Pharmacology of Aldosterone and the Effects of Mineralocorticoid Receptor Blockade on Cardiovascular Systems

Shusuke Yagi,1 Masashi Akaike,2 Ken-ichi Aihara,3 Daiju Fukuda,4 Masayoshi Ishida,1 Takayuki Ise,2 Toshiyuki Niki,1 Yuka Sumitomo-Ueda,1 Koji Yamaguch,1 Takashi iwase,1 Yoshio Taketani,1 Hirotsgu Yamada,1 Takesh Soeki,1 Tetsuzo Wakatsuki,1 Michio Shimabukuro4 and Masataka Sata1

It is well-known that aldosterone plays an important role in reabsorption of sodium and fluid, and in potassium excretion in kidneys via epithelial mineralocorticoid receptor (MR) activation. Recent studies have shown that aldosterone causes cardiovascular remodeling not only in a blood pressure-dependent manner, but also in a blood pressure-independent manner by decreasing nitric oxide bioavailability and modulating oxidative stress, leading to vascular inflammation. In addition, MR blockade does provide beneficial effects associated with cardiovascular protection, resulting in a reduction of cardiovascular morbidity and mortality. A growing body of evidence suggests that MR blockade is a promising therapeutic target to help prevent cardiovascular events.

Key Words: Aldosterone • Mineralocorticoid receptor • Nitrix oxide • Renin-angiotensin-aldosterone system

INTRODUCTION

Aldosterone plays important roles in the reabsorption of sodium and fluid, and in potassium excretion in epithelial cells of the collecting ducts of the kidney via cytosolic mineralocorticoid receptor (MR) activation. Previously, it was believed that the main role of aldosterone was to expand extracellular volume after sodium absorption in renal distal tubules, resulting in elevated blood pressure, as a terminal effector of the renin-angiotensin-aldosterone system (RAAS) cascade. However, recent studies have revealed that aldosterone also has additional physiological and/or pathophysiological effects, such as decreasing nitric oxide (NO) bioavailability and modulating oxidative stress, leading to vascular inflammation. Aldosterone therefore causes organ damage and remodeling not only in a blood pressure-dependent manner, but also in a blood pressure-independent manner. In this review, we focus on the effects of mineralocorticoid receptor blockade on cardiovascular systems and the mechanisms by which aldosterone evokes cardiovascular inflammation through imbalance of NO bioavailability and oxidative stress.

PHYSIOLOGICAL MINERALOCORTICOIDS ARE ESSENTIAL FOR HUMANS

Mineralocorticoids including aldosterone are steroid hormones that are produced in the zona glomerulosa of the adrenal gland. The RAAS first appeared in bony fishes and has been gradually strengthened through the evolutionary process. It is apparent that aldosterone has
an essential role in maintaining body fluid and sodium, and therefore represented a major leap in RAAS evolution in early organisms that transitioned out of the water and onto dry land. If mineralocorticoids had not been secreted appropriately, they would have suffered from salt and water wasting. Thus, aldosterone is an essential hormone for maintaining body fluid homeostasis; however, in this age of relative affluence, humans usually have more salt than is necessary. Therefore, alterations in lifestyle behavior that impact excessive salt intake have made aldosterone a strong risk factor for cardiovascular disease (CVD).

**ALDOSTERONE SYNTHESIS IS STIMULATED AS A RESULT OF THE RAAS ACTIVATION**

RAAS activation is initiated by release of angiotensigen into the circulation by renin. Renin is secreted from the juxtaglomerular apparatus of the kidney to form angiotensin (Ang) I. Ang I is then converted to Ang II by angiotensin-converting enzyme (ACE), which is expressed on pulmonary endothelial cells. Subsequently, Ang II activates Ang II type 1 receptors (AT1R) on vascular smooth muscle cells to induce vasoconstriction. Ang II then stimulates the synthesis and release of aldosterone in the adrenal cortex. Building evidence has shown that aldosterone enhances Ang II expression in neonatal rat cardiomyocytes through up-regulation of ACE messenger ribonucleic acid (mRNA) expression. Aldosterone was also shown to up-regulate Ang II receptors. Therefore, even when the expression of ACE or Ang II receptor is suppressed by ACE inhibitors or Ang II receptor blockers, the RAAS could be enhanced by aldosterone per se, forming a vicious cycle of the RAAS (Figure 1). In addition, the local RAAS also plays a pivotal role in cardiovascular organ damage. Components of the RAAS have been detected in multiple organs, such as the heart and kidney, and in the vasculature. The local RASS is activated independently of the systemic RAAS, indicating that organ damage may occur although the systemic RAAS is not activated. Moreover, inflammatory cells, including monocytes and macrophages, express angiotensigen, renin, ACE, and AT1R, and activation of the RAAS in inflammatory cells promotes monocyte migration into the vascular wall; this leads to differentiation of monocytes into macrophages, resulting in further activation of the local RAAS.

**OTHER REGULATORS OF ALDOSTERONE SYNTHESIS**

In addition to Ang II, recent studies have revealed several stimuli for aldosterone synthesis and secretion: adrenocorticotropic hormone (ACTH) and potassium ion can increase aldosterone secretion acutely by activating the G-protein coupled receptor/cyclic adenosine monophosphate/P450 pathway, as well as increasing aldosterone secretion chronically by increasing gene expression of CYP11B2. Furthermore, it has been shown that aldosterone is involved in biological aging, a pivotal risk factor for CVD, because plasma aldosterone concentration was found to be inversely associated with telomere length. In addition, age-dependent inactivation of 11β hydroxysteroid dehydrogenase type 2 (11β-HSD2), leading to cortisol-mediated MR activation, was seen in elderly hypertensive patients, and age-dependent inactivation of dehydroepiandrosterone sulfate...
(DHEAS), which inhibits Ang II-mediated release of aldosterone, was seen in the elderly. We previously showed that age-dependent reduction of DHEAS was an independent negative factor for intima-media thickness in carotid arteries of males. Inactivation of 11β-HSD and DHEAS might therefore be involved in age-dependent MR activation leading to CVD. Moreover, the results of recent studies have supported an interaction between aldosterone and parathyroid hormone (PTH): PTH increases the secretion of aldosterone from the adrenal glands directly and/or indirectly by activating the RAAS, suggesting that up-regulation of aldosterone synthesis in patients with primary parahyperthyroidism contributes to the development of hypertension and CVD. It has also been shown that 3-beta-hydroxyl-steroid dehydrogenase is involved in aldosterone production as well as aldosterone synthase, the main enzyme for aldosterone production.

**ALDOSTERONE IS A RISK FACTOR FOR CVD**

It is known that aldosterone is a risk factor for CVD in both blood pressure-dependent and independent manners. Patients presenting with primary aldosteronism experienced more cardiovascular events than did essential hypertension patients independent of blood pressure. Aldosterone levels were shown to be correlated with the degree of vascular stiffness and the degree of left ventricular hypertrophy. The Framingham and SUVIMAX studies showed that elevated aldosterone level was positively associated with the incidence of new onset of hypertension. In addition, the CONSENSUS trial showed a relationship between plasma level of aldosterone and mortality in patients with heart failure (HF). Thus, aldosterone and activation of the MR play important roles in the occurrence of CVD and HF.

**MR ACTIVATION BY ALDOSTERONE AND CORTISOL**

Aldosterone binds to the MR, a nuclear receptor located in the cell cytosol. The activated receptor/hormone complex binds to hormone-responsive elements in the S’ untranslated region of aldosterone-responsive genes that activate gene transcription leading to protein synthesis, which is called genomic action of aldosterone. Rapid action of aldosterone is known as a non-genomic action of aldosterone. The MR also binds cortisol, a glucocorticoid. Cortisol has a greater affinity than aldosterone to the MR; however, the enzyme 11βHSD2, detected in epithelial cells in the renal tubule, lung, placenta and vasculature, allows aldosterone to selectively activate the MR by converting cortisol to cortisone, an inactive metabolite. In the myocardium, 11βHSD2 is not abundant; however, under the condition of RAAS activation with increased oxidative stress, the MR could be activated by cortisol, leading to enhanced redox signaling.

**VASCULAR INFLAMMATION MEDIATED BY MR ACTIVATION**

Aldosterone, as a terminal effector in the RAAS, plays a pivotal role in inflammation, leading to fibrosis of cardiovascular tissue. In fact, treatment with eplerenone or other aldosterone antagonists has been shown to reduce vascular inflammation and remodeling.

Aldosterone exacerbates vascular injury through local inflammation in the heart, aorta, and kidney in an MR-dependent manner. Rocha et al. reported that treatment of uninephrectomized rats with aldosterone and salt caused the development of extensive coronary inflammatory lesions with perivascular macrophage infiltration. They assumed that excessive aldosterone and salt increased the expression levels of cyclooxygenase-2, osteopontin, and macrophage chemoattractant protein-1 (MCP-1), and that these effects were attenuated by MR blockade. MR antagonism also decreased aortic inflammation, fibrosis, and hypertrophy in hypertensive rats and decreased oxidative stress and expression levels of inflammatory cytokines and chemokines such as TNF-α and MCP-1, in apolipoprotein E-deficient mice fed a high-cholesterol diet. Interestingly, aldosterone triggers the exocytosis of endothelial cells, the first step in leucocyte trafficking, resulting in the adherence of leucocytes to endothelial cells in a P-selectin-dependent manner. Conversely, aldosterone-induced endothelial exocytosis was inhibited by MR antagonism and knockdown, whereas actinomycin D had no effect, sug-
suggesting that the MR activates vascular inflammation through non-genomic pathways.22

The MR antagonist potassium canrenoate was shown to block markers of vascular inflammation, including osteopontin, cyclooxygenase-2 and ED-1, in uninephrectomized rats treated with deoxycorticosterone and a high salt diet.23 In addition, coronary vascular inflammation and fibrosis caused by mineralocorticoid administration was reversed by eplerenone but not by deoxycorticosterone withdrawal.23 Thus, these findings indicate that MR activation plays a crucial role in the pathogenesis of vascular inflammation and fibrosis.

**REDUCED NO BIOAVAILABILITY IN MR-MEDIATED VASCULAR INFLAMMATION**

RAAS activation increases oxidative stress and decreases NO bioavailability, leading to tissue inflammation: aldosterone breaks the balance between NO bioavailability and oxidative stress leading to inflammation1 (Figure 2). NO bioavailability is decreased in atherosclerosis due to an increase in NO inactivation by reactive oxygen species (ROS) and reduced endothelial NO synthase (eNOS) expression. MR activation also reduces NO bioavailability in endothelial cells through an increase in ROS production. Endothelial-dependent vasorelaxation was improved by eplerenone treatment through suppressed ACE activity and decreased serum MCP-1 levels in hypertensive or atherosclerotic animal models. MR antagonists, together with an ACE inhibitor, normalized NO-mediated relaxation in rats with congestive HF by beneficially modulating the balance between NO bioavailability and superoxide generation.24 In addition, in the presence of aldosterone, a small increase in plasma sodium concentration per se decreased NO release and increased the stiffness of endothelial cells, suggesting that salt intake plays a key role in the decrease in NO bioavailability.25 Nagata et al. showed that aldosterone inhibits NO synthase in human endothelial cells by inducing oxidation of its co-factor 5,6,7,8-tetrahydrobiopterin as well as by activating protein phosphatase 2A.26 Leopold et al. showed that aldosterone decreased the expression and activity of glucose-6-phosphate dehydrogenase (G6PD), which modulates vascular function by reducing oxidant stress to preserve bioavailable NO. Aldosterone antagonism or gene transfer of G6PD improved vascular reactivity by restoring G6PD activity.27 It is therefore likely that eNOS uncoupling and/or G6PD activity mediate the aldosterone-induced decrease in NO bioavailability leading to vascular inflammation.

**INCREASED OXIDATIVE STRESS IN MR-MEDIATED VASCULAR INFLAMMATION**

Systemic administration of aldosterone increases oxidative stress in the heart, vasculature, and kidneys.28 Aldosterone up-regulates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in macrophages and vascular smooth muscle cells through genomic and/or non-genomic pathways, and the up-regulation of NADPH oxidase was attenuated by eplerenone.29,30 MR activation also contributes to Ang II-mediated activation of NADPH oxidase in the heart and aorta:31 aldosterone stimulates aortic expression of p22phox and NOX2 (gp91phox) through an MR-dependent mechanism, and up-regulates p47phox mRNA through both AT1 receptor and MR-dependent mechanisms. It has been reported that superoxide production is also induced by aldosterone through MR-mediated activation of NADPH-oxidase and Rac1 in endothelial cells, contributing to the development of

![Figure 2. Aldosterone decreases NO bioavailability and increases oxidative stress. Imbalance between NO bioavailability and oxidative stress evokes vascular inflammation, leading to cardiovascular events due to cardiovascular remodeling in blood pressure dependent/ independent manners. BP, blood pressure; NO, nitric oxide.](image-url)
Increased oxidative stress stimulates activation of pro-inflammatory transcriptional factors such as activator protein-1 and nuclear transcription factor-κ, resulting in up-regulation of adhesion molecules, chemokines, and inflammatory cytokines that cause vascular inflammation due to the activation of redox signaling. In addition, administration of antioxidant drugs including radical scavengers and NADPH oxidase inhibitors decreased inflammation and injury in aldosterone-treated rodents. Oxidative stress, therefore, is one of the key components of vascular inflammation induced by MR activation as a counterpart to NO.

ADVANTAGEOUS EFFECTS OF MR BLOCKADE IN CLINICAL PRACTICE

Blockade of the MR has advantageous effects on hypertension and the cardiovascular system. Administration of spironolactone to resistant hypertensive patients was effective in decreasing blood pressure along with attenuating cardiovascular damage such as carotid intima-media thickening. In addition, eplelenone was effective as well as enalapril for reducing left ventricular mass.

Two large clinical trials, RALES and EPHESUS, revealed that MR blockade improves morbidity and mortality in patients with moderate to severe HF with left ventricular dysfunction after myocardial infarction. The EMPHASIS-HF trial showed that MR blockade also has advantageous effects in patients with mild symptoms of NYHA class II. In addition, it has been reported that MR blockade with spironolactone improves the echocardiographic indices of diastolic function in patients with diastolic HF. The TOPCAT trial is currently being carried out to clarify whether MR blockade of spironolactone reduces cardiovascular mortality and hospitalization in patients with HF of preserved systolic function (http://clinicaltrials.gov/). In addition, REMINDER and ALBATROSS are also ongoing trials to clarify whether early MR blockade improves clinical outcomes in patients with acute myocardial infarction (http://clinicaltrials.gov/). Taken together, emerging evidence is unveiling the therapeutic value of aldosterone blockade in patients with CVD or HF.

NEW TARGETS FOR MR BLOCKADE TO REVERSE VASCULAR INFLAMMATION

We showed that perivascular adipose tissue plays a crucial role in vascular inflammation and lesion formation. Recent evidence suggests that MR activation in adipose tissue is involved in the pathogenesis of metabolic syndrome and insulin resistance. Serum aldosterone levels were elevated in spontaneously hypertensive rats/NDmcrcp rats, a model of metabolic syndrome, and high MR expression level was detected in adipocytes from obese mice. Conversely, MR blockade decreased the production of ROS and inflammatory cytokine gene expression in adipose tissue of these mice and improved their insulin resistance. Thus, MR blockade in adipose tissue, particularly perivascular adipose tissue, may reduce vascular inflammation. We also showed that bone marrow-derived vascular progenitor cells contribute to vascular repair and remodeling, and that a local RAAS exists in bone marrow and may play a crucial role in vascular inflammation. In addition, it has been shown that aldosterone inhibits the formation of bone marrow-derived endothelial progenitor cells, suggesting that MR blockade and/or co-treatment with antioxidants may enhance vascular regeneration. Thus, blockade of the MR in bone marrow may also have the potential to promote vascular repair and reverse vascular inflammation.

NEW DRUGS FOR MR BLOCKADE

New, non-steroidal mineralocorticoid receptor antagonists with greater selectivity than spironolactone and stronger mineralocorticoid receptor binding affinity than eplelenone are currently being tested in clinical trials (http://clinicaltrials.gov/). Conventional MR blockers have several disadvantages: spironolactone has side effects such as gynecomastia or irregular menstruation, and eplelenone has a half life of only 4 hours. These new drugs therefore are expected to have a strong effect on cardiovascular protection, with fewer side effects.
CONCLUSIONS

Recent experimental evidence has shown the benefits of MR blockade for cardiovascular protection by the mechanism of MR blockade reducing vascular inflammation through improvement of the imbalance of NO bioavailability and oxidative stress. Large clinical trials have also indicated the benefits of MR antagonism in patients with HF. Mounting evidence suggests that MR blockade is a promising therapeutic target for preventing CVD or HF.

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