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Short Communication

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THE EFFECT OF NEBIVOLOL ON ATHEROGENESIS IN apoE - KNOCKOUT MICE

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Nebivolol is a novel beta1-blocker with a nitric oxide (NO) - potentiating, vasodilatory effect that is unique among beta-blockers. It was already shown that nebivolol ameliorates atherosclerosis in cholesterol-fed rabbits. We, therefore, wanted to investigate whether this is the case in the fine experimental model of atherosclerosis: apolipoprotein E (apoE)-knockout mice. Nebivolol attenuated atherogenesis, measured both by "en face" method (9.23±1.8% *vs*. 14.6±2.1%) and "cross-section" method (63125±8455 μm^2 *vs*. 91416±8357 m^2). This is the first report showing the effect of nebivolol on atherogenesis in gene-targeted mice.

Key words: *atherosclerosis, apoE - knockout mice, nebivolol*

INTRODUCTION

The endothelium plays crucial role in vessel wall homeostasis and its inflammatory and proliferative phenotype influences the progression of atherosclerosis (1). Nebivolol, a novel, third generation beta1-selective antagonist has been shown to increase bioavailability of endothelium-derived nitric oxide (NO) and to attenuate inflammatory activation of endothelial cells (2). It has been also shown that nebivolol ameliorates atherosclerosis in old, crude model of atherosclerosis - cholesterol-fed rabbits (3, 4). Importantly, since 1992 the mouse has become an excellent model for experimental atherosclerosis research (5). In 1992 the first line of gene targeted animal model of atherosclerosis (and also Azheimer's disease), namely apolipoprotein E (apoE)- knockout mice was developed (6-8). The creation of apoE- knockout mice, which develop atherosclerotic plaques on chow diet, has changed the face of atherosclerotic research (9). Thus nowadays, this model is considered to be a reference animal setting for testing the anti-atherogenic potential of drugs. This is why we investigated the influence of nebivolol on development of atherosclerosis in apolipoprotein E (apoE)- knockout mice.

MATERIALS AND METHODS

Animals and treatment

Female apoE-knockout mice on the C57BL/6J background were obtained from Taconic (Ejby, Denmark). Mice were maintained on 12-h dark /12-h light cycles in air-conditioned rooms (22.5±0.5°C, 50±5% humidity) and access to diet and water *ad libitum* in Animal House of Chair of Immunology of JUMC. At the age of 8 weeks mice were put on *chow diet* made by Ssniff (Soest, Germany) for 4 months. Experimental group received the same diet, mixed with racemic mixture of D- and L-nebivolol (Janssen Pharmaceutica, Geel, Belgium) at a dose 2.0 μmol per kg of body weight per day. All animal procedures were approved by the Jagiellonian University Ethical Committee on Animal Experiments.

Procedures

At the age of 6 months mice were sacrificed under anesthesia and 1000 UI of fraxiparine (Sanofi-Synthelabo, France) was injected into the peritoneum. The blood was collected from the right ventricle. Plasma was separated by centrifugation at 1000xg at 4°C for 10 min and stored in -80°C. Then, right atrium was incised and the heart was perfused by PBS through the apex of the left ventricle at a constant pressure of 100 mm Hg. Next, the heart and the whole aorta were dissected.

Plasma lipids

Total cholesterol and triglycerides were assayed using commercially available kits (Roche Molecular Biochemical, USA).

Quantitation of atherosclerosis

The heart and ascending aorta were embedded in OCT compound (CellPath, UK) and snap-frozen. Ten micrometer-thick cryosections were cut from the aortic root using a standardized protocol (10-12).

Serial sections were cut from the proximal 1 mm of the aortic root. Eight adjacent sections were collected at 100-μm intervals starting at a 100-μm distance from the appearance of the aortic valves. Sections were thaw-mounted on poly-L-lysine

coated slides and air dried. After fixation in 4% paraformaldehyde (pH 7.0), sections were stained with Meyer's hematoxylin and oil red-O (Sigma-Aldrich, USA). Oil red O-stained sections were examined under Olympus BX50 (Olympus, Tokyo, Japan) microscope and used for quantitative evaluation. Images of the aorta were recorded using Olympus Camedia 5050 digital camera and stored as TIFF files of resolution 1024×768 pixels. Total area of the lesion was measured semiautomatically in each slide using LSM Image Browser 3 software (Zeiss, Jena, Germany). For each animal a mean lesion area was calculated from eight sections, reflecting the cross-section area covered by atherosclerosis.

The aorta from arch to bifurcation was fixed in 4% formaldehyde, opened longitudinally, pinned onto black wax plates and stained with Sudan IV (Sigma-Aldrich, St. Louis, MO, USA). Aortic lesion area and total aortic area were calculated using LSM Image Browser software.

Statistical analysis

Results are expressed as mean±SEM. The nonparametric Mann-Whitney U test was used for analysis of the data. $P<0.05$ was considered as statistically significant.

RESULTS

Nebivolol did not change the level of cholesterol and triglycerides in blood, as compared to the control group (*Table 1*).

Measured by the "en face" method, percentage of area occupied by atherosclerotic lesions in aortas in the control group was 14.6±2.1%, whereas in nebivolol-treated group was 9.23±1.8% ($p<0.05$). Lesion area measured by "cross-section" of aortic roots was 91416±8357 μm² in the control group *vs.* 63125±8455 μm² in nebivolol-treated group ($p<0.05$) (*Fig. 1*).

DISCUSSION

Here, using atherosclerosis model of apoE-knockout mice we confirmed anti-atherogenic action of nebivolol. Our study was not aimed to investigate mechanims, by which it inhibits atherogenesis, but according to previous reports, such action of nebivolol can be partially explained by its beneficial effect on endothelium (2, 3). Although the details of endothelial action remain unclear, it seems that nebivolol augments vascular nitric oxide release via endothelial β_2- or β_3- adrenergic receptors (13, 14). Furthermore, it was shown that nebivolol prevents vascular nitric oxide synthase (NOS) III uncoupling in experimental hyperlipidemia and inhibits NADPH oxidase activity in endothelial and inflammatory cells (14, 15). Recently, nebivolol appeared to be a potent antioxidant and has been shown to reduce expression of inflammatory adhesion molecules (ICAM-1, E-selectin) and cytokines (TNF-α, IL-6) as well as prothrombotic factors (PAI-1) on endothelial and smooth muscle cells (17). Our preliminary data show that inhibition of atherogenesis by nebivolol in apoE knockout mice is associated with its tendency to decrease of plasma sICAM-1 and VCAM-1 levels (unpublished data). Furthermore, Baumhakel *et al*. reported that nebivolol, but not metoprolol, improved endothelial function of the corpus cavernosum in apoE-knockout mice (18).

Interestingly, the third generation of β-adrenoreceptor antagonists with ancillary vasodilator properties (nebivolol and carvedilol) possesses superior clinical efficacy as compared to the classical β-blockers (19, 20). This seems to be related not to their β-blocking properties, but to their ability to reverse endothelial dysfunction. Indeed, nebivolol, but not atenolol reversed endothelial dysfunction in patients with heart failure and hypertension (19, 20). Our data, in agreement with previous reports show strong anti-atherogenic action of nebivolol. We are tempted to speculate that use of nebivolol may offer special benefits in the treatment/prevention of coronary heart disease, however, this has to be confirmed in large, clinical studies.

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Conflict of interests: none declared.

Table 1. Cholesterol (TCH) and triglycerides (TG) levels in control and nebivolol-treated groups, presented as mean±SEM. NS: non-significant difference between groups.

group	TCH (mmol/l)	TG (mmol/l)
control (n=10)	15.7±1.1	1.93±0.1
nebivolol-treated (n=10)	16.2±0.8 (NS)	1.86±0.1 (NS)

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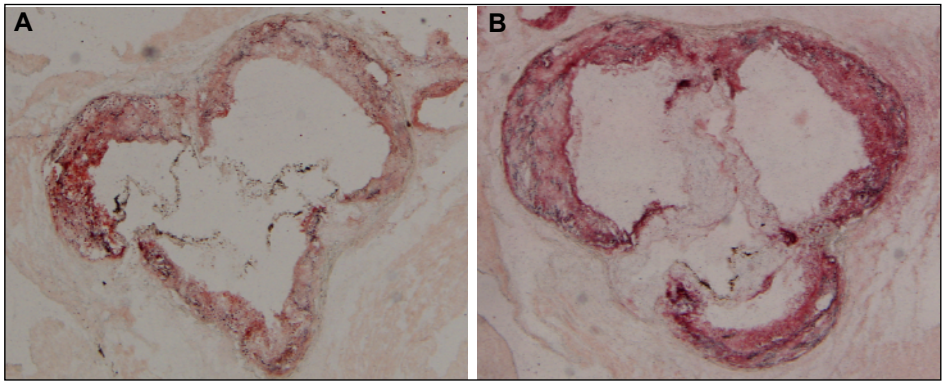

Fig. 1. Representative micrographs showing oil-red O - stained lesions in nebivolol-treated (A) and control (B) apoE-knockout mice (magnification 40).

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