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Efficacy of Aldosterone Receptor Antagonism in Heart Failure: Potential Mechanisms

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Results of the Randomized Aldactone Evaluation Study and the Eplerenone Post-acute Myocardial Infarction Heart Failure Efficacy and Survival Study indicate aldosterone receptor antagonism, together with angiotensin-converting enzyme inhibition and loop diuretics, is a most effective strategy in reducing risk for all-cause and cardiovascular-related mortality and morbidity in patients with symptomatic heart failure. Responsible mechanisms are likely multifactoral. As a circulating hormone, aldosterone has well-known endocrine properties that contribute to the pathophysiology of congestive heart failure. This includes Na^+ resorption at the expense of K^+ excretion in such tissues as kidneys, colon, sweat, and salivary glands. Mg^{2+} excretion at these sites is likewise enhanced by aldosterone, whereas adrenal aldosterone secretion is regulated by extracellular Mg^{2+}. Other endocrine actions of aldosterone receptor-ligand binding include: a reduction in biologically active cytosolic-free Mg^{2+}, with intracellular Ca^{2+} loading in nonepithelial cells such as peripheral blood mononuclear cells; its influence on endothelial cell function; and its central actions, including the choroid plexus, activity of the hypothalamic paraventricular nucleus, and autonomic nervous system. De novo generation of aldosterone within the cardiovasculature is recognized and findings suggest its auto/paracrine properties contribute to tissue repair. Each of these actions is interrupted by aldosterone receptor antagonism and therefore may contribute to its salutary response in heart failure.

Introduction

Aldosterone, an effector hormone of the circulating renin-angiotensin-aldosterone system (RAAS) and the adrenal's most potent mineralocorticoid, is a major contributor to the pathophysiologic scenario that underlies the appearance of congestive heart failure (CHF). CHF, a clinical syndrome, consists of characteristic signs and symptoms that arise from congested organs and hypoperfused tissues. RAAS activation with attendant salt and water retention results in expanded intra- and extravascular volumes and the appearance of this syndrome. Not all patients with left ventricular dysfunction, whether expressed during systolic or diastolic phases of the cardiac cycle, have CHF. For example, reduced ejection fraction does not predict cardiac output, renal perfusion, or activation of the RAAS and therefore does not gauge the severity of chronic cardiac failure.

Several large-scale clinical trials have demonstrated the efficacy of aldosterone receptor antagonism in the overall management of CHF. The Randomized Aldactone Evaluation Study (RALES), conducted in 19 countries on five continents in more than 1600 patients with symptomatic heart failure, demonstrated the efficacy of spironolactone in combination with angiotensin-converting enzyme (ACE) inhibition and loop diuretics. A remarkable 30% reduction in risk for all-cause mortality and cardiovascular-related mortality, including sudden cardiac death, was observed [1••]. More recently, the Eplerenone Post-acute Myocardial Infarction Heart Failure Efficacy and Survival Study (EPHESUS), conducted at 674 centers in 37 countries on three continents in more than 6600 patients with symptomatic heart failure after a recent myocardial infarction (MI), demonstrated the efficacy of eplerenone, in combination with ACE inhibition, loop diuretics, and β-adrenergic receptor antagonism, in significantly reducing risk for the same end points observed in the RALES trial [2••].

Mechanisms responsible for these salutary findings are likely multifactoral. RAAS effector hormones have pleiotropic actions that may be life-saving when dietary Na^+ is markedly restricted or intravascular volume has been compromised by hemorrhage, vomiting, or diarrhea. In patients with heart failure, in whom Na^+ intake and intravascular volume are normal, these same circulating hormones prove pathologic and account for the appearance of CHF. Herein, several receptor-mediated biologic actions of aldosterone are considered (Fig. 1), with a view toward the potential benefits derived from aldosterone receptor antagonism.

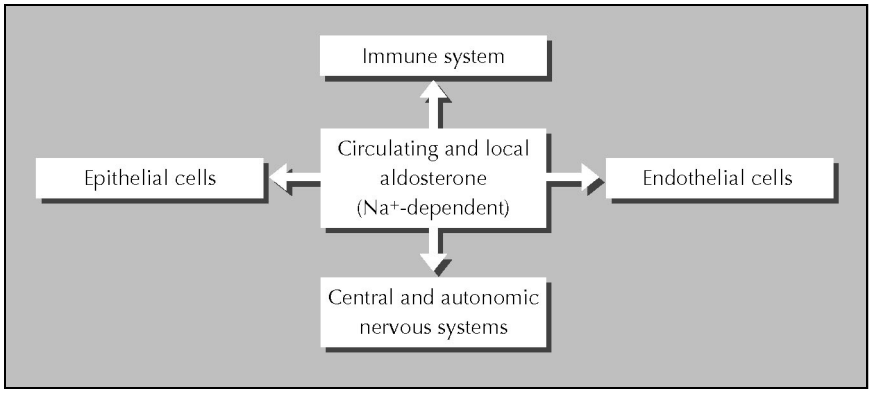

Figure 1. Circulating aldosterone has multiple endocrine properties. Several sites of action are shown.

Aldosterone and Mg^{2+} Balance

As recently reviewed [3,4], aldosterone regulates K^+ and Mg^{2+} excretion by epithelial cells in classic target tissue sites. Urinary K^+ and Mg^{2+} excretion were monitored in patients with primary aldosteronism, each of whom had low normal or reduced serum Mg^{2+} levels. Surgical resection of adrenal adenoma resulted in an immediate and marked decrease in urinary K^+ and Mg^{2+} excretion with a gradual normalization of plasma Mg^{2+}. Spironolactone reduced urinary K^+ and Mg^{2+} excretion in primary aldosteronism, which returned to previous increased basal levels after its discontinuation. Thus, the importance of aldosterone in promoting urinary K^+ and Mg^{2+} excretion is clear. J. W. Conn, who pioneered the discovery of autonomous adrenal aldosterone production, concluded that hypomagnesemia, with hypernatremia, hypochloremia, and metabolic alkalosis, was the cardinal metabolic abnormality of primary aldosteronism. The importance of the adrenal cortex in regulating Mg^{2+} and K^+ excretion is further underscored in the setting of adrenal insufficiency.

The potential for Mg^{2+} loss is increased in patients with CHF, in whom increased plasma aldosterone levels are expected and a loop diuretic is commonly used. The latter creates an independent stimulus to urinary Mg^{2+} excretion. The detection and assessment of intracellular Mg^{2+} deficiency is hindered by current clinical methodology. Nonetheless, it may contribute to morbid and mortal events, such as sudden cardiac death, which may occur in 50% of patients with CHF. The marked reduction in risk for sudden cardiac death observed in the RALES and EPHESUS trials may, in part, be related to the ability of aldosterone receptor antagonists to restore and/or to preserve Mg^{2+} homeostasis.

Aldosterone also regulates Mg^{2+} exchange by nonepithelial cells, such as peripheral blood mononuclear cells (PBMCs). Aldosterone binds to a single class of cytosolic receptors in these cells [5]. In patients with primary aldosteronism or renin-dependent, secondary aldosteronism, aldosterone binding sites are reduced and normalize after surgical removal of adrenal adenoma. Delva *et al.* [6] have suggested aldosterone receptor-ligand binding promotes an efflux of Mg^{2+} from cultured human lymphocytes in exchange for Na^+, a response dependent on extracellular Na^+ and thought to involve transcription and synthesis of a Na^+/Mg^{2+} exchanger. Lymphocyte cytosolic-free $[Mg^{2+}]_i$, its biologically active component, is reduced in patients with primary aldosteronism [6]. In uninephrectomized rats treated with aldosterone, $[Mg^{2+}]_i$ of PBMCs is significantly reduced and is accompanied by immune cell activation [7,8] (vide infra).

Mg^{2+} and Aldosterone Secretion

Extracellular Mg^{2+} ($[Mg^{2+}]_o$) participates in the regulation of adrenal aldosterone secretion to create a pathway of reciprocal regulation in Mg^{2+} homeostasis [3]. An intravenous infusion of $MgSO_4$ of several hours' duration suppresses plasma aldosterone levels in healthy, normotensive men and women. Conversely, diet-induced Mg^{2+} deficiency is accompanied by growth of adrenal zona glomerulosa and renal juxtaglomerulosa cells, and increased adrenal aldosterone secretion and plasma aldosterone levels, with a reduction in urinary Na^+/K^+ ratio to less than 1. Superfusate $[Mg^{2+}]_o$ regulates cultured zona glomerulosa cell aldosterone production: high $[Mg^{2+}]_o$ suppresses, whereas a $[Mg^{2+}]_o$-free media augments, their elaboration of aldosterone. The secondary aldosteronism that accompanies Mg^{2+} deficiency is associated with a time-dependent increase in $[Na^+]_i$ and $[Ca^{2+}]_i$ in heart, skeletal muscle, kidney, and bone, which suggests an inhibition of Na^+/K^+ ATPase, a Mg^{2+}-dependent pump, and increased Na^+/Ca^{2+} exchange at these sites. These iterations in cardiac tissue cation composition in heart failure could contribute to the propensity for arrhythmias.

Vascular Remodeling and Immune Cell Activation

A structural remodeling of the cardiovasculature by fibrous tissue accompanies aldosteronism [3,4]. This fibrogenic phenotype involves intramural arteries of the heart, kidney, and pancreas, the mesenteric circulation and vaso vasorum of aorta and pulmonary artery. Cotreatment with a receptor antagonist (*eg*, spironolactone, eplerenone) in nondepressor or depressor dosage prevents this remodeling,

indicating its independence of elevations in blood pressure. In a substudy to the RALES trial, survival benefit was associated with a reduction in circulating markers of collagen synthesis that presumably reflected an attenuation in ongoing vascular fibrosis [9].

The perivascular fibrosis of the coronary vasculature that ultimately appears in aldosteronism is preceded by a proinflammatory vascular phenotype that is independent of blood pressure. It features invading monocytes/macrophages and lymphocytes and adhesion molecule expression. Molecular mechanisms involved in these cellular responses has been interrogated in animal models, such as rats receiving aldosterone/salt treatment. Plasma renin and angiotensin II are suppressed in this model. Using this model, Sun *et al.* [10] tested the hypothesis that oxidative stress was involved in the appearance of the proinflammatory/fibrogenic cardiac phenotype that first appears in both atria and ventricles at week 4 of aldosterone/salt treatment. Invading cells were found to express NADPH oxidase, a major source of superoxide in leukocytes, and 3-nitrotyrosine, a product of peroxynitrite with stable protein tyrosine residues. Peroxynitrite is formed by the reaction between superoxide and nitric oxide. A redox-sensitive transcription factor integral to inflammatory responses, NFκB, was likewise activated in these cells with upregulated mRNA expression of mediators it regulates: intercellular adhesion molecule-1, monocyte chemoattractant protein-1, and a proinflammatory cytokine tumor necrosis factor (TNF)-α. Cotreatment with spironolactone or an antioxidant prevented the appearance of these cells and associated molecular responses, and the subsequent perivascular fibrosis. Thus, aldosterone/salt treatment is accompanied by oxi/nitrosative stress within inflammatory cells invading the intramural coronary vasculature, and it is this proinflammatory vascular phenotype that leads to intramural coronary artery pathology and subsequent perivascular fibrosis. Why should aldosterone/salt treatment lead to oxi/nitrosative stress in immune cells, and did this first occur at vascular sites or beforehand?

Gerling *et al.* [7] tested the hypothesis that aldosterone/salt treatment results in a reduction in PBMCs $[Mg^{2+}]_i$ before the appearance of cardiac pathology at week 4. This proved to be true, with $[Ca^{2+}]_i$ loading and an early activation of these cells evident in their transcriptome (expressed genes) and proteome (expressed proteins). The interrogation of complex molecular events that account for immune cell activation and subsequent homing of these cells to the heart may explain why cardiac pathology does not appear until week 4 of aldosterone/salt treatment and questions the prospect of an autoimmune response despite there being no prior cardiac injury or exposure to self-antigens in this model. Ahokas *et al.* [8] recently found Ca^{2+} loading-induced oxi/nitrosative stress in PBMCs and where H_2O_2 appears to serve as second messenger to their activation. In abrogating these responses, spironolactone proved to be immunomodulatory. In another state of aldosteronism, induced by dietary Mg^{2+} deficiency, lymphocyte Mg^{2+} is reduced and accompanied by an early (week 1) induction of oxi/nitrosative stress and depletion of antioxidant defenses in PBMCs [11–14]. Lymphocyte activation includes their production of proinflammatory cytokines [15]. Like the aldosterone/salt treatment model, cardiac lesions in dietary Mg^{2+} deficiency are delayed in their appearance and are first seen during week 3 [15].

Immune cell activation accompanies the secondary aldosteronism of human CHF [16–18]. The now-recognized "cytokine storm" of CHF, which features elevations in such circulating proinflammatory cytokines as TNF-α and interleukin-6, likely includes immune cells in its origins. Cells of the monocyte-phagocyte system are a potent source of these cytokines. Another or alternative source of cytokine production in heart failure is the central nervous system [19]. Irrespective of their origin, prolonged elevations in these proinflammatory cytokines contribute to the progressive systemic illness that accompanies CHF and features tissue wasting to eventuate in cardiac cachexia [20]. Immune cell activation with targeting of the heart could contribute to the appearance of atrial and ventricular arrhythmias. The immunomodulatory role of aldosterone receptor antagonism in this setting may likewise contribute to its observed clinical efficacy.

Endothelial Cell Dysfunction

In patients with primary aldosteronism or renal artery stenosis with secondary aldosteronism, forearm vasomotor reactivity to endothelial cell-dependent acetylcholine is diminished compared with normotensive controls, whereas nonendothelial cell-dependent sodium nitroprusside-induced vasodilatation is preserved [21]. Four weeks after surgical removal of adrenal adenoma, the impairment in endothelial cell-dependent vasodilation is restored. In the secondary aldosteronism that accompanies CHF, diminished forearm vasomotor reactivity to acetylcholine is normalized after 1 month of spironolactone treatment [22]. Acetylcholine-induced, nitric oxide–dependent vasorelaxation of aortic rings is reduced in rats after MI [23]. This vasomotor dysfunction, with increased superoxide formation by aortic tissue, is normalized by spironolactone alone or in combination with ACE inhibition [23]. Abnormal Mg^{2+} homeostasis may represent the underlying pathophysiologic basis for endothelial dysfunction seen in primary or secondary aldosteronism.

Central Actions of Aldosterone

Aldosterone has a central action involved in the regulation of blood pressure and cerebrospinal fluid volume and composition [3]. Aldosterone is produced in the brain, where its paracrine properties may contribute to blood pressure regulation [24]. Aldosterone's central actions contribute to the pathophysiology of CHF [25–27]. The hypothalamic paraventricular nucleus (PVN) is involved in the regulation

of extracellular volume and sympathetic nerve activity. It is governed by circulating neurohormones and effector signals originating from the brainstem. In rats with MI induced by coronary artery ligation, the activity of the PVN is increased. Systemic or intracerebroventricular administration of spironolactone reduces this activity, improves baroreflex regulation of renal sympathetic nerve activity (albeit in a time-dependent manner), and prevents the increase in Na^+ appetite and decline in urinary Na^+ and H_2O excretion that appear in this model [25,26]. Plasma levels of TNF-α increase progressively over weeks 1 to 3 after MI, a response abrogated by intracerebroventricular infusion of spironolactone started 24 hours after coronary ligation. This suggests central aldosterone receptor activation is involved in regulating the release of this proinflammatory cytokine from central and/or peripheral tissues [27]. Whether central responses that appear in the weeks after MI were present and abrogated by eplerenone in the EPHESUS trial is unknown.

Autonomic Nervous System

Time and frequency domains of heart rate variability are reduced in heart failure [28–30]. Such a reduction in heart rate variability is a predictor of sudden cardiac death [31]. Spironolactone reduces heart rate and improves heart rate variability and QT dispersion in patients with symptomatic heart failure and reduced systolic function [32].

Aldosterone blocks the uptake of norepinephrine by the myocardium [33]. Using ^{123}I-MIBG scintigraphy, an analog of norepinephrine, spironolactone has been found to increase its uptake in patents with heart failure of diverse causality [33,34].

Extra-adrenal Aldosterone Production and Tissue Repair

During the past decade, Takeda *et al*. [35,36] identified the mRNA expression of aldosterone synthase (CYP11B2) and aldosterone production in rodent vascular tissue. Silvestre *et al*. [37] demonstrated the expression of this enzyme, integral to the biosynthesis of aldosterone, in the rodent heart, where aldosterone generation was regulated by angiotensin II, a low Na^+ or high K^+ diet, or adrenocorticotropin. Subsequently, the expression of this enzyme was identified in human cardiovascular tissue, where it was localized to vascular smooth muscle and endothelial cells [36,38].

If the circulating RAAS is not activated after MI, what is the source of angiotensin II in the infarcted heart? Macrophages and myofibroblasts involved in tissue repair at the infarct site express renin, ACE, and angiotensin-II receptors, predominantly of the AT_1 subtype [39]. This has implicated locally produced angiotensin II in regulating collagen turnover at these sites, which has been further suggested by the cardioprotective actions of AT_1 receptor antagonists in attenuating fibrous tissue formation [40]. Locally produced angiotensin II regulates de novo aldosterone production in the infarcted heart, which likewise contributes to tissue repair [41–43]. Increased expression of aldosterone synthase and aldosterone tissue levels, with increased concentrations of angiotensin II, have been observed in noninfarcted rat myocardium after coronary artery ligation [41,44]. Treatment with losartan prevented these responses related to de novo aldosterone production. Losartan, spironolactone, or eplerenone treatment prevented the accompanying structural remodeling, suggesting the involvement of angiotensin II–driven local aldosterone production in regulating tissue repair [40,41,44,45]. Hayashi *et al*. [46] have reported aldosterone is extracted by the heart after MI, and the transcardiac aldosterone gradient (between aorta and coronary sinus) correlates with coronary venous effluent levels of a serologic marker of collagen turnover (*ie*, procollagen type III aminoterminal peptide, PIIINP) associated with left ventricular dilatation and poor function and prognosis. Aldosterone is extracted by the chronically failing human heart of diverse etiologic origins, a response blocked by spironolactone [47].

Caveats to Angiotensin-converting Enzyme Inhibitor and Aldosterone Receptor Antagonist Cotreatment

Various pharmacologic interventions comprise today's standard of care for the overall management of heart failure. Current guidelines and attendant algorithms, albeit well-intentioned and drawn from controlled clinical trials, have clear limitations, the most resounding of which is one size does not fit all. Any given management strategy must be based on the individual patient and derived from an understanding of pathophysiologic mechanisms operative in that patient, with an assessment of the potential efficacy and safety of the regimen in the patient.

Despite the proven efficacy of adding aldosterone receptor antagonists to the management of symptomatic heart failure, caution is advised. In the RALES and EPHESUS trials, aldosterone receptor antagonism was combined with ACE inhibition in patients who did not have a major reduction in renal function, as reflected by serum creatinine of 2.5 mg/dL or less, or a marked elevation in serum potassium (> 5 mmol/L) during study entry. Additionally, K^+ supplements (pharmacologic and dietary salt substitutes) were restricted or more often discontinued. Finally, regular surveillance of serum electrolytes is mandatory with a frequency based on the individual patient. Episodes of vomiting or diarrhea, with attendant intravascular volume contraction and reduced renal function, may predispose to hyperkalemia, as would the use of various pharmacologic agents. These include nonsteroidal anti-inflammatory agents, which interfere with the formation of prostaglandins integral to renal vasodilatation and intrarenal hemodynamics (and should be avoided in patients with heart failure), heparin, which can behave as an AT_1 receptor antagonist resulting in hypoaldosteronism, and cyclosporine. Hence, reasoned judgment and watchful

anticipation should mitigate against clinically significant hyperkalemia (*ie*, > 5.5 mEq/dL) while aldosterone receptor antagonists might attenuate life-threatening episodes of hypokalemia and retard Mg^{2+} losses.

Contrasted with doses of spironolactone used years ago as sole management of edema formation in chronic heart failure, its dosage in these trials was small (25 or 50 mg once daily) because it was administered in combination with a potent loop diuretic. Moreover, its effects at these doses have been shown to extend beyond salt and water excretion and include less well-appreciated biologic actions of aldosterone noted earlier.

Painful gynecomastia may accompany spironolactone treatment. As seen in Figure 2, spironolactone can occupy androgen receptors, leaving unopposed estrogen receptors and a predisposition to estrogen-like side effects. However, most men do not have biologically significant amounts of estrogen. Accordingly, the incidence of gynecomastia is quite low. This is evident in countries where the combination of aldactone and thiazide is widely prescribed for the management of essential hypertension. However, digoxin has well-established estrogen-like properties. In my experience, the combination of spironolactone and digoxin predisposes to gynecomastia and once present can be managed by discontinuing digoxin.

Conclusions

Elevations in plasma aldosterone are an integral feature of the salt and water retention that accompanies the clinical syndrome of CHF. Results of the RALES and EPHESUS trials, conducted in patients with symptomatic heart failure, indicate the combination of an ACE inhibitor, loop diuretic, and low-dose aldosterone, with or without β-adrenergic receptor blockade, is a very effective strategy in reducing risk for all-cause and cardiovascular-based mortality beyond that seen with ACE inhibitor and loop diuretic alone. This includes a 30% reduction in the risk for sudden cardiac death. The risk for cardiovascular-related morbidities also is significantly reduced with this regimen.

Given the multiple endocrine properties of circulating aldosterone on epithelial and nonepithelial cells, mechanisms responsible for these salutary responses to aldosterone receptor antagonism are multifactoral. They include preserving K^+ and Mg^{2+} homeostasis, preventing adverse vascular remodeling of the heart and systemic organs, re-establishing endothelial vasomotor reactivity, and restoring heart rate variability and myocardial norepinephrine uptake. In addition, aldosterone receptor antagonism will protect the injured cardiovasculature from the auto/paracrine properties of aldosterone generated de novo at these sites.

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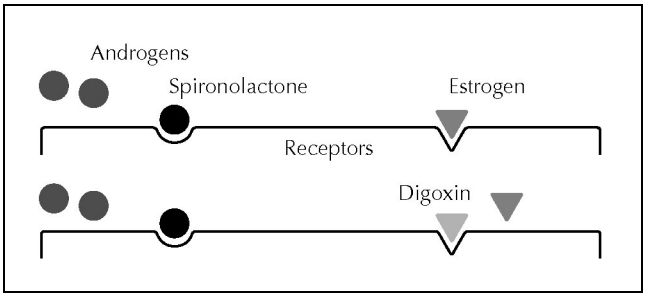

Figure 2. Spironolactone, an aldosterone receptor antagonist, also blocks androgen receptors, which leave unopposed estrogen receptors. Digoxin has estrogen-like properties and can contribute to the appearance of gynecomastia.

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- • Of importance
- •• Of major importance

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