Aldosterone as a Mediator of Cardiovascular Damage

ALDOSTERONE AS A MEDIATOR OF CARDIOVASCULAR DAMAGE

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Abstract

Besides the physiological regulation of water, sodium and potassium homeostasis, aldosterone modulates several physiological and pathological processes in the cardiovascular system. At vascular level, aldosterone excess stimulates endothelial dysfunction and infiltration of inflammatory cells, enhances the development of the atherosclerotic plaque and favours plaque instability, arterial stiffness and calcification. At cardiac level, aldosterone increases cardiac inflammation, fibrosis and myocardial hypertrophy. As clinical consequence, high aldosterone levels are associated with enhanced risk of cardiovascular events and mortality, especially when aldosterone secretion is inappropriate for renin levels and sodium intake, as in primary aldosteronism. Several clinical trials showed that mineralocorticoid receptor antagonists (MRA) reduce cardiovascular mortality in patients with heart failure and reduced ejection fraction, but inconclusive results were reported for other cardiovascular conditions, as heart failure with preserved ejection fraction, myocardial infarction and atrial fibrillation. In patients with primary aldosteronism adrenalectomy or treatment with MRA significantly mitigate adverse aldosterone effects, reducing the risk of cardiovascular events, mortality and incident atrial fibrillation.

In this review, we will summarize the major pre-clinical and clinical studies investigating the cardiovascular damage mediated by aldosterone and the protective effect of MRA for the reduction of cardiovascular risk in patients with cardiovascular diseases and primary aldosteronism.

Introduction

Aldosterone is the main mineralocorticoid hormone synthesized in the zona glomerulosa of the adrenal cortex by aldosterone synthase, converting 11-deoxycorticosterone to aldosterone. Aldosterone mediates its effects trough genomic and non-genomic mechanisms: the first ones are mediated by the activation of the nuclear MR (mineralocorticoid receptor), the second ones by a putative membrane receptor, probably the high-affinity aldosterone-binding membrane protein, GPR30 (G protein-coupled receptor 30)[1]. Aldosterone excess can result from two different conditions: I) the overactivation of the renin-angiotensin system due to the reduction of intravascular volume, as described in heart failure or ascites (secondary aldosteronism), or II) autonomous aldosterone secretion. In the latter condition, aldosterone overproduction is independent of angiotensin II stimulation and its clinical spectrum ranges from the mildest form of renin-independent aldosteronism to the overt forms of PA (primary aldosteronism)[2]. Beside the commonly known effects on the renal tubular epithelium, aldosterone regulates several physiological and pathological process in extra-renal organs. In particular, supraphysiological activation of MR lead to increased cardiac and vascular damage, with consequent increased risk of cardiovascular events and cardiovascular mortality[3,4]. In this review, we summarize the pathological basis of aldosterone-mediated cardiovascular damage, from a pre-clinical and clinical standpoint, and we recapitulate the results of major studies evaluating the impact of mineralocorticoid receptor antagonists (MRA) for treatment and prevention of cardiovascular diseases.

Aldosterone and vessels

Endothelial dysfunction

Endothelial dysfunction is an early feature of the vascular atherosclerotic process caused by impairment of NO (nitric oxide)-mediated vasodilatation[5]. Aldosterone impairs endothelial

function at different levels (Figure 1). In vitro, aldosterone reduces NO production in endothelial cells[6] through inhibition of eNOS (endothelial NO synthase) activity[6] by two mechanisms: via increased phosphatase 2A activity and dephosphorylation of eNOS (Ser1177) and via oxidation of BH_4 (tetrahydrobiopterin), an important cofactor of eNOS, with consequent eNOS uncoupling[6]. Aldosterone also reduce G6PD (glucose-6-phosphate dehydrogenase) in endothelial cells, with increase of ROS (reactive oxygen species) and reduced NO bioavailability[7].

Aldosterone may exert its detrimental effects by indirect mechanisms mediated by EVs (extracellular vesicles): circulating EVs of patients with PA carry *endothelin-1* mRNA that, once transferred to the endothelial cells, may contribute to aldosterone-mediated endothelial dysfunction[8]. Similarly, circulating EVs from patients with PA display differential expression of several surface antigens able to modify gene expression of recipient endothelial cells, potentially affecting endothelial function[9].

In vivo, aldosterone infusion impairs endothelial function in mouse and rat models[7,10,11]. Aldosterone indirectly reduces endothelium-dependent vasorelaxation through endothelial G6PD reduction[7] and COX-2 (cyclooxygenase-2) activation, with increased production of prostacyclin, which act as vasoconstrictors under specific conditions[10], and directly increase vasoreactivity through EGFR (epidermal growth factor receptor) activation[12]. The role of renin-angiotensin-aldosterone system at endothelial and vascular level is more complex than previously thought and aldosterone is a crucial regulator of multiple and interdependent pathways. Knock-out of AT_1R (angiotensin II receptor type 1) in mice or AT_1R inhibition blunts the aldosterone-mediated endothelial dysfunction[11] and this is likely caused by increased overexpression of AT_1R induced by aldosterone, with consequent increase of AngII (angiotensin II)-mediated endothelial dysfunction[13]. Beyond renal epithelia, the ENaC (epithelial sodium channel) is expressed in the vascular endothelium and regulated by

aldosterone through MR[14]. Aldosterone increases ENaC abundance in the plasma membrane and its activity, with increased Na^+ current and activation of multiple pathways leading to impairment of eNOS activity and reduced NO production[15].

In humans, a large observational study reported an association between high aldosterone levels and ARR (aldosterone-to-renin ratio) and impaired FMD (flow mediated dilatation), a surrogate and non-invasive measure of endothelial function[16]. Similarly, in patients with resistant hypertension, FMD is inversely correlated with urinary aldosterone secretion, plasma aldosterone and ARR[17].

In patients with PA, FMD is inversely correlated with the severity of PA phenotype[18,19]. FMD is significantly lower in patients with unilateral PA, compared with matched patients with EH (essential hypertension); whereas patients with bilateral PA, which usually display a milder phenotype, have similar severity of FMD than EH[18]. Circulating EPC (endothelial progenitor cells) mediate functional effects at endothelial level by regulation of endothelial repair and altering the intracellular balance of eNOS activity[20]. Patients with PA have lower number of circulating EPC, compared with patients with EH, and the EPC concentration is inversely correlated with plasma aldosterone levels[21]. Moreover, EPC from patients with PA display reduced migratory potential, which is partially restored by spironolactone treatment[22]. Adrenalectomy significantly restore FMD in patients with unilateral PA[18,19,23]; conversely, the benefit of MRA therapy was inconsistent in different studies[23,24].

Arterial Stiffness

Arterial stiffness is the reduction of arterial distensibility and it is associated with cardiovascular morbidity and mortality. It is estimated by PWV (pulse wave velocity), forward or reflected wave amplitude and augmentation index[25].

Animal studies demonstrated that aldosterone increases arterial stiffness through multiple pathways by MR activation[26] leading to remodeling of the extracellular matrix and cell-matrix attachment proteins[26]. Aldosterone exerts its effect through a profound modulation of gene expression profile at vascular levels, as showed in ex vivo models of mouse aortas[27]. Several genes related to the extracellular matrix remodeling are differentially expressed, including upregulation of connective tissue growth factor and modulation of metalloproteinase/metallopeptidase regulatory proteins[27]. In rat and mouse models, aldosterone increase collagen deposition in the arterial walls through galectin-3[28] and endothelin-1[29] mediated mechanisms, through ENaC activation[15], VEGFR1 (type 1 vascular endothelial growth factor receptor) activation[30] and Nox1 (NADPH [nicotinamide adenine dinucleotide phosphate] oxidase 1) mediated pathways[31].

Aldosterone also induces osteogenic phenotype in VSMC (vascular smooth muscle cells), thereby contributing to calcification and stiffness of the vascular wall[32]. In particular, aldosterone has been shown to induce osteo-inductive signaling through activation of alkaline phosphatase in VSMCs[32], mediated by PIT-1 activation (type III sodium-dependent phosphate transporter)[33]. Moreover, aldosterone reduces autophagy of VSMCs, a process that physiologically inhibits osteogenic differentiation of VSMCs[34]. Beyond these mechanisms, aldosterone may favor indirectly vascular calcification by activating NADPH oxidase that in turn mediate the osteogenic phenotype of vascular SMC[35], by stimulation of vascular inflammation[33], upregulation of parathyroid hormone receptor in SMCs[36], and via activation of aldosterone-induced non-genomic pathway[35]. Blockade of aldosterone effect through spironolactone mitigate the progression of vascular calcification in vitro[32] and in animal models[37].

In individuals with newly diagnosed hypertension or normotension, high aldosterone[38] and high ARR[39] are associated with increased PWV, independently of BP levels[38]. Spironolactone

significantly reduced arterial stiffness in patients with hypertension[40]. Prospective studies reported a stronger reduction of PWV and augmentation index in patients with essential hypertension treated with spironolactone than patients treated with thiazide diuretics[40,41].

A meta-analysis showed that patients with PA have higher PWV than matched patients with EH but no differences in the augmentation index[42]. Forward and reflected wave amplitude are higher in patients with PA than EH, and reflected wave amplitude is correlated with log aldosterone levels, suggesting that arterial stiffness is more pronounced in patients with a florid PA phenotype[43].

Adrenalectomy significantly reduce PWV[44], augmentation index[44] and wave amplitude[43] in patients with unilateral PA while the available literature in patients with bilateral PA treated with MRA provides conflicting results[24,44]. Patients with higher PWV before adrenalectomy have lower probability of normalization of blood pressure levels after surgery[45]. This observation reinforces the recommendation for early identification and treatment of patients with PA.

Aldosterone and heart

Coronary artery disease

Atherosclerosis is the accumulation of fibrous and fatty materials in the intima layer of the vascular wall and is the main cause of acute or chronic coronary artery disease[46]. The initiation of the atherosclerotic process is driven by the progressive engulfment of macrophage with oxidized LDL (low-density lipoprotein), generating foam cells. The accumulation of macrophage in the atherosclerotic lesion is therefore crucial, being the result of an impaired balance between monocyte extravasation and macrophage proliferation on one side, and apoptosis and efferocytosis on the other[46]. In vitro findings suggest that aldosterone can directly impair this delicate balance by several mechanisms (Figure 2). Aldosterone activate endothelial

cells, increasing adhesion molecules, such as the ICAM-1 (intercellular adhesion molecule 1), and promoting leukocyte-endothelium interaction and consequent extravasation[47]. Moreover, aldosterone stimulates human coronary artery SMC (smooth muscle cells) release of pro-inflammatory molecules[36] and enhances monocyte chemotaxis[48]. Once migrated in the intima layer, monocytes differentiate into two types of macrophages: M1, expressing classical macrophage marker and characterized by a pro-inflammatory action, or M2 phenotype, expressing anti-inflammatory markers and reducing the local inflammatory burden[49]. Aldosterone favors M1 differentiation, which increases the secretion of several pro-inflammatory cytokines, including TNFα (tumor necrosis factor α), IL-12 (interleukin-12), CCL5 (chemokine ligand 5)[50], and IL-1 β (interleukin-1β)[51]. The latter is induced by aldosterone-mediated increase of NLRP3 (NLR family pyrin domain containing 3) expression, one of the main component of the NLRP3 inflammasome, a multimeric complex that processes and increase interleukin release in macrophages[51].

Several experiments in animal models corroborated and expanded in vitro findings. Adrenalectomy and eplerenone treatment significantly reduce vascular fibrinoid necrosis observed in a model of secondary aldosteronism (rats treated with AngII and high salt intake) and aldosterone infusion completely reversed the protective effect of adrenalectomy[52]. Aldosterone infusion increase macrophage infiltration in the atherosclerotic plaque of ApoE (apolipoprotein-E) knock out mice[48,53,54]. On one side, aldosterone induce overexpression of ICAM-1[53] and macrophage chemoattractant protein-1[54], enhancing macrophage recruitment; on the other, aldosterone reduce macrophage apoptosis and efferocytosis[55]. As described in vitro, aldosterone enhance macrophage polarization towards a M1 phenotype in mice models[50]. This effect is inhibited by MRA or myeloid selective knock out of MR, which in turns favor polarization towards M2 phenotype[50]. The plaque size and lipid content of the plaque is increased by aldosterone administration in ApoE knock out mice [48,53] and inhibited by MRA

treatment[56]. In the same mouse model, MRA reduce lipid peroxides and oxidation of LDLs within the plaque further contributing to plaque stabilization[57].

Studies in humans demonstrated a significant association between aldosterone levels and subclinical coronary atherosclerosis, assessed by coronary artery calcium assessment[58]. The association is stronger in patients with suppressed plasma renin activity (≤0.5 μg/L/hour)[58]. Aldosterone is independently associate with a higher risk of acute cardiac ischemic events[4] and cardiovascular mortality[4]. However, in patients without known coronary artery disease the association between aldosterone and cardiovascular mortality is significant only in patients with low renin, suggesting that renin independent aldosteronism is a greater predictor of cardiovascular risk than aldosterone levels *per se*[58]. High aldosterone levels are also associated with a higher rate of cardiovascular events, cardiovascular mortality and overall mortality in patients with acute MI (myocardial infarction)[59].

The early administration of MRA in patients with myocardial infarction prevent left ventricular remodeling[60]. On the basis of these findings, two RCT (randomized controlled trial) evaluated the early treatment with MRA after MI in patients without HF (heart failure)[61] o irrespective of HF diagnosis[62](Supplementary Table 1). In the REMINDER (Impact Of Eplerenone On Cardiovascular Outcomes In Patients Post MI) trial[61] eplerenone reduced the primary composite outcome, comprising cardiovascular events and mortality, reduced left ventricular function, prolonged hospitalization or re-hospitalization and natriuretic peptides reduction. However, the difference was driven by the reduction of natriuretic peptides, without significant differences in other components of the primary endpoint[61]. In the ALBATROSS (Aldosterone Lethal effects Blocked in Acute MI Treated with or without Reperfusion to improve Outcome and Survival at Six months follow-up) trial, canrenone intravenous administration followed by oral spironolactone for 6 months failed to improve the composite primary outcome[62].

Nevertheless, an exploratory sub-analysis showed a benefit of mortality in patients with MI with ST-elevation[62].

Patients with PA display greater vascular inflammation than patients with EH, assessed by means of ^{18}F-FDG PET-CT (^{18}fluoro-D-glucose positron emission tomography with computed tomography), a surrogate measure of macrophage vascular infiltration and atherosclerotic burden[63,64]. The results of a wide meta-analysis confirmed that patients with PA display an increased risk of coronary artery disease (1.77-fold higher) compared with patients with EH [65]. In patients with PA, aldosterone levels are associated with the risk of cardiovascular events[66]. Adrenalectomy reduce the risk of cardiovascular events to lower levels than patients with EH[3]. On the other side, MRA treatment reduce the risk of cardiovascular events to values similar to EH patients, but only when renin levels are no longer suppressed (plasma renin activity≥ 1 μg/L/hour)[3], suggesting that a complete blockade of MR is necessary for the reversal of aldosterone-mediated cardiovascular risk.

Heart Failure

MI leads to rapid loss of cardiomyocytes that exceed the cardiac regenerative capacity, leading to a substitution of functional myocardium with fibrotic tissue, with consequent post-infarction ventricular remodeling[67]. In the absence of ischemic events, ventricular remodeling can be the consequence of hemodynamic overload and neurohormonal mechanisms, enhancing LVH (left ventricular hypertrophy) and cardiac fibrosis. In both scenarios aldosterone plays a crucial and detrimental role leading to increased risk of left ventricular systolic and diastolic dysfunction, HF and increased cardiovascular mortality[68] (Figure 2).

In vitro findings showed that aldosterone stimulates fibroblast collagen synthesis[69] and, through MAPK (mitogen-activated protein kinases) cascade[70–72], cardiomyocytes hypertrophy[70], cardiac myofibroblast proliferation[71] and increased myocardiocyte release of matrix metalloproteinase[72].

Aldosterone infusion in rats increase perivascular and interstitial fibrosis by mechanisms that are reversed by MRA administration[73]. Aldosterone-induced cardiac fibrosis is mediated by two mechanisms: perivascular and interstitial inflammation and direct alteration of extracellular matrix deposition[68]. In mouse and rat models, aldosterone increase interstitial oxidative stress[74,75], through NADPH and NFκB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation[74], leading to increased release of inflammatory molecules and infiltration of inflammatory cells[74,75]. In rats, galectin-3[76] and TRAF3IP2 (TRAF3 Interacting Protein 2)[77] expression are upregulated by aldosterone and both proteins mediate aldosterone cardiac fibrosis by regulating collagen deposition and enhancing a pro-inflammatory environment[76,77]. In mice, aldosterone infusion directly alter the extracellular matrix compartment through upregulation of tissue inhibitor of metalloproteinases-1, leading to reduce matrix metalloproteinase activity and cardiac collagen accumulation[78]. On the opposite, after myocardial infarction, aldosterone infusion significantly affect ventricular remodeling in mice, increasing the expression of metalloproteinases via CaMKII (Ca2+/calmodulin-dependent protein kinase II) oxidation, causing cardiac rupture[79].

Cardiac hypertrophy is caused by direct aldosterone-mediated pathways and indirect aldosterone-mediated mechanisms, through increased blood pressure and hemodynamic overload[80]. Aldosterone infusion increase myocardiocyte hypertrophy, through cardiotrophin-1 mediated effect[73], ROS mediated mechanisms[81], PAI-1 (plasminogen activator inhibitor-1) levels[82] and through circadian clock proteins[83]. Most of those mechanisms directly or indirectly promote myocardial fibrosis[73,81,83], further contributing to the ventricular remodeling and increased risk of HF.

In humans, MRAs have been evaluated in patients with HFpEF (HF with preserved ejection fraction), HFmrEF (HF with mildly reduced ejection fraction) and HFrEF (HF with reduced ejection fraction)[84] (Supplementary Table 1). In patients with HFrEF, the RALES

(Randomized Aldactone Evaluation Study) trial[85] and the EPHESUS (Eplerenone Post-Acute MI Heart Failure Efficacy and Survival Study) trial[86] have demonstrated that addition to the standard therapy of long-term treatment with 25 mg of spironolactone[85] or up to 50 mg of eplerenone[86] significantly reduce the overall and cardiovascular mortality. The EMPHASIS-HF (Eplerenone in Mild Patients Hospitalization And Survival Study in HF) trial further demonstrated a benefit of eplerenone add-on therapy in patient with HFrEF and mild symptoms, reducing overall and cardiovascular mortality[86]. The ARTS-HF (MinerAlocorticoid Receptor antagonist Tolerability Study-HF) phase 2b trial compared efficacy and safety of finerenone, a novel and more cardio-selective MRA, *versus* eplerenone in patients with HFrEF and diabetes and/or chronic kidney disease, reporting a similar reduction of natriuretic peptide and a similar safety profile[87].

In patients with HFpEF and diastolic dysfunction, spironolactone improved diastolic function[88,89] with discordant benefit on exercise capacity[88,89]. In the TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist) trial spironolactone treatment of patients with HF and EF≥45% did not reduce the primary composite outcome of cardiovascular mortality, aborted cardiac arrest or hospitalization for HF, but determined a significant reduction of hospitalization for HF[90]. However, a post-hoc analysis showed a reduced primary composite outcome in the subgroup of patients with resistant hypertension[91], further reinforcing the established recommendation of MRA as the most effective add-on treatment for resistant hypertension[92,93].

Patients with PA display increased sign of cardiac fibrosis compared with EH, as assessed trough indirect echocardiographic methods[94] and cardiac magnetic resonance[95]. Left ventricular mass is increased in patients with PA compared with EH, with higher rates of LVH[65]. The severity of LVH correlate with the severity autonomous aldosterone secretion[96] and is inappropriate for the cardiac workload[97]. Diastolic function is significantly impaired in

patients with PA, compared with matched patients with EH[98]. Although no difference in systolic function have been reported between patients with PA and EH[68], preclinical systolic dysfunction, assessed by speckle-tracking echocardiography, is more pronounced in patients with PA[99]. As consequence of the these morpho-functional changes, patients with PA display an increased risk of HF than patients with EH[65].

Aldosterone-induced LVH is partially reversible with adrenalectomy and MRA treatment[96,100]. Similarly, diastolic function is partially restored after adrenalectomy in patients with unilateral PA, although contrasting results have been reported[68].

Atrial Fibrillation

The onset of AF (atrial fibrillation) depends on three mechanisms: automaticity, triggered activity, and re-entry. Triggered activity is caused by additional impulses known as afterdepolarization, favored by cytosolic calcium overload[101]. Pre-clinical studies suggest that aldosterone can directly modulate electrophysiological properties of cardiomyocytes, increasing cytosolic calcium load and facilitating cardiac arrhythmias[102,103] (Figure 2). In vitro, aldosterone increase Ca^{2+} influx via the L-type Ca^{2+} channel[102] and T-type Ca^{2+} channel[104]. L-type Ca^{2+} current activates ryanodine receptor, the main Ca^{2+} channel of the sarcoplasmic reticulum, inducing Ca^{2+} release in the cytosolic space[105]. Aldosterone, through MR activation, increases the activity of ryanodine receptor, contributing to Ca^{2+} overload and facilitating the onset of afterdepolarizations[103]. Rapid depolarizations increase intracellular calcium, MR expression and aldosterone responsiveness in atrial cardiomyocytes, leading to a positive feedback and a vicious cycle that favors AF development[104].

At structural level, increased calcium load enhances profibrotic pathways in atrial tissue, with consequent atrial remodeling and dilatation, which constitutes the main substrate for long-lasting AF stabilization[101]. Moreover, aldosterone directly contributes to atrium remodeling, through increased collagen deposition through MAPK dependent mechanism[106].

In vivo, prolonged infusion of aldosterone in rat models increase P wave duration, total right atrium activation time and atrial anisotropy of conduction, favoring re-entry mechanisms and AF maintenance[107]. Beyond electrophysiological changes, aldosterone infusion directly increases atrial fibroblast proliferation and collagen deposition in the atrium; on the other side, aldosterone reduces active matrix metalloproteinase 13 (MMP13), with consequent reduction of collagen cleavage[107]. The remodeling of atrial tissue favor the stabilization of AF, doubling the time of spontaneous conversion[108]. The aldosterone-mediated structural changes seems to be partially reversible, as suggested by treatment with oral eplerenone in tachypaced sheep models[109]. In this model, MRA treatment reduced atrial fibrosis and atrial dilatation with consequent reduction of progression to persistent AF[109].

In humans, the role of MRAs for AF prevention has been evaluated in patients with HFrEF and HFmrEF-HFpEF. In patients with HFrEF, the EMPHASIS-HF trial showed a significant reduction of new onset AF in the eplerenone group, compared with placebo (2.7% *vs* 4.5%)[110]. On the other hand, a post-hoc analysis of TOPCAT trial, showed no benefit of spironolactone treatment for the prevention of new onset AF in patients with HFmrEF-HFpEF[111]. A meta-analysis, including 5 RCT and 9 observational studies, reported a reduction of new-onset AF and recurrent AF in patients treated with MRA[112], although patients characteristics were very heterogenous. Recently, a RCT including patients with early persistent AF and HF showed that target treatment of underlying conditions, with renin-angiotensin system inhibitors, MRA, statin and cardiac rehabilitation, significantly improved sinus rhythm maintenance, compared with conventional therapy[113]. Intriguingly, the use of renin-angiotensin system inhibitors was similar in target and conventional group, but MRAs were used in 85% of patients in target group *vs* 4% in conventional treatment, suggesting an important role of MRAs in sinus rhythm maintenance[113]. On the basis of these evidences, the recent ESC (European Society of

Cardiology) Guidelines of 2020 introduced MRA as a potential non-antiarrhythmic drugs for upstream therapy of AF[114].

Patients with PA have higher risk of AF, estimated 3.52-fold higher in a large meta-analysis of 31 studies[65]. The prevalence of PA in patients with AF, without underlying cardiac cause of arrythmia, is very high (42%)[115] and therefore these patients should be screened for PA[116]. Adrenalectomy in patients with unilateral PA, reduce AF incident risk to values similar to EH[117]. On the opposite, patients with PA treated with MRA display a persistent higher risk of AF[117]. However, when MRA therapy is titrated to rise renin levels, the risk of AF is similar to patients with EH[118], reinforcing the recommendation that MRA treatment should be up-titrated to achieve a complete blockade of MR.

Perspectives and conclusions

Aldosterone contributes to the development of cardiovascular damage, through multiple arrays of pathways that ultimately lead to increased cardiovascular diseases, events and mortality. Although several pre-clinical studies have investigated the mechanisms that drive aldosterone effects, the elucidation of this complex network is far to be complete. Beyond the direct effects on vascular and cardiac cells, recent evidence suggest that aldosterone excess induces cardiovascular damage through indirect mechanisms, including the alteration of circulating extracellular vesicles. In the future, a complete understanding of the biomolecular processes that drive aldosterone-mediated cardiovascular risk could be crucial to counteract in a synergic fashion the deleterious effects of aldosterone excess.

MRAs have been proposed for the reduction of aldosterone-mediated cardiovascular risk in several conditions with different results. Although the benefit for patients with HFrEF has been clearly established, the benefit in patients with HFmrEF and HFpEF is still debated. Similarly, the benefit for patients with myocardial infarction without heart failure and for prevention of atrial fibrillation is still unclear.

Finally, in patients with PA the blockade of MR is only partially effective for the reversion of the increased cardiovascular risk, compared to adrenalectomy. New highly selective aldosterone-synthase inhibitors and other novel MRAs are currently under investigation and are promising candidates for the treatment of PA in the near future.

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Figure Legends

Figure 1. Molecular pathways mediating aldosterone-induced endothelial dysfunction and arterial fibrosis and calcification.

Aldosterone induces endothelial dysfunction via multiple intracellular pathways in endothelial cells, reducing synthesis of vasodilatory mediators and increasing vasoconstrictor molecules. Aldosterone enhances arterial fibrosis and calcification through activation of several synergic pathways in vascular smooth muscle cells. Aldo=aldosterone, AngII=angiotensin II, AT_1R=angiotensin II receptor type 1, BH_4=tetrahydrobiopterin, COX-2=cyclooxygenase-2, G6PD=glucose-6-phosphate dehydrogenase, EGF=epidermal growth factor, EGFR=epidermal growth factor receptor, ENaC=epithelial sodium channel, eNOS=endothelial nitric oxide synthase, EVs=extracellular vesicles, MR=mineralocorticoid receptor, NO=nitric oxide, Nox1=nicotinamide adenine dinucleotide phosphate] oxidase 1, PA=primary aldosteronism, PIT-1=type III sodium-dependent phosphate transporter, PTH=parathyroid hormone, PTHR= PTH receptor, ROS=reactive oxygen species, SR=sarcoplasmic reticulum, VEGF= vascular endothelial growth factor, VEGFR1= type 1 VEGF receptor. The figure was produced using Servier Medical Art (https://smart.servier.com/).

Figure 2. Pathological mechanisms, anatomical alteration and cardiovascular consequences of aldosterone-mediated cardiovascular damage.

Aldosterone promotes the development of cardiovascular disease by multiple pathological mechanisms, leading to the atherosclerosis of coronary artery, myocardial fibrosis and hypertrophy and electrophysiological alterations with consequent increased risk of

cardiovascular disease and events. The figure was produced using Servier Medical Art (https://smart.servier.com/) and image acquired by iStockphoto (https://istockphoto.com/)