Chapter 3

ALDOSTERONE, FROM (PATHO)PHYSIOLOGY TO TREATMENT IN CARDIOVASCULAR AND RENAL DAMAGE

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ABSTRACT

Aldosterone, a steroid hormone with mineralocorticoid activity, is far more than merely a salt-and-water hormone. Aldosterone has a number of non-classical, mineralocorticoid receptor (MR)-mediated actions, including tissue remodeling, modulation of vascular tone, and stimulating inflammation and fibrosis, which may fuel progression of end organ damage. Aldosterone breakthrough during blockade of the renin-angiotensin aldosterone system (RAAS) may explain why this treatment regimen only confers partial cardiovascular and renal protection. Of major interest, aldosterone is deleterious only if inappropriately high for its sodium status i.e. high aldosterone and high sodium. The mechanism of sodium dependence of aldosterone-induced renal and cardiovascular damage continues to fascinate. Aldosterone excess increases sodium and fluid retention and consequently increases blood pressure, which, in turn, mediates target organ damage. Moreover, blood pressure independent effects play a role with dissociation of low circulating and high tissue aldosterone levels during high sodium intake and possibly enhanced MR signaling. MR blockade is a valuable strategy, which has potency to halt the progressive end organ damage as observed during current treatments. In heart failure, MR blockade on top of RAAS blockade reduces hard clinical endpoints. Despite encouraging results on the intermediate endpoint proteinuria, long-term data on the efficacy and safety of MR blockade in preventing dialysis and/or cardiovascular endpoints in chronic kidney disease are still lacking. It is obligatory that future clinical studies on the effects of MR blockade on end-organ damage take into account the sodium status.
INTRODUCTION

Aldosterone is classically known as a main regulator of sodium and potassium homeostasis. As one of the effector hormones of the renin-angiotensin-aldosterone system (RAAS), it plays a main role in the homeostatic response to volume depletion, leading to increased renal tubular sodium reabsorption in order to restore volume status. It also induces potassium loss, during conditions of high plasma potassium and sodium depletion. In classical experiments, continuous infusion of aldosterone induced sodium retention with a positive volume balance and hence hypertension, followed by the so-called aldosterone-escape, with re-establishment of steady state despite ongoing aldosterone infusion.1

In more recent years, the label “aldosterone-escape” is used to indicate a different phenomenon, namely the restoration of aldosterone towards baseline values after an initial decrease during blockade of the RAAS by angiotensin converting enzyme (ACE) inhibition or angiotensin II receptor blockade (ARB).2 This phenomenon is also known as aldosterone breakthrough. The transient response of circulating aldosterone levels to ACE inhibition is illustrated in Figure 1, showing the day-to-day values of aldosterone in patients with essential hypertension, instituted on the ACE-inhibitor enalapril on low (50 mmol/d) and high (200 mmol/d) sodium intake.3 It shows that the decrease in aldosterone is rapid, occurs on both sodium intakes, and is followed by breakthrough towards baseline values already during the first week of treatment.3 This may well reflect a homeostatic response to the concurrent negative sodium balance, and/or “escape” due to the reactive rise in renin that occurs. Interestingly, the values during high sodium parallel those during low sodium, at an appropriately suppressed level, demonstrating that the relationship between sodium intake and circulating aldosterone remains intact during this pharmacological blockade of the RAAS by ACE inhibition.

ACE inhibitors and ARBs have long been the dominant strategies for RAAS blockade in hypertension, heart failure and renal disease. Aldosterone blockade had a relatively minor role, mainly consisting of the use of spironolactone as a potassium sparing diuretic for conditions characterized by edema and secondary hyperaldosteronism, like heart failure and cirrhosis, conditions in which normal aldosterone escape is impaired.1 However, a shift of paradigm occurred when Pitt et al. reported specific cardioprotective effects in heart failure that could not be attributed to diuretic effects, but rather pointed towards a specific anti-fibrotic action. This observation fueled a new wave of interest in the pathophysiological effects of aldosterone, and its pharmacological blockade as a tool for intervention in cardiovascular and renal disease.4,5
Interestingly, some of the novel, non-classical effects of aldosterone also appear to have a link with sodium status, with more prominent deleterious effects of aldosterone during conditions of sodium excess (Figure 2).

Here, we will review the recent insights of the role of aldosterone in renal and vascular damage and its interaction with sodium status. Furthermore, we will discuss the efficacy of mineralocorticoid receptor (MR) blockade in the treatment of patients with a high cardiovascular risk profile as well as in patients with chronic kidney disease (CKD).

**Figure 1.** Transient response of circulating aldosterone levels to ACE-inhibition. Day-to-day values plasma renin activity (PRA) and plasma aldosterone concentration (PAC) in patients with essential hypertension treated with ACE inhibition, enalapril 10 mg. The continuous lines represent low sodium intake (50 mmol/d) and the broken lines a liberal sodium diet (200 mmol/d). The decrease in aldosterone is rapid, occurs on both sodium intakes, and is followed by breakthrough towards baseline values already during the first week of treatment. Reprinted from reference.3
Classical effects of aldosterone on sodium, potassium, and water homeostasis

Aldosterone is a steroid hormone with mineralocorticoid activity. Not only angiotensin II, but also potassium and adrenocorticotropic hormone (ACTH) stimulate the adrenal zona glomerulosa, the outer layer of the adrenal cortex, to synthesize aldosterone. Aldosterone can also be synthesized locally in tissues such as blood vessels, heart and kidneys. Synthesis at extra-adrenal sites is regulated by the RAAS and the amount produced is <1% compared with the amount produced by the adrenal glands. Consequently, none of these extra-adrenal sources significantly contribute to plasma levels; circulating aldosterone levels fall essentially to zero with adrenalectomy. Circulating aldosterone binds to the inactive cytosolic MR on target cells with epithelial sodium channels. Main target cells in the kidney are located on the principal cells of the cortical collecting duct in the distal nephron. However, the classical effects of aldosterone (i.e. promotion of sodium retention and potassium loss) are not confined to the kidney. Aldosterone exerts similar, but lesser effects on epithelial cells in the colon, sweat and salivary glands. Binding of aldosterone to the MR on target cells results in translocation of the ligand-activated MR into the nucleus, where it exerts its transcriptional effects. It binds to hormone-response elements in the regulatory region of target gene promoters (the MR is a nuclear hormone receptor), resulting in protein synthesis. In the distal nephron of the kidney, MR-induced serum- and glucocorticoid-inducible kinase-1 (sgk-1) gene expression triggers a cascade of molecular events that leads to three important changes. First, it increases the expression of apical epithelial sodium channels, resulting in reabsorption of sodium and water. Second, it increases the expression of basolateral sodium/potassium ATPase for cellular sodium extrusion and potassium entry and third, it increases the expression of apical renal outer medullary potassium channels which are involved in passive excretion of potassium. Collectively, these processes lead to sodium and water reabsorption with subsequent volume expansion and an increase in blood pressure and potassium excretion.

Pre-receptor metabolism

Mineralocorticoid selectivity is achieved through co-expression of the MR and the enzyme 11beta-hydroxysteroid dehydrogenase type 2 (11β-HSD2). 11β-HSD2 metabolizes glucocorticoid hormones into receptor-inactive 11-keto cogeners, preventing occupancy of MR by circulating cortisol (a glucocorticoid hormone). This is also known as ‘pre-receptor metabolism’. The affinity of the MR for aldosterone and glucocorticoids is similar, however cortisol is 100- to 1000-fold more abundantly present in plasma than aldosterone. Clinical importance of this pre-receptor metabolism by 11β-HSD2 is highlighted by inactivating mutations found in the syndrome of apparent mineralocorticoid excess. This is a rare genetic defect characterized by early onset of severe low-renin hypokalemic hypertension. Moreover, consumption of liquorice also
induces hypokalemic hypertension. Its active metabolite, glycyrrhetinic acid, inhibits 11β-HSD2 activity, allowing cortisol to escape pre-receptor metabolism and occupy and activate MR. Reduced activity of 11β-HSD2 can also contribute to the pathogenesis of human essential hypertension. Almost 40% reduction in ex vivo 11β-HSD2 catalytic activity, measured directly on sweat gland cells, was found in patients with essential hypertension as compared to normotensive subjects. Data from a very recent experimental study in rats revealed that 11β-HSD2 expression (and presumably also activity) was >50% reduced in the distal nephron of offspring of protein-restricted mothers. This suggests that the observed elevated blood pressure in the offspring may be the result of insufficient protection of the MR from inappropriate binding by glucocorticoids, as only half the level of glucocorticoids are required to inappropriately activate the MR in the distal nephron.

**Primary hyperaldosteronism**

Primary hyperaldosteronism is a syndrome, in which secretion of aldosterone is relatively autonomous of its normal stimuli, due to hyperplasia, adenoma, or carcinoma of the adrenal cortex. Patients with primary hyperaldosteronism, or presumed idiopathic hyperaldosteronism have an increased risk of stroke, myocardial infarction, and atrial fibrillation and have higher urinary albumin excretion rates than patients with primary hypertension with similar blood pressure levels. In these patients aldosterone excess is associated with increased cardiac mass, fibrosis and cardiovascular tissue remodeling, independent of blood pressure. These data confirm the clinical relevance of blood pressure independent, direct adverse effects of aldosterone excess. Treatment of aldosterone excess by laparoscopic adrenalectomy (for patients with unilateral primary hyperaldosteronism) or by MR blockade reverses the excess in cardiovascular morbidity and improves the metabolic complications related to hyperaldosteronism in these patients.

**Beyond classical actions: profibrotic and proliferative effects of aldosterone**

Over the last decade it has become increasingly clear that aldosterone can directly contribute to processes of tissue remodeling and progressive cardiovascular and renal damage through its pro-fibrotic actions (Figure 2). This appears to affect many cell types, including vascular cells, and different cell types in the heart and kidneys, mediating target organ damage. The cell-signaling pathways of the aldosterone-induced pro-fibrotic effects are not yet fully elucidated, but reactive oxygen species (ROS), NFκB, PAI-1, TGF-β, and increased collagen gene expression and synthesis are likely to be involved and can be abolished by MR blockade. Recent findings showed that the G allele of the common functional genetic polymorphism c.- 2G>C in the MR gene is associated with decreased MR protein levels compared with the C allele.
cally, the G allele of this polymorphism is associated with increased RAAS activation and increased blood pressure.\textsuperscript{31} Mechanisms of aldosterone induced injury, downstream pathways of MR activation and cross-talk between aldosterone and angiotensin II signaling were reviewed recently elsewhere.\textsuperscript{32}

Figure 2. Proposed interaction between the classical pathway of aldosterone release, sodium status, and metabolic status in cardiovascular and renal disease. The left box shows classical associations between aldosterone and sodium intake. These mainly involve increased circulating aldosterone during sodium depletion and suppression of circulating aldosterone during high sodium intake. The right upper and right lower boxes show recent insights on interaction of sodium and metabolic status with this classical pathway. The box shows dissociation of adequately suppressed circulating aldosterone levels with high aldosterone levels in target tissues during high sodium intake.\textsuperscript{7,58} The right lower box shows impaired negative feed-back by high sodium intake on circulating aldosterone in obesity, which is likely to be caused by secretion of aldosterone-releasing factors by adipocytes.\textsuperscript{43,63} Enhanced MR activation by increased levels of both circulating and tissue aldosterone directly contributes to progressive cardiovascular and renal damage through combined effects of blood pressure with fibrosis and inflammation.\textsuperscript{20-23} Abbreviations: BMI, body mass index; RAAS, renin-angiotensin-aldosterone system; MR, mineralocorticoid receptor.
Beyond classical effects: interaction with metabolic status

Classically, glucocorticoids but not mineralocorticoids were thought to exert their effects on metabolic status. However, it has become increasingly clear that cross-talk between both types of steroids and their receptors exists, probably due to structural similarities of the MR and glucocorticoid receptor. Mounting evidence is pointing towards mutual interaction of aldosterone and MR activation with metabolic status (Figure 2). The impact of aldosterone on metabolic status, was first described in patients with primary hyperaldosteronism who also had impaired glucose tolerance. More recent studies performed in obese individuals and patients with primary hyperaldosteronism demonstrate that increased plasma aldosterone levels are independently associated with the metabolic syndrome, but also with its separate metabolic components i.e. impaired glucose metabolism and insulin resistance, high waist circumference and low HDL-cholesterol levels. Reduction of plasma aldosterone levels with either adrenalectomy or MR blockade with spironolactone normalizes plasma glucose and insulin levels.

Several mechanisms have been proposed for the interaction of aldosterone with metabolic status, of which the most important ones are the interaction of aldosterone with adipocytes and its effect on insulin signaling. Chronic exposure to aldosterone promotes adipogenesis in an MR-dependent manner in vitro. Adipocytes are able to secrete cytokines which can induce insulin resistance, such as interleukin-6, TNF-α and MCP-1. MR blockade reduced expression of these pro-inflammatory cytokines in obese diabetic mice. In a model of RAAS activation and insulin resistance in rats, MR blockade improved systemic insulin sensitivity and skeletal muscle glucose uptake, which was accompanied by a decrease in oxidative stress. Aldosterone also exerts inhibitory effects on insulin release by pancreatic β-cells and stimulates hepatic glucose neogenesis. The other way round, adipocytes have been demonstrated to secrete as-yet-unidentified aldosterone-releasing factors which are able to induce synthesis and secretion of aldosterone in adrenal cells. This suggests that interaction of aldosterone with adipose tissue probably is bidirectional (Figure 2). Candidates for adipocyte-derived stimulation of aldosterone synthesis include TNF-α, C1q/TNF-α-related protein, leptin, linoleic acid oxidative products, and interleukin-6. Moreover, free fatty acids can stimulate the adrenal production of aldosterone, reflecting another effect of metabolic status on aldosterone.

Interaction between profibrotic effects of aldosterone and sodium status

Interestingly, aldosterone-induced target organ damage depends on sodium and volume status, even independent of blood pressure (Figure 2). Aldosterone-induced cardiac hypertrophy or cardiac fibrosis is completely prevented by a low sodium diet.
in uninephrectomized rats. Effects of aldosterone on the severity of hypertension, endothelial dysfunction, and cardiac and vascular remodeling are sodium-dependent in stroke-prone spontaneously hypertensive rats. Moreover, aldosterone-induced renal inflammatory cell infiltration and vasculopathy only occurs during high sodium intake. In human, various conditions of hyperaldosteronism secondary to volume depletion, such as habitual low sodium intake in Yanomami Indians or Gitelman or Bartter syndrome with renal sodium loss, have no hypertension or vascular target organ damage. The other way round, in patients with resistant hypertension the deleterious effects of high sodium intake on proteinuria are most pronounced in patients with the highest aldosterone levels. Taken together, these data suggest that aldosterone exerts adverse effects only when its serum concentration is inappropriate for sodium status (i.e. high aldosterone, high sodium). Sodium status might thus have an important role in sensitizing target organs to the damaging effects of aldosterone. The mechanisms of this sensitization are of great interest, in particular because the habitual sodium intake in most populations is high, both in general population and in patient cohorts with renal or cardiac disease.

The classical association between aldosterone and sodium intake involves suppression of aldosterone during high sodium intake (Figure 2), which facilitates renal excretion of the excess sodium. However, recent evidence showed, first, dissociation of adequately suppressed circulating aldosterone levels with increased levels in target tissues in several conditions (Figure 2). In stroke-prone spontaneously hypertensive rats high sodium intake increased vascular aldosterone in association with vascular damage. In healthy rats high sodium intake increases cardiac aldosterone synthase activity and hence cardiac aldosterone, associated with cardiac hypertrophy and perivascular and interstitial fibrosis, independent of blood pressure, along with a reduction in circulating aldosterone levels. Interestingly, aging might represent another major condition with dissociation of circulating and tissue aldosterone effects. Aging is associated with lower circulating aldosterone levels, but higher vascular MR expression. Moreover, sensitivity for aldosterone-induced ERK 1/2 activation is enhanced in aged vascular smooth muscle cells, and MR blockade prevents age-associated upregulation of pro-inflammatory gene expression, supporting relevance of MR and aldosterone signaling in the development of inflammation that is associated with arterial aging. Second, suppression of circulating aldosterone levels by high sodium intake is insufficient in several conditions, and associated with end organ damage. For instance, insufficient sodium-induced suppression of aldosterone is associated with left ventricular structural changes and cardiac dysfunction in hypertensive patients.
Adipocytes are likely to be involved in the impaired negative feed-back regulation by sodium loading on aldosterone, by release of aldosterone-producing factors. In obese spontaneously hypertensive rats (SHR), for instance, high sodium less effectively suppresses aldosterone than in lean littermates. Renal target organ damage in obese SHRs, apparent from proteinuria, podocyte injury and advanced renal lesions, was dramatically ameliorated by MR blockade using eplerenone. Since nuclear MR content and sgk-1 expression in the kidney were also increased in this study, MR signaling might underlie the sodium-induced renal damage in these obese SHRs.

Genetic factors can also affect aldosterone regulation and its response to sodium. The ACE insertion/deletion (I/D) polymorphism affects not only ACE activity levels, but also levels of tissue aldosterone, with higher levels in DD genotype. In healthy DD homozygotes angiotensin I infusion (as a marker of increased tissue ACE activity and angiotensin II generation) led to increased responses of blood pressure during high sodium intake only, which is in line with other studies showing that high sodium conditions induce tissue ACE activity, and possibly aldosterone. This might have clinical relevance, as high sodium intake is associated with resistance to ACE inhibition in renal patients and healthy subjects with the DD genotype. Furthermore, patients with heart failure who have high circulating aldosterone levels despite treatment with ACE inhibition are more likely to have the DD genotype compared to patients lacking this aldosterone breakthrough.

The mechanism of sodium dependence of aldosterone-induced renal and cardiovascular disease continues to fascinate scientists and clinicians. The pathological processes by which aldosterone and high sodium intake promote target organ damage are undoubtedly multifactorial (Figure 2). Aldosterone excess increases sodium and fluid retention through its classical effects and increases blood pressure, which, in turn, mediates target organ damage. Moreover, blood pressure independent effects play a role with dissociation of low circulating and high tissue aldosterone levels during high sodium intake and possibly enhanced MR signaling. It is currently unknown why aldosterone-MR-induced downstream pathways leading to inflammatory cell activation and pro-inflammatory gene upregulation are not activated in the absence of high sodium.

Aldosterone and effects of MR blockade on hypertension and the vasculature
Spironolactone and eplerenone are MR antagonists, which are currently available for the treatment of hypertension. The effects of MR blockade on blood pressure are clearly related to blocking of the genomic effects of aldosterone on renal sodium handling, with consequent a slow-onset natriuretic effect associated with potassium
retention. Interestingly, the effects of mineralocorticoid antagonism on blood pressure are not solely dependent on its diuretic effect. MR blockade during one year reduces stiffness of resistance arteries and decreases the collagen/elastin ratio as compared to treatment with the beta-blocker atenolol in hypertensive patients with similarly well controlled blood pressures. Moreover, pre-dialysis systolic blood pressure is decreased by spironolactone in anuric hemodialysis patients, without differences in serum potassium levels. This strongly suggests direct effects on vascular resistance. The vasculo-protective potential of MR blockade is supported by experimental studies showing reduced neointima formation after coronary angioplasty and coronary stenting in pigs. Furthermore, overexpression of aldosterone synthase in the heart is associated with coronary vascular dysfunction without altering cardiac structure and function in transgenic mice, suggesting direct vascular effects of aldosterone. Direct effects of aldosterone on the vasculature are further supported by experimental data in rats on a high sodium diet infused with angiotensin II and co-treated with L-NAME to inhibit nitric oxide synthesis. Adrenalectomy or MR blockade prevented the associated myocardial necrosis, proteinuria, and vascular lesions without altering systolic blood pressure. This protective effect was lost by aldosterone infusion, suggesting that the noxious effects of NO-depletion plus angiotensin II infusion on a high sodium diet are mediated, at least in part, by aldosterone independent of its classic effects. We recently demonstrated that MR blockade by spironolactone significantly ameliorates transplant vasculopathy (TV) in renal chronic transplant dysfunction (CTD) in rats. TV is characterized by severe neo-intima formation, which leads to impaired perfusion and subsequent allograft dysfunction with reduced survival. Spironolactone ameliorated the development of TV by reducing the number of affected arteries (Figure 3). This suggests that MR activation plays a role in the initiation rather than the progression of neointima formation, possibly by protecting against endothelial activation. The latter plays an important role in the initiation of TV, and MR blockade has been shown to ameliorate aldosterone-associated endothelial activation and dysfunction.

Aldosterone and effects of MR blockade on cardiac fibrosis and heart failure

Direct effects of aldosterone on the heart were suggested already more than a decade ago by the strong correlation of aldosterone levels with left ventricular mass in patients with hypertension, independent of blood pressure. Decreased compliance of the aorta was shown to inversely correlate with aldosterone levels in patients with heart failure. Interestingly, a recent randomized controlled study showed left ventricular mass reduction and improved arterial stiffness by MR blockade in non-diabetic patients with stage 2 and 3 CKD. These effects occurred along with a reduction in systolic blood pressure (placebo vs. spironolactone: 124 vs. 119 mmHg respectively).
Figure 3. Spironolactone ameliorates transplant vasculopathy (TV) by reducing the number of affected arteries in experimental chronic transplant dysfunction (CTD). Effects of MR blockade by spironolactone on TV in the Dark Agouti-to-Wistar Furth renal allograft transplant model of CTD. Treatment with spironolactone or vehicle daily by oral gavage was given from 2 days prior to transplantation (donors and recipients) throughout the experiment of (12 weeks, recipients). Dark Agouti-to-Dark Agouti isografts served as negative controls. [A, B] Photomicrograph of an artery from a vehicle-treated allograft without TV (A) and with TV (B). NI, neointima; arrow head indicates elastin. [C] Percentage of affected arteries present within small (25-49 µm), medium 25 (50-99 µm) and large (≥100 µm) diameter arteries. [D] Total TV index, expressed as the mean occlusion in all elastin-positive arteries with a diameter ≥100 µm. [E] Percent occlusion in TV affected arteries with a with a diameter ≥100 µm. Data are presented as mean ± SE. *P < 0.05 vs. vehicle-treated allografts. Reprinted from reference.
However, these effects on blood pressure were much weaker than expected based on the observed effects on arterial stiffness. So, it is plausible that blockade of the cardiac and vascular MR reduced aldosterone-induced inflammation, fibrosis and hypertrophy in a blood pressure-independent manner.84

Increased aldosterone levels are associated with the development of cardiac fibrosis.85 Atrial fibrosis causes local conduction disturbances, which may lead to the development of arrhythmia. Atrial fibrillation is the most frequently observed arrhythmia, which accounts for substantial morbidity and mortality.86 Aldosterone levels are increased in patients with persistent atrial fibrillation.87 In an experimental study, MR blockade by eplerenone not only prevented development of de novo cardiac fibrosis and inflammation, but also reduced established mineralocorticoid-induced cardiac fibrosis and inflammation.88 It would be of great interest to investigate whether MR blockade can reduce conduction abnormalities by reducing atrial fibrosis. This would offer new approaches for anti-arrhythmic pharmacotherapy in atrial fibrillation.

The MR is present in the heart and aldosterone is produced locally in the heart at low levels.6 However, after myocardial infarction local aldosterone production increases, contributing to cardiac fibrosis,85 which may lead to heart failure. Aldosterone synthase mRNA expression is upregulated in ventricles of patients with heart failure89 and/or hypertrophic cardiomyopathy.90 These observations suggest that cardiac aldosterone may indeed have harmful effects in the myocardium. A recent study highlights the importance of macrophages in cardiac inflammation and fibrosis and assigns a key role to the MR in macrophages.91 Using macrophage-specific MR knockout mice the authors demonstrated that macrophages infiltrating the heart need to express the MR in order to exert their detrimental effects on blood pressure and cardiac fibrosis.91

In line with the cardiovascular effects of aldosterone-MR mentioned above, two large randomized controlled clinical trials (the RALES [randomized aldactone evaluation study] and EPHESUS [eplerenone post-acute myocardial infarction heart failure efficacy and survival study]) have demonstrated the efficacy of MR blockade on top of standard medical treatment in patients with heart failure.4,5 Treatment with spironolactone or eplerenone improved patient survival and reduced cardiovascular events independently of blood pressure.4,5 Results from a recent post-hoc biomarker study from EPHESUS suggest that these beneficial effects of MR blockade may be due to reduced cardiac extracellular matrix remodeling.92 As compared to placebo-treatment, patients treated with eplerenone had lower levels of propeptide of type I procollagen and the amino-terminal propeptide of type III procollagen, which are biomarkers of type I and III collagen synthesis. In contrast, there was no difference in levels of biomarkers for
collagen degradation. Thus MR blockade may prevent cardiac matrix remodeling rather than facilitating degradation of existing deposited collagen. In addition, effects on baroreflex sensitivity and the autonomic nervous system may be involved in the improved survival in patients with heart failure during MR blockade. Aldosterone is a risk factor for decreased heart rate variability and prolonged QT interval, factors that predict sudden cardiac death. MR blockade not only reduces myocardial collagen turnover but also improves time-domain heart rate variability in patients with heart failure.

**Direct renal effects of aldosterone**

There is increasing evidence that aldosterone can be directly involved in the development and progression of renal disease via non-epithelial MR-mediated effects. In vitro studies showed that aldosterone increases the production of TGF-β, ROS, PAI-1 and collagen which can be abolished by MR blockade. Studies in different rat models support a role for non-hemodynamic effects of aldosterone and the MR in renal damage. Uninephrectomized rats develop progressive proteinuria when they are continuously infused with aldosterone and fed a high-salt diet. These effects could be prevented by MR blockade or tempol, a ROS scavenger. In the same study it was shown that addition of aldosterone to cultured podocytes induced sgk-1 via the MR, which could be prevented by the addition of tempol, suggesting that aldosterone-induced podocyte injury is in part caused by an increase in oxidative stress.

MR blockade slows the progression of glomerulosclerosis, and can sometimes induce regression of existing glomerulosclerosis in rats with 5/6 nephrectomy. Despite these glomeruloprotective effects, proteinuria continued to increase in this study, demonstrating that the effects of MR blockade on proteinuria and structural damage can be discordant. It may also be that podocyte injury persists even after regression of glomerulosclerosis. Podocytes have only limited regenerative capacity and may have less ability to heal and/or require longer time for regression of injury than remodeling of the extracellular matrix of sclerotic lesions. We showed that aldosterone induces heparanase expression in podocytes, which could be blocked by spironolactone. Heparanase is an endo-β(1-4)-D-glucuronidase that cleaves heparan sulphate side chains, which is involved in tissue remodeling. Spironolactone reduced glomerular heparanase expression and partially restored heparan sulphate expression in the glomerular basement membrane of rats with adriamycin nephropathy. Despite these beneficial effects on structural damage, MR blockade did not affect proteinuria, again underlining that effects of MR blockade on proteinuria and tissue remodeling can be discordant. MR antagonists also markedly ameliorated tubulo-interstitial injury and/or glomerular damage in several models of nephropathy including spontaneously hypertensive stroke-prone rats without effects on systemic blood pressure or volume
Moreover, exogenous aldosterone infusion completely reversed the renoprotective effects of ACE inhibition in this model. Furthermore, in the remnant kidney model in which rats develop hyperaldosteronism, the renoprotective effect of dual RAAS blockade by the combination of ACE inhibition and ARB was reversed by exogenous aldosterone infusion.

Interestingly, in adriamycin-induced proteinuria mono-therapy MR blockade was ineffective, but in combination with ACE inhibition it effectively reduced blood pressure, proteinuria, interstitial and glomerular damage. It is unclear whether the added effect of MR blockade and ACE inhibition is due to the diuretic effect of MR blockade or by a direct anti-fibrotic effect. In contrast, we found progressive interstitial fibrosis during ACE inhibition in a separate study where dietary sodium restriction was used to induce the volume depletion required to maximize the effects of ACE inhibition, despite similarly effective reduction of blood pressure, proteinuria and glomerulosclerosis. Although a head-to-head comparison of these two different studies is not warranted, the difference in effect on interstitial fibrosis, all other components of the therapy response being equal, is remarkable (Figure 4). Similarly low systolic blood pressure can thus be tolerated without inducing hypoxia-driven interstitial fibrosis, depending on the treatment regimen by which it is achieved.

The mechanisms underlying the adverse effects of aldosterone on the kidney are complex and multifactorial. First, aldosterone increases sodium and fluid retention and accordingly increases blood pressure, which, in turn, mediates target organ damage. Volume overload, moreover, blunts the efficacy of RAAS blockade. Aldosterone also increases intraglomerular pressure due to direct effects on the renal microcirculation. Aldosterone exerts a vasoconstrictor effect on the efferent arteriole and inhibits vasoconstriction in perfused rabbit afferent arterioles, which is abolished by spironolactone. This is consistent with the assumption of aldosterone-induced increased intraglomerular pressure. NO modulates the actions of aldosterone, as endothelial denudation and pharmacological blockade of eNOS increased the sensitivity of the afferent arterioles to aldosterone.

MR blockade on top of treatment with ACE inhibition or ARB induces a larger decrease in urinary albumin excretion in diabetic patients. However, the antiproteinuric effects of MR blockade can be dissociated from circulating aldosterone levels. The syndrome of hyporenin-hypoaldosteronism in diabetic patients with autonomic dysfunction is associated with cardiovascular damage. In these patients with long-standing diabetes the conversion of prorenin to renin is impaired by the diabetic kidney, resulting in lower circulating aldosterone levels. These results suggest that MR blockade
may be effective in preventing the progression of cardiovascular and renal complications in patients with diabetic nephropathy who are known to have low circulating aldosterone levels. This may also suggest the importance of a tissue renin-angiotensin aldosterone system, with high tissue aldosterone levels. Not only diabetes, but also aging is associated with lower circulating aldosterone levels. However, there is no evidence that MR blockade would be less effective in the elderly.

Aldosterone breakthrough is associated with a poor response to anti-proteinuric treatment and an enhanced decline of renal function in patients with (diabetic) nephropathy. It is yet unknown whether this worse clinical prognosis of patients with aldosterone breakthrough is due to the noxious effects of aldosterone, inducing inflammation and fibrosis. In line with this hypothesis, studies in patients with chronic renal failure and early diabetic nephropathy show that MR blockade on top of ACE inhibition and/or ARB exerts added renoprotective effects. The reduction of proteinuria by the addition of spironolactone to ACE inhibitors, ARBs or their combination, is related to aldosterone levels in non-diabetic proteinuric patients with CKD. This strongly suggests that aldosterone is a component of the renal damage that is associated with CKD and that its inhibition by ACE inhibitors, ARBs or their combination can be incomplete. Several small human interventional studies showed greater reductions of proteinuria and blood pressure when MR blockade was added to ACE inhibitors and/or ARBs. In spite of these encouraging results on proteinuria, long-term data on the efficacy of MR blockade on hard end points, for example the development of ESRD or patient survival, are still lacking in CKD.

Aldosterone breakthrough during RAAS blockade and intervention in sodium status
Sodium restriction is known to reduce long-term cardiovascular risk, and is usually required to obtain an effective response to RAAS blockade. However, sodium restriction and diuretic treatment increase aldosterone levels not only in the untreated condition, but also during RAAS blockade by ARB (Figure 5, left panel, proteinuric patients) or by ACE inhibition (Figure 5, right panel, data from healthy volunteers). Short-term treatment with ACE inhibition reduces aldosterone during high and low sodium intake, due to decreased angiotensin II levels. However, this suppression cannot be sustained in 10% to 50% of patients treated with ACE inhibitors and/or ARBs. This aldosterone breakthrough particularly occurs during long-term treatment with ACE inhibition or during sodium restriction, which potentiates the adrenal response to angiotensin II. ARB treatment, on the other hand, did not affect aldosterone levels during either diet in proteinuric patients. Thus, during RAAS blockade aldosterone still responds to changes in volume status. Formerly, this preservation of the homeostatic response of the RAAS was considered neutral from the point of
Figure 4. Combined treatment with ACE inhibition and MR blockade on a normal sodium diet does not induce interstitial fibrosis despite similarly low blood pressure. Two different studies with proteinuria induced renal damage, in which treatment was started six weeks after adriamycin injection, are shown. ACE inhibition with lisinopril combined with MR blockade with spironolactone on a normal sodium diet, given for 12 weeks, similarly reduced blood pressure [A] and proteinuria [B] compared to ACE inhibition with lisinopril on a low sodium diet, given for 3 weeks. The difference in effect on interstitial fibrosis is remarkable [C]. Progressive interstitial fibrosis during ACE inhibition combined with a low sodium diet was seen, while only mild interstitial abnormalities were observed after ACE inhibition combined with MR blockade. Similarly low systolic blood pressure can thus be tolerated without inducing hypoxia-driven interstitial fibrosis, depending on the treatment regimen by which it is achieved. Abbreviations: BL, baseline; NS, normal sodium; ACEi, angiotensin converting enzyme inhibition; SPIR, spironolactone; LS, low sodium; SMA, smooth muscle actin. Data are presented as mean ± SD.
view of therapeutic efficacy. Given the well-established pro-fibrotic effects of aldosterone and the association of aldosterone breakthrough with worse outcome, this point of view should be reconsidered. During RAAS blockade, co-treatment with sodium restriction and/or diuretic is usually required to obtain an effective therapy response of blood pressure and proteinuria. As shown here, this occurs at the expense of elevated circulating aldosterone. The clinical consequences of the rise in aldosterone induced by volume depletion during RAAS blockade are still unclear, but it might be reassuring that this is not a high sodium-high aldosterone condition. The eventual effects on long term outcome might be dependent on the concurrent effect on tissue aldosterone. In theory, overcoming aldosterone breakthrough induced by sodium restriction and/or diuretic treatment might contribute to long-term protection of heart, vasculature and kidneys. It would thus be of interest to investigate whether MR blockade is specifically beneficial during sodium restriction, or conversely, during conditions of volume excess. Moreover, it would be of interest to study whether the benefits of MR blockade derive from its diuretic effects or in its ability to modulate non-volume-mediated effects of aldosterone and whether its renoprotective effects on top of ACE inhibition outweigh the effects of conventional diuretics. Therefore, studies are warranted to investigate extracellular fluid volume in a cross-over design with and without MR blockade during a high and a low sodium diet in proteinuric patients on conventional RAAS blockade and compare these effects to hydrochlorothiazide. This is topic of a current study (ESCAPE study, Dutch Trial Register, NTR2133).

**Pharmacology of MR blocking agents and effects on potassium homeostasis**

Two different MR blocking agents exist, spironolactone and eplerenone. Both are steroid analogues with structural similarity to aldosterone, thereby functioning as competitive antagonists. Spironolactone is absorbed for 80-90% and is rapidly metabolized in the liver into a number of metabolites, including canrenone, 7α-methylspironolactone and 6β-hydroxy-7α-methylspironolactone, having mean half-lives of 1.4, 16.5, 13.8, and 15 hours respectively. The onset of action for spironolactone is slow, with a peak response on natriuresis occurring 48 hours after the first dose. In eplerenone the 17-α-thioacylgroup of spironolactone is replaced with a carbomethoxy group. This confers selectivity of eplerenone to the MR, translating into less affinity of eplerenone for androgen and progestogenic receptors compared to spironolactone. This selectivity results in less endocrine side effects including gynecomastia, menstrual irregularities, loss of libido and impotence. No active metabolites have been identified for eplerenone, resulting in a shorter effective half-life and therefore quicker time to peak response than spironolactone. The maximum concentration of eplerenone is reached in 1.3 hours. The primary route of elimination is through CYP 3A4-mediated metabolism, its elimination half-life being 4-6 hours.
Figure 5. Circulating aldosterone levels are increased by intervention in sodium status. The left panel shows circulating aldosterone levels in proteinuric patients treated during 6 weeks with placebo, ARB (losartan 100 mg/d) and ARB plus diuretic (losartan/hydrochlorothiazide 100/25 mg/d) on a high (200 mmol/d) and a low sodium diet (50 mmol/d) in a randomized double-blind cross-over trial. ARB treatment did not affect aldosterone levels during either diet. Sodium restriction and diuretic treatment both increased aldosterone levels not only in the untreated condition, but also during RAAS blockade by ARB. The right panel shows circulating aldosterone levels in healthy male volunteers treated during one week with ACE inhibition (enalapril 20 mg/d) on a high (200 mmol/d) and a low sodium diet (50 mmol/d) in a randomized double-blind cross-over trial. Short-term treatment with ACE inhibition reduced circulating aldosterone levels during both sodium intakes. Sodium restriction increased aldosterone levels during placebo and during RAAS blockade by ACE inhibition. Abbreviations: HCT, hydrochlorothiazide; ACEi, angiotensin converting enzyme inhibition; LS, low sodium diet. #p<0.05 vs. same treatment on high sodium (effect of LS), †p<0.05 vs. losartan treatment on same diet (effect of HCT), ‡p<0.05 vs. placebo on same diet, *p<0.05 vs. losartan+HCT high sodium (comparison between addition of low sodium and HCT to losartan). Data are presented as mean ± SE.

An important risk of MR blockade is hyperkalemia, which can induce life threatening cardiac arrhythmias. Impaired renal function is the most important risk factor for hyperkalemia. Many patients with hypertension, heart failure or kidney disease are already treated with ACE inhibitors or ARBs, which further increases the risk for MR
blockade-induced hyperkalemia. Both spironolactone and eplerenone are associated with dose-related increases in serum potassium values. Spironolactone dosed 25, 50, and 75 mg/d induced hyperkalemia (serum potassium > 5.5 mmol/L) in 13, 20 and 24% of the participants of a dose-finding study for RALES. Experiences with the RALES and EPHESUS trials on hyperkalemia are somewhat reassuring. Spironolactone dosed 25 mg on top of ACE inhibition increased serum potassium levels with 0.3 mmol/L in the RALES study. There were only 10/841 and 14/822 cases of serious hyperkalemia in the placebo- and spironolactone-treated group, respectively. Importantly, this study excluded patients with renal function impairment (serum creatinine > 221 µmol/L) or a baseline serum potassium level of > 5.0 mmol/L. In the EPHESUS study potassium levels increased significantly more during eplerenone 50 mg (0.3 mmol/L) than during placebo (0.2 mmol/L) and serious hyperkalemia (serum potassium > 6.0 mmol/L) was more prevalent during eplerenone as well (126/3301, 3.9% vs. 180/3307, 5.5%). The incidence of hyperkalemia was higher in patients with renal function impairment (creatinine clearance < 50 mL/min); eplerenone 10.1% vs. placebo 5.9%. A recent post-hoc analysis of the EPHESUS study demonstrates that eplerenone still exerts beneficial effects on mortality regardless of baseline risk factors for development of hyperkalemia (serum potassium > 6.0 mmol/L). The four independent baseline predictors of hyperkalemia were baseline potassium greater than the median of 4.3 mmol/L, impaired renal function (eGFR < 60 mL/min/1.73m2), history of diabetes mellitus, and prior use of antiarrhythmic agents.

To obtain beneficial effects of MR blockade, it is necessary to ensure its safe use. According to current guidelines, this involves measuring both serum potassium and estimating renal function either by eGFR or creatinine clearance before initiating treatment with MR blockade. MR blocking agents should not be prescribed to patients with serum potassium greater than 5.0 mmol/L and/or impaired renal function as defined by serum creatinine > 221 µmol/L and/or creatinine clearance < 30 mL/min. Periodical monitoring of serum potassium and renal function is recommended with dose adjustment of the MR blocking agent as necessary.
CONCLUSIONS

Prevailing evidence suggests that aldosterone is far more than merely a salt-and-water hormone. In addition to its mineralocorticoid function, aldosterone has a number of non-classical, MR mediated actions, including tissue remodeling, modulation of vascular tone, and stimulating inflammation and fibrosis. All these actions may fuel progression of cardiovascular and renal end organ damage. Aldosterone breakthrough during RAAS blockade with ACE inhibition and/or ARB may explain why this treatment regimen only confers partial cardiovascular and renal protection. We have delineated that tissue aldosterone rather than its circulating level is relevant in this respect and that the former and latter do not necessarily run in parallel. Of major interest, sodium status appears to be an important determinant of the end-organ effects of aldosterone. Aldosterone is deleterious only if inappropriately high for its sodium status i.e. high aldosterone and high sodium.

MR blockade is a valuable strategy, which has potency to halt the progressive end organ damage as observed during current treatments. In heart failure, MR blockade on top of RAAS blockade reduces hard clinical endpoints. Despite encouraging results on the intermediate endpoint proteinuria, long-term data on the efficacy and safety of MR blockade in preventing dialysis and/or cardiovascular endpoints in CKD are still lacking. It is obligatory that future clinical studies on the effects of MR blockade on end-organ damage take into account the sodium status.
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