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Protective effects of selective mineralocorticoid receptor antagonist against aortic aneurysm progression in a novel murine model

Hirotsugu Kurobe, MD, PhD,[a] *Yoichiro Hirata, MD, PhD,*[b,c] *Yuki Matsuoka, BS,*[a] *Noriko Sugasawa, BS,*[a] *Mayuko Higashida, MS,*[b] *Taisuke Nakayama, MD,*[a] *Mark Webster Maxfield, MD,*[a] *Yasushi Yoshida, PhD,*[b] *Michio Shimabukuro, MD, PhD,*[b,d] *Tetsuya Kitagawa, MD, PhD,*[a] *and Masataka Sata, MD, PhD*[b,*]

[a] *Department of Cardiovascular Surgery, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan*
[b] *Department of Cardiovascular Medicine, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan*
[c] *Department of Pediatrics, University of Tokyo Graduate School of Medicine, Tokyo, Japan*
[d] *Department of Cardio-Diabetes Medicine, Institute of Health Biosciences, University of Tokushima Graduate School, Tokushima, Japan*

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ABSTRACT

Background: The optimal medical management to delay the progression of aortic aneurysms has not been fully clarified, and the only standard treatment at present is antihypertensive therapy. Previous studies have shown beneficial effects of selective mineralocorticoid receptor (MR) antagonists on cardiovascular remodeling. The aim of the present study was to investigate the effect of a selective MR antagonist on aortic aneurysm progression.

Methods: Seven-week-old C57BL/6J male mice were administered with angiotensin II and β-aminopropionitrile for 4 weeks. The mice received either vehicle or eplerenone, a selective MR antagonist (100 mg/kg daily) every day by gavage, starting at 7 weeks of age. The production of inflammatory cytokines in cultures of high mobility group box-1–stimulated macrophages with or without a MR antagonist was also analyzed using an enzyme-linked immunosorbent assay.

Results: Although no differences were found in the peak systolic blood pressure between the experimental groups, the mice in the eplerenone group showed a significant reduction in aneurysm development. On histologic analysis, coarse and stretched elastic fibers were markedly improved in the aortic wall in the eplerenone group. Real-time polymerase chain reaction of both aortic wall and perivascular adipose tissue demonstrated the expression of tumor necrosis factor-α, interleukin-6, and matrix metalloproteinase-2 was significantly decreased in eplerenone group, and that of monocyte chemoattractant protein-1 in the aortic wall was also significantly decreased. Macrophage infiltration in the aortic wall and perivascular adipose tissue in the eplerenone group was also significantly decreased. The production of tumor necrosis factor-α and interleukin-6 in macrophage culture, which was stimulated by high mobility group box-1 and CpG oligodeoxynucleotides, was also significantly decreased in the eplerenone group.

Drs. Kurobe and Hirata contributed equally to this work.

* *Corresponding author*. Department of Cardiovascular Medicine, Institute of Health Biosciences, The University of Tokushima Graduate School, 3-18-15 Kuramoto-cho, Tokushima 770-8503, Japan. Tel.: +81 88 633 7850; fax: +81 88 633 7894.
E-mail address: sata@clin.med.tokushima-u.ac.jp (M. Sata).

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Conclusions: Eprelenone suppressed aortic aneurysm progression through an anti-inflammatory effect. Thus, selective MR antagonists might be effective in preventing the progression of aortic aneurysms.

1. Introduction

Aortic aneurysm is 1 of the most lethal atherosclerotic diseases, because rupture of an aortic aneurysm leads to a catastrophic cardiovascular collapse, with high morbidity and mortality [1]. Medications for patients who do not fulfill the criteria for surgical intervention for aortic aneurysmal disease are important to delay pathologic progression. However, effective medical management to control the progression of aortic aneurysms has not yet been clarified. This has likely been because of the incomplete understanding of the mechanisms that underlie the development and progression of aortic aneurysmal disease [2].

Infusion of angiotensin II (Ang II) augments the development of atherosclerotic lesions and therefore has been used in animal models. However, in animal models with normal mice in which Ang II has been infused, aortic aneurysm formation has been rare [3]. A new experimental model can produce aortic aneurysms in normal mice using a combination of Ang II infusion with pharmacologically induced degeneration of elastic laminas by β-aminopropionitrile, a lysyl oxidase inhibitor [3]. In this model, the incidence of aortic aneurysms is not inhibited by captopril, an angiotensin-converting enzyme inhibitor, indicating that the resulting aortic aneurysms are not formed solely by the effects of Ang II on the vascular wall.

The cause of the inflammatory process in adipose tissue has been widely recognized in obesity [4–8]. Moreover, perivascular adipose tissue has been recognized as a possible cause of atherosclerotic lesion formation in nonobese subjects [8]. Few reports have investigated the relationships between perivascular adipose tissue and the formation of aortic aneurysms. Withers *et al.* [9] reported that activation of macrophages in the perivascular adipose tissue after aldosterone- or hypoxia-induced inflammation is the key modulator of vascular inflammation. They also showed that eplerenone, a selective mineralocorticoid receptor (MR) antagonist, can ameliorate this inflammatory action [9]. This suggests that blocking the MR can lead to attenuation of inflammation in the perivascular fat and vessel wall, thereby leading to inhibition of atherosclerotic aneurysm formation.

We evaluated whether perivascular inflammation is related to vascular inflammation and aortic aneurysm formation and whether treatment with a selective MR antagonist can protect against progression of aortic aneurysmal disease.

2. Methods

2.1. Animals

Seven-week-old C57BL/6J male mice (Clea Japan, Tokyo, Japan) were used. All animal experiments were performed in compliance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85-23, revised 1996). The institutional review board of Tokushima University approved all animal procedures.

2.2. Model of aortic aneurysm

The model of aortic aneurysm was produced as follows. Kanematsu *et al.* [3] reported that the use of both β-aminopropionitrile (BAPN) and Ang II induced aortic aneurysms in wild-type mice, although the mortality at 4 weeks was very high owing to aortic rupture and dissection. Therefore, we modified this technique to create a model that induces aortic aneurysms at a high frequency and low mortality. By optimizing the doses of BAPN and Ang II strictly as described in the present study, our murine model produced aortic aneurysms with high efficacy (>90%) and high reproducibility. In analyzing the explanted aneurysmal tissue from this model, we observed pathologic changes within the aortic wall similar to those found in patients with aortic aneurysms, in particular, regarding the degenerative changes that occur with aging.

In our protocol, 7-week-old C57BL/6J male mice were administered Ang II (1000 ng/kg/min) to induce hypertension and BAPN, a lysyl oxidase inhibitor (32.5 mg/kg daily) to induce degeneration of the elastic layer through a subcutaneously implanted Alzet osmotic pump (Durect, Cupertino, CA) for 4 weeks [10,11]. The mice received either vehicle 0.5% carboxymethyl cellulose (control group, $n = 19$) or eplerenone, a selective MR antagonist (100 mg/kg daily, eplerenone group, $n = 10$), daily by gavage, starting at 7 weeks of age. The blood pressure and pulse rates were measured using a tail cuff and a pneumatic pulse transducer (AD Instruments, Dunedin, New Zealand), as previously described [10]. A minimum of 10 measurements was obtained from each mouse.

2.3. Tissue harvesting

The mice were killed at 4 weeks after the start of treatment. The mice were anesthetized by intraperitoneal injection of pentobarbital (Dainippon Sumitomo Pharma, Osaka, Japan). Next, the left ventricle was perfused with cold phosphate-buffered saline (20 mL) under physiological pressure with an exit through the incised right atrium. Arterial tissue and perivascular adipose tissue were harvested. The harvested tissues were divided into two groups: one for tissue analysis and one for real-time polymerase chain reaction analysis.

2.4. Morphologic analysis in aortic aneurysm model

The aortic ratio was calculated in each lesion (thoracic and abdominal lesions) as follows: (diameter of most dilated portion of aneurysm)/([(normal aortic diameter proximal to aneurysm) + (normal aortic diameter distal to aneurysm)]/2)