Published in final edited form as:

Curr Atheroscler Rep. 2013 July ; 15(7): 340. doi:10.1007/s11883-013-0340-x.

Mineralocorticoid Receptors in Vascular Disease: Connecting Molecular Pathways to Clinical Implications

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Abstract

The mineralocorticoid receptor (MR), a steroid hormone-activated transcription factor, plays a substantial role in cardiovascular diseases. MR antagonists (MRAs) have long been appreciated as effective treatments for heart failure and hypertension; however, recent research suggests that additional patient populations may also benefit from MRA therapy. Experimental evidence demonstrates that in addition to its classical role in the regulating sodium handling in the kidney, functional MR is expressed in the blood vessels and contributes to hypertension, vascular inflammation and remodeling, and atherogenesis. MR activation drives pathological phenotypes in smooth muscle cells, endothelial cells and inflammatory cells, while MRAs inhibit these effects. Collectively, these studies demonstrate a new role for extra-renal MR in cardiovascular disease. This review summarizes these new lines of evidence and how they contribute to mechanisms of atherosclerosis, pulmonary and systemic hypertension, and vein graft failure, and describes new patient populations that may benefit from MRA therapy.

Keywords

aldosterone; mineralocorticoid receptor (MR); atherosclerosis; vein graft failure; essential hypertension; pulmonary hypertension; inflammation; vascular remodeling

Introduction

The mineralocorticoid receptor (MR) is a member of the steroid receptor family known for its role in blood pressure (BP) control. MR is expressed in renal epithelial cells and

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Disclosures

None.

classically participates in BP regulation by binding the steroid hormone aldosterone (aldo) to induce expression of genes involved in sodium retention, thereby increasing blood volume and BP (reviewed in [1]). MR is the terminal component of the renin-angiotensin-aldosterone system (RAAS), which is activated by hypotension leading to angiotensin II (AngII) production by renin and angiotensin-converting enzyme (ACE). AngII activates angiotensin type 1 receptors (AT1R) on cells of the adrenal gland to promote aldo release. MR antagonists (MRAs) prevent hormone from binding and activating the MR. These MRAs include the available drugs spironolactone and the more MR-specific but less potent eplerenone, as well as new non-steroidal MRAs currently in development [2]. Based on the role MR plays in regulating blood pressure and blood volume, MRAs are used to treat hypertension and congestive heart failure. Interestingly, clinical trials have demonstrated that treatment with RAAS inhibitors affords greater cardiovascular protection than would be predicted solely based on modest reductions in blood pressure. The EPHESUS [3] and RALES [4] trials demonstrated that MRA therapy substantially reduces morbidity and mortality in patients with severe heart failure at doses that are insufficient to exert significant renal effects and yield only small reductions in blood pressure. Patients with milder heart failure symptoms treated with MRA therapy [5] and individuals at risk for coronary disease treated with ACE inhibition or AT1R blockade also have greater cardiovascular benefits than would be expected from modest improvements in hypertension alone [6, 7]. Even patients with hypertension treated with MRA have greater protection from end-organ damage than those treated to the same target blood pressure with other anti-hypertensive agents [8] (Table 1).

These clinical data implicate potential extra-renal actions of MR in the mechanism for the cardiovascular protective effects of MRAs, which has prompted substantial recent basic research on the role of aldo and MR in mechanisms of vascular diseases. We and others have confirmed that MR is expressed in human endothelial cells (EC) and vascular smooth muscle cells (SMC), where it regulates expression of genes involved in vascular cell function. This review will summarize recent experimental data demonstrating novel mechanisms by which MR in the vasculature contributes to atherosclerosis and its complications, vein graft failure, and even systemic and pulmonary hypertension. In each case, we describe how these preclinical studies have now identified new patient populations that may benefit from MRA treatment and also defined potential novel drug targets to treat or to prevent cardiovascular disease that can now be taken from the bench back to the bedside.

Aldosterone and Mineralocorticoid Receptors in Atherosclerosis

Epidemiologic data reveal that circulating aldo levels are independent predictors of cardiovascular ischemia [9, 10]. Compared to individuals with the same degree of essential hypertension but normal aldo levels, patients with primary hyperaldosteronism have a 4-fold increased risk of stroke and a 6-fold increased risk of myocardial infarction (MI) [11]. In a recent study of patients with stable coronary artery disease (CAD), higher serum aldo levels—even within the normal range—predict a 2- to 4-fold increase in subsequent MI or cardiovascular death [12]. These studies cannot determine whether elevated serum aldo is correlative or causative for ischemia; however, clinical trials evaluating the lipid-regulating

drug torcetrapib in patients with dyslipidemia demonstrated an *increased* rate of MI, stroke, and progression of atherosclerosis in patients randomly assigned to receive the drug [13–16]. This outcome was later associated with an off-target increase in serum aldo levels in patients randomized to torcetrapib [16–18]. These clinical data, in addition to the earlier trials linking inhibition of aldo production or MR antagonism to lower mortality and a reduction in cardiovascular ischemic events [3, 4, 6, 7], suggest blood pressure-independent effects of aldo that promote atherosclerosis and plaque rupture in humans. This association has led to recent investigations of the mechanisms by which aldo might contribute to the process of atherogenesis.

Atherosclerosis is a systemic vascular inflammatory disease initiated by cardiovascular risk factors that cause EC damage. Activated EC express surface receptors (such as the intracellular adhesion molecules ICAM, VCAM and selectins) that recruit leukocytes to the vascular wall [19], typically in regions of turbulent blood flow [20, 21]. Activated inflammatory cells within plaques release cytokines and chemokines that further augment protease activity and plaque inflammation, thereby promoting matrix degradation, plaque rupture and thrombosis, the cause of most MIs and strokes [22].

Role of MR in atherogenesis

Animal studies support a role for aldo in atherogenesis, with aldo infusion increasing vascular and macrophage oxidative stress and overall atherosclerotic plaque area [23, 24]. Conversely, MR antagonists [25, 26] and aldo synthase inhibitors [27] decrease atherosclerosis in animal models; however, the detailed molecular mechanisms are only beginning to be elucidated. Several groups have recently demonstrated that MR activation specifically enhances early atherosclerotic lesion formation in areas of non-laminar blood flow, such as the aortic arch and great vessel bifurcations. Aldo also promotes the formation of lipid-rich and inflamed plaques [28–30], a phenotype associated with plaque rupture in humans. In a mouse model of atherosclerosis, infusion of aldo at low doses that do not affect blood pressure resulted in recruitment of activated monocytes and T cells to athero-prone regions of the blood vessel wall prior to promoting plaque formation [30]. This finding supports the notion that blood pressure-independent, direct inflammatory effects of aldo on the blood vessel contribute to atherosclerosis. The mechanism may involve direct activation of vascular MR to modulate local vascular gene expression [31, 32] as pro-atherogenic genes induced by non-laminar blood flow [33] have been shown to be preferentially regulated by aldo in the aortic arch in an oxidative stress-dependent manner [34].

The pro-inflammatory effects of MR in the vasculature involve multiple cell types (Figure 1). In human coronary EC, activation of MR upregulates ICAM1 expression, which increases leukocyte adhesion to the endothelium [32], an initiating step in vascular inflammation and subsequent plaque development. In cultured SMC, aldo promotes vascular calcification, a late stage in atherosclerosis that correlates with the risk of ischemia in humans [35]. In the setting of early atherosclerosis, aldo triggers release of chemotactic factors from human coronary artery SMC that engage the leukocyte vascular endothelial growth factor type 1 receptor (VEGFR1), thereby increasing recruitment of activated human macrophages [30]. *In vivo*, mice lacking the VEGFR1 ligand, placental growth factor

(PLGF), are protected from early aldo-induced vascular inflammation and atherogenesis. Moreover, human aorta specimens explanted from patients undergoing coronary artery bypass graft (CABG) surgery show upregulation of the PLGF/VEGFR1 signaling axis when treated with aldo [36]. Spironolactone inhibits this effect, indicating that these pathways are functional in human vessels and that inhibition of vascular MR-regulated genes by MRAs might contribute to the protective effects of these drugs.

Recent studies indicate that MR modulation of immune cell function may also play a role in atherogenesis. In mouse models of atherosclerosis, low dose aldo infusion causes systemic inflammation with increased spleen size and circulating levels of the cytokine RANTES [30]. Deletion of MR in macrophages shifts cell populations from the classical M1 phenotype to the anti-inflammatory M2 state [37], as does systemic inhibition of MR with eplerenone in atherosclerotic mouse models [28]. Eplerenone also suppresses macrophage activation *in vitro*, further suggesting a direct role for leukocyte MR in inflammation [38]. In models of disease, macrophage MR deletion reduces ischemic stroke infarct size and mineralocorticoid-induced cardiac hypertrophy [37, 39, 40]. In addition to macrophages, T cells are also an integral component of atherosclerotic lesions [19] and are recruited to the vessel in response to aldo [30]. The potential role of MR in adaptive immune responses that contribute to atherogenesis warrants further investigation [41].

Clinical implications

Patients with high plasma aldo levels exhibit higher concentrations of circulating E-, P- and L-type selectins [42], indicating that these individuals have endothelial dysfunction and vascular inflammation and thus may be more prone to plaque rupture, the cause of most MIs and ischemic strokes. More directly, in cardiovascular disease patients, aldo levels correlate with the degree of atherosclerosis and plaque rupture [9, 10, 12]. Thus, in patients at risk for CAD, circulating aldo levels may be a useful biomarker to identify patients that have a particularly high risk for ischemic events, which may warrant more aggressive risk factor modification to prevent adverse clinical events.

The myriad protective effects of MRAs in patients with advanced heart failure have long been appreciated [43], yet the potential for these drugs to prevent the complications of atherosclerosis have not been formally tested clinically. The ALBATROSS trial, which completed data acquisition in February of 2013, is designed to evaluate MRA therapy in CAD patients without heart failure [44]. If this patient population benefits from MRA therapy, future trials will be needed to evaluate MRAs as a preventative treatment in asymptomatic but high-risk individuals. Based on what is known about MR in atherogenesis from basic science studies, MRAs have the potential to reduce early vascular inflammation—an initiating step in atherosclerosis—to prevent inflammation-mediated rupture of existing plaques, and to modulate late stages of vascular calcification, and therefore could benefit individuals at various stages during the progression of vascular disease.

Mineralocorticoid Receptors in Vein Graft Failure

Bypass surgery remains an important therapeutic option for patients with diffuse or severe arterial occlusive disease, with an estimated 400,000 coronary artery bypass graft (CABG)

surgery cases and 75,000 peripheral arterial bypass surgeries performed in the United States annually [45, 46]. Due to their availability, autologous saphenous veins are the most common conduit used during bypass procedures; however, vein graft failure rates remain high with no effective therapy. Arterialized veins undergo rapid adaptive remodeling characterized by smooth muscle cell (SMC) hyperplasia and vessel wall thickening, thereby reducing wall tension [47]. The mechanism involves de-differentiation of medial SMC from a quiescent contractile state into a synthetic phenotype that proliferates and secretes growth factors and cytokines, leading to a robust inflammatory response [48]. Proliferation of SMC and associated inflammation promote histological changes resembling those observed with arterial atherosclerosis, including hyperplasia and the development of inflamed focal lesions that can accumulate oxidized lipids and either occlude blood flow or rupture, leading to thrombus formation and ischemic complications [49, 50].

MR and mechanisms of vein graft failure

While the pathology of failing vein grafts has been characterized [51], less is known about the molecular mechanisms that regulate the process of vein graft remodeling. As roles for MR in arterial remodeling and atherosclerosis have been elucidated, interest has recently developed in whether venous tissue might also express MR that could contribute to vein graft pathology. Human saphenous vein SMC, endothelial cells, and whole vessels have recently been found to express MR mRNA and protein, as well as 11- β -hydroxysteroid dehydrogenase type 2 (11 β HSD2), the enzyme necessary to confer aldo sensitivity [32, 52, 53]. *In vitro*, saphenous vein SMC MR can be activated by aldo at physiologic and pathologically-relevant concentrations to modulate MR transcriptional activity [52, 53]. In addition, aldo regulates expression of the angiotensin type 1 (AT1R) in human venous SMC in a MR-dependent manner [52]. In a recent study, Bafford and coworkers examined failed saphenous vein grafts explanted from peripheral bypass surgery patients undergoing reintervention and found that MR and 11 β HSD2 protein levels are increased compared to ungrafted veins, suggesting enhanced MR signaling in the failing vein graft. These phenomena were also observed in a rabbit vein graft model [52], supporting the potential for venous MR activation by aldo to play a role in the mechanism of vein graft failure.

Recent *in vivo* studies support that MR signaling may play a role in early processes that contribute to graft inflammation, fibrosis and intimal hyperplasia. In a porcine model of carotid artery interposition grafting, low-dose treatment with the MRA spironolactone resulted in vein grafts with greater lumen cross-sectional area as early as 5 days post-procedure, with no change in neointima formation, compared to placebo-treated controls [54]. Using a mouse vena cava to aorta interposition model, we have recently demonstrated that spironolactone dramatically reduces focal vein graft remodeling and vessel fibrosis after grafting [53]. Interestingly, spironolactone treatment reduced the number of inflammatory cells in the grafted vein without changing total SMC content, suggesting that MR signaling may contribute to graft remodeling through inflammatory processes rather than SMC hypertrophy. Additional studies also suggest that circulating inflammatory cells play an underappreciated role in vein graft stenosis [55, 56], and that inflammatory cell MRs may contribute to ischemic vascular disease [39]. Further studies are warranted to determine the molecular mechanisms for the role of MR in vein graft pathology.

Clinical implications

Despite the proven efficacy of bypass surgery for patients with advanced vascular disease, graft failure rates remain high with no effective therapy. In addition to preventing adverse vein graft remodeling, an ideal drug to preserve graft patency should inhibit subsequent clinical complications, such as progression of atherosclerosis and thrombosis. In animal models, blockade of MR signaling attenuates vein graft remodeling, fibrosis and inflammation [53, 54], inhibits SMC proliferation and injury-mediated vascular hypertrophy [36], reduces atherosclerosis, plaque inflammation, and macrophage activation [28, 30], and prevents venous thrombosis (Figure 1) [57]. Additionally, MRAs have a proven history of efficacy and safety in patients with cardiovascular disease, making these drugs attractive candidates for future clinical studies to improve graft patency and long-term survival in patients undergoing vein graft surgery.

MR and hypertension: the vasculature as culprit in elevated blood pressure

In clinical trials, MRA therapy is effective in the treatment of hypertension [8, 58–62]. Many studies in rodent models also demonstrate benefits of chronic MR antagonism in lowering blood pressure and improving hypertension outcomes [63–68]. More recent studies have evaluated the effects of the novel non-steroidal mineralocorticoid receptor (MR) antagonist SM-368229 on rat models of hypertension and found that this drug reduces blood pressure and provides cardio-renal protection [69, 70]. While renal MR regulation of BP continues to be an attractive anti-hypertensive target, data from animal models and human studies support the potential for extra-renal MR to also contribute to BP control. A recent meta-analysis of MRA clinical trials demonstrated that BP reduction with MR blockade does not correlate with changes in plasma potassium, a marker of renal MR activation, thereby supporting the potential for non-renal MR to contribute to BP modulation [71]. Moreover, mice deficient in MR in all tissues die in the neonatal period due to salt wasting, which is consistent with the known role of MR in regulating vascular volume [72, 73]. However, unless challenged with low-salt conditions, mice with renal tubule-specific MR deficiency survive [74, 75], suggesting the possibility that loss of extra-renal MR could also contribute to the hypotension and mortality associated with total MR deficiency. Over the past decade it has become clear that the vasculature is an aldo-responsive MR target tissue, and emerging evidence supports a direct role for the vasculature in BP regulation [76]. New studies utilizing vascular-specific transgenic mouse models have begun to elucidate the direct role of vascular MR in BP control and may provide new insights into novel mechanisms and treatments for hypertension.

Vascular endothelial dysfunction is a hallmark of hypertension. MRA treatment improves endothelial function in animal models of hypertension and in hypertensive patients [77–81]; however, whether antagonism of MR in EC has direct BP benefits is not yet known. Support for this possibility comes from a recent study of a mouse model with inducible over-expression of MR in endothelial cells [82]. These MR-EC mice have elevated systolic and diastolic BP compared to controls, with no difference in renal sodium transport, heart rate, or heart weight. MR-EC mice also showed greater BP rise in response to infusion of AngII or endothelin-1 compared to controls, consistent with *ex vivo* vessel data supporting a role

for MR in potentiating vascular responses to contractile agonists. Further studies in endothelial-specific MR-deficient mice will help clarify the role of endogenous vascular EC MR in the regulation of BP.

A mouse model with inducible, SMC-specific deletion of MR (SMC-MR-KO) has further extended our knowledge of the role of vascular MR in BP regulation [83]. As it does in humans, BP rises with age in SMC-MR-intact mice, yet this age-associated rise in BP is lost in SMC-MR-KO mice. The lower BP in aged SMC-MR-KO mice is independent of sodium loading and renal MR function remains intact in these mice, supporting a renal-independent role for SMC MR in BP regulation. The vasoreactivity and tone of resistance vessels is thought to be critical in the modulation of the total vascular resistance that contributes to systemic BP. Resistance vessels from control mice develop augmented agonist-induced contraction with aging, although this effect is absent in vessels from SMC-MR-KO mice. Similarly, resistance arteries from aged SMC-MR-KO mice have comparable structure and stiffness as those from control mice, but they develop significantly less spontaneous myogenic tone. Voltage-gated calcium channels have a well-characterized role in vascular contraction and in the development of myogenic tone. The expression and activity of the Cav1.2 L-type calcium channel $\alpha 1$ subunit are reduced in vessels from SMC-MR-KO mice, suggesting that MR-mediated regulation of vascular calcium channels in SMC may participate in age-associated alterations in myogenic tone, agonist-induced contraction, and BP regulation.

Previous studies suggest that crosstalk occurs between the AT1R and the MR in vascular SMC during AngII stimulation *in vitro* [31, 84–87]. New evidence for *in vivo* crosstalk between MR activation and AngII signaling comes from studies in aldo synthase-deficient mice. In these studies, aldo deficiency (or treatment with an MRA) prevented AngII-induced cardiac, renal and vascular injury [88]. These findings do not specifically implicate SMC MR signaling; however, we have recently demonstrated that many of the detrimental effects of AngII on the vasculature require SMC MR. AngII infusion causes significant hypertension, vascular contraction, and vascular oxidative stress, all of which are attenuated in young SMC-MR-KO mice and prevented in aged SMC-MR-KO mice [83]. Overall, studies in this mouse model demonstrate a direct contribution of SMC MR to BP control in aged mice likely via modulation of vascular oxidative stress, AngII signaling, and calcium channel function.

Clinical implications

MR antagonism has been beneficial in clinical trials of patients with heart disease when used in combination with standard therapies, including ACE inhibitors and AT1R blockers, and MRAs are as effective for treating hypertension as calcium channel blockers (but with better end-organ protection) [8] or AT1R inhibitors [89]. The emerging evidence for vascular MRs as regulators of BP and their link to calcium channel and AngII signaling indicates a possible benefit for combination therapy in hypertensive patients. A recent study in a rat model of salt-sensitive hypertension found that eplerenone treatment potentiated the protective effects of the L-type calcium channel blocker amlodipine against cardiovascular injury. This finding supports that MRA/L-type calcium channel blocker combination therapy

could potentially reduce the cardiovascular morbidity and mortality in hypertensive patients more effectively than either drug alone and at lower doses that might limit side effects [90]. In addition, hypertension is predominantly a disease of the elderly, affecting more than 60% of people over 60 years of age and up to 80% of the growing population over 80. Recent data in animal models suggests that SMC MR is a unique contributor to the rise in BP with aging and supports clinical studies to evaluate the efficacy of MR antagonism to treat hypertension specifically in the elderly or even to prevent the progression of hypertension with advancing age.

The role of MR in pulmonary arterial hypertension

In addition to its well-known role in regulating systemic BP, a role for MR in pulmonary hypertension has recently been identified. Pulmonary arterial hypertension (PAH) remains a progressive, fatal disease, with a mortality of up to 50% at 5 years, even with recent advances in available therapies [91]. Pathologic abnormalities of the pulmonary vasculature in this disease include medial thickening due to SMC hyperplasia and hypertrophy, muscularization of distal non-muscular arteries, neointimal thickening composed of SMCs or myofibroblasts, and the occurrence of plexiform lesions due to EC and SMC proliferation [92].

While several pathways, such as platelet-derived growth factor (PDGF) [93] or nitric oxide (NO) [94] signaling, have been previously implicated in the pathogenesis of PAH, very recently data has emerged that also support a role for MR in this disease. In three distinct experimental rodent models of pulmonary hypertension, MR antagonism attenuates the severity of the PAH phenotype [78, 95]. MRA treatment initiated at the time of the PAH stimulus prevents the pulmonary vascular hyperplasia and the rise in right ventricular systolic pressure. More importantly, initiation of MRA therapy after establishment of PAH attenuates the progression of disease, supporting a potential for these drugs to be used therapeutically in patients. Mechanistic studies revealed that MR is functional in distal pulmonary artery SMC and that MR inhibition prevents cell proliferation. Exposure of pulmonary artery SMC to hypoxia or to PDGF promotes MR translocation to the nucleus, while MR antagonism blocks the proliferative effects of these PAH activators [95]. In addition, in pulmonary artery EC, aldo-induced oxidative stress impairs endothelin-B receptor signal transduction, resulting in impaired NO synthesis [78]. Moreover, in a cohort of PAH patients, plasma aldo levels were found to be elevated compared to controls and also to correlate with markers of disease severity [96]. Collectively, these recent results support the notion that MR contributes to the development and worsening of pulmonary vascular remodeling and elevation of pulmonary pressure in PAH. Since MRAs are available and their safety profile is well characterized—even in patients with advanced heart failure—they may represent a novel therapeutic target for this devastating disease (Figure 1).

Conclusion

It is now clear that MR signaling plays a much greater role in human physiology and disease than solely controlling electrolyte balance and BP through the kidney. Clinical studies as well as animal models demonstrate that MR activation correlates with and contributes to the

pathophysiology of atherosclerosis, vascular injury, vein graft remodeling, and systemic and pulmonary hypertension (Figure 1). Collectively, these studies show that MR activation in vascular and inflammatory cells exerts a substantial influence on the progression of these diseases and have identified new patient populations that may benefit from MRA therapy (Table 2). New clinical trials evaluating MRA efficacy outside the setting of heart failure and systemic hypertension may establish new strategies to treat or prevent a wide range of cardiovascular diseases.

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Figure 1.

Anticipated effects of MRA therapy in cardiovascular disease patients. Inhibition of mineralocorticoid receptor (MR) signaling in vascular smooth muscle cells (VSMC), endothelial cells (EC) and macrophages induces a variety of cardio-protective actions. Collectively, these effects inhibit the progression of atherosclerosis, vein graft failure, and systemic and pulmonary hypertension, leading to fewer vascular ischemic events and improving patient survival. NO, nitric oxide; Ca^{++} , calcium ion; RV, right ventricle.

Table 1

Current patient populations with proven benefit from MRA therapy: summary of important MRA clinical trials

Clinical Trial	Patient Population	MRA treatment	Outcome
RALES [4]	NYHA class 3+ heart failure, ejection fraction <35%	spironolactone 25–50 mg/day	30% reduction in mortality, 35% reduction in CHF hospitalization at 24 months
EPHESUS [3]	recent MI (within 3–14 days), ejection fraction <40%, evidence of CHF	eplerenone 50 mg/day	15% reduction in mortality at 16 months
4E [61]	cardiac hypertrophy, essential hypertension	eplerenone 200 mg/day	15 g reduction in left ventricular mass, 24/12 mm Hg reduction in systolic/diastolic BP
Eplerenone for older patients with systolic hypertension [8]	age >50, systolic BP>150 mm Hg with pulse pressure>70 mm Hg	eplerenone 50–200 mg/day versus amlodipine	20 mm Hg reduction in systolic BP and decrease in pulse wave velocity (same as amlodipine), 27% reduction in urinary albumin:creatinine (versus 10% for amlodipine)
EMPHASIS-HF [5]	NYHA class 2 heart failure, ejection fraction <35%, CV hospitalization	eplerenone 50 mg/day	37% reduction in CV mortality or CHF hospitalization, 24% reduction in mortality at 21 months
ASPIRANT [62]	resistant hypertension (systolic BP>160 mm Hg on 3 drugs with diuretic)	spironolactone 25 mg/day	10 mm Hg reduction in average 24 hr ambulatory BP

Table 2

Potential new patient populations to benefit from MRA therapy: Future clinical trials

Patients at high-risk for heart attack or stroke
Vein graft surgery patients
Patients at risk for stent failure from adverse vascular remodeling
Cardiac hypertrophy and diastolic heart failure
Age-associated hypertension
Systemic hypertension (in combination with AT1R or L-type calcium channel blocker)
Pulmonary hypertension