COMMENTARY

The main target for inhibiting mineralocorticoid receptor-mediated signaling in cardiovascular diseases

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The renin–angiotensin–aldosterone system has a crucial role in the development and progression of cardiovascular diseases.^{1–3} Angiotensin-converting enzyme inhibitors and angiotensin receptor antagonists are widely used in patients with cardiovascular diseases or those with risk factors (for example, hypertension and diabetes mellitus). Aldosterone is a downstream effector in this system, but its level increases in 30–40% of patients with upstream blockade of this system with an angiotensin-converting enzyme inhibitor and/or angiotensin receptor antagonist over the long term (that is, aldosterone breakthrough).

Following binding to the mineralocorticoid receptor (MR), aldosterone induces detrimental cardiovascular remodeling through processes such as myocyte and smooth muscle cell hypertrophy and collagen accumulation. Previous clinical studies have reported that MR blockade improved the mortality and morbidity of patients with heart failure or myocardial infarction, even if an angiotensin-converting enzyme inhibitor and/or angiotensin receptor antagonist was prescribed.^{4–6} Despite such clinical evidence, MR blockers are underused in clinical practice,⁷ because abrupt increases in the rate of prescriptions for MR blockers after the publication of clinical trials were followed by increased hyperkalemia-associated morbidity and mortality.⁸ Therefore, identifying a master regulator of the MR-mediated pathway could provide more beneficial and/or less adverse effects in treating cardiovascular diseases.

Although MR blockade has been called ‘aldosterone blockade’, this term may be incorrect in reference to several organs. Both glucocorticoids and mineralocorticoids have an affinity for the MR.⁹ The glucocorticoid cortisol/corticosterone is converted by 11 β -hydroxysteroid dehydrogenase type 2 (11 β -HSD2) to an inactive metabolite that cannot bind to MR, which results in the selective binding of aldosterone to the MR. However, 11 β -HSD2 is absent in several organs, such as the heart and hypothalamus, and cortisol/corticosterone is present at a 100–1000-fold greater concentration than aldosterone.^{3,10} Thus, glucocorticoids have been speculated to exert their effects through the MR in the heart; however, the details of this mechanism remain unclear.

Although glucocorticoids induce myocyte hypertrophy, the effects of glucocorticoids can be inhibited by a glucocorticoid receptor blocker, but not by an MR blocker, in cultured myocytes.¹¹ The extent of the contribution of cortisol/corticosterone to MR activation remains unclear, but inhibition of this pathway may be effective in inactivating MR-mediated signaling under certain conditions. Alternatively, previous studies have suggested non-genomic actions of aldosterone independent of MR-mediated signaling.¹² The aldosterone receptors involved in the non-genomic actions have not been identified, which indicates that aldosterone blockade is not equal to MR blockade.

There are several mechanisms to activate MR-mediated signaling without increasing the ligands. First, MR is upregulated in pathological conditions,^{3,13} although the mechanisms for upregulation need to be clarified. Second, MR is degraded by proteasomes, and deubiquitylation may lead to MR stabilization. Third, desumoylation and

cyclin-dependent kinase 5 induce the phosphorylation of multiple serines and threonines on the MR to enhance its trans-activation.^{14,15} Fourth, Rac1 facilitates the nuclear translocation of the MR.¹⁶ Blockade of any of these phenomena may lead to the attenuation of MR-mediated signaling independent of antagonizing ligand binding to the MR.

Recently, He *et al.*¹⁷ showed that MR-induced oxidative activation of Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) has a crucial role in MR-mediated signaling in myocytes (Figure 1). These authors showed that aldosterone activates NADPH oxidase through MR and that this process requires Rac1. Rac1-dependent activation of NADPH oxidase promotes the production of reactive oxygen species and subsequent oxidation of CaMKII in myocytes. This enhanced oxidation of CaMKII facilitates the expression of matrix metalloproteinase-9 (MMP9) through myocyte enhancer factor 2 (MEF2)-dependent transcription, leading to cardiac rupture following myocardial infarction. MR activation is well known to have a crucial role in the development of heart failure induced by pressure overload, and Backs *et al.*¹⁸ showed that knocking out the δ isoform of CaMKII diminished pathological cardiac hypertrophy and remodeling in mice with pressure overload. Therefore, CaMKII may also contribute to the MR-mediated effects in cardiovascular diseases other than myocardial infarction.

In developed countries, the number of patients with heart failure is increasing, and their prognosis remains poor even after the increased prescription rate of angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, MR blockers and β -blockers. Myocardial infarction is a leading cause of heart failure. Several clinical studies

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Figure 1 Signaling pathway for aldosterone-induced activation of the mineralocorticoid receptor (see text).

have proven that the mortality rate in the acute phase of myocardial infarction has decreased, the incidence of heart failure over the long term after myocardial infarction has increased, and coronary revascularization is ineffective in preventing the development of heart failure.¹⁹ Therefore, new therapeutic strategies to prevent cardiovascular remodeling and heart failure are needed.

Several experimental studies have demonstrated that CaMKII is stimulated by β -agonists or angiotensin II and induces deleterious cardiovascular remodeling. Zhang *et al.*²⁰ demonstrated that CaMKII inhibition prevents left ventricular remodeling in catecholamine-induced cardiomyopathy and myocardial infarction. Li *et al.*²¹ demonstrated that CaMKII inhibition blocks angiotensin II-dependent vascular smooth muscle cell hypertrophy. Therefore, CaMKII is likely one of the main targets for the

combined inactivation of MR, β -receptor and angiotensin receptor-mediated signaling, which suggests that CaMKII inhibition is a new therapeutic strategy for cardiovascular diseases as described by He *et al.*¹⁷ However, we must remember that CaMKII activation does not only have adverse effects on cardiac function. Recently, Hamdani *et al.*²² showed that CaMKII enhances myocyte compliance through titin phosphorylation, which is expected to attenuate diastolic stress on the ventricle and to provide beneficial effects for failing hearts. The influence of CaMKII inhibition on the cardiovascular system should be investigated further in various cardiovascular diseases.

We must consider that CaMKII is present in essentially every tissue and that it has various important roles. For example, CaMKII mediates learning and memory in the brain. Enhanced reactive oxygen species

production is responsible for excessive CaMKII activation¹⁷ and is also involved in many other pathological pathways, which suggests the utility of anti-oxidant therapy. This hypothesis was supported by experimental studies and several clinical studies with a limited number of subjects; however, clinical trials have failed to demonstrate the beneficial effects of anti-oxidative therapy. Therefore, cardiovascular-specific inhibition of CaMKII and/or new interventions to inhibit disease-specific oxidative processes may be required.

Many previous experimental studies have suggested the beneficial effect of blocking intracellular signaling pathways in the cardiovascular system. However, many mediators of the intracellular signaling pathway are present in organs other than the heart, and systemic inhibition of these mediators raises concerns about serious adverse effects. To apply the experimental findings regarding the pathological roles of intracellular signaling to the development of new drugs that can be used long term for patients with cardiovascular diseases, an organ-specific drug delivery system may be necessary.

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