Mineralocorticoid receptor activation and blockade: an emerging paradigm in chronic kidney disease

Jean-Philippe Bertocchio[1], David G. Warnock[2] and Frederic Jaisser[1]

[1]*Centre de Recherche des Cordeliers, INSERM U872 Team 1, Paris, France and* [2]*Division of Nephrology, Department of Medicine, University of Alabama at Birmingham, Birmingham, Alabama, USA*

Slowing the progression of chronic kidney diseases (CKDs) requires new and effective treatment approaches. Aldosterone classically acts on the distal nephron: it facilitates sodium reabsorption, potassium secretion, and participates in blood pressure control. Recently, new targets of aldosterone have been described including the heart and the vasculature, and other kidney cells such as mesangial cells, podocytes, and renal fibroblasts. The pathophysiological implications of increased mineralocorticoid receptor (MR) expression and activation (either dependent on aldosterone or direct ligand-independent activation) and its blockade have been illustrated with *ex vivo* in cell cultures and *in vivo* in experimental animal models of CKD, including diabetic and hypertensive nephropathies, and glomerulopathies. The beneficial effects of the MR antagonists are independent of the hypertensive effect of aldosterone, indicating that blocking the activation of the MR may have unique clinical importance. Several studies have reported efficacy and safety studies with spironolactone or eplerenone in patients with kidney diseases. In this review, we discuss the recent results reported in experimental and clinical research in this field, and emphasize the direct activation of the MR that can occur in pathological states associated with CKD, even in the absence of increased circulating levels of aldosterone.

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Correspondence: *Frederic Jaisser, Centre de Recherche des Cordeliers, INSERM U872 Team 1, 15 rue de l'Ecole de Médecine, Paris 75270, France. E-mail: frederic.jaisser@inserm.fr*

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In 1955, Conn[1] described a young female with primary aldosteronism and proteinuria. In 1964, he described the first 145 cases of proven primary aldosteronism: proteinuria was present in 85% of the patients.[2] This proteinuria was attributed to hypertension present in Conn's syndrome until animal studies showed that mineralocorticoid hormones, especially aldosterone, could cause proteinuria in absence of hypertension.[3] In 2001, a brief report of the use of a mineralocorticoid receptor (MR) antagonist in eight proteinuric patients was published; a 54% decrease in proteinuria was reported after 4 weeks of treatment with spironolactone.[4] Several additional studies have explored the role of aldosterone and MR in proteinuria, and in the progression of chronic kidney diseases (CKD).

Prospective randomized controlled trials have demonstrated reduction in mortality in patients with severe heart failure,[5] for those who develop heart failure following acute myocardial infarction,[6] and those with mild heart failure[7] who are treated with MR blockers (MRBs). Patients with CKD have not been included in these large-scale prospective outcome studies, primarily because of concerns about hyperkalemia. The aim of this review is to consider the implications of aldosterone, the MR, and MRBs in the progression and treatment of CKD, and solidify the base upon which prospective outcome studies of CKD can be based.

NEW CONCEPTS OF THE PATHOPHYSIOLOGICAL ROLE OF ALDOSTERONE

Aldosterone controls sodium reabsorption and potassium secretion in the distal nephron, and has a major role in volume and blood pressure homeostasis. The hormone functions in the distal nephron after binding to the MR, a ligand-dependant transcription factor, which binds to specific hormone response elements.[8] Transepithelial sodium reabsorption is regulated acutely via increased expression/activity of the apical epithelial sodium channel, and in the longer term by changes in the expression of the basolateral Na^+/K^+-ATPase.[9,10] Membrane expression of the sodium channel is tightly controlled by aldosterone, through regulation of expression/activity of ubiquinylation pathways.[11] Aldosterone and glucocorticoids (corticosterone in rodents) have similar binding affinities for the MR. Aldosterone

specificity in the distal nephron is ensured by the expression and activity of the enzyme 11-β-hydroxysteroid dehydrogenase type 2, which converts cortisol into cortisone (having a weak affinity for MR), preventing full MR occupancy by cortisol, despite plasma concentrations 100- to 1000-fold greater than those of aldosterone.[12]

Cloning of the MR[13] and development of specific antibodies permitted mapping of the MR expression in the distal nephron (convoluted distal tubule and collecting duct), distal colon, and sweat glands, all sites previously known as classical targets of aldosterone.[12,14] MR is also expressed in tissues and cell types wherein vectorial sodium transport does not occur, indicating novel and yet unknown roles of aldosterone and MR activation that does not serve whole-body sodium homeostasis. In some of these nonclassical targets (like vascular endothelium), MR is co-expressed with 11-β-hydroxysteroid dehydrogenase type 2 indicating that aldosterone is the preferred MR ligand in these cells.[15] In other targets (cardiomyocytes, vascular smooth muscle cells, neurons, adipocytes, and keratinocytes), the absence of 11-β-hydroxysteroid dehydrogenase type 2 expression suggests that the MR ligand is cortisol (or corticosterone) rather than aldosterone.[15,16]

Aldosterone defends the extracellular fluid volume during acute volume loss or salt depletion.[12,14] However, MR activation may become maladaptive,[17] with sustained activation inducing pathological consequences like hypertension, extracellular matrix remodeling, apoptosis, or inflammation. The 'coincidence' model proposes that adding two or more pathological inputs will have a synergic effect resulting in inappropriate activation of MR.[17] These triggers include dietary salt intake, which could affect cellular responses (cellular volume, membrane stiffness, and inflammation), oxidative stress (induced by angiotensin or other hormones), and ligand-independent MR activation. Therefore, during modest, but coincident increases of aldosterone or MR expression, oxidative stress, and sodium load will induce molecular and functional alterations and pathophysiology.

MRs are expressed in heart and blood vessels, posing the question of the pathophysiological role of MR activation in these nonclassical target tissues. The pharmacological model, in which unilateral nephrectomy and chronic aldosterone infusion are associated with a high salt load, induces an increase of blood pressure and pathological consequences with perivascular and extracellular matrix remodeling in kidney and heart.[18,19] The administration of nonhypotensive doses of MR antagonists, has clearly shown differential responses to hemodynamic effects and profibrotic, proinflammatory, and increased oxidative stress of aldosterone and/or activation of MR.[20]

MR expression is not fixed, but can be modulated in various pathophysiological contexts like diabetes,[21–23] CKD with heavy proteinuria,[24] cardiac failure,[25,26] myocardial infarction,[27,28] high blood pressure,[29] vascular aging,[30] or cerebral aneurysm.[31] It has also recently been shown that MR activation can occur independently of ligand. MR activation by Rac1 has been demonstrated in vascular endothelium and podocytes.[32,33] Post-translational modifications of MR like phosphorylation, as demonstrated for the glucocorticoid or estrogen receptors are also possible.[34] A further layer of complexity is offered by the possibility for intracellular 'cross-talk' between the MR and the angiotensin type 1 receptor and the epidermal growth factor receptor, meditated by phosphorylation cascades.[35–37] This novel concept requires that the traditional distinction[38] between 'genomic' and 'non-genomic' effects of aldosterone be viewed in a different perspective. These results indicate that MR activation does not necessarily require increases in circulating aldosterone levels, and can explain the beneficial effects of MR antagonism in various pathological conditions wherein circulating aldosterone levels are not elevated.

MINERALOCORTICOIDS AND RENAL DISEASES: EXPERIMENTAL AND CLINICAL EVIDENCE

MR expression in the kidney

MR expression has been demonstrated in the distal nephron (Figure 1).[12,14] MR immunolocalization has been challenging, but the generation of novel antipeptide monoclonal anti-MR antibodies by CE Gomez-Sanchez[39] has permitted MR immuno-detection in tissues where expression level is lower than in the distal nephron. MR is clearly expressed in vascular endothelial cells and in the vascular smooth muscle cells of inter-lobar arteries in the mouse kidney.[15] MR expression is not normally detected in the glomerulus, but has been demonstrated *ex vivo* in cultured podocytes,[33] mesangial cells,[40] and renal fibroblasts.[41] *In vivo*, MR appears to be expressed in nonclassical targets like podocytes or mesangial cells only during pathological conditions like type 1 diabetes in the rat,[42] and in spontaneous hypertensive rats with metabolic syndrome.[43]

Pathophysiological consequences of MR activation in experimental models of kidney disease

The renal consequences of MR activation have been described during aldosterone infusion, with suppression of aldosterone synthesis after adrenalectomy, and with pharmacological MR blockade (Figure 2).

Hypertensive nephropathy. MR blockade reduces the vascular changes induced by blockade of nitric oxide synthesis during high-sodium intake,[44,45] in the Dahl salt-sensitive rat,[46] in the stroke-prone spontaneous hypertensive rat,[47] and the ren2 transgenic rat.[48] These effects are observed without blood pressure reduction, indicating a nonhemodynamic protective effect of MR blockade. The underlying mechanisms include decreased oxidative stress, inflammation, and extracellular matrix. Podocytes injury is evidenced by decreased expression of podocin, synaptopodine, and nephrin, and increased apoptosis.[49] Intrarenal vessels develop angiosclerosis and microangiopathy secondary to MR activation.[44,50] Aldosterone modulates the expression of proinflammatory and profibrosis molecules, as well as

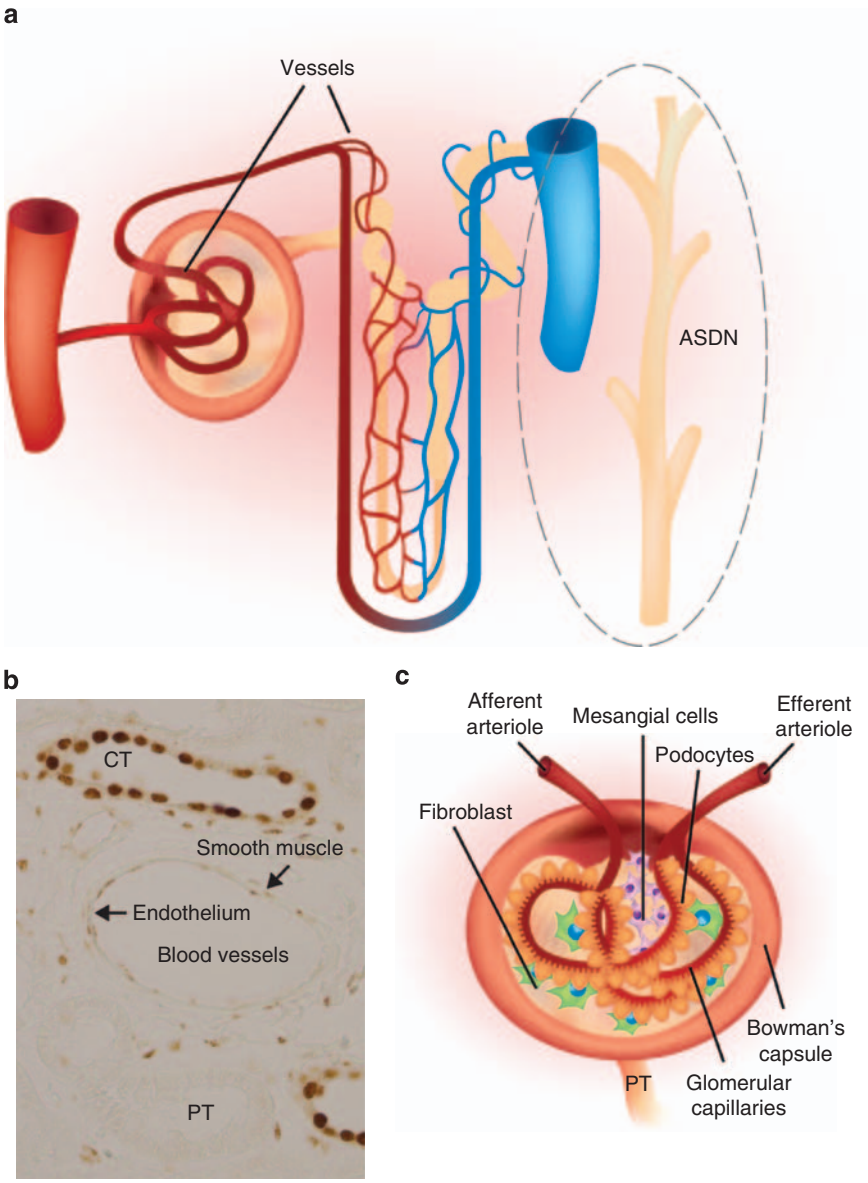

Figure 1 | Mineralocorticoid receptor expression in the kidney. (**a**) The classical target of aldosterone is the aldosterone-sensitive distal nephron (ASDN) but blood vessels have also been shown to express the mineralocortoid receptor (MR). (**b**) Immunolocalization of the MR in the kidney. In addition to collecting tubule (CT), both endothelial and smooth muscle cells physiologically express MR. (**c**) Proposed cellular targets in kidney diseases in the glomerulus: podocytes, mesangial cells, and glomerular capillaries. PT, proximal tubule.

components of the NADPH oxidase complex.[47,49] The endothelium is altered with increased expression of adhesion molecules as well as decreased nitric oxide synthase expression.[46] Eplerenone prevents aldosterone-induced Rac1 activation in the endothelium.[32] Altogether, these various targets could contribute to the beneficial effects of the MR antagonists in hypertensive nephropathy.

Chronic kidney disease. Decreased glomerular filtration, glomerulosclerosis, and proteinuria associated to subtotal nephrectomy are observed after aldosterone infusion[3] and are blunted by adrenalectomy[51] or spironolactone.[52] MR blockade slows the progression of preexisting lesions,[52] and prevents proliferation, interstitial fibrosis, and proteinuria in a model of glomerulonephritis,[53] in adriamycin-induced nephropathy,[54] and in a lupus nephritis model.[55]

Activation of the Rho kinase pathway and Rac1 could be involved in the progression of CKD.[33,46,48,56] In a transgenic model with constitutive activation of the Rac1/Rho kinase pathway presenting with nephrotic syndrome, MR blockade prevented the development of proteinuria in the absence of changes in arterial blood pressure.[33] MR may be a direct target for Rac1, which could modulate MR activation within the podocyte, even in the absence of increased circulating aldosterone.[33]

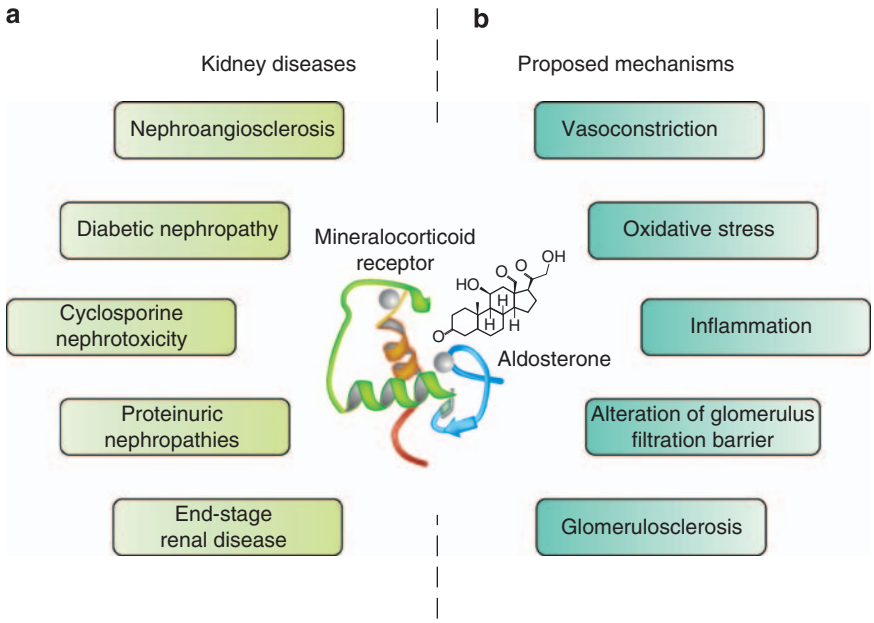

Figure 2 | Implication of aldosterone and the mineralocorticoid receptor (MR) in renal pathophysiology. (**a**) Aldosterone and/or mineralocorticoid receptor are involved in various kidney diseases in human and/or experimental models. (**b**) Proposed pathophysiological mechanisms involved in renal lesions linked to aldosterone/MR activation.

Diabetic nephropathy. The role of aldosterone and/or MR activation in diabetic nephropathy has been demonstrated in experimental models. Renal expression of MR is increased in animals with streptozotocin-induced type 1 diabetes[21,23,42] and in type 2 diabetes (db/db mice,[21] F. Jaisser, personal communication). Spironolactone has beneficial effects on glomerular lesions observed in models of type 1 diabetes,[21,23,57,58] which are associated with aldosterone—MR dependent podocyte apoptosis.[42] Pharmacological MR blockade prevents renal fibrosis and increased expression of transforming growth factor-β1, PAI-1, type 1 and 4 collagens, and fibronectin[23,57,58] as well as local oxidative stress.[58] Type 2 diabetes is associated in the rat and mouse models with renal lesions, including mesangial expansion, albuminuria, tubulointerstitial lesions, macrophage infiltration, inflammation, and increased expression of markers such as MCP-1, osteopontin, transforming growth factor-β1, and PAI-1.[21,40,42,57,59] Decrease podocin and nephrin expression reflects podocyte injury in these models.[40,43] These molecular or functional parameters are improved with MR blockade.[21,40,42,57,59]

Cyclosporine nephrotoxicity. Acute and chronic nephrotoxicity complicates the course of transplant patients treated with cyclosporine A (CsA). Acute nephrotoxicity, includes vascular effects (acute arteriolopathy), tubular damage (toxic tubulopathy), and occasionally, thrombotic microangiopathy.[9,60] Histological lesions related to chronic CsA nephrotoxicity are present after 10 years when analyzed with protocol biopsies.[61] Several irreversible changes have been described: vascular effects (hyaline arteriolopathy), tubular lesions (tubular atrophy and interstitial 'stripped' fibrosis), and glomerular lesions (enlarged Bowman's capsule and segmental and focal hyalinosis).[60] Angiotensin-converting enzyme-inhibitors (ACEI) or angiotensin II receptor blockers (ARB) have been used to limit renal function decline after transplantation.[60,62,63]

Renin activity is reduced during CsA treatment, presumably due to sodium retention and extracellular volume expansion.[64] Paradoxically, aldosterone and MR activation have been proposed as deleterious factors in this setting. However, circulating renin and aldosterone activities do not necessarily reflect tissue activation of MR.[65] Vasoactive and proinflammatory effects of aldosterone could be explain the beneficial effects of MR blockade during CsA nephrotoxicity.[62] Perez-Rojas *et al.*[66] reported that spironolactone prevented renal damage in a model of CsA nephrotoxicity. Vasoconstriction could be due to an 'over-activation' of MR as recently described in transgenic mice with increased MR expression in the vascular endothelium.[15] Endothelial MR regulates vasoconstrictor tone and blood pressure, so MR blockade could limit vasoconstriction and ischemia and thus reduce CsA nephrotoxicity. In rats, spironolactone improved survival,[66,67] prevented decreased renal function,[66–69] afferent arteriolopathy,[68] and interstitial fibrosis.[66–68] Eplerenone prevented renal dysfunction and hyaline arteriolopathy in the chronic CsA nephrotoxicity model.[70] In a model of allotransplantation in the rat, MR antagonism decreased allograft vasculopathy and macrophage infiltration.[69]

Renin–angiotensin–aldosterone system blockade and aldosterone breakthrough in CKD

The importance of renin–angiotensin–aldosterone system (RAAS) blockade for slowing the progression to end-stage renal disease is well recognized.[37] The effects of MR antagonists on the progression of CKD are less well described

than the effects of ACEIs and ARBs. The RALES,[5] EPHESUS,[6] and most recently, the EMPHASIS_HF[7] studies have clearly shown the beneficial impact on outcome of MR blockade in heart failure. Studies of the effects of MR antagonists on the progression of CKD are limited, without any outcome studies examining loss of kidney function, progression to end-stage renal disease, or cardiovascular death as the primary outcome.[37,71]

The use of MR antagonists in CKD is suggested by aldosterone 'breakthrough',[37,72,73] defined as an increase in circulating aldosterone after the initiation of a RAAS blockade as compared with baseline values, despite blockade of the effects of angiotensin II.[37] Aldosterone breakthrough occurs in 10–50% of the patients within 6–12 months after the initiation of a RAAS blocker.[74] This variability may reflect different definitions for the thresholds of plasma aldosterone increase. Furthermore, aldosterone breakthrough does not seem to be correlated with the class of treatment (ACEI or ARB) or the specific agent,[74] and is associated with increased proteinuria,[74,75] or a faster decline in kidney function.[73] Several studies have shown a direct relationship between increases in plasma aldosterone after ACEI treatment and increases in proteinuria and decreases in kidney function.[73,76] The underlying cause of aldosterone breakthrough is not fully defined, but a popular hypothesis invokes activation of non-ACEI enzymes that cleave angiotensin I in angiotensin II,[74] and stimulate aldosterone synthesis. This hypothesis does not explain the equivalent incidence of aldosterone breakthrough when ARBs are used compared with ACEIs. Studies of the angiotensinogen knockout mouse are also relevant.[77] RAAS was presumably totally ablated in this model, and aldosterone secretion was driven by serum potassium, especially during dietary salt restriction. Hyperkalemia needs to be evaluated as a potential contributor to aldosterone breakthrough in patients receiving RAAS blockade,[78] as potassium has long been recognized as an important stimulus for aldosterone secretion.[5,79]

Clinical studies of mineralocorticoids and kidney diseases

Conn[2] described the first prospective cohort of patients with primary aldosteronism, among whom 85% of the patients presented with proteinuria, which was attributed to hypertension and 'kaliopenic nephropathy'.[80] The Primary Aldosteronism Prevalence in Italy Study showed that patients presenting with hyperaldosteronism more often have proteinuria than control patients with essential hypertension.[81]

Plasma aldosterone levels have been correlated with alterations in kidney function in CKD,[82] suggesting an association between kidney dysfunction and MR activation.[80] Patients with a heavy proteinuria (>2 g per day) have increased renal expression of MR and increased plasma aldosterone levels that were correlated with the severity of renal biopsy findings.[24] Chrysostomou *et al.*[4] first proposed the use of MR antagonists in proteinuric patients with CKD, and described a 54% reduction in proteinuria (3.8 ± 2.5 versus 1.8 ± 1.0 g per day) with spironolactone (25 mg per day for 4 weeks). Initial reports[4,83] and more recent analysis describe decreased blood pressure after MR antagonism.[84,85] Other studies showed reduction in proteinuria independent of effects on blood pressure at the doses used.[75,86–89] Whether part of the beneficial effects of MR antagonism in CKD are independent of effects on blood pressure is a point of continuing debate. Table 1 summarizes the number of published studies of MR blockade in CKD and proteinuria.

Diabetic nephropathy. In 2003, a trial in 45 patients with diabetes mellitus and persistent proteinuria during ACEI treatment showed a decrease in urinary albumin/creatinine ratio with the addition of spironolactone, without changes in mean blood pressure,[75] and the decrease in proteinuria was more marked in diabetic patients than in other proteinuric patients.[86] The same year, another team published similar results: proteinuria decreased under MR antagonist; the efficiency was correlated with the aldosterone level before the initiation of the treatment, and after MR antagonist withdrawal, proteinuria reappeared.[90] In this open-label trial, the reduction of proteinuria correlated with MR blockade. This was confirmed in another clinical trial in type II diabetic patients.[91] The first controlled, crossover, versus placebo trial was performed in type I diabetic patients:[92] the authors confirmed that proteinuria was decreased, but there was also a decrease in blood pressure and glomerular filtration rate. These results have been confirmed by at least two other studies.[92,93] The dose of spironolactone used in these studies was 25 mg per day. Higher doses have shown similar efficiency: a trial in 53 diabetic patients used spironolactone 50 mg per day during 1 year and showed a decrease in albuminuria;[94] and a trial comparing the effect of spironolactone 100 mg per day to ACEI (cilazapril) in diabetic patients confirmed the benefit of MR antagonism in addition to the effect of ACEI.[91] Spironolactone decreased albuminuria, renal excretion of MCP-1, and activation of oxidative stress (as estimated with renal excretion of 8-iso-prostaglandin F2α) better than a calcium-channel blocker in diabetic subjects.[95] Only one study has been performed with eplerenone in type 2 diabetic patients: the tolerability was similar to spironolactone and proteinuria was decreased after 3 months.[83]

Nondiabetic proteinurias. In a randomized, controlled, double-blind trial, was decreased by MR blockade in 41 patients with baseline proteinuria >1.5 g per day who had already received ACEI treatment for more than 6 months.[88] Spironolactone (25 mg per day) had a beneficial effect, whereas ARB treatment did not. At 3 and 6 months after starting spironolactone treatment, there were independent effects on proteinuria and blood pressure. Glomerular filtration rate and hyperkalemia were not different in the spironolactone-treated patients compared with the ARB-treated group. In a prospective, randomized, and open-label study of proteinuric patients (>1.0 g/g of creatinine) with nondiabetic forms of CKD and estimated glomerular filtration rate from 34 to 116 ml/min per 1.73 m^2, spironolactone (25 mg per day) decreased proteinuria and slowed the

Table 1 | Effects on proteinuria of mineralocorticoid receptor blockers in clinical studies

							Inclusion criteria					
Author[ref]	Study design	*n*	Months	Kidney disease	Arms (*n*)	Intervention treatment (mg/day)	Proteinuria (g/24 h)	Renal function	Primary end point	Secondary end points	Results	Blood pressure
Chrysostomou[4]	Prospective, open-label, uncontrolled	8	1	Proteinuric nephropathy	Spiro (8)	Spiro 25	3.81 ± 2.5	81 ± 48	Proteinuria	BP, renal function	↘ Proteinuria	↘ Mean BP
Sato[75]	Prospective, open-label, controlled	45	6	Diabetic nephropathy	ACEI without aldo escape (27)/ACEI+aldo escape spiro (18)	Spiro 25	UACR: 389 ± 109 mg/g	>60	UACR	BP	↘ Albuminuria	No change
Sato[86]	Placebo, open-label, uncontrolled	32	3	Proteinuric nephropathy	Diabetic (17)/non-diabetic (15)	Spiro 25	1.18 ± 0.101/ 1.142 ± 0.203	89.1 ± 3.8/94 ± 3.4	Proteinuria	u-NAG, u-β2m, u-collIV	↘ Proteinuria	No change
Rachmani[91]	Prospective, randomized, controlled	46	12	Diabetic nephropathy	Spiro 100: >50 mg/day (23)/Cizalapril+spiro 50 mg/day (23)	Spiro 100/50	UACR: 456/ 451 mg/g	SCr ⩽160 μmol/l	UACR	BP, renal function	↘ Proteinuria	No change
Bianchi[90]	Prospective, open-label, uncontrolled pilot	42	2	Proteinuric nephropathy	Spiro (42)	Spiro 25	2.09 ± 0.16[a]	56.8 ± 4.7[a]	Proteinuria	Renal function, hyperkalemia	↘ Proteinuria	No change
Schjoedt[92]	Prospective, randomized, double-masked, plabeo-controlled, cross-over	20	2	Diabetic nephropathy	Placebo (20)/spiro (20)	Spiro 25	AlbU >300 mg/ 24 h	>30	Albuminuria	BP, renal function	↘ Albuminuria	Independent
Schjoedt[93]	Prospective, randomized, double-masked, plabeo-controlled, crossover	20	2	Diabetic nephropathy	Placebo (20)/spiro (20)	Spiro 25	AlbU >2500 mg/ 24 h	>30	Albuminuria	BP, renal function	↘ Albuminuria	Independent
van den Meiracker[94]	Prospective, double-blind, placebo-controlled, parallel group	53	12	Diabetic nephropathy	ACEI or ARB+placebo (29)/spiro (24)	Spiro 50	AlbU: 1002/770	64/87	Proteinuria	BP, renal function, hyperkalemia	↘ Albuminuria	Independent
Epstein[83]	Prospective, randomized, double-blind, placebo-controlled	268	3	Diabetic nephropathy	Enalapril+placebo (91)/ EPL50 (91)/EPL100 (86)	EPL 50/100	UACR: 280/422/ 240 mg/g	74/73/75	Proteinuria, hyperkalemia	SBP, DBP	↘ Proteinuria	Independent
Chryso-stomou[88]	Prospective, randomized, double-blind, placebo-controlled	41	3	Proteinuric nephropathy	ACEI (10)/ACEI+ARB (10)/ACEI+spiro (10)/ ACEI+ARB+spiro (11)	Spiro 25	2.6 ± 1.6/2.5 ± 1.8/ 20.2 ± 1.4/3.1 ± 1.9	81.6/68/59.4/57.6	Proteinuria	BP, renal function, hyperkalemia	↘ Proteinuria	No change
Bianchi[87]	Prospective, randomized, open-label	165	12	Idiopathic GN	Conventional therapy (82)/+spiro (83)	Spiro 25	2.1 ± 0.08/ 2.0 ± 0.07	62.4 ± 2.4/62.2 ± 2.1	Proteinuria	Renal function	↘ Proteinuria	↘ SBP and DBP
Furumatsu[89]	Prospective, randomized, open-label, multicenter, controlled	32	12	Non-diabetic proteinuric nephropathy	Enalapril+losartan+TZD or furo (15)/spiro (15)	Spiro 25	u-Prot/u-Cr: 1.44 ± 0.28/ 1.42 ± 00.28 g/g	68.9 ± 7.8/91.8 ± 11.8	Proteinuria	Renal function, BP, PRA, u-PAI-1	↘ Proteinuria	Independent
Tylicki[97]	Prospective, randomized, open-label, controlled, crossover	18	2	Non-diabetic proteinuric nephropathy	ACEI+ARB (18)/ ACEI+ARB+spiro (18)	Spiro 25	0.97 ± 0.18	107.8	Proteinuria	u-NAG, u-α1m, u-PIIINP	↘ Proteinuria	Independent

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AlbU, albuminuria; ARB, angiotensin receptor blocker; BP, blood pressure; DBP, diastolic blood pressure; EPL, eplerenone; furo, furosemide; GN, glomerulonephritis; *n*, number of patients; PRA, plasma renin activity; SBP, systolic blood pressure; SCr, serum creatinine; spiro, spironolactone; TZD, thiazide; UACR, urine albumin/creatinine ratio; u-collIV, collagen IV urinary excretion; u-Cr, creatininuria; u-NAG, *N*-acetyl-β-D-glucosaminidase urinary excretion; u-PIIINP, amino-terminal propeptide of type III procollagen urinary excretion; u-Prot, proteinuria; u-PAI-1, plasminogen activator inhibitor-1 urinary excretion; u-α1m, α1microglobulin urinary excretion; u-β2m, β2-microglobulin urinary excretion.
[a]Data are expressed ± s.e.m.

progression of CKD over 12 months duration of follow-up.[87] This study is the only one that clearly demonstrates that spironolactone slowed progression of CKD. A recent systematic review on 15 clinical trials and abstracts evaluated the effect of a MR blockade in patients with CKD and proteinuria who were also receiving another RAAS blocker; there was a uniform decrease in proteinuria.[96] The incidence of hyperkalemia during MR antagonist was between 3 and 17%, depending on the definition used for hyperkalemia.[96] In 2008, a prospective, randomized, multi-centric, open-trial in 32 patients with proteinuric nephropathy (>0.5 g per day) showed that addition of spironolactone (25 mg per day) to ACEI and ARB treatment decreased proteinuria and renal excretion of type 4 collagen (a renal marker of fibrosis) without any modification of blood pressure or kidney function when compared with the control group.[89] Another report of a prospective, open-label, crossover trial, showed that 'triple blockade' during 8 weeks decreased proteinuria as well as urinary excretion of a kidney injury biomarker, decreased fibrosis but increased serum potassium.[97]

In 2009, a meta-analysis of 11 controlled and randomized trials compared the effect of a MR antagonist in patients with CKD, albuminuria, or proteinuria due to diabetic and nondiabetic nephropathy, who were already receiving another RAAS antagonist (ACEI or ARB).[85] All studies, but one, used 25 mg per day spironolactone. This meta-analysis showed that spironolactone significantly decreased proteinuria when added to ACEI and/or ARB treatment without any change in renal function, but with a decrease in systemic blood pressure. The risk of hyperkalemia was higher and a few cases of gynecomastia were reported. Eplerenone (50 mg per day) treatment decreased of proteinuria after 12 weeks associated with a decrease of blood pressure (systolic and diastolic) but without change in kidney function or hyperkalemia. Gynecomastia was not reported with eplerenone.[85]

A large-scale prospective, placebo-controlled evaluation of eplerenone versus conventional agents in hypertensive patients with proteinuria is underway, and is referred to as the EVALUATE Trial.[98] The primary end point is reduction in urine albumin/creatinine ratio after 12 months of active drug treatment, with secondary outcomes that include reduction in blood pressure, hyperkalemia, and slowing of progression of CKD.

End-stage renal disease. In most of the clinical studies, the primary end point was reduction in proteinuria and/or albuminuria. The possibility that the beneficial effects of MR antagonism may not require an effect on proteinuria is raised by studies in anuric end-stage renal disease patients showing cardiovascular effects of MR antagonists.[99–101] Those performed in anuric patients are informative about the effects of MR antagonists that are independent from their action on kidney function and proteinuria. An open-label trial in 14 hemodialyzed patients with a residual renal function (from 2 to 6 ml/min) showed that 25 mg per day spironolactone, three times a week for 2 weeks, did not cause hyperkalemia.[102] Another observational trial reported a similar safety profile in eight chronic hemodialyzed patients.[103] One case of hyperkalemia >6 mmol/l and one with >5.5 mmol/l were reported but without need to modify treatment.[103] Hemodialysis, by effecting potassium removal, seems to limit the risk of hyperkalemia.[104] Another trial in nine hemodialyzed patients examined the risk of hyperkalemia after an oral load of potassium, with or without spironolactone (50 mg per day three times a week): after 2 weeks, hyperkalemia was not worsened by spironolactone.[105] In 2009, an observational study in 50 hemodialyzed patients showed that 25 mg per day spironolactone could be used safely for 6 months.[106]

A single report describes the use of spironolactone in association with an ACEI and a β-blocker in a patient with heart failure who received chronic peritoneal dialysis: there was no particular adverse effect.[107]

Decreased of aortic vascular calcifications has been described with spironolactone treatment in five hemodialysis patients.[99] A controlled, crossover versus placebo trial in eight hemodialysis patients showed a significant blood pressure decrease without hyperkalemia.[100] A prospective, randomized, controlled trial in 30 hemodialysis patients showed decreased carotid intimal thickness with spironolactone and minimal safety issues over 2 years of treatment.[101] These studies have been summarized in a recent meta-analysis.[108]

Pediatric cases and pregnancy. Very few studies are available in children. The etiologies of nephropathies are a bit different than for adults, so it is difficult to extrapolate from the trials performed in adult patients to children. A recent review mentioned two observational trials.[76] The first one described children with Alport's syndrome: spironolactone (25 mg per day) safely decreased proteinuria with 18 months of use.[109] Another retrospective study, in 100 MR antagonist-treated children, reported hypokalemia rather than hyperkalemia.[110] Randomized controlled trials with adequate power have to be done to evaluate the efficiency and the safety of MR antagonists in children as well as adults.

Eplerenone has been assigned to pregnancy risk category B; there are not animal studies with evidence of teratogenicity, and there are no controlled data in human pregnancy. Spironolactone has been assigned to pregnancy risk category C, with adverse effects seen in animal studies and no adequate studies in humans. Both drugs should probably be avoided in pregnancy.

Adverse effects associated with MR antagonists. The adverse effects in MR antagonists could be divided in ionic effects (hyperkalemia and salt depletion related to the diuretic effect) and anti-androgenic effects (gynecomastia, disorders of the menstrual cycle, and so on, related to the nonspecific androgen receptor blockade). The use of MR antagonists has not been recommended in CKD patients because of the concerns about hyperkalemia,[111,112] which often occurs when multiple RAAS blockers are used.[113] Several authors suggest that the MR antagonists are not used often enough in heart failure, based on comparisons to the inclusion criteria for the RALES[5] and EPHESUS[6] studies, and that the biological follow-up (monitoring of serum creatinine

and potassium) have not been optimal.[114] Anti-androgenic adverse effects appeared with high doses spironolactone.[85,115] Spironolactone and eplerenone seem to have different adverse event profiles, particularly the anti-androgenic effects. This difference is due to the lower selectivity for MR of spironolactone; spironolactone is also an antagonist for the androgenic receptor in contrast to eplerenone, which is relatively more MR specific.[115]

The safety issues related to hyperkalemia may have overshadowed the potential beneficial effects of MRBs in patients with CKD.[38,116,117] Indeed, the risks associated with hypokalemia seem to be more consequential than the risks associated with hyperkalemia in patients with CKD.[118,119] What has not been properly emphasized is the possibility that hyperkalemia may stimulate aldosterone secretion and contribute to aldosterone breakthrough, thus limiting the beneficial effects of MRBs and RAAS blockade in patients with CKD.[78] Several approaches to controlling hyperkalemia during RAAS blockade have been recently summarized, and include dietary restriction, increased colonic secretion of potassium, and the use of adjunctive diuretic therapy.[37]

CONCLUSION

Aldosterone and/or direct MR activation are important risk factors for various forms of heart disease. It seems likely that MR activation has similar effects in CKD, beyond any effects on blood pressure. The beneficial effects of MR antagonism have not been fully explored in several clinical situations: in end-stage renal disease patients (with or without anuria, in hemodialysis, and peritoneal dialysis), in proteinuric patients, in kidney transplant patients, and in children. The use of MR blockade to slow the progression of CKD needs to be rigorously explored with properly powered outcome studies. In other clinical conditions, such as cyclosporine and/or transplant vasculopathy, the effects of MRB need to be better defined. As a complement to more aggressive RAAS blockade (with higher doses of ACEIs and ARBs) in proteinuric kidney diseases, there is growing interest in the cardiovascular benefits of adding MR antagonism to RAAS blockade. Concerns about hyperkalemia have prevented the organization of large-scale outcome studies in CKD similar to RALES,[5] EPHESUS,[6] and EMPHASIS_HF.[7] The current recommendations for MR antagonism in heart failure specifically exclude patients with CKD; perhaps this therapeutic window can be extended if effective means of managing hyperkalemia can be developed for use during long-term outcome studies, both from the safety perspective as well as limiting the detrimental effects of hyperkalemia and aldosterone breakthrough on the efficacy of RAAS blockade.

DISCLOSURE

DGW is a consultant for Relypsa Pharmaceuticals, Santa Clara, CA. The remaining authors declared no competing interests.

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DISCLAIMER

The authors take full responsibility for the contents of this review. The services of a medical writer were not used, and the work has not undergone any review by any outside agency or pharmaceutical concern.

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