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Combined effects of HMG-CoA-reductase inhibition and renin–angiotensin system blockade on experimental atherosclerosis

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Abstract

Therapeutic strategies to prevent atherosclerotic plaque progression and achieve plaque stabilization involve 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA)-reductase inhibitors (statins) and renin–angiotensin system (RAS)-blockade, but studies investigating the potentially additive effects of a combined treatment strategy are rare. We hypothesised that the adjunction of atorvastatin with telmisartan or ramipril might achieve additional effects on experimental atherosclerosis though statin-induced lipid-lowering is lacking.

ApoE$^{-/-}$ mice were fed a high-fat diet for 12 weeks and randomized to either placebo (CON), atorvastatin (ATO), ramipril (RAM), telmisartan (TEL) or RAM + ATO and TEL + ATO ($N = 23$ per group). RAS-blockade, but not ATO, reduced systolic blood pressure. None of the treatment regimens lowered systemic cholesterol levels or lipoprotein fractions. RAM, TEL and the combined therapy, but not ATO, significantly reduced aortic lipid deposition. All substances significantly reduced monocyte chemoattracting protein (MCP)-1 concentrations, macrophages and matrixmetalloproteinase (MMP)-9 content and enhanced plaque's content of tissue inhibitor of MMP (TIMP)-1, collagen and fibrous cap thickness, resulting in an overall decrease of advanced plaques (classified as types IV–VI). Additive effects of the adjunction were observed on MMP-9 gelatinolytic activity, interleukin (IL)-6 and IL-10 plasma levels.

These results indicate that a combined treatment with RAS-blockade and statins may have additive effects on systemic cardiovascular risk markers even in the absence of lipid-reduction, although additional effects on atherosclerotic plaque progression and stability were not observed in this model.

Keywords: Atherosclerosis; Plaque stability; HMG-CoA-reductase inhibition; Renin–angiotensin system blockade; ApoE$^{-/-}$ mouse

1. Introduction

Atherosclerosis is a chronic inflammatory process of the cardiovascular system, which may result in a variety of diseases, i.e. coronary artery disease (CAD) or cerebrovascular disease (CVD). Clinical endpoints of chronic CAD include unstable angina, acute myocardial infarction and sudden cardiac death, which may result from fissure, erosion or rupture of a vulnerable atherosclerotic plaque. Morphological characteristics of a vulnerable plaque include an increased content of lipids, inflammatory cells and an enhanced activity of extracellular matrix degrading components, i.e. matrixmetalloproteinases (MMPs), resulting in a reduced collagen content and a low smooth muscle cell proportion [1]. Although the extent of clinical symptoms of CAD depends on the degree of lumen stenosis, it is likely that many lesions grow through rupture [2]. Therefore, plaque stabilization may also reduce the incidence of acute coronary syndromes (ACS).

Hypertension induced by chronic activation of the renin–angiotensin system (RAS) and elevated cholesterol levels have been identified as independent risk factors for the development of CAD [3]. In patients suffering from hyperten-

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sion or dyslipidemia, therapeutic strategies to achieve plaque stabilization involve inhibition of the RAS with angiotensin II receptor type 1 (AT_1)-antagonists or angiotensin-converting enzyme (ACE)-inhibitors and lipid-lowering agents such as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)-reductase inhibitors (statins). Both RAS-inhibitors and statins have been shown to reduce the mortality and morbidity of patients suffering from CAD in large-scale clinical trial, i.e. ASCOT, HOPE, HPS and EUROPA [4–7].

Moreover, experimental studies in various atherosclerosis models showed the direct impact of statins and RAS-inhibitors on atherosclerotic plaque formation and processes involved in plaque stability [8,9]. As hypertension and dyslipidemia are often present in the same patient [10] their treatment frequently involves both statins and RAS-inhibitors. However, studies evaluating the potentially additional anti-atherosclerotic impact of combining statin with RAS-blockade remain controversial and rare [11–13]. The aim of this study was to investigate the impact of a RAS-blockade with either AT_1-antagonist or ACE-inhibitor in combination with a statin on morphological markers of plaque stability and systemic inflammation in the apoE$^{-/-}$ mouse, an established model of atherosclerosis [14]. As statins seemingly develop anti-atherosclerotic effects despite a lack of cholesterol reduction in this mouse model [18], we hypothesised that additive effects of a combined therapy may occur even in the absence of systemic lipid-lowering.

2. Methods

2.1. Experimental animals

One hundred and thirty-eight male apoE$^{-/-}$ mice on a C57BL/6 background (Charles River, 8 weeks of age) were housed on a regular chow diet. At week 10, animals were randomly assigned to six groups ($n = 23$ per group) and fed a high-fat western diet (20% total fat, 1.5% cholesterol, 50% carbohydrates, Altromin GmbH, Germany) for 12 weeks while receiving placebo (CON), atorvastatin (ATO, 1 mg/kg bw) [17], telmisartan (TEL, 1 mg/kg bw) [18], ramipril (RAM, 5 mg/kg bw) [19] or the combination of RAM + ATO and TEL + ATO.

TEL and RAM were administered to chow (Altromin GmbH, Lage, Germany). ATO was administered to drinking water. A small pilot study was performed to evaluate the average chow and water uptake of apoE$^{-/-}$ mice. The study was approved by the Institutional Animal Care and Use committee.

2.2. Blood pressure measurements

Systolic blood pressure (SBP) was measured by a non-invasive computerised tail cuff system (Blood Pressure Analysis System BP-200, Visitech Systems, Apex, NC, USA). All animals were acclimated to tail cuff measurements prior to randomisation. SBP values were derived from an average of three to five measurements per animal per day starting 12–14 days prior to sacrification.

2.3. Tissue preparation and blood samples

After a 12 h over night fast, animals were heavily sedated (ketamin 0.001 ml/g and xylazine 0.0025 ml/g, i.p.). Blood samples (0.5–1 ml) were obtained by canulating the left ventricle. Animals were sacrificed by exsanguination. Samples were centrifuged at 3000 × g for 15 min at 4 °C and stored at −80 °C. Tissues were harvested, rinsed in ice-cold phosphate-buffered saline (PBS, pH 7.4), shock frozen and stored at −80 °C.

2.4. In situ fixation and morphometrical analysis

In situ perfusion for morphological analysis was performed as reported recently [20]. In brief, the chest was opened and the left ventricle was canulated. Perfusion under standardized pressure (100 mmHg) was performed with ice-cold PBS, pH 7.4, followed by a 5 min fixation with 10% phosphate-buffered formalin (pH 7.4, containing 0.1 mg/ml sodium nitroprusside, Sigma–Aldrich, USA). Vessels were dissected, immersion fixed in 10% phosphate-buffered formalin, pH 7.4, over night, cut in four segments (aortic arch, thoracic aorta, abdominal aorta, and abdominal aorta bifurcation) and paraffin embedded. Serial sections (6 μm) over a total length of 600 μm per segment per animal ($n = 5$ animals/group) were used and stained with hematoxylin eosin or elastica van Gieson. Atherosclerotic lesions were classified according to the guidelines given by the American Heart Association (AHA) [21]. Every morphological parameter was quantified using computer-assisted morphometry (Leica Qwin 500, Leica, Heidelberg, Germany) and performed by one investigator blinded for the treatment regimens. Intraobserver variation was <5%. Each measurement was performed in triplicate.

2.5. Plasma lipid and lipoprotein analysis

Plasma samples were obtained as indicated above. Total cholesterol and triglyceride levels as well as cholesterol within lipoprotein fractions following ultracentrifugation were determined using a commercially available assay modified for microtiter plates (Wako Pure Chemical Industries, Japan) as described previously [22].

2.6. Gelatin zymography

Matrix metalloproteinase (MMP)-9 activity was assayed by gelatin zymography as reported previously [23]. Aortas ($n = 5$ per group) were homogenized in PBS supplemented with 0.5 % (v/v) Triton X-100. Protein homogenates were separated by 10% SDS-PAGE containing 1% gelatin. The gel was renaturated by 2.5% Triton X-100 solution, followed