Medical Advances

A New Connection: Myeloid Mineralocorticoid Receptor and Cardiovascular Disease

Sheng Zhong Duan, MD, PhD, Richard M. Mortensen, MD, PhD

Abstract

Mineralocorticoid Receptor (MR) is a classic steroid hormone receptor. Its traditional role is to mediate aldosterone to control electrolyte homeostasis and blood pressure via renin-angiotensin system. Besides aldosterone, MR can also bind to glucocorticoids. In aldosterone sensitive tissues such as kidney, 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) inactivates glucocorticoids and makes MR binding to aldosterone possible. In tissues lack 11βHSD2, MR is presumably occupied by glucocorticoids. The functions of MR in these tissues are largely unknown. Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) successfully demonstrated cardiovascular benefits of blocking MR with antagonists. However, the mechanisms have not been clearly delineated. Macrophage polarization, a phenotype that macrophages polarize to distinct functional states such as classically activation and alternatively activation, has emerged as an important control element in cardiovascular diseases (CVD). Recent studies have shown that MR controls macrophage polarization and that deletion of MR in myeloid cells protects cardiac and vascular damages under pathological stress. These studies present a great opportunity for developing new antagonists to target myeloid MR specifically in order to improve specificity and effectiveness of this class of drug in CVD. *[N A J Med Sci. 2010;3(4):167-170.]*

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Randomized Aldactone Evaluation Study (RALES) and Eplerenone Post-Acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS) clearly demonstrated blocking of a classic nuclear receptor – mineralocorticoid receptor (MR) – effectively improves the outcome of heart failure.[1-3] These studies provoked strong research interests, both basic and clinical, on MR. However, a great deal of the mechanisms remains uncertain and some key questions remain unanswered. For instance, why are MR antagonists beneficial in patients without hyperaldosteronism? Since cardiomyocytes are considered as aldosterone insensitive and MR in these cells are most likely occupied by glucocorticoids, how do MR antagonists have effects in heart? Recent findings on myeloid MR reveal an important new target for these antagonists and may pave the way to elucidate the molecular mechanisms of MR antagonism. In particular, the modulation of myeloid MR on macrophage polarization (macrophages polarize to different states including classically activation or alternatively activation) has been demonstrated to have crucial roles in controlling CVD.

MR and its Classical Actions

MR is a member of the steroid hormone receptor family.[4] It is widely expressed in a variety of tissues such as kidney, central nervous system, heart, colon, brown adipose tissue, sweat glands, as well as myeloid cells.[5,6] Both mineralocorticoids (aldosterone) and glucocorticoids (cortisol in humans and corticosterone in rodents) can bind to MR as agonists with high affinity.[7]

MR is a critical control element of fluid and electrolyte homeostasis and therefore blood pressure via renin-angiotensin system.[8] When blood pressure falls due to significant decrease of plasma sodium level or circulating volume, the amount of renin secreted by renal juxtaglomerular cells increases accordingly. Renin cleaves angiotensinogen to generate angiotensin I, which is subsequently cleaved by angiotensin converting enzyme to

generate angiotensin II (Ang-II). Ang-II then constricts blood vessels, increases the secretion of aldosterone from adrenal gland. Aldosterone in turn increases sodium and water retention by activating MR in renal and colonic epithelium. Both blood vessel constriction and salt retention lead to increase blood pressure. Through this negative feedback loop, blood pressure and plasma electrolytes are maintained relatively constant.

An important enzyme in the process is 11β-hydroxysteroid dehydrogenase type 2 (11βHSD2) that convert corticosterone and cortisol to 11-dehydrocorticosterone and cortisone respectively, which do not bind MR. This inactivation of glucocorticoids allows aldosterone bind to MR to exert their biological function, because otherwise MR would be occupied by glucocorticoids, which circulating at over 100 times the concentration of mineralocorticoids and yet have the same affinity.[9] Expression of 11βHSD2 is limited to tissues such as renal epithelium, colon, and vascular endothelium.[7] Pathological increases in MR activation such as in the setting of hyperaldosteronism result in excessive salt and water retention and hypertension with hypokalemia.[8]

RALES and EPHESUS Studies

The cloning of MR in 1987 moved MR out of the shadow of glucocorticoid receptor (GR) and made it an intriguing research subject.[10] Some 12 years later MR became more clinically well known when two successful clinical studies demonstrated that antagonists of MR remarkably reduced cardiovascular death.[1-3] These two studies are RALES and EPHESUS that investigated spironolactone and eplerenone respectively.[1-3] In RALES, spironolactone in combination with standard care showed a 30% improvement in survival and a 35% decrease in hospitalisation in patients with progressive cardiac failure. Similarly in EPHESUS, eplerenone in addition to optimal medical therapy significantly reduced cardiovascular morbidity and mortality.

Although the success of RALES and EPHESUS is widely attributed to the ability of spironolactone and eplerenone to block aldosterone in the cardiovascular system, the exact mechanisms of the beneficial effects remain unknown. Two interesting features of the two studies deserve a second look and may point to new directions of the mechanistic studies on MR. First, both studies used low average dose of the drugs. Secondly, the plasma levels of aldosterone in the patients were not high to start with. One would question what spironolactone and eplerenone were antagonizing. Furthermore, 11βHSD2 is either not expressed or expressed at extremely low level in the heart, presumably leaving cardiac MR essentially occupied by glucocorticoids.[8] It is very worth to explore how spironolactone and eplerenone have their effects on glucocorticoid occupied MR in the heart.

Myeloid Cell Polarization and Cardiovascular Disease

Immune cells have been known for some time to have an important role in atherosclerosis and other CVD. These include myeloid cells such as neutrophils, monocytes, and macrophages, as well as lymphoid cells, as major contributors. Macrophages and their precursor monocytes play a particularly central role, e.g. lipid-laden macrophages are one of the first pathologic hallmarks of the fatty streak, the beginning of atherosclerosis.

More recently, subsets macrophages and monocytes have been recognized to have distinctly different phenotypes and functions. The normal evolution of an inflammatory response requires carefully coordinated recruitment of functionally distinct subclasses of macrophages. In its simplest description, the macrophage phenotype falls within a spectrum between classically activated macrophages (M1) expressing a high level of pro-inflammatory cytokines and reactive oxygen species and alternatively activated macrophages (AAM) involved in pathogen sequestration, wound healing, and phagocytosis of apoptotic cells.[11,12]

Although originally used to describe the activation by Th2 cytokines, interleukin (IL)-4/IL-13, the use of the term AAM has been broadened to include a host of related but different macrophage phenotypes.[13,14] Macrophages that have been called AAM have different subtypes with different properties and functions depending on the exact cytokine stimuli [15]. Further, the stimuli in CVD will likely include endogenous stimulators of this innate immune system that would result in even further diversity.[13,14] Perturbation of this balance of macrophage subtypes has been associated with disorders such as fibrotic diseases and thus is an important consideration to the development of therapies to disorders with an inflammatory component. Similarly, other perturbations could be protective. A somewhat more complex view holds that there are inflammatory, wound healing and regulatory macrophages. Other macrophage types may be a combination.

In addition to the macrophage subsets, circulating monocytes display heterogeneity. Lymphocyte antigen 6 complex (Ly-6C) is expressed on monocytes/macrophages, endothelial cells and granulocytes as well as a subset of lymphocytes. Two main groups of monocytes have been identified by the expression level of Ly-6C, Ly-$6C^{hi}$ (high) and Ly-$6C^{lo}$ (low). Other markers have been used to further group monocytes. These markers include granulocyte-differentiation antigen-1 (Gr-1) that is expressed on mature granulocytes, chemokine (C-C motif) receptor 2 (CCR2) and chemokine (C-X3-C motif) receptor 1 (CX_3CR1) that are both important in mediating inflammation. Interestingly, subsets of monocytes that are associated with adipose infiltration in obesity are also the same types that migrate to atherosclerotic plaques (Ly-$6C^{hi}$ Gr-1^{+} $CCR2^{hi}$ CX_3CR1^{lo}) and are induced by high fat diets.[15,16] It is harder to show migration from the circulation to the resident macrophages and these populations may be sustained by dividing.[17] Ly-$6C^{lo}$ cells may arise from Ly-$6C^{hi}$ cells in the tissue [18] or be directly recruited to the tissue from the circulation.[19] These Ly-$6C^{lo}$ cells can be CX_3CR1^{hi} and are associated with repair and may be precursors for alternatively activated macrophages subtypes.

Recent Findings of Myeloid MR and CVD

Two recent studies have utilized tissue-specific gene inactivation strategy to explore the function of myeloid MR in CVD.[21,22] Both studies showed selective deletion of MR in myeloid cells led to cardiovascular protection in pathological mouse models, although the results from the two studies do not completely agree with each other. Using a uninephrectomy/deoxycorticosterone model, Rickard et al. showed that inflammatory gene expression and cardiac fibrosis were mitigated in the myeloid MR knockout mice, but cardiac macrophage recruitment was not different between knockout and wild type mice.[21] Further, myeloid MR deficiency attenuated the hypertensive effect of uninephrectomy/deoxycorticosterone treatment.

Usher et al. treated myeloid MR knockout mice with a combination of a NOS inhibitor, N (G)-nitro-L- arginine methyl ester (L-NAME), and Ang-II to generate cardiac and vascular damage and fibrosis.[22] MR deficiency in myeloid cells mimics MR antagonists and protects against cardiac and vascular hypertrophy, inflammation, and fibrosis caused by L-NAME/Ang-II. In contrast to the previous study that used a different model, both cardiac and vascular macrophage infiltration was almost completely blocked by myeloid MR deletion. Moreover, blood pressure and heart rates were higher in the knockout mice and had less circadian variation, suggesting that the cardiovascular protection of myeloid MR deficiency was not due to its impact on blood pressure. In vitro study showed that MR was necessary for efficient M1 activation and that MR deficient macrophages from the myeloid MR knockout mice resulted in an AAM transcriptional profile. Further, MR deficiency in macrophages synergizes with Th2 cytokines (IL-4) and other nuclear receptors (Peroxisome-Proliferator-Activated-Receptor-γ and GR) to enhance alternative macrophage activation. Myeloid MR presents as a strong control point for macrophage polarization that may ultimately explain the phenotype of cardiovascular protection (**Figure 1**).

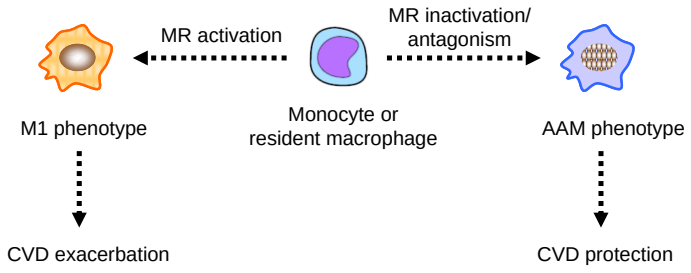

Figure 1. Summary of myeloid MR controlling macrophage polarization and its role in cardiovascular disease. MR: mineralocorticoid receptor; M1: classically activated macrophages; AAM: alternatively activated macrophages; CVD: cardiovascular disease.

It is worth mentioning that macrophages do not express 11βHSD2 and are therefore considered aldosterone insensitive. Although not directly demonstrated due to technical difficulty (if not impossibility), macrophage MR is also presumably occupied by glucocorticoids. The fact that MR reserved high affinity for glucocorticoids during evolution indicates that glucocorticoid occupied MR has important biological functions. The recent two studies on macrophage MR have shed some light on such functions of MR. These findings are critical in understanding the molecular signalling that directs macrophages to respond and control cardiovascular events following pathological stress.

Furthermore, the cardiovascular protection by MR deficiency in macrophages in experimental models of cardiac and vascular remodelling largely mimics the effects of MR antagonists. Therefore, blocking MR in macrophages may be a critical mechanism of MR antagonists and this explains the benefit of MR antagonists in the absence of hyperaldosteronism.

Future Directions and Clinical Implications

Studying other CVD may provide more evidence and possibly a universal protective phenotype by myeloid MR deficiency in the cardiovascular system. On the other hand, detailed molecular mechanisms how MR controls macrophage polarization responding to cardiovascular insult need to be delineated.

Current MR antagonists in clinical use include spironolactone (the first generation) and eplerenone (the second generation). In comparison, eplerenone has lower affinity for other steroid receptors such as progesterone, androgen, and glucocorticoid receptors. As a result, eplerenone has fewer side effects, particularly sexual side effects. However, both drugs have substantial side effects such as hyperkalemia caused by unwanted renal tubular effects. Targeting myeloid MR may provide a more specific and effective therapeutic strategy. Besides their effectiveness in heart failure reported by RALES and EPHESUS studies, these antagonists have also been reported to have other cardiovascular benefits at least in animal models. For instance, eplerenone can inhibit atherosclerosis in mouse, rabbit, and primate models.[23-25] More specific and effective MR antagonists will also help the next generation of these drugs have a broader range of indications in CVD. However, more studies are warranted to further prove the effectiveness of blocking myeloid MR specifically.

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