann Arbor, MI, USA), analytes were solid-phase extracted, their pentafluorobenzyl (PFB) derivatives were prepared, separated by thin-layer chromatography, converted to their trimethylsilyl derivatives, and analysed by GC-tandem MS. Quantification was performed by selected reaction monitoring of the product ions at a mass/charge ratio (m/z) of 299 for endogenous 8-iso-$PGF_{2\alpha}$ and m/z of 303 for the internal standard which were generated by collisionally activated dissociation of the parent ions $[M - PFB]^-$ at m/z 569 and 573, respectively.

Urinary creatinine levels were determined spectrophotometrically by the alkaline picric acid reaction with an automatic analyser (Beckmann 6641, Galway, Ireland). Urinary excretion rates of 8-iso-$PGF_{2\alpha}$ were corrected for creatinine excretion.

Statistical analyses

All data are given as means $\pm$ s.e.mean. Statistical analyses were performed using paired Student's t-test (two-tailed). Statistical significance was assumed for $P<0.05$. Data analysis was performed with SPSS (release 9.0.1 for windows).

Results

Following nebivolol administration for a 7 day period urinary excretion of 8-iso-$PGF_{2\alpha}$ decreased significantly from 55.3 ± 5.1 pmol $mmol^{-1}$ creatinine to 42.3 ± 4.7 pmol $mmol^{-1}$ creatinine ($P<0.05$; Figure 1), a mean decrease of 13 pmol $mmol^{-1}$ creatinine (95% CI: -22.8; -3.1). Urinary excretion of creatinine was similar in both groups (13.1 ± 1.1 [nebivolol] *vs* 13.2 ± 1.1 [placebo] mmol/24 h; $P=0.82$). Urinary 8-iso-$PGF_{2\alpha}$ excretion rates did not correlate with gender or body mass index. Basal levels of urinary 8-iso-$PGF_{2\alpha}$ were in accordance with previously published data [11].

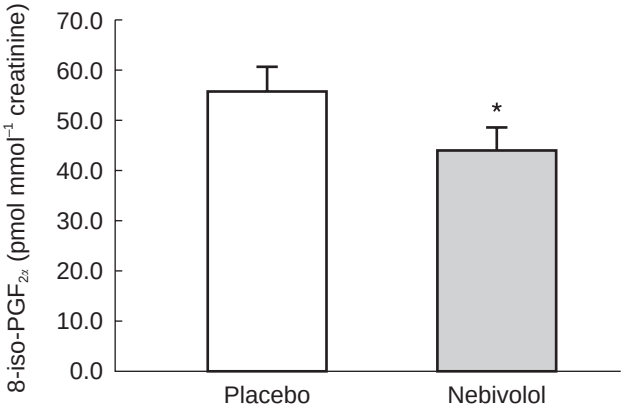

Figure 1 Creatinine-corrected urinary excretion + s.e. mean of 8-iso-$PGF_{2\alpha}$ in 12 healthy subjects receiving nebivolol 5 mg once daily or placebo. Asterisks indicate significance, i.e. $P<0.05$ for nebivolol *vs* placebo.

Discussion

This study demonstrates that oral administration of nebivolol at standard antihypertensive doses decreases significantly urinary excretion of the isoprostane 8-iso-$PGF_{2\alpha}$. This finding strongly supports the hypothesis that nebivolol exerts sytemic antioxidative effects. These results are in accordance with a previous *in vitro* study which showed that nebivolol can effectively prevent a large part of OH induced systolic and diastolic dysfunction in right ventricular rabbit cardiac trabeculae [3].

There is conflicting evidence as to whether other factors such as vitamin E or NSAIDs could possibly effect formation and urinary excretion of 8-iso-$PGF_{2\alpha}$. Davi *et al.* found a dose-dependent suppression of enhanced 8-iso-$PGF_{2\alpha}$ formation by vitamin E supplementation in hypercholesterolemic patients [7]. In contrast, supplementation with vitamin E in healthy humans did not affect urinary excretion of 8-iso-$PGF_{2\alpha}$ [12]. In principal the formation of 8-iso-$PGF_{2\alpha}$ in humans seems to be COX-independent [13, 14]. However, in our study supplementation with vitamins or use of NSAIDS was excluded.

Nebivolol has vasodilator activity and there is indirect evidence that this is mediated via the L-arginine/NO system [2]. The finding of the present study that nebivolol decreases systemic oxidative stress supports an interaction between nebivolol and L-arginine/NO system in favour of NO. This interaction could involve inhibition of the production or amplification of the degradation of oxidizing species such as superoxide anion by nebivolol. The consequence of such an interaction would be the availability of NO at higher concentrations despite unchanged NO synthase activity, which would lead to

enhanced NO-dependent vasodilation. Such an explanation is attractive since both enantiomers of nebivolol cause vasodilation in human resistance vasculature [2]. This lack of stereospecificity would be consistent with a chemical antioxidant activity of nebivolol [1–3] rather than with mechanisms which involve enzymes or receptors.

Increased oxidative stress, as determined by increased excretion of 8-iso-$PGF_{2\alpha}$, appears to be an important event in several forms of cardiovascular disease. In human atherosclerosis [8], hypercholesterolemia [7] and coronary reperfusion [6] urinary excretion of 8-iso-$PGF_{2\alpha}$ has been shown to be elevated. Our study shows that oxidative stress expressed in terms of urinary excretion of 8-iso-$PGF_{2\alpha}$ occurs even in healthy humans under basal conditions and can significantly be reduced pharmacologically, for instance by use of nebivolol at therapeutically relevant doses.

The results of the present study provide a rationale for further clinical studies on the antioxidative effects of nebivolol in cardiovascular disease.

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