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Effects of nebivolol on biomarkers in elderly patients with heart failure

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ABSTRACT

Background: Heart failure activates neurohormones, and elevated levels of brain natriuretic peptide (BNP) are associated with adverse outcomes. The SENIORS trial showed that nebivolol, a highly selective beta-1 antagonist with vasodilating properties, reduced the composite outcome of all cause mortality or cardiovascular hospital admissions in older patients with heart failure. We explored the effects of nebivolol on a range of neurohormones, cytokines and markers of nitric oxide activity in heart failure.
Methods: In a subset of patients in SENIORS we measured N-terminal pro-brain natriuretic peptide (NT-BNP), pro atrial natriuretic peptide (Pro-ANP), endothelin-1 (ET-1), peripheral norepinephrine (PNE), soluble Fas (sFas), soluble Fas-ligand (sFas-L), tumour necrosis factor-alpha (TNF-α), serum uric acid (SUA), symmetrical dimethyl arginine (SDMA), arginine, citrulline and asymmetrical dimethyl arginine (ADMA) at baseline (before study drug), at 6 months and 12 months in a prespecified substudy.
Results: One hundred and six patients were enrolled and 75 had a baseline and at least one follow-up sample. There were no significant differences in neurohormone cytokines or nitric oxide markers measured between the two groups at six or twelve months. NT-ProBNP showed a numerical increase in the nebivolol group compared to placebo ($P = 0.08$) and sFas showed a numerical increase in patients on placebo ($P = 0.08$). Mean baseline LVEF was 35% in both groups and at 12 months was 43% on nebivolol group and 34% on placebo group ($P = 0.01$).
Conclusion: There were trends but no clear changes associated with nebivolol in neurohormones, cytokines or markers of nitric oxide activity in this study of elderly patients with heart failure. Further studies are needed to understand the mechanistic effects of beta blockers on biomarkers in heart failure.

1. Introduction

Heart failure (HF) is a common condition in the elderly which is associated with a high risk of mortality and morbidity. Several large randomised trials have shown that beta blockers reduce the risk of death and heart failure hospital admissions in patients with HF [1]. This has also been shown in elderly patients in the SENIORS trial [2]. However the underlying mechanisms of benefit of beta blockers in HF are poorly understood especially in the elderly. Beta blockers are thought to exert their benefits by reducing sympathetic activity, by reducing the workload of the heart and by exerting beneficial effects on the ventricular remodelling process [3,4]. However there is very little randomised trial evidence to demonstrate these effects. Cardiomyocyte

Abbreviations: NT-Pro BNP, N-terminal brain natriuretic peptide; Pro-ANP, Pro atrial natriuretic peptide; ET-1, endothelin-1; sFas, soluble Fas; sFas-L, soluble Fas-ligand; TNF, tumour necrosis factor; TNF-α, tumour necrosis factor-alpha; SUA, serum uric acid; SDMA, symmetrical dimethyl arginine; ADMA, asymmetrical dimethyl arginine; PNE, peripheral norepinephrine; HF, heart failure; NO, nitric oxide; SENIORS, Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalisation in seniors with heart failure; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association; STARS-BNP, systolic heart failure treatment supported by BNP.

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necrosis and/or apoptosis may be induced via activation of TNF and the Fas/FasL system and inflammatory cytokines play an important role in the pathogenesis and progression of heart failure [5]. It is not known if beta-blockers would reduce levels of natriuretic peptides, and/or markers of apoptosis and of ventricular remodelling, and sympathetic activation in HF, especially in the elderly. Nebivolol has a vasodilating action mediated by a nitric oxide enhancing mechanism with possible beneficial effects on endothelial function. The precise mechanism by which nebivolol enhances NO bioavailability is not known. Nebivolol influences phospholipase C activity, which raises intracellular free calcium concentrations which in turn [6] could stimulate nitric oxide synthase (NOS III). Asymmetrical dimethyl arginine (ADMA) is an endogenous competitive inhibitor of NO synthase [7] and elevation of ADMA is accompanied with impaired endothelium-dependent NO mediated vasodilation in the brachial artery [8]. L-Arginine forms substrate for formation of NO with citrulline formed as byproduct in a reaction catalysed by NO synthase enzyme. SDMA impairs uptake of L-arginine and inhibits NO synthase effecting NO formation. The possible effects of nebivolol on these mechanisms have not been tested in randomised controlled outcome clinical trials [9]. We undertook a pre-specified sub-study of SENIORS [2], to explore the effects of nebivolol on several key biomarkers (Table 1) that reflect neurohormonal activation and apoptosis, and also to assess effects the nitric oxide pathway in elderly HF patients with both reduced and preserved LV ejection fraction. The main hypothesis was that nebivolol therapy could be associated with a reduction in NT-ProBNP compared to placebo.

2. Methods

The main SENIORS study was a parallel group, randomised, double-blind, multicentre, multinational trial comparing nebivolol with placebo in 2128 elderly patients with chronic heart failure on optimum standard therapy [10]. Patients aged >70 years with a history of stable chronic heart failure were eligible. This neurohormonal sub-study was conducted at 21 participating sites from 4 European countries [Romania, Czech Republic, Germany and UK; Fig. 1] and enrolled 106 patients from the main SENIORS population. Patients were randomised into the main SENIORS study [10] either to nebivolol or placebo and centres that participated [Fig. 1] in the neurohormonal substudy followed the substudy protocol as well. The drug was started at 1.25 mg and the dose was doubled every 2 weeks up to 10 mg daily or the maximum tolerated dose. The study was performed in compliance with good clinical practice and followed the recommendations of the Declaration of Helsinki. The relevant national and local ethics review boards and regulatory authorities approved the protocol. Written informed consent was obtained from all patients before enrolment.

Blood samples were obtained at baseline (prior to first dose of active study drug), and at 6 months and 12 months. Samples were centrifuged and stored below −20 °C for a maximum of 12 weeks and were then sent in dry ice to a central laboratory at St George's Hospital, London for storage and analysis. Samples were also sent to the Institute of Clinical Pharmacology, University Hospital Magdeburg, Germany for analysis of ADMA, SDMA, arginine and citrulline. On receipt at the central laboratory, the samples were logged and stored at −70 °C with strict temperature quality control. Samples from different time points [baseline, 6 and 12 months] were all analysed at the same time. Parameters were analysed using the following quality approved methods.

2.1. Laboratory methods

2.1.1. NT-ProBNP

NT-ProBNP: Used Elecsys 2010 electrochemiluminescent immunoassay (Roche Diagnostics). The NT-ProBNP assay is an 18-minute sandwich immunoassay, in which 20 μL of serum, a biotinylated polyclonal NT-ProBNP specific antibody and polyclonal NT-ProBNP specific antibody labelled with a ruthenium complex reacted to form a sandwich complex. The intra-assay coefficient of variation (CV%) as reported by the manufacturer was 1.8 to 2.7% at 4.67 to 86.9 ng/L. The assay analytical sensitivity was 5 ng/L with a functional sensitivity of 50 ng/L. The measuring range was 5 to 35,000 ng/L. The upper reference intervals of 125 pg/mL and 425 pg/mL are for the young and elderly, respectively.

2.1.2. Pro-ANP

Pro-ANP was determined by an enzyme-linked immunosorbent assay (ELISA, Biomedica Medizinprodukte GmbH & Co). Total CV% was 6.0–7.0% at 427–436 fmol/mL. The detection limit was 50 fmol/mL with an analytical range of 0–5000 fmol/mL. The upper reference interval based on 336 apparently healthy persons was <1945 fmol/mL.

2.1.3. TNF-alpha

TNF-alpha was determined by chemiluminescent immunoassay automated on the IMMULITE immunoassay analyser. The TNF-α assay is a solid phase two site chemiluminescent assay with two 30 minute incubation cycles. The intra- and inter-assay CV was 2.6%–3.6% at 34–800 pg/mL and 4.0%–6.5% at 17–788 pg/mL, respectively. The analytical sensitivity is 1.7 pg/mL with a calibration range up to 1000 pg/mL. There is no reported cross reactivity with IL-1β, IL-2, IL-2 receptor, IL-4, IL-6, IL-8, IL-10 or IL-13. The absolute range of values reported by the manufacturer from 58 apparently healthy individuals was from undetectable to 8.1 pg/mL.

2.1.4. sTNFR-1

sTNFR-1 was determined by ELISA (Quantikine Immunoassay, R&D Systems, Europe, Oxfordshire, UK). Total CV% was 3.6–5.0% at 69–355 pg/mL. The detection limit was 0.7 pg/mL with an analytical range of 0.7–500 pg/mL. Reference interval based on 40 apparently healthy persons was 749–1966 pg/mL.

2.1.5. Soluble Fas ligand

Soluble Fas ligand was determined by ELISA (Quantikine Immunoassay, R&D Systems, Europe, Oxfordshire, UK). Total CV% was 2.9–4.6% at 130–1424 pg/mL. The detection limit was 20 pg/mL with an analytical range of 20–20,000 pg/mL. Reference interval based on 60 apparently healthy persons was 4792–17,150 pg/mL.

2.1.6. Human Fas ligand

Human Fas ligand was determined by ELISA (Quantikine Immunoassay, R&D Systems, Europe, Oxfordshire, UK). Total CV% was 4.1–5.4% at 103–624 pg/mL. Detection limit was 2.66 pg/mL with an analytical range of 2.66–10,000 pg/mL. Reference interval based on 56 apparently healthy persons was 39.8–145 pg/mL.

2.1.7. Endothelin-1

Endothelin-1 was determined by ELISA (Quantikine Immunoassay, R&D Systems, Europe, Oxfordshire, UK). Total CV% was 4.2–6.50% at 14.4–70.0 pg/mL. Detection limit was 1.0 pg/mL with an analytical range of 1.0–117 pg/mL. Reference interval based on 25 apparently healthy persons was 0.3–0.9 pg/mL.

2.1.8. Serum uric acid

Serum uric acid was determined on a Synchron LX® automated clinical chemistry system (Beckman Coulter UK Ltd., High Wycombe, UK). Detection limit was 0.03 mmol/L and had a linear range up to 1.19 mmol/L. Reference intervals are 0.25–0.45 mmol/L and 0.15–0.35 mmol/L for males and females, respectively.

2.1.9. ADMA & SDMA & citrulline & arginine assays

ADMA & SDMA & citrulline & arginine assays: By liquid chromatography/mass spectrometry with the isotope dilution technique. Normal values: <0.5 pg/mL.

2.1.10. PNE assay

PNE assay: High-performance liquid chromatography method. Kit used: Causon RC et al. 1981. Normal levels: below 0.2 nmol/L as <0.2.

Table 1
Summary of biomarkers analysed and their roles.

Biomarkers	Roles in heart failure
Natriuretic peptides	Natriuresis and vasodilation. Secreted by myocardial stretching
Tumour necrosis factor and TNF receptor	Cytokine with role in cell death by cardiac apoptosis
sFas and Fas-L	Ventricular remodelling and apoptosis
PNE	Sympathetic neurotransmitter. Vasopressor and positive inotropic agent
ET-1	Endothelial function and vasodilator
ADMA and SDMA	Endothelial function and antagonist of nitric oxide. Reduces production of NO.
SUA	Endothelial function marker.
Citrulline	Endothelial function marker. Formed as byproduct during synthesis of NO from L-arginine
Arginine	Precursor of nitric oxide [NO]. Endothelial function.

NT = N-terminal. BNP = brain natriuretic peptide, ANP = atrial natriuretic peptide, TNF = tumour necrosis factor, TNFR = tumour necrosis factor receptor, SUA = serum uric acid, sFas = CD95, Fas-L = CD95L, ADMA = asymmetric dimethyl arginine, SDMA = symmetric dimethyl arginine, PNE = peripheral norepinephrine, and ET = endothelin.