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AVE 0991 - ANGIOTENSIN-(1-7) RECEPTOR AGONIST, INHIBITS ATHEROGENESIS IN APOE - KNOCKOUT MICE

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Recent evidence shows that the renin-angiotensin system is a crucial player in atherosclerotic processes. It was also proved that Ang II promotes atherogenesis. Angiotensin-(1-7) [Ang-(1-7)] opposites Ang II action. Therefore, we would like to find out whether Ang-(1-7) receptor agonist: AVE 0991, could ameliorate atherosclerosis progression in an experimental model of atherosclerosis: apolipoprotein E (apoE) - knockout mice. AVE 0991 inhibited atherogenesis, measured both by "en face" method (7.63±1.6% *vs*. 14.6±2.1%) and "cross-section" method (47 235±7 546 μm^2 *vs*. 91 416±8 357 μm^2). This is the first report showing the effect of AVE 0991 on atherogenesis in gene-targeted mice.

Key words: *atherosclerosis, apoE - knockout mice, angiotensin-(1-7), AVE 0991, angiotensin II, Mas receptor*

INTRODUCTION

Angiotensin II (Ang II) is involved in physiology and pathology of cardiovascular system (1). Recent evidence shows that the renin-angiotensin system is a crucial player in atherosclerotic processes (2). It was also proved that Ang II promotes atherogenesis (3). On the other hand, angiotensin-(1-7) [Ang-(1-7)] opposites Ang II action (4, 5). Therefore, we would like to find out whether angiotensin-(1-7) receptor agonist: AVE 0991, could ameliorate atherosclerosis progression in an experimental model of atherosclerosis: apolipoprotein E (apoE) – knockout mice (6).

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*. 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 AVE 0991 (Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany) at a dose 0,58 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 1000×g 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 (7, 8).

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), 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

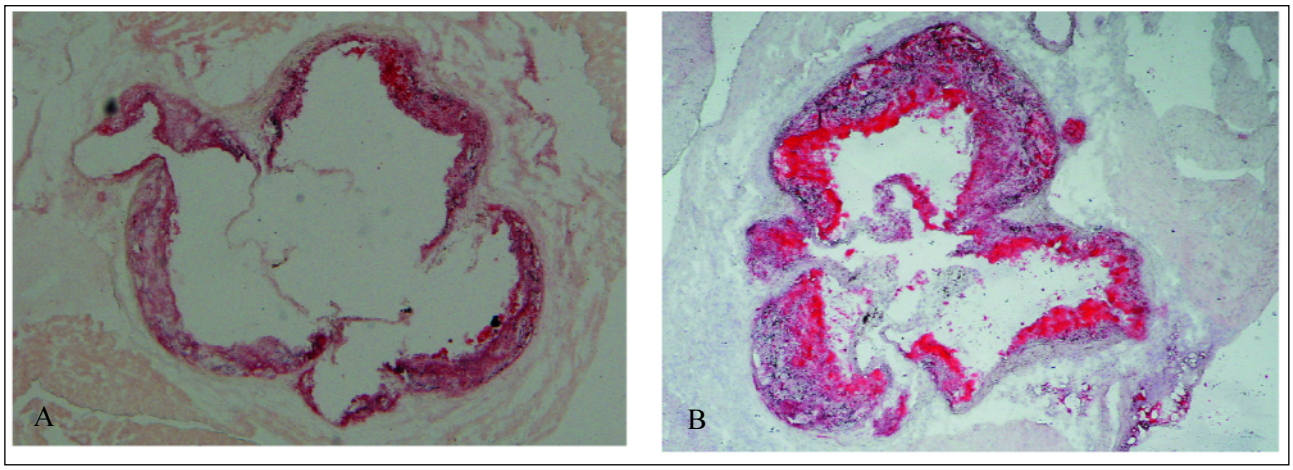

Fig. 1. Representative micrographs showing oil-red O – stained lesions in AVE 0991-treated (A) and control (B) apoE - knockout mice (magnification × 40).

Table 1. Cholesterol (TCH) and triglycerides (TG) levels in control and AVE 0991-treated groups, presented as mean±SEM. NS: non-significant difference between groups.

group	TCH (mmol/l)	TG (mmol/l)
control (n=10)	25.7±1.1	1.93±0.1
AVE 0991-treated (n=10)	27.1±0.9 (NS)	1.97±0.2 (NS)

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

AVE 0991 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 AVE 0991-treated group was 7.63±1.6% ($p<0.05$). Lesion area measured by "cross-section" of aortic roots was 91 416±8 357 μm^2 in the control group *vs.* 47 235±7 546 μm^2 in AVE 0991-treated group ($p<0.05$) (*Fig. 1*).

DISCUSSION

The renin-angiotensin system (RAS) is highly complicated hormonal system controlling cardiovascular system, kidney and adrenal glands, thus crucial for hydro-electrolyte balance and blood pressure regulation (1). Ang II is the best described peptide of RAS. Ang II increases activity of sympathetic nervous system, acts as a vasoconstrictor, increases aldosterone release and sodium retention (9). Additionally, Ang II stimulates free radical production, plasminogen activator inhibitor-1 (PAI-1) release, tissue factor (TF) and adhesion molecules (VCAM-1) expression. Moreover, in blood vessels it stimulates smooth muscle cells proliferation and leukocyte adhesion. What is important, Ang II inhibits nitric oxide synthase (NOS), thus diminishing all beneficial effects of nitric oxide (NO).

Ang-(1-7) is an active peptide of RAS. It counteracts vasoconstriction by releasing nitric oxide and prostacyclin (10). Moreover, it opposites Ang II mitogenic, arrythmogenic and procoagulant activities (11). Enhancing natriuresis and diuresis it inhibits water and sodium retention caused by Ang II. Recently it has been shown that vasodilatative and diuretic activities of Ang-(1-7) are mediated *via* Mas, G-coupled protein receptor (12). Furthermore, some activities of Ang-(1-7) are blocked by AT1 and AT2 receptors antagonists (13). On the other hand, Ang-(1-7) independently to Mas-receptor increases bradykinin activity and antagonizes hypertrophic action of Ang II. In 2002 non-peptide antagonist of Ang-(1-7) receptor: AVE 0991, has been described (14-16).

Ang II has several potential mechanisms that may increase the atherogenic process (3, 17). First, Ang II may indirectly influence the atherogenic process *via* hemodynamic effects resulting from increased arterial blood pressure. Marked increases in arterial blood pressure have been demonstrated to increase the severity of experimental atherosclerosis. Second, Ang II has been demonstrated to exert several direct effects relevant to the development of atherosclerosis including stimulation of monocyte recruitment, activation of macrophages, and enhanced oxidative stress, all of which have been linked to an increase in the atherogenesis process. These effects of Ang II would occur independent of elevations in arterial blood pressure.

In our experiment we have shown that AVE 0991 inhibits atherogenesis in mouse model of atherosclerosis. This phenomenon is in agreement with general "anti-Ang II" action of Ang-(1-7). Since Ang II is a potent proatherogenic agent, its functional antagonist should ameliorate atherogenesis. However, we have not completed yet data, concering the exact molecular action of AVE 0991 on apoE-knockout mice. This issue needs further investigation.

To our knowledge, this is the first report that shows the effect of angiotensin-(1-7) receptor agonist: AVE 0991, on atherogenesis in apoE-knockout mice.

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Conflict of interests: None declared.

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