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# Impact of aldosterone antagonists on the substrate for atrial fibrillation: Aldosterone promotes oxidative stress and atrial structural/electrical remodeling

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## Abstract

Atrial fibrillation (AF), the most common cardiac arrhythmia, is an electrocardiographic description of a condition with multiple and complex underlying mechanisms. Oxidative stress is an important driver of structural remodeling that creates a substrate for AF. Oxidant radicals may promote increase of atrial oxidative damage, electrical and structural remodeling, and atrial inflammation. AF and other cardiovascular morbidities activate angiotensin (Ang-II)-dependent and independent cascades. A key component of the renin–angiotensin-aldosterone system (RAAS) is the mineralocorticoid aldosterone. Recent studies provide evidence of myocardial aldosterone synthesis. Aldosterone promotes cardiac oxidative stress, inflammation and structural/electrical remodeling via multiple mechanisms. In HF patients, aldosterone production is enhanced. In patients and in experimental HF and AF models, aldosterone receptor antagonists have favorable influences on cardiac remodeling and oxidative stress. Therapeutic approaches that seek to reduce AF burden by modulating the aldosterone system are likely beneficial but underutilized.

### Keywords

Aldosterone; Aldosterone antagonist; Atrial fibrillation; Oxidative stress

## 1. Introduction

Atrial fibrillation (AF), the most common cardiac arrhythmia, currently affects more than 3 million Americans, and more than 12 million Americans are projected to suffer from AF by 2050 [1]. Major complications associated with AF include thromboembolic events and impaired cardiac function, resulting in increased risk of heart failure (HF), stroke and



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mortality [2]. AF is frequently associated with co-morbid conditions such as hypertension (HTN), HF, valvular diseases, and cardiomyopathy [3].

The concept of substrates and triggers is useful to frame discussions of arrhythmic mechanisms and antiarrhythmic targets. An arrhythmia substrate constitutes a persistent change in atrial structure or function (e.g., myocyte hypertrophy, chamber dilatation, interstitial fibrosis and ion channel remodeling) that increases the persistence of arrhythmia episodes once they have been initiated; triggers are acute events that promote the initiation of arrhythmic episodes that may either spontaneously terminate, or persist, depending on the arrhythmia substrate. Triggers are spontaneous or evoked sources of depolarization, caused by exposure to high levels of catecholamines, spontaneous release of calcium from intracellular stores, or high rates of electrical activation.

Current pharmacologic strategies for controlling AF rely mainly on blocking cardiac ion channels either to slow electrical conduction (sodium channels) or prolong atrial refractoriness (potassium channels). These interventions have been largely unsuccessful, with most patients having recurrent AF within a year of treatment [4]. Thus, treatment efforts have increasingly focused on the use of anticoagulants to reduce risk of stroke, and invasive surgical (Maze) or endovascular ablation procedures to suppress AF [5]. The high rate of AF recurrence reflects in part our poor understanding of the mechanisms and causes of atrial arrhythmia substrates that initiate and maintain AF. The mechanisms underlying AF are complex and multiple, including electrical, structural, inflammatory, and metabolic factors [6] (Fig. 1).

In addition to generic cardiac ion channel blockade, some studies have considered development of new antiarrhythmic drugs and upstream therapies for AF that seek to be more effective and safer, particularly for patients with structural heart disease. These new drugs include selective atrial specific ion channel blockers and agents that target the underlying substrates that promote AF [7]. Among the latter category are upstream drugs that suppress activation of renin–angiotensin-aldosterone system (RAAS), which includes several known and novel therapeutic targets for AF [8].

The RAAS is involved in myocardial fibrosis, inflammation, oxidative stress and electrical abnormalities in HTN, HF, AF, myocardial infarction (MI), and cardiomyopathy [9]. Aldosterone, an adrenal hormone secreted after activation of the renin–angiotensin-aldosterone system (RAAS), is a critical regulator of blood pressure and electrolyte homeostasis [10,11]. HF is associated with increased production of aldosterone [12,13], and the use of an aldosterone receptor antagonist has been shown to attenuate atrial remodeling [14] and oxidative stress in HF [15]. Aldosterone receptor antagonists such as spironolactone and eplerenone have demonstrated several effects on cardiac diseases that are unrelated to their effects on blood pressure [16].

Aldosterone production is mainly regulated by the action of angiotensin II (Ang-II) on aldosterone producing cells of the adrenal cortex [17]. Evidence of myocardial aldosterone synthesis has led to a generation of new hypotheses regarding the physiological and the pathophysiologic significance of this hormone. Both cardiomyocytes and cardiac fibroblasts

express mineralocorticoid receptors (MRs) that have a high affinity for aldosterone [18]. Genomic effects of aldosterone are mediated by its interaction with MRs [18]. Aldosterone also amplifies Ang-II signaling and induces expression of ventricular and vascular Angiotensin type-1 receptors ($AT_1R$) [19,20] and angiotensin converting enzyme (ACE) [21]. Aldosterone also regulates vascular transcription of several pro-atherogenic and oxidant genes [22]. Aldosterone also induces rapid changes (nongenomic) [23] that are not prevented by MR antagonists, but which are likely mediated by still uncharacterized plasma membrane receptors [24].

In this review, we summarize the role of aldosterone on promoting substrates of AF with focus on oxidative stress and its relation to inflammation and structural/electrical remodeling. The potential use of aldosterone antagonists to prevent onset or progress of AF will also be discussed.

## 2. Oxidative stress and atrial fibrillation

There are multiple cardiac sources of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which contribute to the loss of redox homeostasis and oxidative stress [15]. Prominent sources of oxidant stress in the atria include the mitochondrial electron transport chain, nicotinamide adenine dinucleotide phosphate oxidases (NADPH oxidases), and xanthine oxidase, and uncoupled nitric oxide synthase (NOS) [15]. The body also has a system of antioxidant defense enzymes to protect from an excess of reactive species, including superoxide dismutases, glutathione reductase and thioredoxin reductase [15].

ROS generation modulates the redox signaling of molecules such as NF-κB. NF-κB signaling stimulates many downstream inflammatory responses, including upregulation of tumor necrosis factor-alpha (TNF-α), C reactive protein (CRP), interleukin-6 (IL-6) and a reduction in endothelial NOS (eNOS) activity and NO production [25].

Several studies have documented evidence of increased oxidative stress in the atria of AF patients [26,27]. Rapid atrial pacing is also associated with increased nitrotyrosine abundance and decreased $Ca^{2+}$ current [28], suggesting an important role of ROS in AF pathogenesis. Use of glutathione [28] and ascorbic acid [29], a water-soluble antioxidant, attenuated acute atrial electrical remodeling. Studies have also documented increased oxidative damage and ROS production in AF. Enhanced ROS generation via NADPH oxidase (NOX) is also associated with the development of post operative AF (POAF) [30]. In addition, increased NOX activity promotes cardiac remodeling and dysfunction [31].

NOS uncoupling promotes ROS generation [15]. NOS 1 and 3 forms are expressed under normal conditions. However, under pathological conditions associated with inflammation such as in HF, an induction of the inducible form of NOS (NOS 2, iNOS) occurs, which can become uncoupled, shifting the balance from a predominant synthesis of nitric oxide to that of superoxide anion [15].

ROS are also produced by the mitochondria, and AF is associated with mitochondrial injury and dysfunction [32]. Myocardial injury promotes the production of inflammatory cytokines including TNF-α. TNF-α is associated with mitochondrial dysfunction, apoptosis, and

oxidative stress [33]. Similarly, in a goat model of AF, atrial tissues showed signs of mitochondrial dysfunction [32], decreased oxidative phosphorylation, and increased proton leak [34]. This was associated with increased ROS from the mitochondria. In addition to uncoupling of electron transport, NADPH oxidase 4 (NOX4) is thought to be localized to the mitochondria and may contribute to the production of $H_2O_2$ and $O_2^-$ under conditions of aging [35]. Oxidative stress can promote a cycle of oxidative damage in which the mitochondrial dysfunction promotes impaired energy production, which can impair contractile and electrical activity and promote cell death.

## 3. Aldosterone and oxidative stress

The RAAS is a primary modulator of cardiac oxidative damage [25]. AngII increases the generation of ROS in the vascular system via activation of the membrane-bound NADPH oxidase enzymes, endoplasmic reticulum stress and mitochondrial oxidative stress [25]. Several studies suggested that MR activation might potentiate the pro-inflammatory/fibrotic effects of AngII-AT1R signaling by increasing cardiac oxidative stress [19,36,37]. Aldosterone infusion in rats resulted in impaired endothelial relaxation, and increased oxidative stress in the vessel [36,38]. In the former study, use of eplerenone improved endothelial function in ischemic rat by reducing NADPH oxidase subunits, and increasing protein expression of eNOS and angiogenic factors such as VEGF and angiopoietin-1&2 [36]. Aldosterone antagonist treatments increased the levels of NO and prostacyclin, and reduced the levels of Ang-II, aldosterone, glutathione [39], and superoxide [40]. Spironolactone reduces oxidative cardiac damage and remodeling via generation of Ang-I, the less active form of angiotensin, in macrophages of HF patients. Aldosterone also induced oxidative stress independent of Ang via ET-1 signaling [41] and the Rho kinase pathway [42].

Interestingly, low dose spironolactone treatment (1 mg/kg for 3 weeks) suppressed diastolic dysfunction, oxidative stress and fibrosis without affecting blood pressure [43,44] suggesting that the pleiotropic effects of aldosterone antagonists are independent of blood pressure lowering.

In addition to its effect on NADPH oxidase activity and NO levels, aldosterone increased the levels of the NOX subunit p22phox, urinary 8-isoprostane levels (a marker of renal oxidative stress), superoxide dismutase (SOD) mRNA levels, and plasma oxidants markers such as malonyl dialdehyde (MDA) and hydrogen peroxide ($H_2O_2$) [45–48]. Interestingly, eplerenone only partially attenuated these effects, suggesting that additional mechanisms are involved in aldosterone effects that may be mediated by receptors other than the MRs [45]. Interestingly, apocynin, a superoxide production inhibitor, appeared to reverse the effects of aldosterone on protein expression of selective components of the NADPH oxidase complexes and cardiac remodeling, suggesting that therapeutic targeting of NOX isoforms might present an alternative approach to attenuate the oxidant-stress associated with aldosterone generation [47,49].

Mitochondrial dysfunction appears to be a critical factor in the development of several cardiovascular conditions such as arrhythmia [50] and HF [51]. Aldosterone increases

mitochondrial-ROS generation independent of NADPH oxidase via the ERK1/2 pathway [52]. In the isolated mitochondria and in cardiac myocytes, $Ca^{2+}$ serves as pro-oxidant while $Zn^{2+}$ serves as antioxidant. Aldosterone causes dyshomeostasis of intracellular $Ca^{2+}$ and $Zn^{2+}$ via respective induction of oxidative stress and generation of antioxidant defenses [53].

Loss of intracellular $Mg^{2+}$ has been associated with risk of cardiovascular diseases and arrhythmia [54]. Aldosterone was reported to cause a reduction in the cytosolic free $Mg^{2+}$ concentration in rats resulting in intramural coronary artery remodeling accompanied by increased markers of cardiac and plasma oxidative stress, arterial inflammation and evidence of perivascular fibrosis [55]. Intriguingly, spironolactone has been shown to prevent AF in HF patients by modulating $Mg^{2+}$ homeostasis [56].

The RAAS is implicated as a primary pathway underlying cardiorenal syndrome, and the use of aldosterone antagonists has been shown to reduce kidney oxidative damage, and also to prevent cardiac hypertrophy and oxidative stress in renal failure [46–48,57].

## 4. Aldosterone and inflammation

Inflammation is mechanistically related to oxidative stress. A role for inflammation in AF can be inferred from the common association of AF with inflammatory conditions like pericarditis and myocarditis [58]. Impaired atrial contractility promotes pro-inflammatory changes such as platelet adhesion, cytokine and neurohormone production. Monocytes and leukocytes are abundant in atria specimens from AF patients [59]. Increased levels of CRP-complement 4B complex have also been associated with increased risk of POAF, and the time of peak elevation of CRP 2–3 days post-surgery coincides with the peak incidence of POAF [60]. Elevated levels of IL-6 have been identified as an independent predictor of stroke and death in high risk AF patients, linking abnormal inflammation with risk of thromboembolism [61]. Elevated plasma IL-6 levels have also been identified as a risk marker for AF [62], and polymorphisms in both IL6 [62] and the IL-6 receptor [63] have been associated with AF risk. Interestingly, aldosterone has been suggested as a mediator of the increased levels of IL-6 in the DOCA-salt model of hypertension [64].

### 4.1. Role of aldosterone and inflammation in the development of fibrosis

Aldosterone promotes tissue inflammation leading to fibrosis and remodeling in the heart, vascular system, and the kidney [65]. Several studies suggested cell-specific effects of MR activation on inflammatory cell adhesion and infiltration. Aldosterone promotes kidney macrophage infiltration, complement C3 deposition and formation of nitrotyrosine, collagens, chemokines, adhesion molecules and profibrotic cytokines. These effects were all reduced by use of spironolactone [66]. Aldosterone also induces several inflammatory processes in adipocytes and contributes to insulin resistance by promoting oxidative stress, suggesting that it may also contribute to development of diabetes mellitus [25].

In rat heart, aldosterone treatment increased perivascular levels of the inflammatory mediators cyclooxygenase-2 and osteopontin, leading to perivascular fibrosis [67]. These changes were attenuated by eplerenone treatment. In rat aortic tissues, aldosterone induced Ang-II expression and expression of several pro-inflammatory genes such as TNF-α,

monocyte chemotactic protein-1 (MCP-1) and NADPH oxidase [68]. In addition, aldosterone induced expression of adhesion molecules such as soluble intercellular and vascular adhesion molecule-1 (ICAM-1 and VCAM-1), matrix metalloproteinase-2 (MMP-2), platelet derived growth factor A (PDGF) and plasminogen activator inhibitor (PAI) [69]. Interestingly, use of eplerenone suppressed all of these effects, while candesartan, an angiotensin-II receptor blocker, and tempol, a mimetic for superoxide dismutase only partially suppressed these effects, suggesting a more important role for aldosterone in promoting inflammation and oxidative stress [69]. Intriguingly, tempol has been shown to significantly attenuate cardiac oxidative stress and transforming growth factor (TGF) beta expression [70].

NF-κB signaling is associated with both inflammatory and pro-fibrotic responses during AF [71]. In a Dahl salt-sensitive rat model of HF, eplerenone reduced iNOS and activated NF-κB [72]. Activation of NF-κB in vascular SMCs involves complex interaction between the $AT_1$-R and MR [25].

In human HF, aldosterone levels were closely correlated with levels of pro-inflammatory markers such as 8-isoprostaglandin F-2alpha (8-iso-PGF), I-CAM and tissue inhibitor metalloproteinase-1 (TIMP-1) [73]. Aldosterone and Ang-II infusion increased levels of human IL-6 but not CRP, and use of spironolactone attenuated these effects suggesting that Ang-II induces IL-6 expression through a MR-dependent pathway [74]. Similar to IL-6, IL-18 also promotes myocardial hypertrophy, impaired contractility and apoptosis. Aldosterone promotes IL-18 expression in cardiac myocytes via parallel activation of Ang-II, ET-1, Rho/Rho kinase and peroxisome proliferator activated receptors (PPARs) [75].

## 5. Aldosterone and cardiac fibrotic remodeling

Fibrosis is a hallmark of atrial structural remodeling and a common feature of clinical AF [10]. Increased interstitial fibrosis can physically separate myocytes, decreasing myocyte electrical coupling and creating a barrier to rapid impulse propagation [10]. AF is associated with marked structural changes in atrial tissues that, to a large extent, depend on increased activity of the RAAS [71]. In a HF model, development of atrial fibrosis was associated with increased atrial Ang-II levels [13]. Increased Ang-II production in transgenic mice with cardiac-restricted ACE over expression causes marked atrial dilation with focal fibrosis and AF [76]. In this experimental system, treatment with ACE inhibitors improved left ventricular function and attenuated the development cardiac fibrosis [13,77]. In the RALES clinical trial [78], use of spironolactone led to a significant improvement of LV function, and a reduction in cardiac fibrosis and sudden death in patients with severe HF.

Elevated plasma and myocardial aldosterone levels are reported in HF patients and are associated with development of cardiac fibrosis [12,13]. An increase in plasma and myocardial aldosterone has been shown also to cause cardiac fibrosis [11,79,80]. Interestingly, in normal rat hearts, atrial aldosterone levels are higher than those in the ventricles, suggesting a differential role of aldosterone in the atria [81]. The antioxidant effects of spironolactone decreased MMP-2 [69], and improved vascular fibrosis found in rat models of HTN and HF [40,72]. Cardiac fibrosis induced by aldosterone and a high salt diet

was exaggerated in $AT_1$-R knockout mice [42]. This effect was attenuated by antioxidants effects of eplerenone [42]. Eplerenone also attenuated the transition from ventricular hypertrophy to failure associated with chronic pressure overload [37]. Intriguingly, mineralocorticoid receptor antagonism has been shown to prevent cardiac remodeling associated with hypertension, but not with aging [82], suggesting a more dominant role of other profibrotic neurohormones that may increase with aging.

Cardiac apoptosis is a complex process that is mediated in part by redox signaling [83]. Spironolactone prevented cardiac apoptosis and remodeling in aldosterone infused rats [83]. Although aldosterone has been shown to cause cardiac interstitial fibrosis, it is still unclear whether the mechanism is solely related to specific cardiac effects of aldosterone, to the combined effects of aldosterone with that of other local mediators (e.g., angiotensin-II, endothelin-1 or oxidant stress), or if it is also related to extra-cardiac effects, such as those on the kidney. Interestingly, in a cardiac-specific conditional MR knock-down model, mice developed severe HF and cardiac fibrosis in the absence of HTN or hyperaldosteronemia. These changes were fully reversed when MR antisense mRNA was suppressed [84]. Intriguingly, use of spironolactone potentiates cardiac fibrosis in this model, raising questions about the physiologic/pathophysiologic role of MR in the heart, and the potential role of receptors other than MR.

It is important to know whether Ang-II or aldosterone effects predominate. In a large study using rats, Milliez et al. [14] evaluated the role spironolactone, lisinopril (ACE inhibitor), and atenolol (β blocker) or their combination on the development of atrial fibrosis in their HF model. Only spironolactone attenuated atrial fibrosis. This result was further validated in a study by Yang et al. [85], where spironolactone also attenuated atrial fibrosis in a canine HF model. These observations raise the interesting possibility of using an aldosterone/MR antagonist to reverse atrial fibrosis.

The RAAS activates key downstream profibrotic mediators like transforming growth factor (TGF)-β [9]. Another potential factor is PDGF, which has recently become of great interest [9,86,87]. MR antagonists attenuate Ang-II induced cardiac hypertrophy and fibrosis [88,89]. Nishioka et al. [90], reported that myocardial fibrosis promoted by AngII induced PDGF-A, B and PDGFR-α, was ameliorated by use of eplerenone. Similarly, aldosterone increases PDGF B protein [91] in hepatocytes, and use of a MR antagonist reduced PDGF and TGF-β induced cell proliferation and migration [92,93].

We and others have shown that connective tissue growth factor (CTGF) is increased in the atria of patients with AF [94,95]. Interestingly, CTGF has been shown to promote atrial remodeling via Ang-II induced NADPH oxidase/Rac-1 dependent pathway [96]. Aldosterone also induces CTGF expression by activation of the NF-κB [97].

## 6. Aldosterone and electrical remodeling

Many studies suggest that improper $Ca^{2+}$ handling is an important arrhythmogenic factor and a candidate mechanism to underlie AF-generating ectopic foci.

During AF, profound changes in $Ca^{2+}$ cycling occur in the atria. At the single channel level, increased single L-type $Ca^{2+}$ channel ($Ca_v1.2$) activity due to an increase of channel open probability has been observed in human AF [98]. In human and animal models of AF [99,100], whole cell L-type $Ca^{2+}$ current densities are decreased by 60–70%, possibly as an adaptive response to arrhythmia-induced $Ca^{2+}$ overload.

In rat ventricular myocytes, aldosterone treatment increased the density of L- and T-type $Ca^{2+}$ currents, and mRNA expression of alpha1C and beta2 subunits of cardiac $Ca_v1.2$ channels [101,102]. Use of spironolactone reduced $Ca^{2+}$ current density. In rabbits, eplerenone also attenuated the decrease in atrial $Ca^{2+}$ current following atrial tachypacing [103]. Eplerenone suppressed AngII induced-inward $Ca^{2+}$ current, and this effect was reversed by an addition of aldosterone, suggesting that the intracellular effects of AngII on $Ca^{2+}$ current of cardiac mycocytes are mediated through the MR [104].

AF is associated with cellular changes of several potassium currents [105]. Aldosterone decreases the transient outward $K^+$ current, $I_{to1}$ density secondary to the rise in $Ca^{2+}$ current [106]. Both spironolactone and canrenoic acid (active metabolite of spironolactone) directly blocked Kv1.5, Kv4.3 and Kv7.1 channels that generate the human $I_{Kur}$, $I_{to1}$ and $I_{Ks}$ [106]. In a rat model of inducible AF, aldosterone promotes AF via both increased structural remodeling and shortening of action potential via alteration of $K^+$ currents [107]. These data suggest that aldosterone antagonists may be helpful in treatment of supraventricular arrhythmias via modulation of both $K^+$ and $Ca^{2+}$ channels.

Cardiac myocytes contain inositol 1,4,5 trisphosphate receptors (IP3R), which also contribute to $Ca^{2+}$ release from intracellular stores when activated by IP3. Enhanced IP3 signaling increases the likelihood of spontaneous $Ca^{2+}$ release/overload. In addition, $Ca^{2+}$ release through plasmalemmal IP3Rs may sensitize RyRs. Interestingly, in human mononuclear leukocytes, aldosterone caused a concentration-dependent increase in IP3 levels through sodium hydrogen antiporter ($Na^+$/H) [108,109]. In addition, aldosterone increased generation of diacylglycerol (DAG) and IP3 via increase activity of phospholipase C (PLC) [110]. These studies suggest that aldosterone may mediate rapid effects by membrane receptors similar to GPCRs.

Gap junctions underlie impulse propagation in cardiac myocytes. Spironolactone prevented gap junction remodeling and reversed the down regulation of connexin-43 (Cx-43), phosphorylated Cx-43 expression, and the progressive slowing of impulse propagation in a rat model of HF [111].

## 7. Aldosterone system expression in atrial fibrillation

Patients with primary aldosteronism have a 12-fold higher risk of developing AF when compared to blood pressure-matched controls [14], suggesting that aldosterone strongly contributes to AF development. Additional evidence documenting a causal impact of aldosterone on AF substrates has been recently reported [112]. Aldosterone contributes to a substrate for AF by promoting atrial fibrosis/hypertrophy, and by causing conduction disturbances without affecting ventricular hemodynamics [112].

Elevated plasma and myocardial aldosterone levels are routinely reported in HF and HTN patients [21,12,13]. Low plasma aldosterone post-MI is an independent predictor of survival and reduction in hospitalization in HF patients [113]. In addition, plasma aldosterone is independently associated with LV structure and hypertrophy in patients with cardiovascular disease risk and preserved ejection fraction [114]. Changes in mRNA expression of elements of the aldosterone system have been also shown in AF. Both the MR and 11beta-hydroxysteroid dehydrogenase type 2 (11betaHSD2) proteins are increased in the cytoplasm of atrial myocytes from patients with AF [115]. In a canine model of AF, atrial tachypacing increased plasma and atrial aldosterone levels [116]. Use of spironolactone or perindopril (an ACE inhibitor) attenuated atrial remodeling and improved atrial function by reducing plasma and atrial aldosterone levels, suggesting a critical role of aldosterone in AF pathogenesis and progression [116].

The aldosterone synthase (CYP11B2) T-344C gene polymorphism, which is associated with increased aldosterone activity, is also associated with AF in HF patients [117].

In patients with established AF, AF at long-term follow-up visit was associated with elevated plasma aldosterone levels [118]. Electrical cardioversion of atrial arrhythmia significantly lowers plasma levels of aldosterone in patients with persistent AF [119]. Similarly, the decrease in plasma aldosterone is associated with maintenance of sinus rhythm following cardioversion of AF [120]. These data suggests that aldosterone may be involved in the development of AF in patients, and that modulation of aldosterone levels may modify the atrial substrates for AF.

Surprisingly, data from the Framingham cohort study revealed that plasma aldosterone was not associated with AF incidence [121]. Further studies are required to explain the discrepancy in findings.

## 8. Aldosterone antagonists and atrial fibrillation

Accumulating evidence supports a critical pathophysiologic role of aldosterone in AF and has led to increased interest in the development of aldosterone/MR antagonists to treat or prevent the progression of cardiovascular diseases including HF, MI and AF.

In an experimental canine model of persistent AF, spironolactone treatment markedly prevented AF-related changes in atrial structure and function [122]. Spironolactone maintained left atrial ejection fraction and attenuated apoptosis, myolysis, fibrotic pathways, and mitochondrial swelling [122]. In a canine HF model, eplerenone attenuated the inducibility of sustained atrial tachyarrhythmias, prolonged atrial effective refractory periods, and attenuated LV remodeling [123].

In human studies, spironolactone therapy was associated with a reduction in the burden of AF and a reduction in hospitalizations for AF direct current cardioversion [124]. A recent study showed that eplerenone markedly improved maintenance of sinus rhythm after catheter ablation in patients with long term persistent AF [125]. Aldosterone antagonists also prevented onset of AF and flutter in patients with systolic HF [126]. In the RALES study [78], use of spironolactone led to a marked improvement of LV function and a reduction in

cardiac fibrosis and sudden death in patients with severe HF. This may be due to the reduction in norepinephrine levels and the increase in the threshold of ventricular fibrillation [127]. In addition, 6 months use of spironolactone markedly decreased the 24-hour mean heart rate, the frequency of atrial and ventricular premature beats, and the risk of AF/flutter in patients with HF, in part by maintaining magnesium homeostasis [56]. The mechanism may due also to improving atrial conduction and remodeling in patients with HF [128]. These data suggest that aldosterone/MR antagonists may provide a preferential effect on the development of AF in the setting of HF.

Close monitoring of serum potassium and creatinine is important in patients when administering aldosterone antagonists, especially in the presence of comorbid conditions associated with impaired kidney function. Eplerenone, the newer selective aldosterone antagonist, has fewer side effects than spironolactone and holds greater promise and safer therapeutic target in patients with HF and other comorbidities [41]. Aldosterone synthase inhibition may also serve as potential future drug therapy similar to MR antagonists. Use of aldosterone synthase inhibitor (FAD286) improves left ventricular (LV) hemodynamics, function and remodeling similar to spironolactone, but only FAD286 persistently reduced LV oxidative stress [129]. Another recent novel MR antagonist with fewer side effects has also been tested and may have promise as a future drug [130].

Several studies suggested that MR antagonists provide additional antiarrhythmic effects to Ang-II or catecholamines, and it may be helpful to add an aldosterone/MR antagonist to ACEi/ARBs or beta blockers in patients. In human MI patients, MR antagonists combined with ACE inhibitor prevented post-infarct LV remodeling more effectively than ACE inhibitor alone [131]. In human AF patients, 12 month combination of spironolactone and beta-blocker prevented AF episodes in patients with normal LV function and history of recurrent paroxysmal AF [132]. Interestingly, beta-blocker alone or in combination with enalapril was significantly less effective in preventing AF relative to a combination that included spironolactone [132]. Combination of spironolactone and atenolol also attenuated atrial and ventricular remodeling in patients with permanent AF [133]. Addition of an aldosterone antagonist to optimal medical therapy reduces morbidity and mortality in patients with acute MI and HF [78,134]. This practice is important because it’s unclear if routine use of statins, ACEi, or ARBs does improve the outcome of AF ablation [135]. Surprisingly, in a retrospective study of human HF, use of aldosterone antagonists did not appear to prevent onset of AF, unlike ACEi and ARBs [136]. These results suggest that aldosterone antagonists may be more helpful in preventing the progression of AF than in suppressing the onset of AF. This suggests that aldosterone has a greater effect on AF substrate than triggers. However, further studies are required to investigate this hypothesis.

Several ongoing clinical trials are designed to evaluate the efficacy of aldosterone antagonists in AF and other cardiovascular disease such as HF. The treatment of preserved cardiac function heart failure with an aldosterone antagonist trial (TOPCAT) is designed to evaluate the effect of spironolactone on morbidity, mortality, and quality of life in patients with HF and preserved ejection fraction [137]. On the other hand, the Prospective Appraisal of the Prevalence of Primary aldosteronism in Hypertensive patients presenting with atrial flutter or fibrillation study (PAPPHY) will prospectively study prevalence of primary

aldosteronism in hypertensive patients presenting with atrial flutter or fibrillation [138]. Results from these ongoing clinical trials should provide further insights into the impact of aldosterone antagonists on the prevention of atrial arrhythmias.

## 9. Conclusions

Strong clinical and preclinical evidence suggest an important role for aldosterone in the setting of cardiovascular pathology. Elevated plasma aldosterone levels in patients with HF and AF suggest a role of aldosterone in the etiology of these diseases. Further studies evaluating the biosynthesis and localization of aldosterone enzymes and receptors would provide improved insights into the functional integration of this system in the heart.

Aldosterone seems especially likely to contribute to AF development in the setting of HF. Activation of the aldosterone system has an important role in atrial oxidative stress and structural remodeling. Aldosterone antagonists appear to be effective in reducing circulating levels of oxidants, inflammatory, fibrotic and neurohormonal factors that influence atrial molecular biology (Fig. 2). The clinical impact of aldosterone antagonists has not yet been adequately studied in AF patients after onset of AF. Further studies to evaluate the impact aldosterone/MR antagonists on AF patients or patients with risk of AF are warranted.

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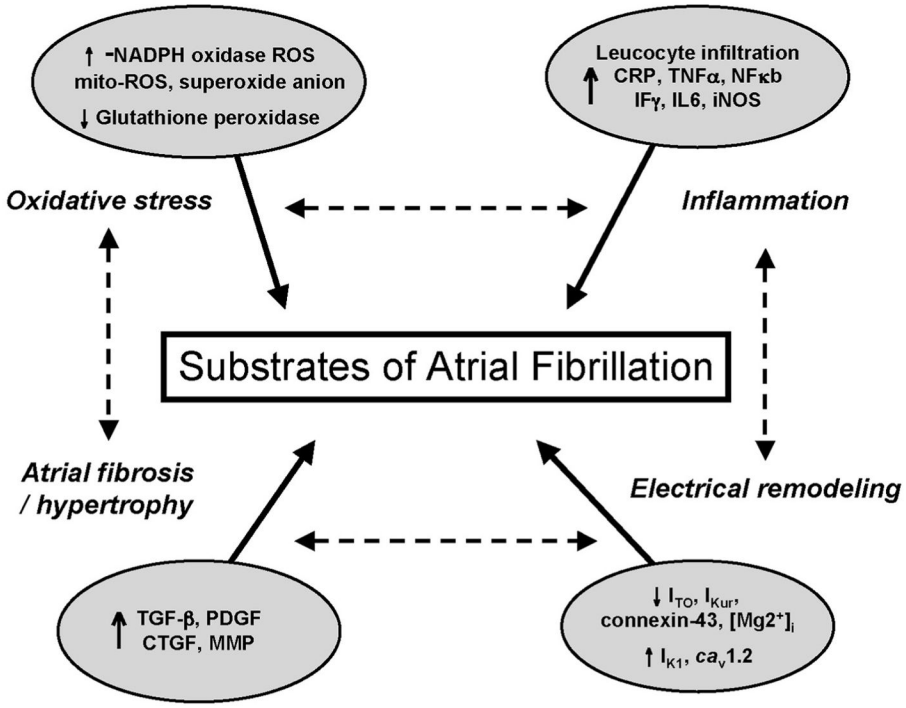


**Fig. 1.**

The substrates of atrial fibrillation. During AF, profound increases in expression of inflammatory proteins such as C reactive protein (CRP), tumor necrosis factor α (TNFα), nuclear factor κb (NFκb), interferon γ (Fγ), interleukin 6 (IL6), and inducible nitric oxide synthase (iNOS) occur. Generation of reactive oxygen species (ROS) is also increased in AF via activation of NADPH oxidase activity and expression, mitochondrial (mit) ROS generation, superoxide generation as well as reduction in antioxidants defenses such as glutathione peroxidase. Atrial fibrosis and hypertrophy also promotes atrial arrhythmogenesis through increased expression of profibrotic proteins such as tumor growth factor β (TGF β), platelet derived growth factor (PDGF), connective tissue growth factor (CTGF), and metalloproteinase (MMP). The decrease in outward (Ito) or rectifier (kur) K + current, the down regulation in connexin 43 (Cx 43) or intracellular Mg+, and the increase in native I (K1) current and L-type Ca + channel current ($Ca_v1.2$) promote atrial electrical remodeling leading to AF. These substrates are highly related where one substrate promotes generation of other substrates (AF begets AF).

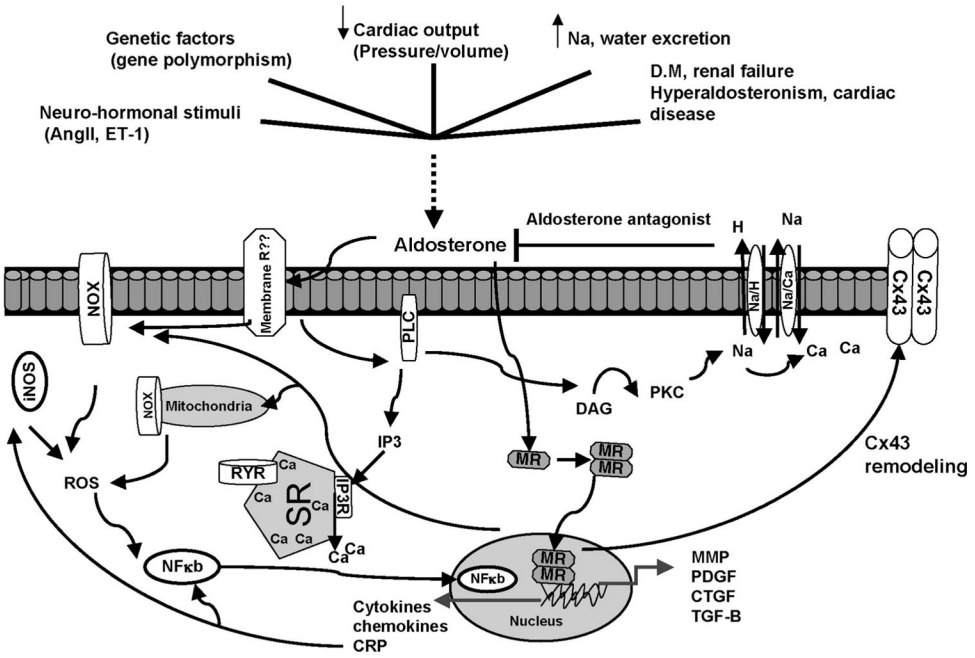


**Fig. 2.**

The primary intracellular pathways activated downstream of aldosterone. Several stimuli increase aldosterone expression and release. Aldosterone acts through the mineralocorticoid receptor (MR) and a putative unknown membrane receptor (membrane R??). Aldosterone diffuses through the plasma membrane to the cytosol where it binds the MR and induces MR homodimerization, activation, and translocation to the nucleus. Activated MR promotes gene transcription of fibrotic and hypertrophic proteins (PDGF, CTGF, TGF β), and inflammatory cytokines and chemokines. Aldosterone acts through the MR and membrane Rs and promotes ROS generation via NADPH oxidase (NOX) and the mitochondria. ROS generation and inflammatory molecules activate the iNOS and NFκb signaling. Activated NFκb translocates to the nucleus promoting transcription of genes involved in atrial oxidative damage and inflammation. Aldosterone rapid signaling on membrane receptors also leads to phospholipase C (PLC) activation and other cellular signal-transduction cascades such as those mediated by inositol 1,4,5 trisphosphate (IP3), protein kinase C (PKC). Phosphorylation of membrane $Na^+/H^+$ antiporters increases intracellular pH and modulates cardiac myocyte myofilament $Ca^{2+}$ sensitivity. Influx of $Na^+$ can activate reverse mode activity of the $Na^+/Ca^{2+}$ exchanger (NCX), resulting in elevated intracellular $Ca^{2+}$, positive inotropy, and arrhythmogenesis. Hydrolysis of phosphatidylinositol 4,5-bis-phosphate (PIP2) increases intracellular IP3, which subsequently promotes $Ca^{2+}$ release through IP3 receptors (IP3Rs) that are located under the plasma-lemma adjacent to ryanodine receptors (RyRs). $Ca^{2+}$ release through the IP3Rs may also contribute to the inotropic and arrhythmogenic activity of aldosterone, by sensitizing RyRs located there. Gene transcription pathways are also activated resulting in atrial apoptosis and connexin remodeling. During atrial fibrillation, aldosterone signaling may be enhanced, promoting oxidative damage, increased intracellular $Ca^{2+}$and hypertrophic/fibrotic remodeling.