

Atherosclerosis and Arterial Blood Pressure in Mice

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Abstract: Increased blood pressure is a consistent risk factor for the development of atherosclerotic diseases in humans, although the basis for this relationship is unknown. Genetically engineered mice are now commonly used to study mechanisms of atherosclerosis. More recently, blood pressure can be reliably measured in conscious mice using either tail cuff or telemetric techniques. Thus, mouse models permit the investigation of the complex interactions of blood pressure and atherogenesis. Most mouse models exhibiting hypertension have increased atherosclerotic lesion size, although there have been exceptions to these findings. Also, there are several reports that have used methods to decrease blood pressure and demonstrated reduced atherosclerosis. In contrast, there are many studies in which atherosclerosis has been altered without changes in blood pressure, and conversely, studies in which blood pressure changes did not alter atherosclerosis. Studies that have specifically defined the role of elevated systolic blood pressure on the development of atherosclerosis have uniformly demonstrated that pressure *per se* is not responsible for changes in lesion development. Thus, while increased systolic blood pressure is frequently associated with atherosclerosis, the stimulus for the hypertension appears to be the major determinant of atherogenesis rather than pressure *per se*. A consistent theme in the literature has been that perturbations of the renin angiotensin system display the strongest correlations between blood pressure and atherosclerosis.

INTRODUCTION

As noted in a recent statement from the American Heart Association, blood pressure is a powerful, consistent, and independent risk factor for atherosclerosis-based cardiovascular diseases in humans [1]. Hypertension in humans is defined as a systolic blood pressure of ≥ 140 mmHg or a diastolic blood pressure of ≥ 90 mmHg. The recent Joint National Committee 7 report on hypertension included a new classification of "prehypertension" (systolic 120-139; diastolic 80-89 mmHg), to recognize the impressive increase in risk of cardiovascular complications, including atherosclerosis, associated with levels of blood pressure previously considered to be normal [2]. Decisions regarding the treatment of hypertension are influenced by the severity of hypertension and the presence of other cardiovascular risk factors. It is generally recognized that lowering blood pressure by either lifestyle or pharmacologic therapy is extremely beneficial in decreasing the risk of myocardial infarction. However, it should be noted that the affliction of atherosclerosis-based cardiovascular diseases increases as a continuum of pressure, rather than being associated with a specific blood pressure threshold.

Similar to hypertension, atherosclerosis is a disease that is almost exclusively confined to the arterial side of the circulation. This distinctive localization provides clear evidence that blood pressure is a component of the development of atherosclerotic lesions. For example, the level of blood pressure can influence the structure of blood vessels. These pressure-induced structural properties of arteries could indirectly predispose to the development of atherosclerosis. Moreover,

there is a potential for direct physical effects of pressure on different aspects of the atherogenic process. For example, pressure may have a direct effect on increased low density lipoprotein influx into the arterial wall [3]. Overall, the etiology of the most common forms of human hypertension is complex and multifactorial. Thus, the manifestations of high blood pressure on atherosclerosis in humans could involve multiple mechanisms that have variable effects on the atherogenic process.

The relatively recent availability of mouse models of atherosclerosis and the technology to measure arterial blood pressure in mice has enabled the study of the interrelationships between these two parameters [4, 5]. This chapter will overview these studies to determine whether there is current evidence using mouse models to support the hypothesis that elevated blood pressure increases atherosclerosis. Moreover, we will focus in this review on the mode of altering blood pressure to determine its impact on lesion formation and whether pressure *per se* is a direct contributor to the atherogenic process.

MEASUREMENT OF ATHEROSCLEROSIS AND BLOOD PRESSURE IN MICE

Prior to reviewing studies in which atherosclerosis and blood pressure have been measured in experimental mouse models, it should be noted that precision and standardization of the measurements will influence the definition of their inter-relationship. In this regard, each measurement has been determined using a range of protocols and modalities.

Atherosclerosis studies are now commonly performed in mice since the development of strains that are susceptible to lesion formation, predominantly those deficient in apolipoprotein E or LDL receptors [6-8]. The absence of apoE or LDL receptors have profoundly different effects on lipopro-

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tein metabolism. The distribution of cholesterol among lipoprotein fractions differs markedly between these two strains with apoE^{-/-} mice transporting the majority in VLDL versus LDL in LDL receptor^{-/-} mice. Despite these divergent lipoprotein characteristics, atherosclerotic lesions develop in the same location with similar general characteristics [8, 9]. Also, there have been few drug or genetic interventions that have affected atherosclerosis differentially between these two models. Moreover, currently there are no obvious differences in arterial blood pressure-related mechanisms on atherosclerosis in these two mouse models.

The size of atherosclerotic lesions in these mouse strains can be measured in a number of vascular beds. A common method measures lesion size in tissue sections cut from aortic roots [10]. Variable numbers of sections are measured in these studies and different modes have been used to measure lesion size [11]. Since lesion size varies throughout the aortic root, it is advisable to acquire sections at multiple locations, for example at 100 μ M intervals with quantitation of 4 to 10 sections, depending on the severity of the disease [11]. The other most frequently used approach is to measure the area of lesions on the intimal surface of the aorta [12]. More recently, there is an increasing number of studies in which atherosclerotic lesion size is determined in the innominate artery [13]. In addition to the methodological variances for quantifying atherosclerosis in mice, it is not uncommon for an intervention to have different effects on the progression of atherosclerosis in different vascular regions of the same mouse [14].

The measurement of blood pressure is generally performed in conscious mice using either noninvasive tail cuff systems or by radiotelemetry with surgically implanted catheters. The cuff system detects arterial blood flow in the tail, with systolic pressure being determined by the pressure that is applied to a cuff positioned at the base of the tail. The most commonly used tail cuff blood pressure machine in atherosclerosis studies has been the one developed by Visitech [5]. This approach is readily amenable to screening large numbers of mice and detecting substantial increases in blood pressure. However, there have been several concerns expressed about the use of tail cuff for blood pressure measurement, including its inability to dynamically measure blood pressure (24 hours a day), the potential stress induced by the need to constrain the mice, and the accuracy of the data [15]. An alternative approach is the use of radiotelemetry for blood pressure measurement. This technique has the benefit of enabling the continuous acquisition of blood pressure through the day in freely moving mice. However, there are caveats about the use of radiotelemetry, especially as it relates to studies of atherosclerosis in mouse models. First, a practical concern is the expense of radiotelemetry, which may prohibit studies involving large numbers of mice per experimental group. Second, atherosclerosis studies are typically of long duration (3 or more months), but carotid artery catheters for telemetry measurements may only remain patent for approximately 2-3 weeks. Thus, it is difficult to obtain blood pressure measurements throughout the entire duration of a study. For these reasons, the majority of publications examining inter-relationships between hypertension and atherosclerosis have used a tail cuff approach to measure arterial pressure (see Tables 1-3).

INDUCTION OF HYPERTENSION IN MOUSE MODELS OF ATHEROSCLEROSIS

Several procedures have been performed in mice to increase blood pressure with the primary end point for determining the effect on atherosclerosis. These have included genetic, pharmacological, and surgical approaches (Table 1).

Several studies have focused on the role of the renin-angiotensin system (RAS) on the development of atherosclerosis, using either gene targeted mice or pharmacologic infusion of angiotensin II (AngII). The first study to examine AngII was performed in the Tsukuba hypertensive mouse. These C57BL/6 mice have transgenes that express both human angiotensinogen and renin, resulting in increased blood pressure (by 23 mmHg) [16]. To study atherosclerosis in this mouse model, mice were rendered hyperlipidemic by a diet containing cholesterol, saturated fat, and cholate. The small lesions that formed in nontransgenic control C57BL/6 mice fed this diet were increased 4 times in the Tsukuba hypertensive mice.

Many recent studies have infused AngII into lesion-susceptible mice to determine its effects on atherogenesis. The most common mode for chronic delivery of AngII is *via* osmotic minipumps that are implanted in the subcutaneous space [17-20]. Early studies demonstrated that AngII infusion promoted atherogenesis in both LDL receptor^{-/-} and apoE^{-/-} mice [17, 18]. Interestingly, a one-month infusion of AngII was able to increase atherosclerosis in both of these mouse models, demonstrating that AngII rapidly induced the atherogenic process [17, 18]. Our initial report infusing AngII into apoE^{-/-} mice failed to demonstrate that the AngII-induced atherosclerosis was associated with increases in blood pressure [18]. However, this was probably related to the use of anesthesia to obtain blood pressure measurements in mice and the crude blood pressure measuring device. Subsequent studies, in many laboratories, have uniformly demonstrated that sufficiently high doses of AngII infusions can increase arterial pressure and promote atherosclerosis. These AngII doses can be 500 ng/kg/min or higher. This would imply that the effects of AngII on the atherogenic process could be mediated through hypertension. However, some studies have used lower doses of AngII that increased atherosclerosis without a detectable increase in blood pressure, suggesting that AngII can promote atherosclerosis through pressure-independent mechanisms [21, 22].

Surgical approaches have also been used to increase arterial pressure and study its effects on atherosclerosis. A 2-kidney, 1-clip surgical procedure generates a greater than 60 mmHg increase in arterial pressure in apoE^{-/-} mice which is associated with a stimulation of the RAS [23]. This procedure led to a large increase in the size of aortic root atherosclerotic lesions. Hypertension can also be induced by the 1-kidney, 1-clip surgical procedure. However, this is not presumed to be related to RAS activation based on the lack of effect on plasma concentrations of renin and AngII. This procedure led to a similar increase (60 mmHg) in arterial pressure as obtained with 2-kidney, 1-clip. Interestingly, aortic root atherosclerosis was increased by 1-kidney, 1-clip hypertension; however, the extent of the increase was much less than in the 2-kidney, 1-clip model [23].

Table 1. Studies in which Changes in Blood Pressure were Associated with Changes in the Size of Atherosclerotic Lesions

Category	Drug/Approach	Mouse	BP Method	Reference	
A. Increased both BP and atherosclerosis					
Renin angiotensin system	renin and angiotensinogen Tg	C57BL/6	TC	[16]	
	AngII infusion	apoE ^{-/-}	TC	[18, 19]	
		LDL R ^{-/-}	TC	[17]	
Surgery	2-kidney, 1-clip	apoE ^{-/-}	TC	[23]	
	aortic constriction	apoE ^{-/-}	CC	[24, 25]	
Nitric oxide	eNOS ^{-/-}	apoE ^{-/-}	AC	[75]	
			TC	[29]	
Natriuretic peptide receptor A	NPR1 ^{-/-}	apoE ^{-/-}	TC	[76]	
Transgenic	growth factor (bGH)	apoE ^{-/-}	TC	[77]	
	SMC specific UCP Tg	apoE ^{-/-}	TC & T	[78]	
B. Decreased both BP and atherosclerosis					
ACE inhibitors	ramipril	apoE ^{-/-}	TC	[26, 27]	
	quinapril	diabetic apoE ^{-/-}	TC	[28]	
	captopril	NZ0/BL6F1	TC	[40]	
	enalapril	nephrectomy apoE ^{-/-}	TC	[30]	
		apoE ^{-/-} x eNOS ^{-/-}	TC	[29]	
	fosinopril	apoE ^{-/-}	TC	[70]	
Ang receptor antagonists/deficiency	losartan	nephrectomy apoE ^{-/-}	TC	[30]	
		apoE ^{-/-} AngII	TC	[31]	
	losartan and prazosin	apoE ^{-/-}	TC	[32]	
	candesartan	apoE ^{-/-}	TC	[33]	
	olmesartan	apoE ^{-/-}	TC	[34]	
	telmisartan, 3 mg/kg/d	apoE ^{-/-}	TC	[35]	
	telmisartan	apoE ^{-/-}	TC	[27]	
		irbesartan	diabetic apoE ^{-/-}	TC	[36]
			apoE ^{-/-}	TC	[37]
	NZ0/BL6F1	TC	[40]		
	AT1aR ^{-/-} or irbesartan	apoE ^{-/-}	TC	[38]	
Aldosterone antagonists	eplerenone	apoE ^{-/-}	TC	[39]	
Calcium channel blockers	lacidipine	apoE ^{-/-}	TC	[41]	
Neutral endopeptidase inhibitor	omapatrilat	diabetic apoE ^{-/-}	TC	[28]	
		apoE ^{-/-}	TC	[49]	
PPAR alpha deficiency		apoE ^{-/-}	TC	[42]	
Nitric oxide	eNOS Tg	apoE ^{-/-}	AC	[43]	
Gonadectomy	estrogen	apoE ^{-/-} , eNOS ^{-/-}	TC	[44]	
Weight loss		ob/ob LDLR ^{-/-}	T	[45]	

Abbreviations: Tg, transgenic; TC, tail cuff; AC, anesthetized cannulation; CC, conscious cannulation; T, telemetry.

Hypertension has also been induced surgically by aortic constriction at the level of the suprarenal aorta. This procedure, applied to apoE^{-/-} mice, generated a relatively mild increase (of 16 mmHg) in carotid artery pressure [24, 25] that was associated with increased lesion size in both the aortic root and the thoracic aorta that was proximal to the aortic constriction. Interestingly, Johansson *et al.* [25] reported that the aortic constriction increased plasma renin concentrations, although losartan failed to attenuate the increased lesion size in aortic constricted mice.

To summarize, there are several reports in which blood pressure has been altered by genetic, pharmacological, or surgical approaches. There is a reasonable consistency in that these methods of increasing blood pressure have increased the size of atherosclerotic lesions. However, a confounding variable in these studies is that the induction of a similar increase in arterial pressure has not led to equivalent changes in atherosclerotic lesion size.

CORRELATIONS OF ATHEROSCLEROSIS AND ARTERIAL BLOOD PRESSURE

The data summarized in the Tables represents the current literature in which arterial pressure and atherosclerosis have been measured in the same study. As summarized in Table 1B, many studies have demonstrated that arterial blood pressure is decreased in mice that have decreased atherosclerosis. This includes several studies that have administered inhibitors of the RAS including ACE inhibitors [26-32], AngII receptor antagonists [27, 30-38], or aldosterone receptor antagonists [39]. Moreover, these studies have been performed in a variety of mouse atherosclerosis models including apoE^{-/-} mice that have also been combined with AngII infusion, endothelial nitric oxide deficiency (eNOS), diabetes, or nephrectomy. NZ0/BL6F1 mice have also demonstrated both decreased blood pressure and atherosclerosis when administered with either ACE inhibitors or AngII receptor antagonists [40]. In addition to inhibition of the RAS, decreases in both arterial pressure and atherosclerosis have also been noted in apoE^{-/-} mice using a variety of other approaches. These include administration of the calcium channel antagonist lacidipine [41], a neutral endopeptidase inhibitor omapatrilat [28, 49], mice genetically engineered to either be deficient in PPAR alpha [42], or overexpressing eNOS [43], surgical gonadectomy [44], or weight loss [45].

In contrast to the studies listed above that have noted both reduced arterial pressure and atherosclerotic lesion size, there have been many studies that have failed to demonstrate a relationship between these two measurements, as listed in Tables 2 and 3. Surprisingly, many of the drugs that have been shown to decrease arterial pressure and atherosclerosis, as described above and detailed in Table 1B, were also used in studies that did not show a relationship between blood pressure and atherosclerosis (Table 2). A practical issue to explain these disparities may be the mode of blood pressure measurement. In all these studies, systolic blood pressure was measured by tail cuff using systems described above. Moreover, several of these studies measured systolic pressure a limited number of times using protocols that did not include acclimation of mice to the tail cuff system.

Table 2 summarizes the studies in which no blood pressure changes were observed following various treatments that might be predicted to influence blood pressure; however, atherosclerotic lesion size was either increased or decreased. Included in this Table are studies using modulators of the RAS that did not influence blood pressure, but did decrease atherosclerotic lesion size. These have included ACE inhibitors, AngII receptor antagonists, and aldosterone antagonists. One possible explanation of this disparity is that the mechanisms of the substance under study that regulate lesion formation may be more sensitive compared to the mechanisms regulating blood pressure. Supporting this premise, the dosage used in these studies provided a discrimination between the effect of the RAS on these two processes. For example, the study of Takaya *et al.* [35] demonstrated that a 3 mg/kg/day dose of telmisartan reduced both arterial pressure and atherosclerosis, while a 10-fold lower dose of 0.3 mg/kg/day had no measurable effect on blood pressure, but decreased atherosclerotic lesion size. Currently, the mechanisms by which AngII promotes atherosclerosis that have been suggested include increased oxidative stress, secretion of monocyte chemoattractants, and adhesion of monocytes [46].

In addition to drugs that regulate the RAS, there is a wide range of other drugs that have been studied and exhibited no effect on arterial pressure, but decreased atherosclerosis (Table 2A). These include calcium channel blockers [53, 83, 86] endothelin receptor antagonists [47, 48], neutral endopeptidase inhibitors [49], free radical scavengers [50, 51], TXA2 receptor antagonists [52], statins [27, 53, 54], and insulin [55]. In addition, genetically engineered deficiency of either microsomal prostaglandin synthase-1 [56] or p21 [57] reduced atherosclerosis without any measurable effects on arterial pressure. As with the results listed in Table 1, the studies have predominantly been performed with apoE^{-/-} mice, but have also included studies using LDL receptor^{-/-} and apoE Leiden transgenics.

Several studies have used experimental procedures resulting in no effect on blood pressure, but increased atherosclerosis (Table 2B). Again, the predominance of studies have focused on manipulation of the RAS. Low-dose infusions or injections of AngII did not increase blood pressure, but increased atherosclerosis in both aortic root and aortic tree [22, 58]. Administration of the AT2 receptor antagonist, PD123319, caused only a transient increase in arterial pressure during the first few days of administration, but caused a profound increase in aortic atherosclerosis [21]. Deficiency of AT2 receptors has also been shown to increase atherosclerosis, with no effect on blood pressure [59]. However, other studies have failed to demonstrate an effect of AT2 receptor deficiency on atherosclerosis in either apoE^{-/-} or LDL receptor^{-/-} mice [60, 61]. In addition to RAS inhibitors, administration of L-NAME or infusion of isoprostanine F2alphaIII has also increased atherosclerosis without effect on arterial pressure [62, 63].

In addition to pharmacological studies, increased atherosclerosis in the absence of any blood pressure changes have been noted in mice that were nephrectomized, and in genetically engineered mice with deficiencies of NOS, glutathione

Table 2. Studies in which Changes in the Size of Atherosclerotic Lesions Occurred in the Absence of Changes in Blood Pressure

Category	Drug/Approach	Mouse	BP Method	Reference
A. Decreased atherosclerosis with no effect on BP				
ACE inhibitors	ramipril	apoE ^{-/-}	TC	[26]
	fosinopril	apoE ^{-/-}	TC	[70]
	captopril	apoE ^{-/-}	TC	[79]
	enalapril	apoE ^{-/-} AngII	TC	[80]
Ang receptor antagonists/deficiency	losartan	apoE ^{-/-} AngII	TC	[31]
		apoE ^{-/-}	TC	[70]
	valsartan	apoE ^{-/-}	TC	[81]
		apoE ^{-/-}	TC	[59]
		apoE ^{-/-}	NS	[82]
	olmesartan alone or with azelnidipine	apoE ^{-/-}	TC	[83, 84]
	Telmisartan (0.3 mg/kg/d)	apoE ^{-/-}	TC	[35]
AT1a receptor ^{-/-}	LDLR ^{-/-}	TC	[61]	
Aldosterone receptor antagonists	eplerenone alone or with valsartan	apoE ^{-/-}	TC	[81]
Calcium channel blockers	azelnidipine	apoE ^{-/-}	TC	[85]
		apoE ^{-/-}	TC	[83]
	amlodipine	apoE ^{Leiden} /CRP Tg	TC	[53]
		apoE ^{-/-}	TC	[86]
Endothelin antagonist	LU135252	apoE ^{-/-}	TC	[47]
	LU224332	LDLR ^{-/-}	AC	[48]
Neutral endopeptidase inhibitor	omapatrilat	apoE ^{-/-}	TC	[49]
Free radical scavengers	edaravone	apoE ^{-/-}	TC	[50]
	N-acetylcysteine	apoE ^{-/-}	AC	[51]
TXA2 receptor antagonist	BM-573	LDLR ^{-/-}	TC	[52]
Statins	atorvastatin alone or with amlodipine	apoE ^{Leiden} /CRP Tg	TC	[53]
		apoE*3 ^{Leiden} Tg	TC	[54]
	atorvastatin	apoE ^{-/-}	TC	[27]
	fluvastatin and/or valsartan	apoE ^{-/-}	NS	[82]
Insulin		apoE ^{-/-}	TC	[55]
prostaglandin (PGE) synthase	mPGES-1 ^{-/-}	LDLR ^{-/-}	TC	[56]
p21	p21 ^{-/-}	apoE ^{-/-} AngII & BMT	TC	[57]
B. Increased atherosclerosis with no effect on BP				
Renin angiotensin system	AngII infusion	apoE ^{-/-}	TC	[22]
		apoE ^{-/-} to +/- BMT	TC	[58]
	AngII injection i.p.	apoE ^{-/-}	TC	[87]
	PD123319	apoE ^{-/-}	TC	[21]
	AT2 receptor ^{-/-}	apoE ^{-/-}	TC	[59]

(Table 2) contd....

Category	Drug/Approach	Mouse	BP Method	Reference
B. Increased atherosclerosis with no effect on BP				
Surgery	Nephrectomy	apoE ^{-/-}	AC	[88]
			TC	[64]
			CC	[65]
Nitric oxide	L-NAME	apoE ^{-/-}	CC	[62]
	nNOS ^{α-/-}	apoE ^{-/-}	AC	[66]
Glutathione peroxidase-1	GPx-1 ^{-/-}	apoE ^{-/-}	TC	[67]
Isoprostanes	iPF2 ^α -III	apoE ^{-/-}	TC	[63]
Interleukins (IL)	IL-6 ^{-/-}	apoE ^{-/-}	TC	[68]
C-reactive protein	CRP Tg	apoE ^{-/-}	TC	[69]

Abbreviations: Tg, transgenic; BMT, bone marrow transplantation; TC, tail cuff; AC, anesthetized cannulation; CC, conscious cannulation; NS, not stated.

peroxidase, interleukin-6, or mice that were transgenic for C-reactive protein (Table 2B) [51, 64-69].

Table 3 summarizes studies in which no effects on atherosclerotic lesion size were noted, despite either increases or decreases in arterial pressure. This includes two studies in which the AT1 receptor antagonist, losartan, lowered blood pressure, but had no effect on atherosclerosis [31, 32]. The calcium channel blocker, amlodipine, has also decreased arterial pressure without significant effects on atherosclerosis [33, 36]. The most common approach in which arterial pressure has been lowered without a concomitant effect on the development of atherosclerosis is by administration of the vasodilator, hydralazine [38, 70-72]. This has been a consistent result in apoE^{-/-} mice, apoE and eNOS compound deficient mice, and in nephrectomized apoE^{-/-} mice. One potential confounding issue in the results from these studies is that hydralazine, through baroreflex mechanisms to autoregulate blood pressure, activates the RAS. As noted above, AngII is strongly implicated in the atherogenic process. Irrespective of this consideration, the use of hydralazine demonstrates the ability to dissociate arterial pressure from the development of atherosclerosis. There are also a limited number of studies that have increased arterial pressure through various experimental methods without effect on atherosclerosis (Table 3B). These have included high dietary salt intake in C57BL/6 mice, and administration of the inhibitor of nitric oxide, L-NAME, to apoE^{-/-} mice [73, 74].

To summarize, there are now a relatively large number of studies that have measured both arterial pressure and atherosclerosis. While many studies have shown an association between changes in arterial pressure and atherosclerosis, there is approximately an equal number that have not discerned any association. However, as noted at the outset, the studies described in this section did not specifically address the question of the contribution of blood pressure to the development of atherosclerotic lesions.

MECHANISTIC STUDIES ON ARTERIAL BLOOD PRESSURE AND ATHEROSCLEROSIS

There are relatively few studies in mice that were designed specifically to address the issue of the contribution of arterial blood pressure to the development of atherosclerosis. These studies have used two general approaches. One approach to determine the link of arterial blood pressure to atherosclerosis has been to lower blood pressure and determine its effect on lesion size. One series of studies have been performed in apoE and endothelial nitric oxide (eNOS) compound deficient mice. Deficiency of eNOS increases mean arterial blood pressure, measured using a femoral catheter in anesthetized mice, by 30 mmHg and leads to a large increase in aortic atherosclerosis in both male and female mice [72]. Blood pressure was also lowered in these mice by the administration of hydralazine in the drinking water. The addition of 250 mg/L of hydralazine to the drinking water reduced mean arterial blood pressure by 26 mmHg. This was an equivalent reduction of arterial blood pressure to that measured in the mice with a single genetic deficiency of apoE. Despite the reduction of blood pressure with hydralazine, there was no effect on the augmented atherosclerosis resulting from the eNOS deficiency [72].

Other studies have lowered blood pressure using multiple drugs to determine the equivalency of effects on atherosclerotic lesion size. Using apoE^{-/-} mice fed a saturated fat enriched diet, systolic blood pressure was reduced by administration of either candesartan or amlodipine [33]. The dosages of candesartan and amlodipine were adjusted to induce equivalent reductions in systolic blood pressure (27 and 30 mmHg decreases, respectively). Despite the equivalent reductions in blood pressure, amlodipine did not reduce the aortic atherosclerosis, while candesartan strikingly blocked the development of lesions.

A converse approach has been to increase systolic blood pressure with multiple approaches and define the effects on

Table 3. Studies in which Changes in Blood Pressure were not Associated with Changes in the Size of Atherosclerotic Lesions

Category	Drug/Approach	Mouse	BP Method	Reference
A. No effect on atherosclerosis but decreased BP				
Ang receptor antagonists	losartan	apoE ^{-/-} AngII	TC	[31]
		apoE ^{-/-}	TC	[32]
Calcium channel blockers	amlodipine	apoE ^{-/-}	TC	[33]
		diabetic apoE ^{-/-}	TC	[36]
Hydralazine	nephrectomy	apoE ^{-/-}	TC	[38, 70, 89]
		apoE ^{-/-}	TC	[30]
	eNOS ^{-/-}	apoE ^{-/-}	TC	[72]
NADPH oxidase	p47 ^{phox} ^{-/-}	apoE ^{-/-}	TC	[90]
B. No effect on atherosclerosis but increased BP				
Sodium	high sodium	C57BL/6	TC	[73]
L-NAME	ovariectomy	apoE ^{-/-}	TC	[32]
		apoE ^{-/-}	AC	[74]

Abbreviations: TC, tail cuff; AC, anesthetized cannulation.

atherosclerosis. Many studies have demonstrated that subcutaneous AngII infusion accelerates the development of atherosclerosis in both LDL receptor ^{-/-} and apoE ^{-/-} mice [18-20]. AngII has been infused at doses up to 2,500 ng/kg/min, resulting in an increase in systolic blood pressure of approximately 30 mmHg, with augmented lesion sizes in both the aortic root and the aortic tree. In a side-by-side comparison, subcutaneous infusions of norepinephrine (5.6 mg/kg/day) also induced increased systolic blood pressure by approximately 30 mmHg in apoE^{-/-} mice [19]. Norepinephrine infusion did augment lesion size in apoE^{-/-} mice. However, the magnitude of the effect was minor compared to AngII, despite an equivalent increase in systolic blood pressure.

CONCLUSIONS

As can be appreciated from a review of the literature, the effects of blood pressure on the development of atherosclerosis in mice are complex. The current literature has an array of data in which coincident measurements of blood pressure and atherosclerosis in mouse models have revealed both an association and a lack of association between hypertension and atherosclerosis. Unfortunately, there are relatively few studies that have directly addressed the question of whether increases in arterial blood pressure *per se* determines atherosclerotic lesion size. In the studies performed to date, the majority report that arterial blood pressure changes *per se* do not have a direct major consequence on the development of atherosclerosis. Taken together, a synopsis of all the published studies is that arterial blood pressure may have some modest effects on the development of atherosclerosis. More importantly, the underlying mechanisms for an effect of blood pressure on the atherosclerotic process is a more critical determinant. In this regard, a consistent finding is that

blood pressure changes resulting from manipulation of the RAS have a direct consequence on the size of lesions. Thus, AngII appears to be able to influence the atherogenic process through direct as well as potentially indirect (i.e., pressure-mediated) mechanisms. The relative paucity of literature in mouse models that directly addresses the effects of blood pressure on atherosclerosis suggests that additional studies should be performed. Many of the future developments are likely to center on the mode of measuring blood pressure. As can be seen from Tables 1, 2, and 3, the vast majority of the published studies have measured systolic blood pressure using tail cuff methods. When used in experienced hands, this technique can provide authentic measurements. However, given the dynamic nature of blood pressure, which fluctuates according to circadian rhythms, the use of tail cuff systems for static once/day blood pressure measurements limits the information. Therefore, the use of ambulatory monitoring has the advantage of providing continuous measurements throughout a protracted interval. Consequently, the field would benefit from chronic telemetry studies in which multiple interventions are studied for their effects on both lesion size and a systematic evaluation of lesion characteristics.

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