Chapter

Aldosterone/MR Signaling, Oxidative Stress, and Vascular Dysfunction

Ana M. Briones and Rhian M. Touyz

Abstract

The mineralocorticoid receptor (MR) is a transcription factor of the family of steroid receptors that classically binds the hormone aldosterone. The contribution of MR in the regulation of sodium retention and blood pressure is well known. However, MR is expressed in extrarenal tissues including endothelial and vascular smooth muscle cells, and its activation leads to vascular remodeling, vascular stiffness, and endothelial dysfunction leading to vascular damage, an important pathophysiological process in hypertension and other cardiovascular diseases. Moreover, MR is expressed in nonvascular cells in close contact with the vascular wall including immune cells and adipocytes that might influence vascular function and structure. MR activation involves its translocation to the nucleus and regulation of gene transcription. In addition, aldosterone exerts rapid non-genomic effects mediated by MR-dependent and MR-independent mechanisms. Both genomic and non-genomic effects facilitate reactive oxygen species (ROS) production (particularly by the enzyme NADPH oxidase), inflammation, and fibrosis, which, in turn, promote tissue remodeling, vascular stiffening, and endothelial dysfunction. Studies with MR antagonists and experimental models with cell-specific knockout or overexpression of MR further support a role for aldosterone/MR-mediated oxidative stress-dependent processes in vascular damage. This review focuses on the relationship between aldosterone/MR signaling and oxidative stress and the implications in vascular regulation in health and disease.

Keywords: aldosterone, mineralocorticoid receptor, oxidative stress, NADPH oxidase

1. Introduction

The mineralocorticoid receptor (MR) classically binds the hormone aldosterone and in the kidney regulates sodium retention, volume homeostasis, and blood pressure. The MR, originally thought to be expressed only in the kidney, is now known to have an extensive extrarenal distribution and is functionally active in the cardiovascular and immune systems. MR activation is involved in various cardiovascular diseases [1, 2] and has also been implicated in metabolic disorders and insulin resistance. At the vascular level, MR is expressed in endothelial and vascular smooth muscle cells (VSMC), and its activation leads to vascular remodeling, vascular fibrosis, and endothelial dysfunction leading to vascular damage, arterial stiffness, and hypertension [1–3]. However, MR is also expressed in nonvascular

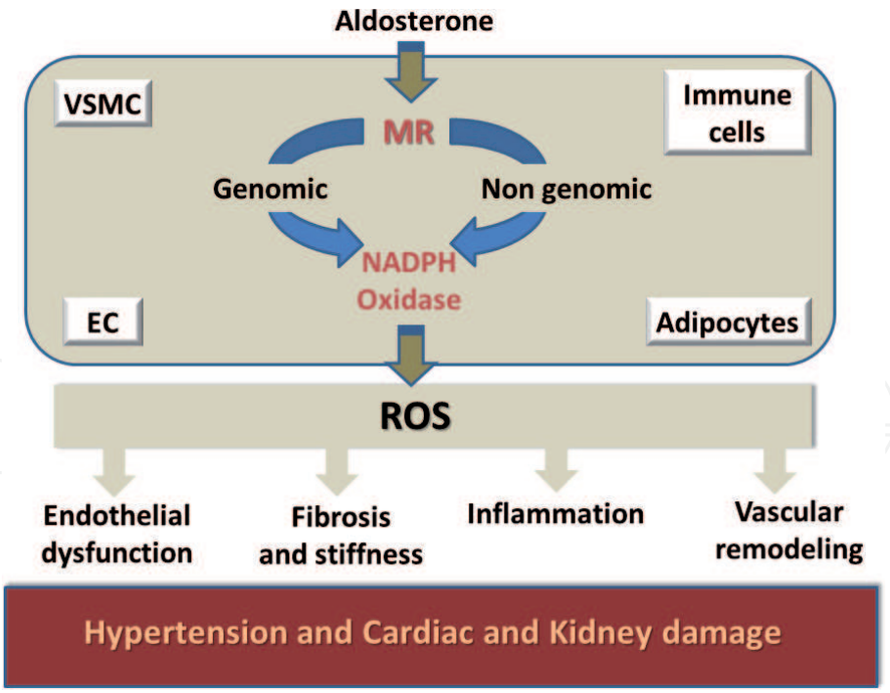

Figure 1.
At the vascular level, MR is expressed in endothelial (EC) and vascular smooth muscle cells (VSMC). Its activation by genomic and rapid non-genomic effects mediated by MR-dependent and MR-independent mechanisms leads to activation of NADPH oxidase that produced reactive oxygen species (ROS) leading to vascular remodeling, fibrosis, inflammation, and endothelial dysfunction that produces vascular damage and arterial stiffness and might participate in hypertension and cardiac and kidney damage.

cells in close contact with the vascular wall including immune cells and adipocytes where it influences inflammatory and metabolic processes [4, 5].

The MR is an intracellular receptor that has three domains: (i) an N-terminal domain that controls transcriptional activity of the receptor, (ii) the DNA-binding domain that influences binding of the specific response element on the promoter of MR target genes, and (iii) the ligand-binding domain for aldosterone. Upon activation, the MR is translocated to the nucleus and further regulates gene transcription and translation of proteins by binding to DNA hormone/steroid stimulatory or negative response elements [1, 2]. In addition, aldosterone exerts rapid non-genomic effects mediated by MR-dependent and MR-independent mechanisms [6], and recent studies uncovered an MR-dependent suppression of miRNA expression resulting in upregulation of vascular miRNA targets [7]. Both genomic and non-genomic effects promote reactive oxygen species (ROS) production particularly by the enzyme NADPH oxidase, as well as inflammation and fibrosis, which, in turn, leads to tissue remodeling and vascular stiffening and endothelial dysfunction. This review focuses on the relationship between aldosterone/MR signaling and oxidative stress and its vascular effects (**Figure 1**).

2. MR and oxidative stress in vascular cells

Extensive evidence has demonstrated a relationship between MR and redox signaling in vascular cells. Animal models including the deoxycorticosterone acetate (DOCA)/salt model and the aldosterone/salt model with or without nephrectomy exhibit vascular oxidative stress. The significance of ROS in these models is supported by studies that demonstrated that MR blockers reduce ROS levels in cardiovascular pathologies including hypertension, obesity, atherosclerosis, or heart failure. In many of the experimental and human studies, evidence of

altered oxidative stress was often based on a single method to determine oxidative stress, i.e., altered gene or protein expression of NADPH oxidase subunits, NADPH oxidase activity, lipid peroxidation determinations, fluorescence-based studies, etc., and this might explain, at least in part, some divergent findings. Because of the complexity of redox biology and difficulties in accurately measuring ROS, expert recommendations have been published suggesting that multiple different assays need to be used to accurately assess redox status in biological systems and experimental models [8]. Here we will not discuss specific methods to measure ROS in the context of aldosterone/MR, and the reader is referred to comprehensive reviews [8, 9].

Aldosterone increases ROS production in cultured VSMC [10–15] and endothelial cells [16–21]. Moreover, aldosterone infusion into mice or rats increases plasma and vascular oxidative stress, and MR blockade reduces ROS production in the setting of hypertension, obesity, and other cardiovascular diseases [21–29]. Earlier studies identified NADPH oxidase as responsible for increased production of vascular ROS, specifically superoxide (O_2^-), in the aorta from mineralocorticoid (DOCA-salt) hypertensive rats excluding other ROS sources (i.e., uncoupled eNOS or xanthine oxidase) as potential contributors [30]. NADPH oxidase is considered the major source of ROS in response to aldosterone/MR stimulation in vessels.

The NADPH oxidase (Nox) family is composed of seven Nox isoforms (Nox1–Nox5 and Duox1 and Duox2); several regulatory subunits p22phox, p47phox, Noxo1, p67phox, Noxa1, and p40phox; and the major binding partner Rac. The main catalytic function of NADPH oxidases is the generation of ROS. NADPH oxidase reduces oxygen to O_2^-, with NADPH being the electron donor. Nox-2 is the classical Nox that was characterized initially in leukocytes. Nox-1, Nox-2, Nox-4, and Nox-5 are expressed in the cardiovascular system with Nox5 not being present in rodents. Nox-1, Nox-2, Nox-3, and Nox-5 produce O_2^-, while Nox-4, Duox-1, and Duox-2 produce H_2O_2 [31, 32]. In vessels, in addition to vascular cells possessing functional Noxes, resident macrophages, neutrophils, and platelets express NADPH oxidase, particularly in pathological states. Accordingly, these cells can also contribute to vascular oxidative stress in disease [32, 33].

2.1 Genomic and non-genomic effects of aldosterone/MR on ROS production

At the vascular level, a combination of aldosterone and high salt caused O_2^- production in VSMC through upregulation of Nox1 without affecting expression of mRNA Nox4, p22phox, and p47phox [11]. In human vein endothelial cells (HUVEC), aldosterone increased p47phox transcription, but no effect on transcription levels of Nox1, Nox2, Nox4, p22phox, p40phox, or p67phox was observed [16]. However, other studies showed that incubation with aldosterone for 24 h dose-dependently increased Nox4 mRNA expression in HUVEC [17]. In human pulmonary artery endothelial cells, aldosterone increased protein levels of Nox4 and p22phox as well as H_2O_2 production [20]. Similarly, in bovine retinal endothelial cells, aldosterone increased mRNA for Nox4 [19], and other studies showed that aldosterone administration modulated exclusively p22phox mRNA expression in freshly isolated aortic endothelial cells [21]. Aldosterone plus salt infusion into rats increased vascular NADPH oxidase activity and expression of p47phox, gp91phox, and p22phox [34], and recently, Jia et al. [29] found vascular upregulation of Nox2 expression and nitrotyrosine (a marker of nitrosative stress) formation after 3 weeks of aldosterone infusion in mice. Together, these findings clearly show a pattern of vascular NADPH oxidase upregulation by aldosterone at the vascular level. This is also supported by the fact that MR antagonists decrease NADPH oxidase subunit expression [9]. For example, eplerenone treatment decreased the

expression of p22phox, p47phox, and p40phox in a model of high-fat diet [21]. Moreover, deletion of MR in specific vascular cell types also downregulates NADPH oxidase isoforms (discussed below).

For the rapid MR-dependent aldosterone effects, the MR seems to be localized near the plasma membrane, but not directly inserted into it. It is located at the cytosolic site associated with scaffolding proteins that are associated with or inserted in the cell membrane such as striatin or caveolin-1 [6]. In this location, aldosterone can also interact with receptors such as receptor tyrosine kinases including epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), and insulin-like growth factor 1 receptor (IGF1R) or G protein-coupled receptors (GPCR) such as angiotensin type 1 receptor (AT1) or G protein-coupled estrogen receptor 1 (GPER1) [6].

The Src family of non-receptor tyrosine kinases seems to be involved in non-genomic ROS generation by aldosterone [9]. In cultured VSMC, NADPH oxidase-dependent ROS generation through non-genomic effects of aldosterone is increased in spontaneously hypertensive rats and is dependent on c-Src [10]. A role for c-Src and Rac-1 in NADPH oxidase activation in endothelial cells has also been described although this effect might be mediated via genomic actions because long incubation times were tested [18]. In VSMC, EGFR and PDGFR, but not IGFR, transactivation by MR and AT1 activates c-Src that in turn facilitates activation of NADPH oxidase and ROS production leading to VSMC migration [35].

Besides receptor tyrosine kinases, GPCR are important partners involved in the non-genomic actions of aldosterone. Thus, MR/AT1 receptor interaction has been implicated, because MR blockade can inhibit angiotensin II-induced ROS production in vascular tissue [22], and more recently, it has been shown that AT1a is required for MR-induced endothelial dysfunction and vascular remodeling, oxidative stress, and inflammation [27], although a genomic effect cannot be excluded in these studies. In cardiac myocytes, an interaction between MR and AT1 participates in aldosterone-induced ROS generation by Nox4 via G protein-coupled receptor kinase (GRK) 2 likely via non-genomic actions [36], but whether this GRK2-dependent mechanism also occurs in vascular cells is unknown. Similarly, there is a paucity of information regarding the novel putative aldosterone receptor GPER1 (also known as GPR30) in aldosterone-induced ROS production in vascular cells, and the evidence supporting this possibility comes from cardiac cells [37, 38].

Non-genomic MR signaling can modulate MR genomic effects [6], thus further perpetuating ROS generation in vascular cells.

2.2 Aldosterone/MR/oxidative stress pathway and endothelial and smooth muscle cells

Extensive experimental evidence has demonstrated a beneficial effect of aldosterone/MR blockade in vascular damage (i.e., endothelial dysfunction, vascular remodeling, and stiffness) and oxidative stress [1–3, 21, 22, 39–42]. As such, it has been suggested that many of the beneficial effects of MR antagonist rely, at least in part, on its ability to decrease oxidative stress. This is supported by direct evidence emerging from studies using models of aldosterone or mineralocorticoid infusion together with antioxidant treatments and by the use of transgenic mouse models of MR overexpression or deletion.

MR expressed in cerebral artery endothelial cells mediates increased capacity for O_2^- production in response to chronically increased systemic levels of aldosterone [26]. Moreover, mRNA expression of p22phox, but not gp91phox,

was upregulated by aldosterone, and this effect was abolished in endothelial cell-specific MR knockout mice (EC-MR-KO) [21], concomitant with improved endothelial function. However, in the absence of stimuli, conditional overexpression of the MR in endothelial cells is not sufficient to increase local vascular or systemic oxidative stress [43], suggesting that upregulation of the MR alone is not enough to produce increased oxidative stress generation. This is consistent with the idea that EC-MR might be vasoprotective in healthy states and that this protection is lost when cardiovascular risk factors such as hypertension, obesity, or increased aldosterone levels are present, as suggested recently [2, 44] (**Figure 2**). In support of this hypothesis, EC-MR deficiency prevented western diet-induced Nox2, Nox4, p22phox, and 3-nitrotyrosine expression, and this was concomitant with reduced aortic fibrosis and stiffness and restoration of endothelial nitric oxide synthase activation [28]. Similarly, EC-MR deletion prevented resistance vessel endothelial dysfunction associated with hyperlipidemia in females, but not in males, and this was associated with decreased O_2^- generation [45].

Although adult SMC-MR-KO mice show no difference in basal vascular ROS, aged SMC-MR-KO mice vessels produce significantly less vascular ROS [7, 46]. Moreover, both young and aged SMC-MR-KO mice show attenuated angiotensin II-stimulated ROS production [46] which might have contributed to the lower blood pressure observed in these mice via improved vascular contraction. More recently, an inverse relationship between SMC-MR and miR-155 has been described in aging [7], whereby this miRNA would repress the SMC-MR-associated oxidative stress also having an impact in vascular function [7]. However, the specific ROS source that is modulated by miR-155 is unknown. VSMC-MR was also shown to be involved in the progression of heart failure post myocardial infarction, through its direct role in oxidative stress-induced coronary endothelial dysfunction and in decreased coronary reserve [47]. In this study the antioxidants apocynin and superoxide dismutase (SOD) improved endothelium-dependent relaxation of

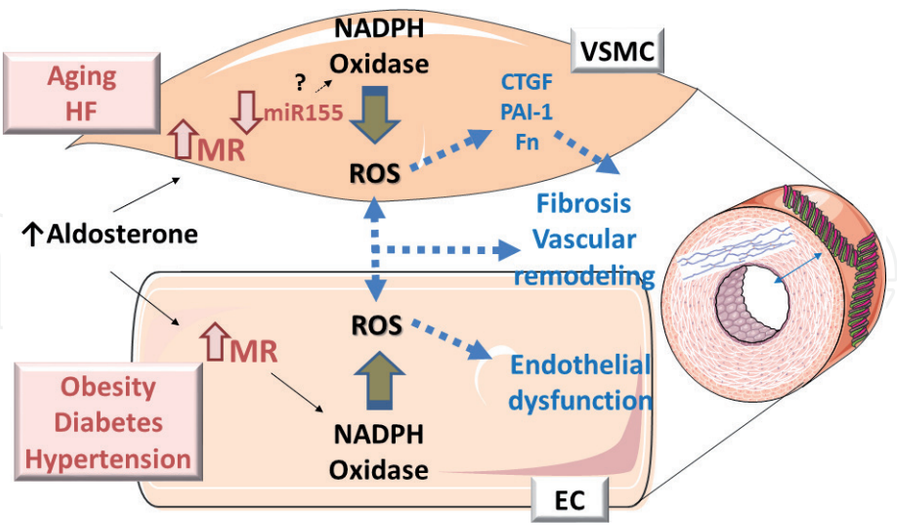

Figure 2.
MR expressed in endothelial cells mediates increased capacity for O_2^- production in response to chronically increased systemic levels of aldosterone such as those occurring in hypertension, obesity, or diabetes. In aging or heart failure after myocardial infarction (HF), SMC-MR facilitates increased ROS production. In addition, an inverse relationship between SMC-MR and miR-155 has been described in aging, whereby this miRNA would repress the SMC-MR-associated oxidative stress. Whether this is via NADPH oxidase is unknown. In these pathologies, both EC and SMC-MR activation facilitate ROS formation that participates in endothelial dysfunction, vasoconstriction, vascular remodeling, and fibrosis, the latter being mediated by the increased expression of different extracellular matrix proteins and profibrotic factors (CTFG, connective tissue growth factor; PAI-1, plasminogen activator inhibitor 1; Fn, fibronectin).

coronary arteries from myocardial infarction mice without affecting relaxation of arteries from myocardial infarction-MR-SMC-knockout or treated with finerenone, indicating a lower effect of oxidative stress when MR is absent in VSMCs or after general MR blockade [47]. Together, these findings point to both endothelial cells and VSMC as potential sources of ROS in response to aldosterone or pathological conditions that impact vascular function (**Figure 2**).

Regarding the role of oxidative stress in vascular remodeling and stiffness, it has been demonstrated that a combination of aldosterone and high salt caused O_2^- production and VSMC hypertrophy through the upregulation of Nox1 [11] and antioxidants attenuated aldosterone-induced VSMC senescence and Ki-ras2A expression [48]. In addition, it has been suggested that aldosterone augments vascular hypertrophic effects of insulin via an MR- and oxidative stress-mediated pathways [49]. Aldosterone-induced hypertrophy and perivascular fibrosis were significantly ameliorated by long-term treatment with spironolactone or antioxidants [50, 51]. However, the profibrotic, but not the hypertrophic, action of aldosterone in resistance arteries was blocked by the antioxidant tempol treatment [23]. Mechanisms responsible for these effects likely rely on the ability of aldosterone-derived ROS to modulate a number of genes involved in vascular injury including placental growth factor, metallothioneins 1 and 2, or connective tissue growth factor [52]. In addition, aldosterone increased expression of profibrotic factors fibronectin and plasminogen activator inhibitor (PAI)-1 in wild type but not in Nox-1 knockout mice [53, 62] (**Figure 2**). Moreover, tempol treatment inhibited other proinflammatory and profibrotic markers such as osteopontin, intracellular adhesion molecule 1, vascular cell adhesion molecule 1, or PAI-1 mRNA expressions that were induced by aldosterone infusion in rats [54].

Among the mechanisms responsible for endothelial dysfunction, it is generally assumed that the interaction between NO and O_2^-, usually from NADPH oxidase, leads to $ONOO^-$ formation or eNOS uncoupling, thus decreasing NO availability, among other mechanisms (for detailed reviews, see [31–33, 55, 56]) (**Figure 3**). In this scenario, aldosterone-induced inhibition of NO production in endothelial cells was partially restored by p47phox knockdown using siRNA [16]. Other potential mechanisms responsible for aldosterone/MR/ROS-induced endothelial dysfunction include (i) oxidative posttranslational modification(s) of guanylyl cyclase activity that impair sensing of this enzyme by NO [12], (ii) downregulation of the antioxidant enzyme glucose-6-phosphate dehydrogenase [57], or (iii) oxidative modification of the redox sensitive, functional cysteinyl thiol(s) in the endothelin receptor (ETBR) (Cys405) by Nox-4-dependent H_2O_2, to impair ETB-dependent activation of eNOS and decrease synthesis of NO [20]. In this sense, during renal ischemia, activation of MR signals Rac1 to increase ROS production in the SMCs that diffuse to ECs to induce posttranslational sulfenic acid modification in ETBR that impairs eNOS activation and diminishes NO production leading to sustained vasoconstriction and reduced kidney perfusion [58]. In addition, cardiomyocyte-specific overexpression of human MR induces severe coronary endothelial dysfunction with decreased NO-mediated relaxing responses to acetylcholine in coronary arteries (but not in peripheral arteries), effects prevented by 1-month treatment with an MR antagonist, vitamin E/vitamin C, or a NADPH oxidase inhibitor [59]. Finally, a role for the epithelial sodium channel in aldosterone-induced oxidative stress and in endothelium stiffness and endothelial dysfunction and fibrosis has also been described [29] (**Figure 3**). Interestingly, Rac1 is not only one of the NADPH oxidase components but also serves as the upregulator of MR signaling in the kidney [60]. This Rac1-MR pathway is activated by ROS in cardiomyocytes [61] and also plays a crucial role in ROS production and cardiac dysfunction [62].

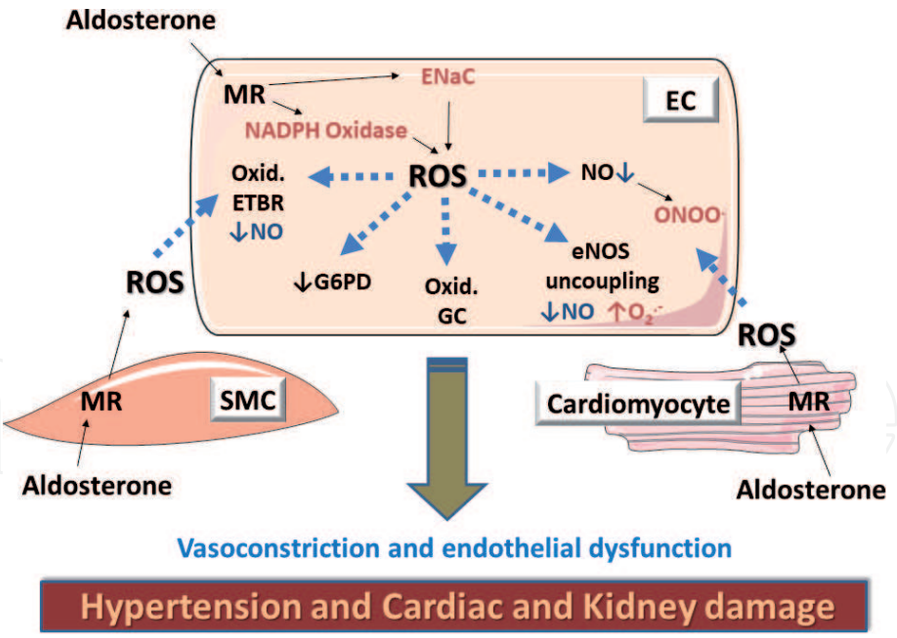

Figure 3.
Mechanisms responsible for aldosterone/MR-induced endothelial dysfunction. The interaction between NO and O_2^-, usually from NADPH oxidase, leads to $ONOO^-$ formation or eNOS uncoupling, thus decreasing NO availability. Other potential mechanisms include oxidative posttranslational modification(s) of guanylyl cyclase (GC) activity that impair sensing of this enzyme by NO, downregulation of the antioxidant enzyme glucose-6-phosphate dehydrogenase (G6PD), or oxidative modification of the endothelin receptor (ETBR) to impair ETB-dependent activation of eNOS and decrease synthesis of NO. In renal ischemia, ROS production in the SMCs diffuses to ECs to induce modifications in ETBR that diminishes NO production leading to sustained vasoconstriction and reduced kidney perfusion. Also, cardiomyocyte-specific overexpression of human MR induces severe coronary endothelial dysfunction with decreased NO-mediated relaxing responses via NADPH oxidase-dependent ROS. Epithelial sodium channel (ENac) also participates in aldosterone-induced oxidative stress and endothelial dysfunction.

3. MR and oxidative stress in immune cells

Growing evidence suggests that aldosterone induces vascular monocyte/macrophage and T-cell infiltration in different pathological states [24, 63, 64]. MR is expressed in both macrophages and T cells, where it functions as an important transcriptional regulator of cellular phenotype and function and can be activated even with normal or low aldosterone levels in pathological conditions [5, 65].

The relationship between immune cells, MR, and oxidative stress was demonstrated in uninephrectomized rats treated for 4 weeks with dietary 1% NaCl and aldosterone, where there was an increased H_2O_2 production by monocytes and lymphocytes, upregulation of oxidative stress-inducible tyrosine phosphatase and Mn-SOD genes in peripheral blood mononuclear cells, and the presence of 3-nitrotyrosine in CD4+ inflammatory cells invading intramural coronary arteries [66]. Guzik et al. [67] showed that DOCA/salt-induced hypertension and O_2^- production in the aorta were blunted in $rag^{-/-}$ mice deficient in T and B lymphocytes. Notably, enhanced suppressor regulatory T lymphocytes, which are suppressors of the innate and adaptive immune responses, prevented aldosterone-induced endothelial dysfunction, vascular remodeling, and oxidative stress [25]. More recently, the key role of immune cell MR in oxidative stress generation was demonstrated by Sun and coworkers [68] that showed that blood vessels from T-cell MR knockout mice had suppressed O_2^- production, and this was paralleled by attenuated target organ damage including better endothelial function and less vascular hypertrophy and fibrosis after angiotensin II infusion. This may be due to a lower proportion of IFN-γ-producing T cells in the arteries [68]. In fact, T-cell MR facilitates activation of T

cells modulating the production of inflammatory cytokines such as IFNγ and IL-6 [69] that can induce ROS production at vascular level.

Regarding macrophages, it has been shown that aldosterone stimulation of macrophages induces a proinflammatory M1 phenotype [5]. Macrophages from mice lacking MR in myeloid cells exhibited a transcription profile of alternative activation from a M1 phenotype toward a M2 more anti-inflammatory phenotype [70]. This might modulate vascular function as NLRP3 inflammasome in macrophages explains aldosterone-induced hypercontractility, endothelial dysfunction, and hypertrophic remodeling [71], although in this study the specific contribution of macrophage-derived ROS was not evaluated. Interestingly, a shift in polarization to a M2 phenotype in the EC-MR-KO mice exposed to a western diet was also observed [28], suggesting a role for endothelial cells-MR in macrophage function. Regarding oxidative stress, aldosterone increases O_2^- production and NADPH oxidase activation in macrophages both in vivo and in vitro [72] and also mitochondrial ROS generation [71] that might contribute to the activation of inflammasome in this cell type as suggested previously [73]. Notably, aldosterone-induced endothelial dysfunction and vascular oxidative stress were decreased in mcsfOp/+, which have a low monocyte/macrophage number in the vessel wall [24], suggesting that MR activation in macrophages modulates vascular oxidative stress. Interestingly, oxidative stress assessed as Nox2 and p22phox gene expression is equivalently increased in the heart of wild-type and mac-MR-KO with L-NAME/salt treatment [74], and MR deficiency in macrophages did not influence their oxidative status in the context of atherosclerosis [75], suggesting a different contribution of MR-derived ROS in different tissues and pathologies. In vivo, MR deficiency in macrophages mimicked the effects of MR antagonists and protected against vascular damage

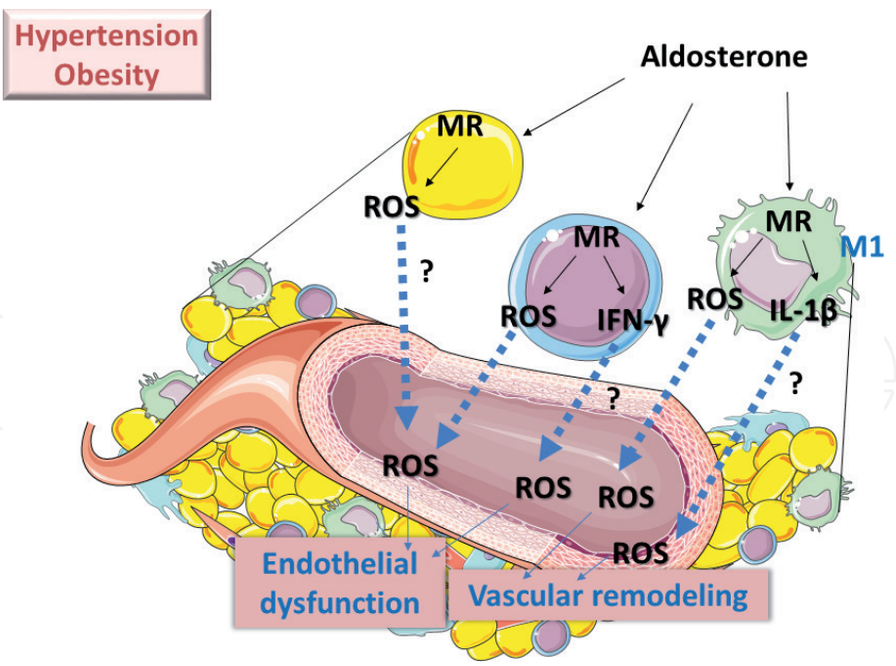

Figure 4.
Aldosterone induces vascular monocyte/macrophage and T-cell infiltration in different pathological states such as hypertension and obesity. MR is expressed in both macrophages and T cells. T-cell MR facilitates activation of T cells modulating the production of inflammatory cytokines such as IFNγ and IL-6 that can induce ROS production at vascular level. MR activation in macrophages induces a proinflammatory M1 phenotype leading to the production of IL-1β. Either directly or through the production of these proinflammatory cytokines, immune cell-derived ROS seems to facilitate vascular remodeling and endothelial dysfunction. MR expression is increased in the adipose tissue in obesity. MR in the different cell types included in the adipose tissue (i.e., adipocytes, preadipocytes, macrophages) might facilitate oxidative stress and vascular alterations associated with obesity.

caused by L-NAME/angiotensin II [70], and selective deletion of MR in myeloid cells limits macrophage accumulation that leads to less VSMC activation and vascular inflammation and inhibits neointimal hyperplasia and vascular remodeling [76]. In both studies, again the specific contribution of macrophage-derived ROS was not evaluated.

Together, these findings highlight a key contribution of MR-dependent ROS from immune cells in vascular damage. Whether this is due to locally produced ROS by infiltrated cells or through the release of proinflammatory cytokines affecting the underlying VSMC or endothelial cells to induce oxidative stress, or both, remains unclear (**Figure 4**).

4. MR and oxidative stress in adipose tissue

Other extrarenal tissue that express MR is the adipose tissue where MR is involved in essential processes such as differentiation, autophagy, and adipokine secretion [4, 77]. MR expression is increased in adipose tissue of murine models of obesity and in obese human subjects, and different studies using MR antagonists and also adipocyte-specific MR transgenic mice have demonstrated a key role of MR in insulin signaling and inflammation, as reviewed previously [4, 77, 78].

It is well accepted that adipose tissue, particularly perivascular adipose tissue (PVAT), modulates vascular health and disease through the release of a number of adipokines that affect contractile and relaxant properties, vascular smooth muscle cell proliferation and hypertrophy, fibrosis, and inflammation [79]. Among the many substances released, ROS such as H_2O_2 seems to have a pivotal role both in physiological and pathological conditions; however, in some disease states such as obesity or hypertension, proinflammatory cytokines such as IL-1, IL-6, or TNF-α released from PVAT clearly affect vascular tone [79], probably in part through increased oxidative stress generation. Thus, in healthy rat mesenteric arteries, the anticontractile effect of PVAT was lost following incubation with aldosterone (10 minutes and 3 hours), and this was restored by a combination of SOD and catalase and by eplerenone, and this is likely dependent on macrophage infiltration in the PVAT [80]. Moreover, MR blockade reduced ROS production in 3 T3-L1 adipocytes [81, 82].

Earlier studies showed that the NADPH oxidase subunits p22 and p47phox were significantly increased in adipose tissue from ob/ob and db/db obese mice compared with lean control mice and that eplerenone treatment suppressed this increase [81]. The increase in ROS levels observed in adipose tissue of these models of obesity could also be due to the decreased gene expression of the ROS-eliminating enzymes, catalase, and Cu/Zn-SOD, which were reduced in both ob/ob and db/db mice and which were also restored by administration of eplerenone [81]. Other studies also reported upregulation of antioxidant enzymes (SOD-1 and catalase) at vascular level by MR blockade in obesity/diabetes [41]. Finally, in the adipose tissue of nephrectomized rats, oxidative stress increased, and this was reversed by spironolactone [83].

In vivo conditional upregulation of MR in mouse adipocytes led to increased production of H_2O_2 from epididymal adipose tissue likely due to the decrease in catalase mRNA levels and the increased Nox-4 mRNA levels without changes in Nox-1 and Nox-2 expressions which likely explain changes in vascular contractility [84]. Interestingly, adipocyte-specific MR knockout mice (AdipoMR-KO) fed with high-fat/high-sucrose diet showed similar levels of 8-isoprostane, p22phox, SOD-1, or catalase mRNA levels, and they did not show differences in body weight, fat weight, glucose tolerance, insulin sensitivity, or inflammation [85]. Similar results

were reported by Feraco and coworkers [86] using inducible adipocyte-specific deletion of MR fed a 45% high-fat diet although in this study oxidative stress was not evaluated. Further studies are warranted to identify the specific contribution of MR in the different cell types included in the adipose tissue (i.e., adipocytes, preadipocytes, macrophages) as responsible for the MR-dependent inflammation, oxidative stress, and metabolic alterations associated with obesity (**Figure 4**).

5. Clinical relevance

While there is extensive preclinical data indicating that aldosterone-MR signals through redox-dependent pathways, there is a paucity of information in humans. However, a few clinical studies have suggested that hyperaldosteronism is associated with increased concentration of circulating markers of oxidative stress. In patients with stable heart failure and in patients with hypertension, higher aldosterone levels were associated with systemic evidence of oxidative stress, inflammation, and matrix turnover [87]. In heart failure patients, aldosterone-associated cardiovascular damage and renal fibrosis were linked to decreased production of NO, increased oxidative stress, and activation of proinflammatory transcription factors, including NF-κB [9]. At the cellular level, there is also some suggestion that aldosterone stimulates ROS production in humans. In human endothelial cells, spironolactone inhibited Nox-induced oxidative stress and increased eNOS activity [88], indicating a role for MR-mediated regulation of ROS in human vessels. In patients with hyperaldosteronism and adrenal adenomas, a number of studies have reported increased expression of redox-related genes and proteins including Nrf2, p22phox, HO-1, and proinflammatory transcription factors [89–91]. In human cardiomyocytes, aldosterone impairs mitochondrial function, important in redox regulation [91]. Despite suggestions that hyperaldosteronism promotes oxidative stress in human cardiovascular disease, studies using MR antagonists have not shown significant improvement in oxidative stress markers. Thus, Hwang and coworkers [92] demonstrated that eplerenone-related improvement in flow-mediated dilation (a marker of endothelial function) was not associated with oxidative stress markers, plasma F2-isoprostanes, and vascular endothelial cell protein expression of nitrotyrosine and p47phox. In a small group of older adults with metabolic syndrome, flow-mediated dilation, levels of oxidized low-density lipoproteins, or F2-isoprostanes did not improve in response to MR blockade, despite a large reduction (10 mmHg) in systolic blood pressure [93]. In addition, there was no effect of 1-month treatment with eplerenone on oxidative stress (oxidized LDL) and arterial stiffness in healthy older adults [94]. However, Chen et al. [95] recently demonstrated that increased NADPH oxidase-dependent oxidative stress, oxidative BH4 degradation, eNOS uncoupling, and reduced NO generation were responsible for the impaired in vivo endothelial repair capacity of early endothelial progenitor cells from hypertensive patients with primary hyperaldosteronism [95]. Further clinical studies are needed to confirm the role of ROS in aldosterone-mediated cardiovascular injury. Moreover, trials that assess effects of MR antagonists on ROS levels rather than markers of oxidative stress (oxidized LDL, F2-isoprostanes) are warranted.

6. Conclusions

Experimental evidence clearly shows that MR/aldosterone blockade decreases vascular oxidative stress and improves vascular function, structure, and mechanical properties in different experimental models. Among the cell types involved

in aldosterone/MR/oxidative-associated vascular damage are endothelial cells and vascular smooth muscle cells. However, growing evidence suggests that these vascular effects can also be modulated by MR expressed in infiltrating immune cells, i.e., lymphocytes and macrophages, and in the surrounding perivascular adipose tissue that might release ROS directly impacting the endothelium and vascular wall. Alternatively, these cells can generate MR-dependent inflammatory cytokines (or adipokines) that act in a paracrine manner in the underlying vessels to induce oxidative stress and hence vascular damage. Thus, MR-associated oxidative stress in different cell types emerges as an important pathway contributing to vascular dysfunction and injury associated with conditions of high aldosterone/MR activation. Accordingly, it is suggested that some of the vasoprotective effects of MR antagonists used clinically may be mediated by inhibiting ROS-induced vascular damage.

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Conflicts of interest/disclosures

The authors declare no conflict of interest.

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