Interfering with mineralocorticoid receptor activation: the past, present, and future
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Abstract
Aldosterone is a potent mineralocorticoid produced by the adrenal gland. Aldosterone binds to and activates the mineralocorticoid receptor (MR) in a plethora of tissues, but the cardiovascular actions of aldosterone are of primary interest clinically. Although MR antagonists were developed as antihypertensive agents, they are now considered to be important therapeutic options for patients with heart failure. Specifically, blocking only the MR has proven to be a difficult task because of its similarity to other steroid receptors, including the androgen and progesterone receptors. This lack of specificity caused the use of the first-generation mineralocorticoid receptor antagonists to be fraught with difficulty because of the side effects produced by drug administration. However, in recent years, several advances have been made that could potentially increase the clinical use of agents that inhibit the actions of aldosterone. These will be discussed here along with some examples of the beneficial effects of these new therapeutic agents.

Introduction
Aldosterone, a mineralocorticoid produced primarily in the adrenal gland, is classically considered to regulate sodium and water balance in the kidney and to control blood pressure. Increases in plasma aldosterone lead to sodium retention, potassium excretion, and hypertension. In recent years, it has become clear that aldosterone, or activation of its receptor, the MR, has several extrarenal effects that are largely detrimental, at least in the setting of heart disease [1-3] and hypertension [4,5]. The increasing knowledge of the effects of aldosterone on the cardiovascular system in particular has led to a renewed interest in developing ways to block its actions. This has led to the development of several new drugs that can potentially interfere with MR signaling. These will be discussed here; for each drug class, I have selected recent studies describing the effects of the drug to highlight their potential usefulness in the treatment of cardiovascular conditions. I will discuss the classic steroidal MR antagonists—spironolactone and eplerenone—and the newer non-steroidal antagonists. I will also discuss the progress in the development of aldosterone synthase inhibitors and will consider the rapid non-genomic effects of aldosterone and their inhibition. The potential sites for inhibition of the actions of aldosterone are summarized in Figure 1.

The basics of adrenal biology and the mineralocorticoid receptor
Before discussing the ways to interfere with the aldosterone/MR system, it is first necessary to describe a little of the basic biology of the system. Aldosterone is produced primarily in the adrenal zona glomerulosa. There is some evidence that other tissues, including the vasculature, heart, brain, and adipose tissues, produce aldosterone [6-13]. However, these findings are controversial and have largely been refuted [14,15].

Aldosterone secretion is controlled by several factors. The most prominent are angiotensin II and potassium. Increases in both of these factors cause an increase in the production of aldosterone, but the actions of angiotensin II and potassium are independent of each other [16]. Acute
increases in the adrenocorticotropic hormone (ACTH) also increase aldosterone production, but sustained stimulation of the adrenal gland with ACTH inhibits aldosterone production [16]. There are several other aldosterone secretagogues, which include endothelin, vasopressin, and serotonin; these are less potent than angiotensin II and potassium and their physiological roles remain ill-defined [17].

Aldosterone causes its effects by binding to the MR. The MR belongs to the steroid receptor superfamily that contains the progesterone, estrogen, androgen, and glucocorticoid receptors [18]. These receptors have a common structure, and this has made the development of highly specific MR antagonists difficult. The MR is unique in this family in that it has two ligands—aldosterone and cortisol (or corticosterone in rodents)—that bind to the MR with the same affinity [19]. In epithelial tissues, the 11β hydroxysteroid dehydrogenase type II (11βHSD2) protects the MR from being occupied by glucocorticoids that circulate at much higher concentrations than aldosterone [20]. The 11βHSD2 metabolizes cortisol to cortisone in humans, cortisone cannot bind to the MR, therefore, when 11βHSD2 is active, aldosterone can bind to and activate the MR. If 11βHSD2 is not present or not functional, the ligand binding site on the MR is occupied by cortisol. Several non-epithelial MR-expressing tissues, including the heart, adipocytes, and macrophages, do not express 11βHSD2 and, therefore, in these tissues, the MR is occupied predominantly by cortisol [21]. There have been several excellent review articles describing the pre-receptor regulation of MR signaling [20,22]. The MR has been the least studied of the steroid receptor family (for reviews of MR signaling, see [18,23]). The status of the MR as the “Cinderella” of the steroid receptors changed when two groundbreaking clinical studies showed that MR activation is involved in the pathogenesis of cardiovascular disease [3,24]. These trials showing that spironolactone and eplerenone reduced the morbidity and mortality in patients with heart failure and left ventricular dysfunction led to a renewed interest in MR biology and to a new search for novel ways to inhibit the system. There is a real interest in finding ways to inhibit the cardiovascular effects of MR activation, while leaving the physiological
effects on the kidney intact. Interestingly, although the scientific community is convinced of the beneficial effects of MR antagonism, the molecular mechanisms responsible for the effects of aldosterone/MR activation have not been completely elucidated. It is clear, however, that aldosterone/MR activation increases reactive oxygen species production and vascular inflammation [25].

**Classic mineralocorticoid receptor antagonists**

The competitive MR antagonist spironolactone was developed as an antihypertensive agent in the 1950s and became commercially available in 1960. Although spironolactone is a potent MR antagonist, it also binds to other members of the steroid receptor family. It has significant anti-androgenic and progestogenic effects [26] that lead to gynecomastia, impotence, and menstrual irregularities [27-29]. The side effects associated with spironolactone meant that there was a need for a more selective MR antagonist that could mimic the beneficial effects of spironolactone in the population with essential hypertension without the related side effects [30]. The search for this drug led to the development of eplerenone, which entered phase 1 clinical trials in 1986 [31] and was first marketed in the United States in 2002. Although eplerenone is a more potent antagonist than spironolactone, it has a 40-fold lower affinity for the receptor, which makes it a selective MR antagonist than spironolactone [32,33]. It seems that the 42 years from the approval of spironolactone to the approval of the second-generation drug is a pharmaceutical industry record [34]. Even with this really long wait, it is not clear that the successor is any better than the parent compound for the treatment of human hypertension [35,36]. However, it should be noted that the long search for new MR blockers yielded some other useful drugs, including drospirenone, which has progestogenic activity and is included in several forms of birth control pills [37].

One of the differences between spironolactone and eplerenone is their metabolism. Spironolactone is metabolized to two compounds, which also have anti-MR activity [32], whereas eplerenone has no active metabolites [38]. One of the active metabolites of spironolactone, canrenone [39], is currently in clinical use and effectively reduces blood pressure, insulin resistance, and markers of inflammation in patients with metabolic syndrome [40]. From an experimental standpoint, several labs, including our own, have begun to utilize canrenone or potassium canrenoate, which is converted to canrenone, in long-term animal studies because of their ease of administration. Both can be administered in the drinking water without the need for additional vehicles [41,42]. Importantly, canrenone has less anti-androgenic effects than spironolactone [43-45].

One of the suggested mechanisms responsible for the beneficial effects of MR antagonists is a reduction in vascular inflammation. It has been clear for some time that inflammation is increased in mineralocorticoid-dependent hypertension, such as the deoxycorticosterone (DOCA)-salt hypertensive rats and much of this research has focused on macrophages. Early studies showed that MR activation increases intracellular adhesion molecule-1 (ICAM-1) expression and leukocyte adhesion in endothelial cells [46], and we have shown that spironolactone reduces ICAM-1 messenger RNA (mRNA) expression in cerebral arteries from hypertensive rats [47]. Vascular smooth muscle and endothelial cells express 11βHSD2; therefore, in these studies, the MR is likely to be occupied and activated by aldosterone [48-51]. Several studies have shown that MR activation is linked to macrophage infiltration into the heart in DOCA-salt rats and that eplerenone inhibits this action [52-54]. More recent studies of the effects of MR activation on macrophages have focused on the MR within the macrophage itself and on the concept that MR activation can alter macrophage polarity, leading to a more proinflammatory phenotype. The first studies using myeloid cell-specific MR knockout mice were published by Rickard and colleagues [55] in 2009. Later studies using myeloid cell-specific MR knockout mice showed that, in the absence of the MR, the macrophages took on an anti-inflammatory wound-healing phenotype [56]. Macrophages do not express 11βHSD2; therefore, in these cells, it is likely that the MR is occupied by glucocorticoids [57,58]. It is, however, interesting that MR antagonism with eplerenone in macrophages from healthy patients and patients with heart failure does not completely recapitulate the effects of genetic ablation of the MR [59,60].

Although most studies have focused on macrophages, some studies suggest that T cells are also an important part of the vascular inflammatory response associated with hypertension [61]. Recent studies assessed the mechanisms responsible for T cell activation in DOCA-salt hypertensive rats. These studies showed that MR activation caused the activation of T helper 17 (Th17) cells and a downregulation of regulatory T (Treg) cells; this occurred in a blood pressure-independent manner. Spironolactone ameliorated the response to DOCA-salt, but blood pressure lowering through a non-renin angiotensin II-dependent mechanism had no effect. This suggests that the effects of spironolactone on the T cell populations are MR – and not blood pressure – dependent. Interestingly, the authors suggest that the Th17/interleukin-17 response to MR activation may precede the macrophage infiltration described above [62].

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Non-steroidal mineralocorticoid receptor antagonists

MR blockade is useful in patients with heart failure and chronic kidney disease [3,24,63]. However, the risk of side effects, hyperkalemia development, and renal dysfunction makes eplerenone and spironolactone the least frequently prescribed medications among all those recommended for the treatment of heart failure [64-68]. This means that the search for new MR antagonists has been focused on finding drugs that have greater effects on the heart than on the kidney. This is essentially a repurposing of MR antagonists; they have moved on from their initial roles as blood pressure-lowering agents to become cardioprotective agents. Several pharmaceutical companies, including Pfizer (New York, USA), Bayer (Leverkusen, Germany), Novartis (Basel, Switzerland), and Takeda (Osaka, Japan), have developed non-steroidal MR antagonists with varying degrees of success [69]. The goal of these companies was to identify compounds with greater specificity at the MR than spironolactone but that were more efficacious than eplerenone.

Interestingly, some of the answers to this question were found in a class of drug that was already in use clinically. The dihydropyridines, which are L-type calcium channel blockers, were found to act as MR antagonists in vitro and in vivo [70-73]. This led to a surge of drug development activity using the basic structure of the dihydropyridines as a backbone for the development of new drugs. For Bayer, this led to the development of BAY 94-8862 [74]. This drug, also known as finerenone, is a potent antagonist at the human MR; it has a half maximal inhibitory concentration (IC50) of 18 nM for the MR and no activity at any of the other steroid hormone receptors or at 65 other receptors and ion channels [74]. Phase 2 clinical trials in patients with heart failure and chronic kidney disease have been conducted [75] (NCT01345656). Thus far, the results of this trial appear favorable. The mineralocorticoid Receptor Antagonist Tolerability Study (ARTS) assessed the safety and tolerability of BAY 94-8862 in patients with reduced left ventricular ejection fraction and chronic kidney disease [76]. Although the trial was too short to assess mortality from heart failure, the analysis of cardiac markers of failure, including brain natriuretic peptide (BNP) and N-terminal pro-BNP, suggests beneficial cardiovascular effects of BAY 94-8862. The incidence of hyperkalemia and reduced renal function was lower in the patients treated with BAY 94-8862 than it was in patients treated with spironolactone [76]. The choice of spironolactone as the comparator drug is, however, considered to be a negative feature of this study. In the future, it will be important to conduct a head-to-head comparison between BAY 94-8862 and eplerenone [77]. Preclinical studies using BAY 94-8862 are also producing promising results. A recent study using rats with mineralocorticoid-dependent hypertension and rats with heart failure showed that BAY 94-8862 has remarkable effects at very low doses. This study was a head-to-head comparison with eplerenone, and the authors matched the drugs for natriuretic effects. The authors found that the tissue distribution of BAY 94-8862 is different than that for eplerenone. Spironolactone and eplerenone preferentially accumulate in the kidney compared with the heart [69], whereas BAY 94-8862 accumulates in both organs to a similar extent [78]. This differential accumulation pattern may be part of the reason why the patients in the ARTS trial experienced less renal dysfunction when taking BAY 94-8862 compared with spironolactone [76]. Interestingly, BAY 94-8862 does not appear to be as good an antihypertensive agent as eplerenone. It appears that, although BAY 94-8862 does not lower blood pressure, its effects on the heart are more marked than those of eplerenone: cardiac hypertrophy, fibrosis, and pro-BNP expression were diminished by BAY 94-8862. BAY 94-8862 also had marked beneficial effects on the kidney. The cardiac injury in a chronic myocardial infarction model was also significantly reduced by BAY 94-8862 [78]. In this study, the fact that BAY 94-8862 did not cause as large a reduction in blood pressure as spironolactone was considered a positive because in patients with worsening heart failure, the blood pressure is already low [66].

Aldosterone synthase inhibitors

There are several reasons to consider aldosterone synthase inhibitors as potential therapeutic agents for hypertension and heart failure. The issues with side effects from the steroidal MR antagonists and the need to identify a way to inhibit both the genomic and non-genomic actions (see below) of aldosterone make inhibiting aldosterone production an attractive concept [79]. Also, some patients receiving angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) experience aldosterone breakthrough, where aldosterone levels increase with drug administration [80,81]; this negative effect of ACEIs and ARBs could be negated by blocking the production of aldosterone. The development of an aldosterone synthase inhibitor has been a particularly difficult task to achieve. The enzyme aldosterone synthase, encoded by the CYP11B2 gene, catalyzes the rate-limiting step in aldosterone production, the conversion of deoxycorticosterone to aldosterone. One of the major issues associated with producing a direct inhibitor of this enzyme is the homology it shares with the 11β hydroxylase enzyme (CYP11B1), which is the rate-limiting step in cortisol production. At the amino acid level, there is 93% homology between the two enzymes in humans [82]. Thus, a non-specific inhibitor could interfere with cortisol production and this could impair the stress response...
while also impacting the inflammatory response and metabolism.

The search for a specific aldosterone synthase blocker has led to the development of two potential therapeutic candidates. FAD286 showed great potential initially in preclinical trials where it was shown to reduce aldosterone production [83] and to have beneficial effects in various models of hypertension and heart failure [84-86]. Unfortunately, FAD286 was also found to have significant inhibitory effects on cortisol production and this limits the clinical usefulness of this compound [87].

This led to the development of LCI699, an aldosterone synthase inhibitor that is similar in structure to FAD286 but is approved for human use. This inhibitor is currently in phase 2 clinical trials, and the results are mixed. Although LCI699 inhibits aldosterone production, there is some evidence that it might inhibit 11β hydroxylase but that the inhibition is not significant enough to reduce baseline plasma cortisol levels. This was evidenced by an increase in plasma ACTH levels and an impairment in cortisol production in response to ACTH stimulation [88]. Despite the potential effects on cortisol production, LCI699 was deemed safe and well tolerated. A recent clinical trial comparing the effects of LCI699 and eplerenone was conducted in 534 patients with mild to moderate primary hypertension. LCI699 produced a dose-dependent reduction in systolic blood pressure but was no better at reducing blood pressure than eplerenone. The same potential effects on cortisol production were observed in this study [89]. A second study of patients with essential hypertension confirmed the antihypertensive effects of LCI699 [90]. Other studies, however, have failed to show a significant reduction in blood pressure with LCI699-treated patients with resistant hypertension [91]. In a head-to-head comparison, it seems that at least at the level of reducing blood pressure, LCI699 is no better than eplerenone [89,92]. This finding, combined with the fact the LCI699 may have significant effects on cortisol production and, therefore, the stress response, suggests that the drug may be of limited clinical use for the treatment of hypertension. However, LCI699 is currently being promoted as a drug for the treatment of Cushing’s syndrome [93]. It is possible that second-generation inhibitors will be more specific with fewer effects on cortisol synthesis.

Non-genomic actions of aldosterone and G protein-coupled estrogen receptor

Several groups have proposed that aldosterone also induces rapid responses that are non-genomic in nature; that is, they do not require gene transcription and translation; these have recently been reviewed [94-97]. Second messenger pathways such as cyclic adenosine monophosphate, diacylglycerol, inositol triphosphate, and calcium are activated by aldosterone within seconds to minutes of the initial exposure to the steroid [98-101]. Similarly, activation of protein kinases occurs rapidly [102-106]. The receptors responsible for these actions have been difficult to identify. This area of MR receptor biology is reviewed particularly well by Grossmann and Gekle [96], who present a clear argument for most of the non-genomic effects of aldosterone occurring through the classic MR, and as such, these effects can be blocked by classic MR antagonists.

However, recent studies have shown that the G protein-coupled estrogen receptor (GPER), which was initially known as GPR30, may be responsible for some of the rapid non-genomic responses to aldosterone in endothelial [107] and vascular smooth muscle cells [108]. Specific agonists and antagonists have been developed for GPER. The agonist G-1 was identified in 2006 by a combination of virtual and molecular screening using COS7 cells. G-1 is a non-steroidal molecule that has a high affinity and selectivity for the GPER [109]. G-1 mimics the rapid actions of aldosterone in endothelial and smooth muscle cells. These effects include ERK (extracellular-signal-regulated kinases) phosphorylation and apoptosis [107,108]. The GPER antagonist, known as G-15, is a G-1 analogue that was first identified in 2009 [110]. This compound effectively inhibits the rapid actions of aldosterone on vascular smooth muscle and endothelial cells [107,108]. Having both of these drugs available is a significant advance in helping delineate the rapid non-genomic effects of aldosterone from the slower classic effects requiring gene transcription and translation. These drugs have been used primarily in cell culture, and, therefore, it is not clear how important the rapid non-genomic responses mediated by aldosterone are in the pathology of the aldosterone-mediated conditions. G-1 has been chronically administered to female hypertensive rats; this activation of the GPER caused a reduction in blood pressure [111]. The mechanism responsible for this antihypertensive effect of G-1 appears to be direct dilation of resistance arteries [112]. It is difficult to reconcile these findings with the accepted pro-hypertensive effects of aldosterone; clearly, additional studies need to be conducted to delineate the estrogen-mediated effects from the aldosterone-mediated effects of GPER activation. There are several other studies that show direct vasodilator properties of aldosterone, but there appear to be an equal number of studies showing direct effects of aldosterone to inhibit dilation or cause constriction [113,114]. A recent review of the literature in this area suggests that aldosterone causes nitric oxide...
(NO)-mediated dilation in arteries from healthy individuals; but when endothelial dysfunction is present, aldosterone promotes vasoconstriction [115]. This concept is well described in a study by Heylen and colleagues [116]. This study utilized arterioles from normotensive Wistar rats, aldosterone produced an endothelium-dependent dilation that could be inhibited by spironolactone. Inhibiting NO production also prevented the aldosterone-mediated dilation, thus it appears that aldosterone stimulates vasodilation by activating NO production. However, when the authors removed the endothelium from the arteriole to simulate endothelial dysfunction, they observed a small contractile response to aldosterone administration.

The future of aldosterone/mineralocorticoid receptor blockade

The publication of the Randomized Aldactone Evaluation Study (RALES) trial in 1999 [24] and the work of Karl Weber (for selected references, see [117-121]) sparked the renaissance of aldosterone research. Around this time, it also became clear that hyperaldosteronism, or an increase in the renin-to-aldosterone ratio, was more prevalent in patients with hypertension than previously predicted [122-126]. Since then, the field has taken huge leaps forward with the identification of non-steroidal MR antagonists, aldosterone synthease blockers, and blockers of the non-genomic actions of aldosterone. However, several unknowns remain. The physiological relevance of the rapid non-genomic responses to aldosterone is not clear. Definitely identifying the receptor for the non-genomic actions of aldosterone is a necessary first step in developing highly specific drugs to inhibit these actions.

The evidence that aldosterone has detrimental effects on the vasculature and the heart has certainly been clear for some time [5,25], but aldosterone has also been recently implicated in other conditions, including cognitive impairment [127]. It seems that exciting times are ahead for aldosterone/MR biology.

Abbreviations

11βHSD2, 11β hydroxysteroid dehydrogenase type II; ACEI, angiotensin-converting enzyme inhibitor; ACTH, adrenocorticotropic hormone; ARB, angiotensin receptor blocker; ARTS, minerAlcorticoid Receptor Antagonist Tolerability Study; BNP, brain natriuretic peptide; DOCA, deoxycorticosterone; GPER, G protein-coupled estrogen receptor; ICAM-1, intracellular adhesion molecule-1; MR, mineralocorticoid receptor; NO, nitric oxide; Th17, T helper 17.

Disclosures

The author declares that she has no disclosures.

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