Review

An Update on Actions of Mineralocorticoid Receptor in the Cardiovascular System: From Clinical Studies to Basic Research

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Recent clinical studies continued to show beneficial outcome of mineralocorticoid receptor (MR) antagonists for treating heart failure. However, the side effects of these drugs, particularly in the kidney, justified more studies to explore the cell type-specific effects of this receptor. Experimental data from cell type-specific knockout mouse models revealed that deletion of MR in myeloid cells, cardiomyocytes, or vascular smooth muscle cells in general protected mice from cardiovascular disease. Overexpression of MR in cardiomyocytes or endothelial cells, on the other hand, exacerbated cardiovascular disease. These results indicated direct function of MR in these cells. In all these cell types, oxidative stress and inflammation may be unified mechanisms of MR actions. Future clinical trials may broaden the indications of MR antagonists and basic research may provide more insightful understanding of MR actions in the cardiovascular system.

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INTRODUCTION

Mineralocorticoid receptor (MR) has been a great research topic in the past decade. It is increasingly appreciated that understanding the function of MR is far beyond its traditional roles in epithelial cells to control water-electrolyte homeostasis. The positive results of clinical studies evaluating MR antagonists (spironolactone and eplerenone) in heart failure patients encourage more basic research to understand the molecular mechanisms. The expression and function of MR in different cell types of the cardiovascular system have drawn close attentions in the past few years.

CLINICAL STUDIES

While the Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) showing the beneficial effects of spironolactone and eplerenone respectively in patients with severe systolic heart failure, a recent clinical study, termed as the Eplerenone in Mild Patients Hospitalization and Survival Study in Heart Failure (EMPHASIS-HF), aimed to evaluate the therapeutic effects of eplerenone in heart failure patients with mild symptoms.[1] The results were remarkably positive, with a 29% reduction in the rate of the primary outcome, a composite of death from cardiovascular causes or hospitalization for heart failure, in the eplerenone group compared with the placebo group. Similar to the previous two studies, this one was stopped prematurely because the interim analyses showed overwhelmingly beneficial effects of eplerenone. These results clearly demonstrated that the aldosterone blocker was effective not only in patients with severe symptoms of heart failure but also in those with mild symptoms, expanding the indications of this category of drugs.

These results from the clinical studies continue to present MR as an attractive drug target for cardiovascular disease (CVD). Other clinical data implied that these drugs may be effective in treating other CVD such as atherosclerosis,[2] although more clinical trials were required.

However, the antagonists of MR also have serious side effects. Hyperkalemia, a condition with the risk of potentially fatal arrhythmia, was observed in RALES, EPHESUS, and EMPHASIS-HF as expected because of the action of blocking MR in the kidney.[1,3,4] Furthermore, a recent report analyzing EPHESUS showed that although it did not affect its clinical benefit on cardiovascular outcomes, eplerenone caused more decline in estimated glomerular filtration rate (eGFR) compared with placebo, indicating the renal side effects of this drug.[5] Therefore, it is important to dissect out the beneficial actions of MR blockade on the cardiovascular system and the deleterious effects on the kidney and other organs. Basic medical research that combines genetic mouse models and disease models has been an effective approach and has produced meaningful results in recent years.

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RECENT ADVANCE IN STUDYING MR IN MYELOID CELLS USING GENETIC MOUSE MODELS

Since our previous review on the actions of MR in myeloid cells,[6] several other reports have continued to demonstrate the important roles of myeloid MR in the cardiovascular system. First, in a mouse stroke model induced by middle cerebral artery occlusion, both eplerenone and myeloid-specific knockout of MR (MMRKO) significantly reduced the infarct volume and inflammatory response.[7,8] Second, in a model of cardiac fibrosis induced by the combination of uninephrectomy and the nitric oxide synthase (NOS) inhibitor NG-nitro-l-arginine methyl ester (L-NAME), MMRKO significantly inhibited fibrosis, infiltration of inflammatory macrophages, and gene expression of tumor necrosis factor alpha (TNFα).[9] These results were similar to the phenotype seen in previous studies using different disease models,[10,11] except that the overall inflammatory response was not altered by MMRKO in this model.[9] Further, this study demonstrated that the protective roles of MMRKO can be independent of aldosterone.

RECENT ADVANCE IN STUDYING MR IN CARDIOMYOCYTES USING GENETIC MOUSE MODELS

In the past few years, data generated using cell-specific knockout mouse models have further demonstrated cardiomyocyte MR as an important player in the CVD. In a mouse model of myocardial infarction (MI) induced by coronary artery ligation, cardiomyocyte-specific knockout of MR (CMMRKO) improved infarct healing, cardiac remodeling, and contractile function.[12] Mechanistically, CMMRKO may have inhibited oxidative stress and inflammation in the myocardium. Interestingly, myocardial recruitment of monocytes and neutrophils was increased 1 day after MI in CMMRKO mice, while that of neutrophils was decreased 3 days after MI when compared with control mice. This indicates that MR deletion in cardiomyocytes may have promoted the recruitment and clearance of myeloid cells. This in turn may have prompted the acute inflammatory response that is important in stimulating neovascularization, improving cardiac remodeling and function. Another consequence of this is the suppression of chronic inflammation that is deleterious for cardiac repair. Consistently, MR antagonist eplerenone showed similar effects in a rat model of MI.[13] In a pressure overload model induced by transverse aortic constriction, CMMRKO protected mice from left ventricular dilatation and dysfunction but not cardiac hypertrophy or fibrosis, while fibroblast-specific knockout of MR did not have these effects.[14] Elevated levels of phospho-extracellular signal–regulated kinase (p-Erk) 1/2 may be part of the mechanisms in this model. The mild cardiac hypertrophy observed in CMMRKO mice at baseline, a result seemed contra-intuitive, may contribute to reduced wall stress and thus protection during pressure overload. In a deoxycorticosterone/salt model, CMMRKO suppressed cardiac fibrosis and inflammation.[15] All these data pointed to protective roles of MR blockade in cardiomyocytes, although the exact effects were somewhat different in different mouse models.

Conversely, data generated using cardiomyocyte-specific MR overexpression (CMMROE) mouse model have illustrated the deleterious effects of super-physiological activation of MR. Previous studies demonstrated that CMMROE resulted in lethal arrhythmias because of ion channel remodeling and that the effects of CMMROE and Ang II were additive.[16,17] More recent data suggested that CMMROE caused coronary endothelial dysfunction that was associated with increased reactive oxygen species (ROS).[18] Using the same mouse model combined with the stimulation of aldosterone, connective tissue growth factor, a molecule critically involved in fibrotic disease, was identified as a putative target gene of MR in cardiomyocytes,[19] providing more mechanistic explanation why CMMRKO had inhibited cardiac fibrosis.

RECENT ADVANCE IN STUDYING MR IN VASCULAR SMOOTH MUSCLE CELLS USING GENETIC MOUSE MODELS

Recent data demonstrated that MR in vascular smooth muscle cells (VSMCs) also played direct roles in CVD. VSMC-specific knockout of MR (SMRKO) effectively blocked age-associated hypertension through decreasing vascular myogenic tone and vasoconstriction response, without alterations in vascular structure or renal function.[20] Further analyses found that SMRKO decreased the expression and activity of L-type calcium channels in isolated VSMC or blood vessels. When challenged with Angiotensin II (Ang II), the SMRKO mice also had lower vascular contraction and blood pressure. Ang II-induced ROS production was significantly lower in the blood vessels of SMRKO mice, indicating that vascular oxidative stress may be part of the mechanisms. It would be interesting to see whether MR in VSMCs plays direct roles in other models of CVD.

RECENT ADVANCE IN STUDYING MR IN ENDOTHELIAL CELLS USING GENETIC MOUSE MODELS

Studies using endothelial cell-specific MR overexpression (ECMROE) showed that endothelial MR was also critical in the cardiovascular system. ECMROE mice developed hypertension that was reversed by MR antagonist.[21] When treated with Ang II or endothelin 1, the blood pressure of ECMROE mice was higher than that of control mice. Ex vivo data showed that the resistance arteries from ECMROE mice had increased contractile response to vasoconstrictors.

So far, loss-of-function studies using EC-specific MR knockouts have not been reported, although they may be emerging soon. Such studies may reveal more detailed function of endothelial MR in CVD.

OXIDATIVE STRESS AND INFLAMMATION: THE RECURRING THEME OF MR ACTIONS IN CARDIOVASCULAR SYSTEM

Oxidative stress and inflammation form a 'vicious' perpetuating cycle in the process of CVD such as heart failure and atherosclerosis.[22,23] On one hand, ROS stimulates the release of inflammatory cytokines by activating

downstream pathways involving nuclear factor-kappa B (NF-κB), activator protein 1 (AP-1), and p38 mitogen-activated protein kinase. On the other hand, pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) suppress antioxidants while stimulate pro-oxidants. By contrast, anti-inflammatory cytokines such as interleukin 10 (IL-10) stimulate antioxidants while suppress pro-oxidants.[22]

Numerous studies with convincing data have demonstrated that activation of MR causes oxidative stress and inflammation in different cell types of the cardiovascular system.[24,25] MR activation upregulates oxidative stress by increasing the expression and activity of nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase and therefore enhances ROS generation, as well as mitochondrial electron transport uncoupling. Meanwhile, MR activation stimulates infiltration of inflammatory cells and promotes expression of inflammatory markers in the cardiovascular system. Furthermore, the inflammatory response is at least partially dependent on oxidative stress, in agreement with the 'vicious' cycle.[24,25] Conversely, blockade of MR by antagonists or gene knockouts as described above leads to reduced oxidative stress and/or inflammation, break the "vicious" cycle, and therefore demonstrate beneficial effects in the cardiovascular system. Taken together, oxidative stress and inflammation are a recurring theme of MR actions, presenting a unified hypothesis for exploring the mechanisms of MR blockade.

Table 1. Summary of recent studies on MR actions in the cardiovascular system using genetic mouse models.

Cell type	Genetic model	Disease model	Major outcome	Possible mechanism	Reference
Myeloid cell	MR knockout	Middle cerebral artery occlusion	Reduced infarct volume	Decreased inflammation	8
Myeloid cell	MR knockout	Uninephrectomy/L-NAME	Reduced fibrosis	Decreased inflammation	9
Cardiomyocyte	MR knockout	Coronary artery ligation	Improved infarct healing, cardiac remodeling, and contractile function	Decreased inflammation and oxidative stress	12
		Transverse aortic constriction	Protected from left ventricular dilatation and dysfunction	Increased phosphorylation of Erk1/2	14
Fibroblast	MR knockout	Transverse aortic constriction	No effect	NA	14
Cardiomyocyte	MR knockout	Deoxycorticosterone/salt	Decreased fibrosis	Decreased inflammation	15
Cardiomyocyte	MR overexpression	NA	Arrhythmia	Ion chamel remodeling	17
Vascular smooth muscle cell	MR knockout	Aging /Angiotension II	Protected from hypertension	Decreased expression and activity of L-type calcium chamels/decreased oxidative stress	20
Endothelial cell	MR knockout	NA/Angiotension II/endothelin 1	Hypertension	NA	21

*NA; not applicable

CONCLUSIONS AND FUTURE DIRECTIONS

Recent clinical studies continued to show tremendously beneficial effects of MR antagonists for heart failure despite concerns on the renal side effects. Findings from recent basic research further confirmed direct roles of MR in the cardiovascular system (**Table 1**), supporting new MR antagonists with cell type-specific effects to circumvent the side effects and improve the effectiveness.

MR antagonists will continue to be a strong interest in clinical trials. On-going and future studies that aim to investigate the effectiveness of MR antagonists in heart failure patients with preserved systolic function, or early administration right after MI, or in other CVD besides heart failure will likely broaden the indications of these drugs. Efforts to develop and test new antagonists with cell type specificity are a new direction worth pursuing.

With more mechanistic explorations, basic research on MR will paint a more complete picture of how this nuclear receptor and its blockade work. For example, the roles of MR in endothelial cells, a critical cell type in the cardiovascular system, needs to be further explored. For another example, more molecular and mechanistic studies are needed to

understand in details how MR activation or blockade affects oxidative stress and inflammation. In addition, it is worthwhile to differentiate the genomic and non-genomic effects of MR activation in the cardiovascular system. Ultimately, these efforts will bring out more insightful understanding and clinical usage of MR in CVD.

CONFLICT OF INTEREST

None.

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