The Role of the Mineralocorticoid Receptor in Inflammation: Focus on Kidney and Vasculature

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Abstract

Background: The remarkable success of clinical trials in mineralocorticoid receptor (MR) inhibition in heart failure has driven research on the physiological and pathological role(s) of nonepithelial MR expression. MR is widely expressed in the cardiovascular system and is a major determinant of endothelial function, smooth muscle tone, vascular remodeling, fibrosis, and blood pressure. An important new dimension is the appreciation of the role MR plays in immune cells and target organ damage in the heart, kidney and vasculature, and in the development of insulin resistance. ***Summary:*** The mechanism for MR activation in tissue injury continues to evolve with the evidence to date suggesting that activation of MR results in a complex repertoire of effects involving both macrophages and T cells. MR is an important transcriptional regulator of macrophage phenotype and function. Another important feature of MR activation is that it can occur even with normal or low aldosterone levels in pathological conditions. Tissue-specific conditional models of MR expression in myeloid cells, endothelial cells, smooth muscle cells and cardiomyocytes have been very informative and have firmly demonstrated a critical role of MR as a key pathophysiologic variable in cardiac hypertrophy, transition to heart failure, adipose inflammation, and atherosclerosis. Finally, the central nervous system activation of MR in permeable regions of the blood–brain barrier may play a role in peripheral inflammation. ***Key Message:*** Ongoing clinical trials will help clarify the role of MR blockade in conditions, such as atherosclerosis and chronic kidney disease.

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Introduction

In the 1950s, Selye et al. [1] presciently characterized the effects of aldosterone on nonepithelial tissues and postulated that spironolactone is protective in conditions of aldosterone excess. The cloning of the human mineralocorticoid receptor (MR) by Arriza et al. [2] exactly 30 years ago, spurred interest in the nonclassical aspects of aldosterone including the role of aldosterone in cardiac remodeling and fibrosis [3]. MRs are members of a superfamily of intracellular ligand-operated steroid recep-

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tors that regulate transcription of multiple genes and other transcription factors resulting in a complex repertoire of effects. The remarkable success of clinical trials in MR inhibition through their effects on the cardiovascular system have driven research on the physiological and pathological role(s) of nonepithelial MR expression [4–6]. An important new dimension is the evolving appreciation of the role MR plays in immune cells as it relates to target organ damage (Fig. 1). In this review, we attempt to review the most significant developments related to MR modulation of immune expression and new studies over the last decade that have paved the way for renewed understanding of MR-mediated cardiorenal disease.

MR Structure and Expression

The MR receptor is a 984-amino acid intracytoplasmic receptor divided into 3 domains: the N-terminal domain that regulates transcriptional activity of the receptor; the DNA-binding domain involved in the binding of the specific response element found on the promoter of MR target genes; and finally, a ligand-binding domain responsible for the selectivity of hormone binding. MR also binds to a number of chaperones that play a pivotal role in maintaining MR in an appropriate conformation for ligand binding. Upon hormone binding, the MR dissociates from chaperone proteins, undergoes nuclear translocation, and interacts with numerous molecular partners in a coordinated and sequential manner to ensure appropriate transcriptional regulation. In the nucleus, MR recruits co-regulators (cofactors and/or corepressors) to induce the transactivation and regulation of hundreds of target genes [7]. These genes present a palindromic DNA sequence common for GR and MR called the glucocorticoid response element within their promoter. Although MR is regulated transcriptionally, posttranscriptional mechanisms of regulation are important and include phosphorylation and sumoylation. Very recently, ubiquitination, another posttranslational modification of MR, has been reported [8].

MR Activation/Antagonism and Specificity-Conferring Mechanisms

MR has been shown to bind to aldosterone at low concentrations (high picomolar) and with high affinity. MR also binds with equally high affinity to cortisol, corticosterone, deoxycorticosterone, and progesterone [9].
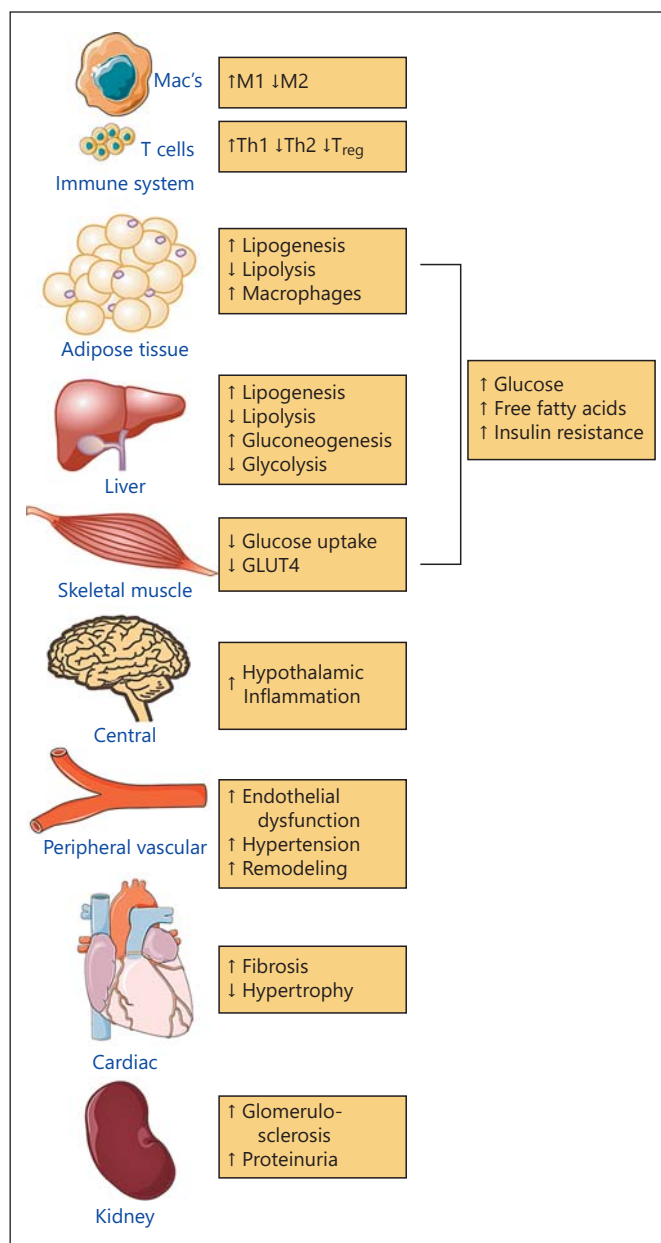

Fig. 1. Mineralocorticoid agonism or elevated aldosterone levels have a deleterious effect on organ systems and cells relevant to cardiometabolic diseases.

Since circulating physiologic glucocorticoid levels are ~1,000-fold higher than those of aldosterone on a normal diet, the issue of MR selectivity to aldosterone has been a topic of contention for decades. Studies by Funder and Minireview [9] suggested that 11β-HSD type 2 (11β-HSD2), a high-affinity (nanomolar K_M), low-capacity NAD-dependent dehydrogenase is expressed alongside MR, where its activity reduces the availability

of glucocorticoids, permitting aldosterone to bind to the MR with relative exclusivity. However, the idea that the presence of 11β-HSD2 is enough to provide unfettered access to aldosterone, through "debulking" cortisol (conversion to cortisone) would require a tremendously efficient system that converts vast excesses of cortisol to cortisone in the area proximal to the MR. This mechanism is not supported by in vivo evidence in the cardiovascular, immune, and central nervous systems. Indeed, the preponderance of evidence suggests little or no 11β-HSD2 activity in the heart, inflammatory cells, and regions of the central nervous system; yet there is extensive evidence to support MR binding in these organs [10]. In addition, in vivo competition studies in adrenalectomized animals show high MR selectivity in these organs. For instance, cortisol has ~30% of the apparent affinity of aldosterone in the heart. These studies firmly suggest that specificity-conferring mechanisms, other than pure enzymatic mechanisms are essential for selective aldosterone action. 11β-HSD1, the enzyme that converts inactive cortisone to cortisol in humans, has low affinity for glucocorticoids (micromolar K_M) relative to 11β-HSD2, and functions both as an oxidoreductase (transfers electrons from one molecule to another) and as a dehydrogenase. The latter activity is dependent on the supply of NADPH through coupled expression of the enzyme hexose-6-phosphate dehydrogenase. Although there is plentiful evidence that myocardial cells express 11β-HSD1, reactivation of glucocorticoid is apparently limited under physiological conditions both in mice and humans. In an in vivo study in humans, the stable isotope tracer 9, 11, 12, 12-[2H]4-cortisol underwent little metabolism across the human heart [11]. Administration of the MR antagonist canrenoate in the same patients resulted in the elevation of cortisol collected from the coronary sinus, suggesting displacement of endogenous glucocorticoids. These data support the view that the cardiac MR is normally occupied by glucocorticoids rather than by aldosterone. A model that provides an explanation for glucocorticoid-mediated MR signaling in the setting of a protected MR (presence of 11β-HSD2) is the concept that the activity of the enzyme 11β-HSD2 results in a decrease in the NAD/NADH ratio (owing to generation of NADH) which alters the redox state, resulting in blocking activity of MR. There is direct evidence for redox stress/state regulating the activation of other nuclear transactivating factors, and it is likely that similar changes in redox state are operant for cortisol [10]. There are undoubtedly other aspects such as conformation of the ligand-binding interactions and co-regulator recruitment that may contribute to between-ligand (cortisol vs. aldosterone) differentiation in signaling [7, 12].

MR Activation in Kidneys and Cardiovascular Tissues

The mechanism for MR activation in tissue injury continues to evolve and suggests a complex repertoire of effects involving a multitude of mediators that are cell and context dependent. While in both animal models and humans, there is evidence that both plasma and urinary aldosterone concentrations are increased in a variety of cardiometabolic conditions, MR activation may occur in the absence of elevated aldosterone levels [13]. Recently, several studies have suggested that MR activity is also affected by factors other than its ligands including PKA, Rac-1, ubiquitin conjugating enzymes and other factors involved in the regulation of diverse nuclear receptors [14–17]. MR is widely expressed in the cardiovascular system such as in the endothelium, smooth muscle cells, and fibroblasts, and is a major determinant of endothelial function, smooth muscle tone, vascular remodeling, fibrosis, and blood pressure (BP) [18–25]. Endothelial, vascular smooth muscle, and cardiomyocyte-specific overexpression/deletion studies in animals and studies in humans, support a role for MR activation in promoting vascular oxidative stress, inflammation, proliferation, migration, vasoconstriction, vascular remodeling, and fibrosis [26–34], (online suppl. Table for all online suppl. material, see www.karger.com/doi/10.1159/000480652).

In the kidneys, the classical effects of MR activation are to increase epithelial sodium channel (ENaC) density in the distal convoluted tubule via increased expression and activation of serum and glucocorticoid regulated kinase-1 (SGK1). Phosphorylated SGK1 in turn "inhibits an inhibitor" of ENaC, NEDD4, a protein involved constitutively in the ubiquitination of ENaC [35]. Chronically, SGK1 may also play a role by promoting ENaC transcription through inhibition of H3K79 methyltransferase, which blocks transcription of ENaC. Mineralocorticoid-sensitive inflammation and fibrosis involves the upregulation of the inflammatory transcription factor NFκB, which in turn stimulates the expression of diverse mediators including connective tissue growth factor. SGK1 also inhibits the degradation of the transforming growth factor beta (TGFβ)-dependent transcription factors Smad2/3, further promoting a profibrotic signal [36].

Aldosterone promotes the proliferation of renal fibroblasts and mesangial cells via transactivation of epidermal

growth factor receptor and platelet-derived growth factor receptor, induces myofibroblastic transdifferentiation of mesangial and tubular epithelial cells, and directly stimulates the synthesis of profibrotic cytokines and matrix proteins [37–40]. The profibrotic response of aldosterone at least in animal models, clearly requires sodium. Studies by Shibata et al. [15, 41] have shown that salt can lead to the activation of MR, even in the absence of ligand resulting in a profibrotic response in the kidney and heart. Recent studies have additionally implicated an important role for the immune system in aldosterone-mediated fibrosis and tissue injury. As detailed below, the activation of the immune system appears to be an important mediator of MR-mediated effects and is a requisite for its profibrotic effects.

Role of the MR in Macrophage/Monocytes

MR appears to play a central role in regulating macrophage phenotype and function broadly through transcriptional reprogramming of monocytes/macrophages. Many of the phenotypic effects reported to be regulated by MR may reflect a broad repurposing of cellular function. Thus, while the effects of MR activation/antagonism are reported discretely, they may reflect related and connected effects. In this regard, deletion of MR in myeloid cells (MR knock out or MRKO) has been very useful in ascribing MR-dependent mechanisms in macrophages. Table 1 details the cell-specific and phenotypic effects of conditional tissue-specific deletion of MR in cardiovascular cells, including in myeloid cells.

Transcriptional Reprogramming of Macrophages
PPARγ, PPARδ, and KLF4 are major regulators of alternate activation and are required for the maintenance of alternatively activated macrophages (Fig. 2) [42]. Macrophages in mice can be rendered pro-inflammatory (M1) in the presence of aldosterone, an effect prevented by MR antagonism which favors an anti-inflammatory, alternatively activated (M2) phenotype [43]. Conversely, MRKO in myeloid cells recapitulated the effects of MR antagonism, by shifting the phenotype to M2 (alternatively activated macrophage) and downregulating pro-inflammatory and profibrotic genes (TGFβ and PAI-1). In vivo, myeloid MRKO reduced aortic and cardiac macrophage recruitment, cardiac hypertrophy, fibrosis, and fetal gene reprogramming in response to AngII and L-NAME (model of MR activation) suggesting that myeloid MR is crucial to adverse, fibrotic cardiovascular re-

modeling [43]. Gene expression analysis revealed significant similarity between MRKO and PPARγ activation. These findings were similar to another study investigating macrophages from the heart in myeloid MRKO and wild-type (WT) animals treated with vehicle or DOCA/salt. A pro-inflammatory and profibrotic profile in response to DOCA salt was prevented in the heart of mice with myeloid MR-null macrophages [44]. Further, MRKO in macrophages diminished the activation of both AP1 and NFκB in restenosis models with effects dependent on SGK1, consistent with other studies [45, 46].

Regulation of Myeloid Inflammatory Numbers and Chemotaxis
MRKO macrophages demonstrate reduced migration de novo in response to a chemokine gradient with restenotic injury, resulting in lower macrophage content in MRKO animals vs. WT [47]. Corning Transwell® assays with conditioned media derived from LPS-stimulated MRKO macrophages induced markedly less migration of vascular smooth muscle cells and lower expression of pro-inflammatory cytokines, compared to conditional media from control macrophages [47]. Several in vivo studies involving hypertension and stroke models have demonstrated a reduction in macrophage content in mice transplanted with MRKO myeloid cells [43, 48]. Similarly, in a study of cardiac hypertrophy (transaortic constriction), a reduction in macrophages in the heart with myeloid MR deletion was noted [49]. However, two studies using a severe hypertension model (unilateral nephrectomy with 0.9% salt and L-NAME) and a uninephrectomized mouse model of DOCA salt excess, demonstrated no change in the number of tissue macrophages in the heart [44, 50]. In many of these studies, macrophage recruitment seems to play a critical role in BP response, with evidence suggesting a CCL2-dependent movement of myeloid cells to the heart required for myeloid MR-dependent effects [51]. However, all studies demonstrated rather consistent reduction in tissue fibrosis and inflammation suggesting that MR deficiency critically regulates fibrosis [43, 47, 48, 50].

Regulation of ROS and Inflammatory Kinases
Aldosterone has been shown to activate components of NAPDH oxidase in various cell types [27, 28, 52–54], and reductions in NADPH oxidase have been noted in response to MR blockade [54–56]. The degree to which these are direct, nongenomic effects is difficult to understand and is currently uncertain [57]. Rac1, a Rho family

Table 1. Lineage-specific MR knockout effects in animal models

Author, model	Cell-specific effects of MRKO	Phenotype
Myeloid MRKO		
Shen et al. [44], 2016 Uninephrectomized DOCA-salt	↑ Cardiac M2 macrophage markers PPARγ, PDK4, MRC2, CXCL9, ↓ TNFα, MMP12 expression	↓ Cardiac fibrosis
Rickard et al. [93], 2009 Uninephrectomized DOCA-salt	↓ Cardiac macrophage infiltration, collagen, PAI-1, NADPH oxidase	↓ Cardiac fibrosis and hypertrophy
Usher et al. [94], 2010 Uninephrectomized L-NAME/salt treated	↓ Peritoneal macrophage TNFα, rantes, IL12, IL1β, MCP2 expression ↑ Arg1, IL10, Ym1, FIZZ1, F13a1, CCL-17, CCL-7 expression ↓ Cardiac macrophage infiltration ↓ ANP, BNP, collagen III, TGFβ, PAI-1	↓ L-NAME/AngII-induced cardiac interstitial fibrosis, aortic fibrosis, and thickening
Bienvenu et al. [50], 2012 Uninephrectomized L-NAME/salt treated	↓ CTGF, collagen III, TNFα expression MRKO made no difference in macrophage infiltration	↓ Cardiac and large vessel fibrosis
Li et al. [49], 2014 AAC mice	↓ Cardiomyocyte hypertrophy, ANP, βMHC, collagen I/III, CTGF, fibronectin 1, TGFβ1, and TGFβ2 expression, aortic collagen I/III, CTGF staining ↓ Cardiac Nox2, Nox4, p40, p47 ↑MnSOD expression Rescued AAC-induced cardiac β-oxidation (Acox1, Acadm, and Acadvl) and oxidative phosphorylation (Sdhb, Cox4i1, Atp5j), ↑ Cardiac PPARα and PGC1α expression ↓ Cardiac macrophage M1 markers F4/80 and CD68, M1 cytokines TNFα, MIP1β, COX2, MCP1, and IL6 staining ↓ Cardiac and aortic macrophage infiltration	↓↓ Cardiac fibrosis and hypertrophy ↓ Aortic fibrosis
Shen et al. [45], 2017 LDLR null mice	↓ Plaque necrotic core area, macrophage accumulation, in vitro foam cell formation ↑ Plaque collagen area	↓ Atherosclerotic lesion area (aorta)
Zhang et al. [95], 2017 Lep^ob/ob	↑ ERα and ESR1 gene expression ↑ HGF expression ↑ Hepatocyte Met signaling ↑ Insulin sensitivity ↓ Hepatic triglyceride storage and lipogenesis genes - SCD1, Ly6d, and Cidea	↑ Glucose homeostasis ↓ Hepatic steatosis
Sun et al. [47], 2016 Femoral artery wire injury in MRKO mice	↓ Injury-induced vascular macrophage infiltration and proliferation, smooth muscle proliferation ↓ Injury-induced vascular AP-1, NFκB, SGK1 signaling, IL6, IL1β, ICAM1, MIP1α, MIP1β, MIP2α, NOS2, MMP9, CXCL1, MCP1, CCR2, CCR4, osteopontin	↓ Injury-induced intimal hyperplasia and fibrosis
Frieler et al. [96], 2011 Focal cerebral ischemia	↓ Infarct-induced myeloid TNFα, IL1β, MCP1, MIP1α, and IL6 ↑ Myeloid Arg1 and Ym1	65% reduction in infarct volume ↓ Infarct-induced microglial activation in the ischemic core

Table 1. (continued)

Author, model	Cell-specific effects of MRKO	Phenotype
Endothelial MRKO		
Rickard et al. [97], 2014 Uninephrectomized DOCA-salt or aldosterone Duration: 8 weeks	↓ Cardiac CCR5 expression at 8 days ↓ Cardiac CTGF expression at 8 weeks ↓ DOCA-salt-induced cardiac macrophage infiltration and fibrosis at 8 weeks Eplerenone inhibited aldosterone-induced ICAM1 and CTGF expression in HUVEC cells	↓ Aldo-induced endothelial dysfunction in aorta but not mesenteric resistance vessels, as measured by Ach-induced relaxation
Schäfer et al. [98], 2013 ND or a HFD and Aldosterone infused mice	↓ Diet-induced endothelial dysfunction ↓ Aldo-induced COX1 expression Indomethacin reduced aldosterone infusion-induced endothelial dysfunction to same extent as MRKO	EC MR−/− had no effect on WAT inflammatory state or glucose tolerance of obese or aldo infused mice EC MR−/− prevented HFD and aldo infusion-induced endothelial dysfunction
Jia et al. [99], 2015 Control vs. high fat and carbohydrate diet (western; WD)	↓ WD-induced cardiomyocyte stiffness (by atomic force microscopy), cardiac TGFβ1, and phospho-Smad2/3, collagen I, CTGF, and fibronectin immunostaining ↓ WD-induced cardiac 3-nitrotyrosine staining ↓ WD-induced cardiac pS6K, pIRS1, pERK1/2, M1 markers MCP1, IL17, CD11b immunostaining CD206 and IL10 immunostaining	↓ WD-induced diastolic dysfunction (evaluated by relaxation time and Doppler), cardiac interstitial fibrosis and hypertrophy
Jia et al. [100], 2016 Chow or western diet for 16 weeks	KO attenuated WD-induced aortic endothelial decreases in p-AKT and p-eNOS ↑ Mesenteric artery flow-induced vasodilation ↓ WD-induced aortic endothelial 3-nitrotyrosine immunostaining ↓ WD-induced aortic pERK immunoblot, and osteopontin, FGF23 immunostaining ↑ M2: M1 marker ratios (↓M1 markers CD86, and CD11c; ↑M2 markers CD206 and IL10) ↓ WD-induced aortic ENaC expression	↓WD-induced aortic stiffness (evaluated by pulse wave velocity and atomic force microscopy), aortic endothelial dysfunction, aortic medial thickening, and fibrosis
VSMC MRKO		
McCurley et al. [101], 2012 Tamoxifen-induced MRf/f/SMA-Cre-ERT2+ and tamoxifen-induced MRf/f/ SMA-Cre-ERT2− littermate controls with no alterations in feeding or growth	↓ Mesenteric artery L-type Ca-channel Cav1.2 expression ↓ AngII-induced ROS production	Age-dependent decrease in systolic blood pressure, becoming significant at 7 months of age ↓ Age-related cardiac hypertrophy ↓ Mesenteric artery myogenic tone ↓ AngII-induced pressor response
Amador et al. [102], 2016 CsA-induced nephrotoxicity model	↑ CsA-induced NGAL expression, marker of tubular injury ↓ CsA-induced pMLCK:MLCK, pMLC2:MLC2 ratios, markers of SMC contractility ↓ CsA-induced L-type Ca-channel Cav1.2 activation	SMC MR inactivation but not EC MR inactivation prevents cyclosporine-induced uremia, creatininemia, and tubular vacuolization ↓ Renal vasculature AngII and KCl-induced contractile response
Galmiche et al. [103], 2014 NAS hypertension model	↓ NAS-induced carotid α5-integrin expression	↓ NAS-induced carotid stiffness No changes in vascular structure
Pruthi et al. [104], 2014 Wire-induced carotid injury model and aldosterone enhanced vascular fibrosis model	↓ Wire injury-induced VEGFR1 expression ↓ Aldo-induced PlGF expression	Prevented aldosterone-induced 79% increase in SMC proliferation post wire injury Prevents aldosterone-induced vascular fibrosis post wire injury ↓Injury-induced medial hypertrophy

Table 1. (continued)

Author, model	Cell-specific effects of MRKO	Phenotype
Cardiomyocyte MRKO Rickard et al. [105], 2012 Uninephrectomized DOCA-salt	↑ Cardiac MMP9: TIMP-1 mRNA expression ratio ↓ DOCA-salt-induced cardiac PAI-1, CCR5, Nox2, p22Phox, TGFβ1, VEGF, VEGFR2, MCP1, CD14, CD81 expression ↓ DOCA-salt-induced cardiac macrophage infiltration, CD45+ leukocytes, CD8+ T cells	↓ DOCA-salt-induced cardiac fibrosis, positive inotropic state as assessed by Langendorff apparatus measured pressure and contraction/relaxation times
Fraccarollo et al. [106], 2011 Cardiomyocytes LCA ligation model	↓MI-induced collagen, ACE, CTGF, fibronectin, periostin, vimentin expression ↓MI-induced NADPH oxidase subunits Nox2, Nox4, and chronic MI-induced mitochondrial ROS production ↑ Acute MI-induced NF-κB activation ↓ IκBα and cardiomyocyte apoptosis (TUNEL assay) ↑ Neutrophil and macrophage infiltration 1 day post-acute MI	↓Post-MI rightward shift in PV loop, infarct area expansion, Post-MI cardiac hypertrophy, and attenuation of progressive LV dilation ↑ Scar thickness, post-MI ejection fraction, post-MI capillary density at 1 day post-acute MI
Lother et al. [107], 2011 Cardiomyocytes Chronic left ventricular pressure overload by TAC model	↑ TAC-induced ANP, βMHC gene expression ↑ ERK1/2 phosphorylation and ↓ TAC-induced increase in SGK1 mRNA expression	Prevented TAC-induced ventricular dilation Prevented TAC-induced LV wall stress Prevented TAC-induced decline in ejection fraction TAC led to cardiac fibrosis and was unaffected by MRKO
CD4+ T cell MRKO Li et al. [108], 2017 T cells AAC mouse model	↓ Cardiomyocyte hypertrophy ↓ Cardiac CD11b+Ly6C+, CD11b+Ly6C-, Ly6Chi, macrophage, neutrophil, CD4+ & CD8+ T cell, CD4+CD69+, CD8+CD69+, CD4+CD44hiCD62low, CD8+CD44hiCD62low T cell infiltration, ANP, βMHC, collagen I/III, CTGF, TGFβ1 expression ↓ Anti-CD3 stimulated IL2, IFNγ, and IL6	↓ AAC-induced cardiac hypertrophy, fibrosis, LV dilation Improved ejection fraction
Sun et al. [66], 2017 T cells AngII-induced hypertensive mouse model	↓ AngII-induced renal CD11b macrophage, CD4+ and CD8+, CD4+IFNγ+, CD8+IFNγ+ T cell infiltration, ↓ NGAL, osteopontin, MCP1, and VCAM1 ↓ AngII-induced aortic macrophage infiltration, CD4+ and CD8+, CD4+IFNγ+, and CD8+IFNγ+ T cell infiltration ↓ RANTES & MCP1	↓ AngII-induced hypertension, glomerular hypertrophy, renal fibrosis, albuminuria, aortic fibrosis, endothelial dysfunction

AAC, abdominal aortic constriction; Ach, acetylcholine; AngI, angiotensin I; ACE, angiotensin I converting enzyme (peptidyl-dipeptidase A) 1; Aldo, aldosterone; ANP, natriuretic peptide precursor type A; Arg1, arginase 1; BNP, natriuretic peptide precursor type B/brain natriuretic peptide; Cav1.2, calcium channel, voltage-dependent, L type, alpha 1C subunit; CCL, chemokine (C-C motif) ligand; Cidea, cell death-inducing DNA fragmentation factor; alpha subunit-like effector A; COX, cytochrome c oxidase; CTGF, connective tissue growth factor; CsA, cyclosporine A; DOCA, deoxycorticosterone acetate; CXCL, chemokine (C-X-C motif) ligand; EC, endothelial cell; ENaC, endothelial sodium channel; ER, estrogen receptor; ERK, extracellular regulated MAP kinase; F13a1, coagulation factor XIII, A1 subunit; ESR1, estrogen receptor 1; FIZZ1, found in inflammatory zone 1; HFD, high fat diet; HGF, hepatocyte growth factor; IFNγ, interferon gamma; IL, interleukin; L-NAME, L-NG-nitroarginine methyl ester; MMP, matrix metalloproteinase; MnSOD, manganese superoxide dismutase; MCP1, chemokine (C-C motif) ligand 2; M2, macrophage phenotype 2 – alternatively activated; βMHC, beta myosin heavy chain; MIP1β, chemokine (C-C motif) ligand 4 (CCL4); NGAL, lipocalin 2; MRC2, mannose receptor, C type 2; NAS, Nephrectomy–aldosterone–salt; ND, normal chow diet; Nox2, NADPH oxidase 2/cytochrome b-245, beta polypeptide; NOX4, NADPH oxidase 4; PAI-1, plasminogen activator inhibitor–1; RANTES, chemokine (C-C motif) ligand 5; SGK1, serum/glucocorticoid regulated kinase 1; TAC, transverse aortic constriction; ROS, reactive oxygen species; SCD1, stearoyl-Coenzyme A desaturase 1; TNF, tumor necrosis factor; TGF, transforming growth factor; TIMP1, tissue inhibitor of metalloproteinase 1; VCAM1, vascular cell adhesion molecule 1; VEGF, vascular endothelial growth factor; VEGFR, endothelial growth factor receptor; VSMC, vascular smooth muscle cell; WD, western diet.
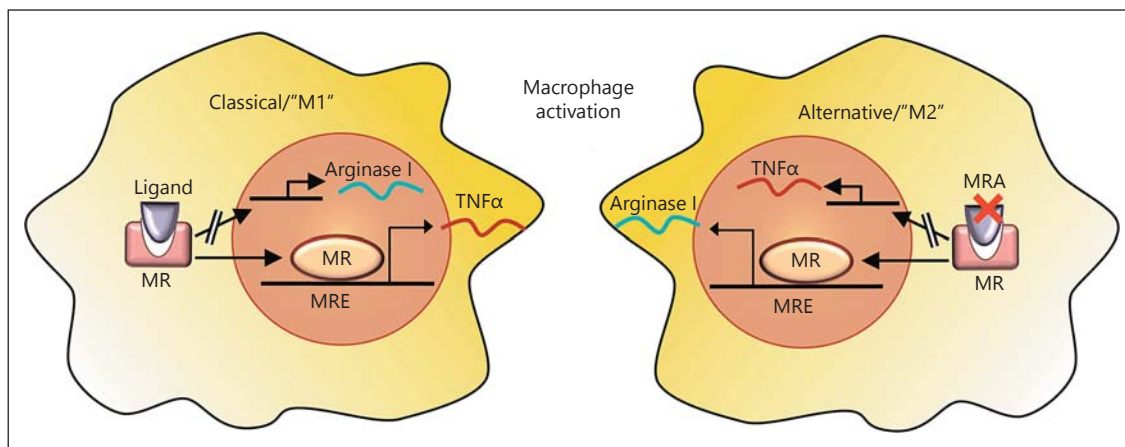
Fig. 2. Mineralocorticoid receptor agonism (ligand binding, left side) increases the classical activation of macrophages, while antagonism MRA promotes an alternative activation (right side).
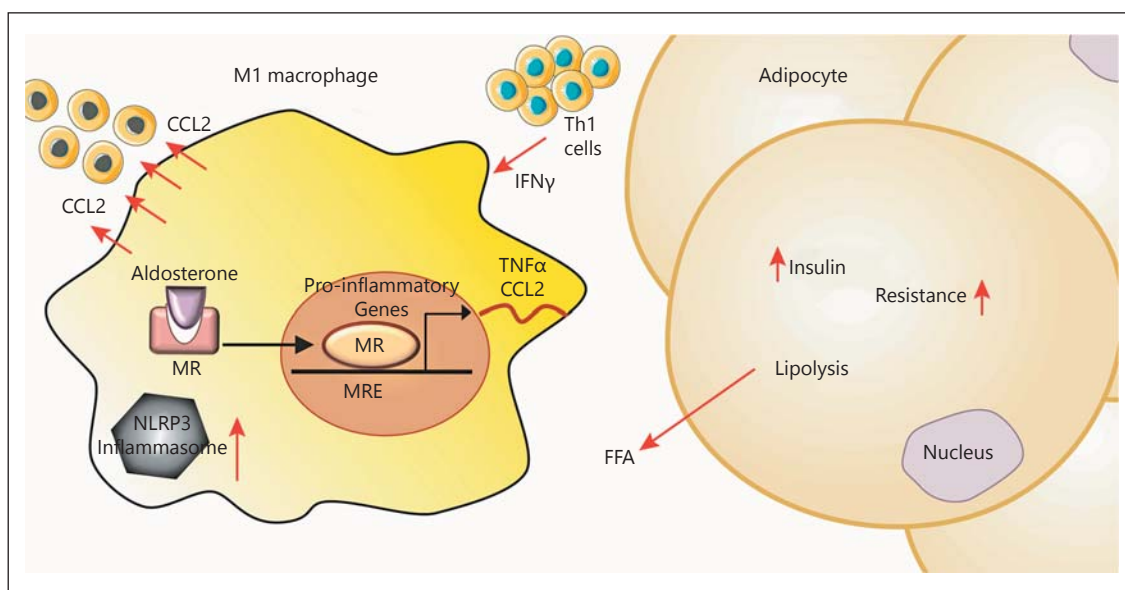

Fig. 3. Aldosterone stimulation of adipose tissue macrophages may potentiate local tissue inflammation exacerbating insulin resistance, diabetes, and atherosclerotic processes.

small GTPase, is a novel modulator of MR activity and demonstrated the pathological role of Rac1-mediated MR activation in the kidney and in salt-sensitive hypertension [58]. In prior studies, overexpression of constitutively active mutant Rac1 in rat cardiomyocytes promoted nuclear accumulation of MR and increased MR-dependent transcriptional activity regardless of the ligand level implicating a potential contribution of Rac1-MR signaling in cardiac diseases [16, 41]. Rac1 associates with Nox isoforms such as Nox4 (present in endothelial cells) rather than Nox2 (present in macrophages and neutro-

phils), at least in the myocardium and contributes to heart failure in response to pressure overload hypertrophy. Thus, the activation of Rac1 and subsequent activation of MR in addition to ROS generation through Nox4 may contribute to tissue injury and transition to heart failure [41]. cJun N-terminal kinases (JNK) may also be a downstream target of MR, as bone marrow-derived macrophages exposed to LPS (classic type 1 proinflammatory mediator) significantly increased the phosphorylation of JNK, while phosphorylation of JNK is attenuated in MR-null bone marrow-derived macrophages. Analysis of oth-

er MAPK pathways such as p38 and ERK1/2 showed equivalent phosphorylation. In this study there were no differences in phosphorylation of the NFκB pathway and IκBα.

Evidence for Inflammasome Activation in Myeloid Cells in Response to Aldosterone and MR Involvement

Chronic inflammation caused by inflammasome activation is involved in many diseases including atherosclerosis, diabetes, and obesity (Fig. 3). Recently, a role for inflammasome activation in kidney injury via MR activation has been proposed; aldosterone stimulates various components of the inflammasome complex and products of inflammasome activation (IL18) lead to podocyte injury while blockade of MR reverses these effects [59]. Mice deficient in apoptosis-associated speck-like protein (ASC) had reduced renal fibrosis and inflammation without affecting macrophage numbers. Bone marrow transplantation using ASC-deficient mice marrow reduced inflammasome activation, implicating myeloid cell-derived ASC in tubulointerstitial damage and subsequent fibrotic changes in the kidney [60]. The mechanism of activation was attributed to mitochondrial-driven ROS as mitochondrial-directed antioxidant mito-TEMPO interrupted caspase activation in response to aldosterone in cultured macrophages [60]. A model of anti-glomerular basement membrane glomerulonephritis (anti-GBM GN) was used to interrogate the contribution of podocyte versus myeloid MR. The absence of MR in podocytes did not reduce immune injury in anti-GBM GN. In contrast, injury glomerular crescents, myofibroblast accumulation, and gene expression of profibrotic molecules (COL1A1, FN1, PAI-1) were all decreased in MyMRKO mice versus WT [61].

Role of Central Nervous System MR in Regulation of Peripheral Inflammation

MR activation in the brain has been linked to sympathetic hyperactivity and an increase in peripheral tissue aldosterone levels, while central MR blockade attenuates sympathetic hyperactivity [62, 63]. In an interesting study, the time course of macrophage infiltration and apoptosis in the heart in response to central MR blockade (intracerebroventricular infusion of eplerenone, 5 μg/day) was evaluated post-myocardial infarction. Central MR blockade significantly decreased CD80-positive pro-inflammatory M1 macrophages and increased CD163-positive anti-inflammatory M2 macrophages in the infarct. Central MR blockade also reduced apoptosis of myocytes by 40–50% in the peri-infarct zone [64].

Role of the MR in T Cells

T lymphocytes play an important role in target organ damage in hypertension and atherosclerosis. Indeed, MR in T cells is critical in mediating fibrosis in the heart and kidney. T cell deficient in MR reduces ventricular remodeling in hypertension caused by trans-aortic constriction. This was associated with reduced T cell activation markers and inflammation in heart. Activation of MR resulting in increased Th17 T cells could contribute to cardiac fibrosis. Conversely, treatment with IL-17 blocking antibodies prevented DOCA/salt induced cardiac and renal fibrosis in rats [65]. Recently, it has also been shown that MR may interact with a critical transcription factor in T cells, NFAT1, and activator protein-1 to control interferon gamma in T cells and regulate BP and target organ damage in an AngII-infusion murine model [66]. MRA (mineralocorticoid receptor antagonism) by eplerenone and T cell-specific MR ablation (TMRKO) in mice resulted in reduced abdominal aortic constriction (AAC)-induced cardiac hypertrophy, with reduced measures of cardiac fibrosis (% fibrotic area, βMHC, collagen I/III, connective tissue growth factor, and TGFβ1; Table 1) [67]. Measures of cardiac function (e.g., LV end-systolic volume) were partially preserved in TMRKO-AAC versus WT-AAC and LV-dilation/LVH was attenuated. Post-ACC cardiac neutrophil and monocyte/macrophage (CD11b$^+$Ly6G$^+$; CD11b$^+$Ly6C^{hi} respectively) content was dramatically lower in TMRKO mice.

Role of the MR and Effect of MR Antagonism in the Treatment of Cardiovascular and Renal Diseases

Pharmacological and clinical studies over >20 years have defined the importance of the MR in hypertension and heart failure which will not be discussed here. There are studies that demonstrated an impact of MRA in patients with risk factors, with improvement in surrogate outcomes related to LV mass and hypertrophy (Table 2).

Effect of MRA in Cardiac Hypertrophy, Fibrosis, and Diastolic Dysfunction

Online supplementary Table details studies involving MRA that have demonstrated efficacy in reducing fibrosis and hypertrophy in a variety of animal models irrespective of aldosterone levels. A consistent effect of MRA is its impact on fibrosis and reduction of left ventricular

Table 2. Cardiovascular effects of MRA in humans

Author, study cohorts	Patient population	End point, measurements	Results
Pitt et al. [109], 2003 n = 202 Duration: 9 months	Patients with left ventricular hypertrophy and hypertension (mean age 58 ± 12 years) Eplerenone 200 mg/day; enalapril 40 mg/day; eplerenone 200 mg/day, and enalapril 10 mg/day	Change in left ventricular mass (MRI), changes in systolic and diastolic BP	Eplerenone significantly reduced LV mass from baseline (p = 0.001); eplerenone-enalapril combination was more effective than eplerenone alone (p = 0.007) Eplerenone significantly reduced systolic and diastolic BP from baseline and more effectively than eplerenone alone (p = 0.048)
Kosmala et al. [69], 2013 n = 113 Duration: 6 months	Patients with BMI ≥30 without comorbidities with impaired early diastolic mitral annular velocity (mean age 58 ± 8 years) Spironolactone 25 mg/day; placebo	LV systolic and diastolic function, myocardial reflectivity and serological fibrosis markers (procollagen type III N-terminal propeptide [PIIINP] and procollagen type I C-terminal propeptide [PICP])	Significant reduction in PICP (p = 0.04), E/e' ratio (p = 0.005), LV systolic function (p = 0.02), and myocardial reflectivity (p = 0.02)
Montalescot et al. [110], 2014 n = 1,012 Duration: 10.5 months	Patients with acute STEMI without a history of HF receiving standard therapy Eplerenone 25–50 mg/day in addition to standard therapy; versus standard therapy control (mean age 58 ± 11 years)	Composite of CV mortality, rehospitalization or extended initial hospital stay due to diagnosis of HF, sustained ventricular tachycardia or fibrillation, LVEF ≤40% or elevated BNP/NT-proBNP levels (primary end point)	The incidence of primary end point was reduced by 42% in the eplerenone group relative to control (p < 0.0001) Primary end point difference was driven by increased BNP/NT-proBNP in control group Adverse event rates were similar in both groups
Garg et al. [111], 2014 n = 64 Duration: 6 months	Patients with diabetes on chronic ACE inhibition (enalapril 20 mg/day, mean age 55 years) Spironolactone 25 mg/day; HCTZ 12.5 mg/day; placebo	Coronary flow reserve (CFR) was assessed by cardiac PET at baseline, and at the end of treatment	Spironolactone significantly improved CFR compared to control (p = 0.04)
Renal Effects of MRA			
Epstein et al. [112], 2006 n = 268 Duration: 12 weeks	Patients with diabetes mellitus and UACR ≥ on enalapril (50 mg/day) (mean age of 59 years) Eplerenone 50 mg/day, 100 mg/day or placebo on top of Enalapril therapy	Percentage change from baseline in UACR and incidence of hyperkalemia (primary end points)	By week 12, UACR was reduced 7.4% in placebo group compared to 41.0% in eplerenone (50 mg) group (p = 0.001) and 48.4% in eplerenone 100 mg group (p = 0.001). No significant difference in the incidences of sustained and severe hyperkalemia
Bianchi et al. [113], 2006 n = 165 Duration: 1 year	Patients with CKD receiving ACE inhibitors and/or ARBs (mean age 59 years) Spironolactone 25 mg/day on top background therapy compared with background treatment alone	Proteinuria and eGFR	Spironolactone treatment significantly reduced proteinuria from 2.10 to 0.89 g/g creatinine (p < 0.001), and did not change in patients receiving ACE inhibitors and ARB alone By the end of 1 year, the monthly rate of the decrease in eGFR from baseline was lower in patients treated with spironolactone than in controls

Table 2. (continued)

Author, study cohorts	Patient population	End point, measurements	Results
Furumatsu et al. [114], 2008 $n = 32$ Duration: 1 year Trichloromethiazide 1 mg/day or furosemide 20 mg/day in addition to enalapril 5 mg/day and losartan 50 mg/day	Patients with nondiabetic nephropathy and proteinuria >0.5 mg/day after receiving an ACE inhibitor and ARB in combination for more than 12 weeks (mean age 52 ± 3 years) Spironolactone 25 mg/day in addition to enalapril 5 mg/day and losartan 50 mg/day (triple blockade)	Proteinuria, urinary type IV collagen level	Urinary protein level decreased by 58% in triple blockade group compared with no change in the control group ($p < 0.05$) Urinary collagen IV levels decreased by 40% from baseline in triple blockade group compared with no change in the control group ($p < 0.05$)
Mehdi et al. [115], 2009 $n = 81$ Duration: 48 weeks	Patients with diabetes mellitus, hypertension and UACR ≥300 mg/g receiving Lisinopril 80 mg/day Spironolactone 25 mg/day; losartan 100 mg/day; placebo	Urine albumin to creatinine ratio, clinic and ambulatory BP, creatinine clearance, and glycemic control	Spironolactone group UACR decreased 34.0% ($p = 0.007$ vs. placebo) Blood pressure, creatinine clearance, and glycemic control did not change significantly between groups
Boesby et al. [116], 2011 $n = 40$ Duration: 8 weeks	Patients with nondiabetic CKD and urinary albumin excretion greater than 300 mg/day Eplerenone 25–50 mg/day Mean age 45 years	24-h urinary albumin excretion, BP, plasma potassium, creatinine clearance	Eplerenone treatment lowered mean urinary albumin excretion by 14% ($p = 0.008$)
Ando et al. [117], 2014 $n = 304$ Duration: 1 year	Hypertensive patients with albuminuria (UACR = 30–599 mg/g), eGFR >50 mL/min who had received ACE inhibitor or ARB or both for ≥8 weeks Eplerenone 50 mg/day vs. placebo	Mean percent change in UACR	UACR decreased by 27.6% in eplerenone treatment group ($p = 0.022$ vs. placebo)
Matsumoto et al. [118], 2014 $n = 309$ Duration: 3 years	Patients with oligo-anuria undergoing hemodialysis (mean age 67 years ± 12 years) Spironolactone, 25 mg/day or placebo	Composite of death from CV causes and hospitalization for CV causes (primary outcome), and death from all causes (secondary outcome)	Spironolactone decreased the rate of incidence of primary outcome by 62% relative to control ($p = 0.016$), and of the secondary outcome by 67% ($p = 0.003$)
Bakris et al. [91], 2014 $n = 821$ Duration: 90 days	Patients with diabetes and high or very high albuminuria receiving ACE inhibition or ARB (mean age 64 years) Finerenone 1.25–20 mg in graded dose increments (7 different doses) or placebo	Ratio of urinary albumin-creatinine ratio (UACR) at day 90 to baseline	UACR was reduced relative to baseline at each dosage: 7.5 mg/day, 0.79 ($p = 0.004$) 10 mg/day, 0.76 ($p = 0.001$) 15 mg/day, 0.67 ($p < 0.001$) 20 mg/day, 0.62 ($p < 0.001$)

UACR, urine albumin creatinine ratio; ACEI, angiotensin converting enzyme inhibitor; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide; PET, positron emission tomography.

hypertrophy. In humans, MRA reduced LVH in patients on top of ACE inhibition [68]. While these effects may rely on BP reduction, data from animal models seem to support an effect that may occur independently of BP. A small study in obese patients demonstrated that MRA (spironolactone) for 6 months can improve diastolic dysfunction, subclinical markers of systolic dysfunction (global longitudinal strain), a surrogate marker of myocardial fibrosis (integrated backscatter, echo) and circulating markers of fibrosis (PICP) [69]. Although results from the Treatment of Preserved Cardiac Function Heart Failure with an aldosterone antagonist trial were negative, subset analysis suggests benefits in patients with evidence of definitive heart failure who were medication compliant [70–72].

Effect of MRA in Atherosclerosis

Proof-of-concept experiments from our group using eplerenone in a rabbit model of atherosclerosis provided one of the first lines of evidence that MRA may improve vascular function and redox stress independent of aldosterone levels. New Zealand white male rabbits were fed 1% cholesterol chow (HL) or normal chow for 6 weeks to induce endothelial dysfunction and then randomized to receive eplerenone or placebo (100 mg/kg) for 6 additional weeks [56]. Eplerenone normalized peak endothelium-dependent relaxation and reduced O_2^- in the aorta of high cholesterol fed animals. A number of studies have demonstrated an impact of MRA in reducing atherosclerosis in mouse models, including reduction of inflammatory cell infiltration, increased M2 markers, smooth muscle proliferation, and reduced pro-inflammatory cytokines in plaques (online suppl. Table). Studies in monkeys have also demonstrated important effects of eplerenone in reducing aortic intimal volume (intravascular ultrasound) and improving acetylcholine-induced vasorelaxation [73]. In models of experimental thrombosis, aldosterone enhances thrombosis while spironolactone reverses this effect [74]. In recent studies, $LDLR^{-/-}$ chimeric mice with bone marrow cells from floxed (control) mice or from myeloid $MR^{-/-}$ demonstrated reduced atherogenesis, suggesting that MR in myeloid cells likely promotes atherogenesis. Further chimeric $ApoE^{-/-}$ mice with myeloid MRKO also exhibited reduced atherogenesis in response to AngII, an effect mediated in part by reduced foam cell formation and enhanced cholesterol efflux [45].

While the role of MRA in secondary prevention in patients with post-myocardial infarction and symptoms of heart failure is well known, there are data suggesting that use of MRA early post-MI in patients with STEMI and LV dysfunction may be beneficial [75]. In the REMINDER study ($n = 1,012$), in patients with STEMI without HF, eplerenone reduced the composite end point of CV mortality, rehospitalization, extended hospital stay, due to HF, sustained ventricular tachycardia or fibrillation, ejection fraction ≤40%, or elevated BNP/NT-proBNP at 1 month. The end point was primarily driven by persistent elevation of BNP/NT-ProBNP in the placebo group. In contrast to earlier studies such as EMPHASIS-HF, this was a low risk population without HF or low EF with a low event rate (0.4% morality rate through the trial). Further the drug was administered early on after presentation, with the first dose of study drug administered within 24 h of the onset of symptoms of acute MI and preferably within 12 h. There is limited data supporting a role for aldosterone in the progression of atherosclerosis. In human studies, polymorphisms of the aldosterone synthase gene (Cyp11β2) have been associated with plaque size on MRI. Plasma aldosterone levels have been associated with nonfatal cardiovascular events and CV death. In a study of 848 patients, plasma aldosterone was the only independent predictor of plaque progression (carotid ultrasound) in the first 2 years of the study [76].

Effect of MRA on Proteinuria and Progression of Chronic Kidney Disease

The beneficial impact of renin-angiotensin-aldosterone system (RAS) blockade with ACE-I and ARB in both diabetic and nondiabetic chronic kidney disease (CKD) has been demonstrated in multiple animal models and human studies (online suppl. Table). Two previously published meta-analysis studies published initially in 2009 and updated in 2014 demonstrated that the addition of MRA to RAS blockade reduced BP and proteinuria in CKD [77, 78]. An updated meta-analysis that included previously unpublished data as well as including data from 3 studies which were not considered in the previous meta-analysis supported previous findings [79]. A total of 19 trials (1,646 patients) were included, of which 8 were done in patients with diabetic nephropathy. Fourteen (889 patients) compared spironolactone plus ACE-I or ARB with ACE-I or ARB alone, and 5 trials (757 patients) compared eplerenone plus ACE-I or ARB to ACE-I or ARB alone. The follow-up period of included trials was <1 year and the mean baseline eGFR was >35 mL/min/1.73 m², therefore the impact of addition of MRA to

RAS blockade on long-term renal outcomes or mortality in the later stages of CKD cannot be evaluated. In random effects meta-analysis, addition of MRA to RAS inhibitors resulted in a reduction in systolic BP from baseline of –5.7 and diastolic BP of –1.7 mm Hg, respectively. The GFR fell by –3.2 mL/min/1.73 m^2. MR antagonism reduced the weighted mean protein/albumin excretion by 38.7%. MRA was associated with 3-fold increased risk of hyperkalemia above the predefined trial limit. Diabetic CKD patients, however, were not at a greater risk of developing hyperkalemia than patients with CKD of alternative etiology ($p = 0.38$). Number needed to harm for 1 year of treatment, calculated from trials reporting at least one case of hyperkalemia, was 10 (95% CI 5–27). The addition of MRA to RAS blockers led to a moderate increase from baseline potassium compared to ACE-I and/or ARB alone, both at end-of-trial visit (0.19 mmol/L [95% CI 0.07–0.31]; 16 trials; $n = 1,356$; $I^2 = 83.8\%$).

MRA and Insulin Resistance and Type 2 Diabetes

A number of reviews have already detailed in vitro, experimental and human evidence linking aldosterone/MR activation with IR [80–82]. The visceral adipose RAS system synthesizes aldosterone, expresses MR, and predicts IR in both humans and animal models [82]. Adipocyte overexpression of MR results in metabolic syndrome and enhanced vascular contractility, and suggests an independent contribution outside of MR in inflammatory cells in adipose [83]. In patients with nondiabetic stages 2–5 CKD, treatment with spironolactone ameliorated insulin resistance. In the same study, insulin resistance in nephrectomized rats was improved with spironolactone presumably via adipose overexpression of the rate limiting enzyme for aldosterone, CYP11β2 and the downstream effector of MR, SGK-1 [84]. Spironolactone has also been shown to prevent insulin resistance in response to diuretics [85]. However, treatment of individuals with uncomplicated obesity over 6 weeks with spironolactone 50 mg, did not appear to improve insulin sensitivity index assessed by Matsuda method. It is possible that this study was performed in metabolically healthy obesity, although the insulin sensitivity index was <5 indicating potential insulin resistance [86]. MR activation may affect IR through multiple mechanisms that include attenuation of insulin signaling in the heart, vasculature, and skeletal muscle. This may include impairment of expression of insulin receptor and substrate, decreased GLUT4 expression, abnormal phosphorylation of IRS, and activation of multiple stress kinases downstream of insulin receptor/IRS leading to the attenuation of insulin signaling [87]. Recently, caveolin-1 appears to be an additional mediator of MR action. Caveolin-1 knockout mice exhibit features of insulin resistance and oxidative stress with the effects being ameliorated by MR blockade. In humans, individuals with a specific mutation of caveolin-1 also appear to be more insulin-resistant [88].

Conclusion

Based on the central role MR plays in the pathogenesis of target organ damage, there is optimism that the use of MRA could benefit patients with a variety of cardiometabolic diseases to prevent complications. The advantages of MRA use for a broad spectrum of patients are far beyond theoretical and are supported by a vast body of data over the last 5 decades. However, there are challenges in the use of these agents. The identification of patients who may truly benefit from MRA is an important issue, as demonstrated in Treatment of Preserved Cardiac Function Heart Failure [71, 89]. Additionally, hyperkalemia is a concern, particularly in patients with advanced CKD. Whether the use of nonsteroidal MRA's can minimize hyperkalemia, while allowing continued benefit of MRA is an exciting area of investigation. Quantitative whole-body labeling studies with [^{14}C]-labeled finerenone, a novel nonsteroidal MRA, show equal distribution in heart and kidney tissues in contrast to disproportionate renal deposition for steroidal MRAs such as eplerenone or spironolactone, an aspect believed to be important in the lower incidence of hyperkalemia despite comparable IC$_{50}$ (24 nM for spironolactone vs. 18 nM for finerenone) [90]. Different dose strengths of finerenone have been investigated in 823 randomized patients with type 2 diabetes mellitus (T2DM) and diabetic kidney disease receiving standard of care (i.e., ACEIs/ARBs) and either once-daily finerenone or placebo [91]. FIGARO-DKD (NCT2545049, $n = 6,400$) and FIDELIO-DKD (NCT2540993, $n = 4,800$) are 2 randomized, double-blind, placebo-controlled, parallel-group, multicenter, phase 3 studies that investigate the safety and efficacy of finerenone in the reduction of cardiovascular morbidity and mortality, and progression of CKD in subjects with T2DM and diabetic kidney disease. Apararenone, a novel nonsteroidal MRA (MT-3995), is being tested in nonalcoholic steatohepatitis (NCT2923154) and in diabetic nephropathy (NCT2676401). The Mineralocorticoid Receptor Antagonism in Diabetic Atherosclerosis study will test the util-

ity of MR antagonism in patients with T2DM with CKD, at high risk for cardiovascular complications [92]. The co-primary efficacy end point will be percentage change in total atheroma volume in thoracic aorta and left ventricular mass at 52 weeks in patients treated with spironolactone versus placebo. Secondary outcomes include 24-h mean systolic BP, central aortic BP, and insulin resistance at 6 weeks. A novel measure in the study will be changes in candidate miRNAs that regulate expression of NR3C2 (MR gene) as well as measuring monocyte/macrophage polarization in response to therapy with spironolactone. These studies may extend the utility of MRA and make these agents attractive adjuncts to statins in the prevention of cardiometabolic complications.

Disclosure Statement

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