Supplemental Table 1. Animal and *ex vivo* human studies of cardiovascular and kidney diseases providing direct (hormone infusion, transgenic animal models) and indirect (RAAS suppression) evidence of the cardio- and nephrotoxicity of chronic and/or excess angiotensin II and aldosterone production. The studies are presented in chronological order.

Experimental Model (or Disease)	Cardiovascular and/or Renal Effects (Control or Pre-treatment)	Treatment(s) Used in Experiments	Outcomes	Key Points
Unilateral renal artery clipping Rats were fed a normal salt diet	Renovascular hypertension,	Captopril, hydrochlorothiazide, captopril and hydrochlorothiazide, hydralazine (vs. untreated control) Therapy started 6 weeks after renal artery clipping and was continued for 6 months	Captopril and captopril with hydrochlorothiazide lowered blood pressure consistently where rats appeared to develop a tolerance to hydralazine after about 4 weeks of therapy; Survival was best in the captopril and hydrochlorothiazide treated rats, yet captopril alone also prolonged survival and hydrochlorothiazide or hydralazine alone did not affect survival when compared to untreated rats	Captopril (alone or in combination with hydrochlorothiazide) benefited survival in these rats; this was early evidence that RAAS suppression impacted survival.(1)
Spontaneously hypertensive rats Rats were fed a normal salt diet	Systemic hypertension, left ventricular hypertrophy, heart failure	Captopril, guanethidine, or hydralazine (vs. control group) Captopril: 14 month old rats for 10 months; guanethidine or hydralazine: 12 month old rats for 6 months	Left ventricular hypertrophy could be reversed and left ventricular (LV) dysfunction (decreased cardiac output) was prevented by all 3 anti-hypertensive therapies	Barotrauma of systemic hypertension leads to left ventricular hypertrophy and LV dysfunction; normalization of blood pressure reduces LV mass and prevents deterioration of LV function (2-5)
Chronic myocardial infarction (coronary artery ligation) in rats	LV dilation and dysfunction	Captopril (vs. control group) Treatment duration 3 months	Captopril treatment attenuated LV dilation and dysfunction	This was early evidence that Ang II associated with pathologic remodeling after myocardial infarction (4)
Spontaneously	Systemic hypertension, Left	High and low-dosage	High dosage, but not low dosage	Fibrosis attends left ventricular

hypertensive rats Rats were fed a normal salt diet	ventricular hypertrophy, myocardial fibrosis, diastolic dysfunction	isinopril (vs. control group) Treatment started at 14 and 26 weeks of age for a duration of 12 weeks	lisinopril treatment lowered systemic blood pressure and reversed left ventricular hypertrophy, *both* dosages prevented myocardial fibrosis	hypertrophy secondary to systemic hypertension and benefit, derived from RAAS suppression is independent from blood pressure control (6,7)
infra-renal aortic banding with right renal artery banding (IRB+RRAB) in rats Rats were fed a normal salt diet	Renovascular hypertension, left ventricular hypertrophy, and myocardial fibrosis, increased LV stiffness	Captopril (vs. control group) Treatment duration 8 weeks (started 2 days prior to banding)	Captopril treatment prevented development of systemic hypertension, myocardial fibrosis, and increased LV stiffness	Implicates activation of RAAS in the development of myocardial fibrosis in renovascular hypertension {(8-10)
Unilateral nephrectomy (UN) with aldosterone infusion and high sodium diet or IRB alone or IRB+RRAB in rats	Renovascular hypertension or LV pressure load (IRB); left ventricular hypertrophy in all groups, myocardidal fibrosis attended the left ventricular hypertrophy in the IRB+RRAB and aldosterone infusion groups	None	ATII levels increased in the IRB+RRAB group; aldosterone levels increased in IRB+RRAB and aldosterone infusion group	Ang II *and* aldosterone are associated with development of myocardial fibrosis (see Figure 3) {Brilla:1992un, Brilla:1990jm}
Ang II infusion to rats Rats were fed a normal salt diet	Moderate Systemic hypertension	None Infusion duration was 2 weeks	Renal changes included focal tubulointerstitial injury, cast formation, interstitial monocyte infiltration, and mild interstitial fibrosis. Changes to the glomerulus included a mild increase in glomerular cell proliferation and mesangial cell proliferation. In both tissues there was a phenotypic modulation with increased alpha-smooth muscle actin expression.	Ang II-infused rats not only develop chronic hypertension and vascular remodeling, but also glomerular and tubulointerstitial injury (11) An early study also showed that very short term (< 1 day), high dosages of Ang II infusion can cause hilar arterial necrosis, capillary aneurysms, and focal glomerular necrosis {Byrom:1964kt}
IRB+RRAB with and without aldosterone infusion or UN with	Renovascular hypertension, left ventricular hypertrophy, myocardial	Spironolactone (vs. control group)	Spironolactone treatment led to reduction (but not normalization) of blood pressure and did not	Further support for aldosterone's key role in the development of myocardial fibrosis

high sodium diet or aldosterone infusion in rats	fibrosis	Treatment duration 8 weeks	prevent left ventricular hypertrophy; Spironolactone did prevent the development of myocardial fibrosis	{Brilla:1993iz}
Essential hypertension in people – biopsies of resistance arterioles before and after treatment	Prior to therapy – medial hypertrophy of arterioles and impaired endothelium-dependent relaxation	Losartan or atenolol ± hydrochlorothiazide, if needed Treatment duration was 1 year	Blood pressure normalized in both treatment groups, yet only patients treated with losartan had significant reduction in arteriolar hypertrophy and normalization of endothelium dependent-relaxation	Both medial hypertrophy and vascular dysfunction were reversed in the patients receiving losartan-induced RAAS suppression, but not those made normotensive *without* RAAS suppression (atenolol) (12)
People with NYHA class II or III heart failure – endothelial function assessed by bilateral forearm venous occlusion and plethysmography	Patients' forearm blood flow tested after both acetylcholine, Ang I, or Ang II administration	Spironolactone or placebo with standard diuretic therapy and ACEI Treatment duration was 1 month	Increased forearm blood flow in response to acetylcholine and decreased vasoconstrictor response to AngI, but not AngII administration in patients treated with spironolactone	This was interpreted as indirect evidence that spironolactone inhibits further vascular conversion of AngI to AngII, even in the presence of chronic ACE inhibition (13)
Aldosterone infusion and normal salt diet in rats	Increased plasma endothelin levels, medial hypertrophy and collagen deposition, and increased oxidative stress within the vascular walls	Endothelin A (ET_A) receptor antagonist, spironolactone, or hydralazine concurrent with infusion (vs. control group) Treatment duration was 6 weeks	All 3 treatments led to significant blood pressure reduction; treatment with either the ET_A-receptor antagonist or spironolactone led to normalization of hypertrophy and decreased collagen deposition, all treatments led to a reduction in markers of oxidative stress, though this reduction was greatest in the ET_A-receptor antagonist and spironolactone groups	Aldosterone and a high salt diet leads to increased plasma endothelin levels and increased oxidative stress, mechanisms that likely underlie the pathologic remodeling (hypertrophy and fibrosis) in the vascular smooth muscle (14) Another study in a similar model also showed that an ET_A receptor antagonist, reduced blood pressure and prevented vascular remodeling.(15)
Ang II or aldosterone infusion in rats Rats were fed a normal salt diet	Systemic hypertension, impaired endothelium-dependent relaxation (Ang II group only), concentric arteriolar hypertrophy,	Spironolactone or hydralazine concurrent with infusion Treatment duration was	Both treatments led to equivalent reduction in blood pressure; only spironolactone treatment attenuated development of arteriolar hypertrophy and	MR activation mediates some of the Ang II-induced vascular remodeling and dysfunction in Systemic hypertension, with oxidative stress being one likely

	increased markers of oxidative stress	2 weeks	impaired endothelium-dependent relaxation, and reduced markers of oxidative stress	mechanism (16)
Deoxycorticosterone acetate and high sodium diet in rats	Myocardial fibrosis, and increased markers of coronary vascular inflammation and oxidative stress	Eplerenone concurrent with deoxycorticosterone acetate /diet (vs. control group) Treatment duration last 4 weeks of 8 week study	Eplerenone treatment led to resolution of myocardial fibrosis, and led to a reduction in markers of coronary vascular inflammation and oxidative stress	Aldosterone and a high salt diet leads to myocardial fibrosis and coronary artery inflammation and oxidative stress; the MRA, eplerenone, reversed established myocardial fibrosis, and reduced coronary arterial markers of inflammation and oxidative stress {Young:2004ho}
Spontaneously hypertensive – stroke prone rats (SHR-SP) Rats were fed a high salt diet	Systemic hypertension, proteinuria, and death due to stroke; cerebrovascular lesions include edema, spongiosis, liquifactive necrosis, and hemorrhage; tubular and glomerular lesions also noted	Spironolactone (vs. untreated control) Treatment was started at 7.5 weeks of age and continued for 3-4 weeks in phase I of the study and was continued until natural death in phase II	Blood pressure did not vary between control and treated rats; spironolactone led to a significant reduction in cerebrovascular and renal histologic lesions; urine protein excretion was significantly lower in the spironolactone group; the spironolactone-treated rats lived significantly longer than controls	MRA led to a reduction in proteinuria and cerebrovascular and renal tubular and glomerular lesions independently of blood pressure lowering; aldosterone and a high salt diet plays an important role in the development of vascular injury in this model.(17) A similar study also showed that the MRA eplerenone attenuated the pathologic remodeling that developed during aldosterone excess and a high salt load in SHR-SP rats.(18)
Transgenic rat model overexpressing human AT_1R on podocytes Rats were fed a normal salt diet	Blood pressure remained normal, rates developed severe proteinuria starting at 8 to 15 weeks of age	None The rats were studied throughout their natural life span	Rats developed severe proteinuria and pseudocyst formation on podocytes, then foot process detachment at a young age; ultimately nephron loss developed, recapitulating the well known pathway typical of focal segmental glomerulosclerosis	Increased AT_1R signaling in the podocyte damages the podocytes, leads to proteinuria and causes focal segmental glomerulosclerosis (19)
5/6 Nephrectomy in rats	Systemic hypertension, proteinuria,	Spironolactone alone and spironolactone with	Spironolactone therapy alone did not significantly reduce blood	MR blockade slows the development of

Rats were fed a normal salt diet	glomerulosclerosis, tubular atrophy, dilation, and interstitial fibrosis	antihypertensive therapy (reserpine, hydralazine, hydrochlorothiazide) (vs. untreated control) Treated started 8 weeks after 5/6 nephrectomy and continued for 4 weeks	pressure; spironolactone treatment did lead to a reduction in glomerular injury, a benefit that was amplified by concurrent control of systemic blood pressure via triple therapy Neither spironolactone alone, nor the combination of spironolactone and anti-hypertensive therapy, led to significant improvement in the severity of proteinuria	glomerulosclerosis, and also may reduce existing glomerulosclerosis, a benefit which is amplified by concurrent blood pressure control. The failure to significantly affect the degree of proteinuria was postulated to be due to irreversible podocyte damage and/or unique pathophysiology of this rat model. (20)
Unilateral nephrectomy, aldosterone infusion, and high salt diet in rats	Progressive proteinuria, increased markers oxidative stress, and damage to glomerular podocytes	Eplerenone, tempol (an antioxidant), or hydralazine (vs. untreated control) Therapy started 5 days prior to infusion start, the continued for 2 weeks concurrently with infusion/diet	Eplerenone treatment lowered but did not normalize blood pressure, attenuated markers of oxidative stress in podocytes and prevented the development of proteinuria and podocyte damage; tempol also led to a reduction in proteinuria and podocyte damage and attenuated markers of oxidative stress; hydralazine nearly normalized blood pressure, yet did not reduce proteinuria, podocyte damage, or makers of oxidative stress	Aldosterone excess and high salt diet leads to glomerular injury and proteinuria, which appears to be mediated in part by the formation of reactive oxygen species; this damage occurs/persists even when blood pressure is normalized(21) Additional studies have also demonstrated that podocyte injury underlies the pathogenesis of proteinuria in aldosterone-infused rats.(19,22)
Adriamycin induced nephrosis in rats	Mild Systemic hypertension, proteinuria, markers of tubular injury increase; glomerular and interstitial lesions include influx of interstitial macrophages, increased alpha smooth muscle actin, and collagen IV deposition at tubular basement membranes, focal	Lisinopril, spironolactone, or lisinopril and spironolactone (vs. untreated control) Treated was initiated 6 weeks after adriamycin administration and continued for 12 weeks	Lisinopril alone and in combination with spironolactone led to a significant reduction in blood pressure, proteinuria, and prevented the increase in alpha smooth muscle actin, and collagen IV deposition at tubular basement membranes; all therapies reduced the number of interstitial macrophages	ACEI and MRA reduce proteinuria, and tubular and interstitial injury to a greater extent than ACEI alone in this model (23)

	glomerulosclerosis and interstitial fibrosis			
Essential hypertension in people – biopsies of resistance arterioles before and after treatment	Prior to therapy – medial hypertrophy of arterioles, increased vascular collagen content, increased arteriolar stiffness, increased inflammatory mediators, and impaired endothelium-dependent relaxation	Eplerenone or atenolol ± hydrochlorothiazide, if needed Treatment duration was 1 year	Blood pressure normalized in both treatment groups, yet only patients treated with eplerenone had a reduction in arterial stiffness, a reduction in the media collagen to elastin ratio, and a decrease in circulating inflammatory mediators; Neither endothelial function nor arteriolar hypertrophy changed either group	MR activation mediates the vascular remodeling and stiffening associated with essential hypertension; increased inflammation likely plays a role in the development of this remodeling and dysfunction (24)
Aldosterone infusion and high sodium diet, electrical stimulation of the right ventricle	Right ventricular and LV myocardial fibrosis, increased susceptibility to induction of ventricular arrhythmia	Spironolactone concurrent with infusion/diet (vs. control group) Treatment duration was 4-6 weeks	Spironolactone prevented development of myocardial fibrosis and led to a reduction in susceptibility to ventricular arrhythmia	Aldosterone and a high salt diet leads to myocardial fibrosis, spironolactone's anti-fibrotic effects reduced susceptibility to arrhythmia in this model {Deshmukh:2011ga}
Rat model for type-2 diabetes mellitus and nephropathy Rats were fed a normal salt diet	Glomerulosclerosis and proteinuria, increased renal deposition of type I and IV collagen, and increased expression of pro-fibrotic and pro-inflammatory cytokines, including plasminogen activator inhibitor-1, transforming growth factor-beta, connective tissue growth factor, and fibronectin	Lisinopril, eplerenone, lisinopril and eplerenone (vs. untreated control) Treatment was started at 14 weeks of age and continued for 26 weeks	The combination of lisinopril and eplerenone led to a reduction in proteinuria, glomerulosclerosis, and renal desposition of type I and IV collagen greater than that seen with ACEI monotherapy; Mono- and dual-therapy with epleronone and lisinopril also led to a significant reduction in renal expression of pro-fibrotic and pro-inflammatory cytokines; Dual therapy was associated with a greater increase in creatinine when compared to monotherapy	ACEI and MRA alone, or in combination, reduce the expression in pro-fibrotic and –inflammatory mediators; ACEI monotherapy reduces proteinuria, yet efficacy was improved when an MRA was added; MRA therapy alone did not have a significant effect on proteinuria in this model (25)
Part I: Deoxycorticosterone acetate, unilateral	Part I: myocardial fibrosis and vasculopathy, proteinuria,	Part I: high, intermediate, and low dosages of finerenone	Part I: Finerenone, at the highest dosage tested and both dosages of eplerenone, reduced blood	The next generation, non-steroidal MRA, finerenone, conferred better end organ protection in both

nephrectomy and high sodium diet in rats Part II: chronic myocardial infarction (coronary artery ligation) in rats	glomerulosclerosis, tubulointerstitial injury, and vascular damage Part II: LV systolic dysfunction, increased LV diastolic pressure, diastolic dysfunction, increased plasma pro-BNP levels Plasma aldosterone was significantly increased by both finerenone and eplerenone, which supports in vivo MR blocking activities of these MRAs	or eplerenone (vs. untreated control) Treatment was initiated at 9 weeks of age, one week after nephrectomy and continued for 10 weeks Part II: Finerenone or eplerenone (vs. untreated control) Treatment was initiated 1 week post-myocardial infarction and continued for 8 weeks	pressure; finerenone led to dose-dependent protection from structural heart injury whereas eplerenone had weaker protective effects at both dosages studied; Finerenone, at the intermediate and high dosages resulted in a significant reduction in renal structural changes when compared to eplerenone; both finerenone and eplerenone led to a reduction in proteinuria, yet the reduction was significantly greater in the high dose finerenone group when compared to the high dose eplerenone group Part II: Finerenone led to improvement in LV systolic and diastolic dysfunction and a reduction in plasma pro-BNP levels whereas eplerenone did not	models when compared to the steroidal MRA, eplerenone.(26)
Chronic myocardial infarction (coronary artery ligation) in rats	Decreased LV compliance and elastance and increased interstitial fibrosis; reduction in coronary reserve (assessed by magnetic resonance imaging)	Rats with deletion, preventing expression of the MR in vascular smooth muscle cells or finerenone (vs. control group) Finerenone started 1 day post-infarct and continued for 2 months	Both the MR-deletion and finerenone-treated rats had less reduction in LV compliance, elastance, and fibrosis; and coronary artery reserve was preserved	MR blockade mitigated the LV dysfunction and remodeling post-myocardial infarction (27)

ACEI, angiotensin converting enzyme inhibitor; Ang I, angiotensin I; Ang II, angiotensin II; AT_1R angiotensin II type-1 receptor; BNP, B-type natriuretic peptide; IRB infra-renal aortic band; LV, left ventricular; MR, mineralocorticoid receptor; MRA, mineralocorticoid receptor antagonist; RAAS, renin angiotensin aldosterone system; RRAB, right renal artery banding; SHR-SP, spontaneously hypertensive rat-stroke

prone; UN, unilateral nephrectomy

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