Published in final edited form as:

Future Lipidol. 2008 December ; 3(6): 625–636. doi:10.2217/17460875.3.6.625.

Augmentation of the renin–angiotensin system by hypercholesterolemia promotes vascular diseases

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Abstract

Activation of the renin–angiotensin system (RAS) and aberrant cholesterol metabolism have generally been considered as independent mechanisms in the development of several vascular diseases. However, it is becoming increasingly apparent in both human and animal studies that many aspects of the RAS may be augmented by hypercholesterolemia, resulting in enhancement of the severity and occurrence of several vascular diseases, including hypertension, atherosclerosis and abdominal aortic aneurysms. Some potential hypercholesterolemia-induced mechanisms have been demonstrated to increase activity of specific components of the RAS. These include increased AT1-receptor expression, increased responsiveness to Ang II and increased synthesis of angiotensin peptides. Future studies need to validate mechanisms of hypercholesterolemia-induced RAS activation in different vascular diseases.

Keywords

aneurysms; angiotensin; atherosclerosis; cholesterol; hypertension

Activation of the renin–angiotensin system (RAS) is considered a major cause of hypertension. Conversely, defective cholesterol metabolism is a principal risk factor for the development of atherosclerotic-based disease. There has been a traditional view that these two systems operate independently. However, concomitant dysfunction of the RAS and cholesterol

metabolism is common within patients afflicted with cardiovascular diseases. In agreement with these associations, there are an increasing number of studies that directly demonstrate that hypercholesterolemia increases activity of the RAS in promoting several vascular diseases.

The RAS was identified over 100 years ago and has progressively become more complex with the identification of several bioactive angiotensin peptides that act on multiple receptors (Figure 1). The synthesis of the peptide angiotensin II (Ang II) begins with a single protein precursor, angiotensinogen. Renin cleaves angiotensinogen to generate the biologically inactive decapeptide Ang I. This is subsequently cleaved by angiotensin converting enzyme (ACE) to the octapeptide Ang II, which is the most extensively characterized peptide in this system. Many of the effects of Ang II are mediated through activation of Ang II type 1 (AT1) receptors. Ang II also activates AT2 receptors, which frequently generate responses that oppose the consequences of AT1-receptor stimulation. Ang II can be cleaved by amino-peptidases to smaller bioactive peptides. More recently, a novel enzyme has been identified in this system, termed ACE2, which catabolizes primarily Ang II to generate Ang(1–7), a functional antagonist of Ang II [1]. Therefore, the RAS has many complexities, although Ang II remains the peptide that exerts many of the well characterized biological effects of this system [2].

Cholesterol is principally synthesized in the liver and is packaged into lipoproteins to permit its transport in the aqueous media of plasma. The majority of cholesterol in plasma is usually carried in LDL, which delivers cholesterol to peripheral cells following interaction with LDL receptors (LDLRs) and delivery to the intracellular compartment via endocytosis [3]. This transport into cells is a tightly regulated homeostatic process that downregulates following sufficient sterol accumulation and, thus, prevents cellular cholesterol engorgement. However, this regulation is circumvented to generate lipid-laden macrophages that are present in atherosclerotic lesions. Although not unequivocally proven, a major hypothesis to explain excessive cholesterol deposition in atherosclerosis is lipoprotein modification leading to recognition by an array of receptors that are collectively referred to as scavenger receptors [4].

Overall, there are evolving data to link the RAS and aberrant cholesterol metabolism in the development of many vascular diseases. For the diseases that are the focus of this selective review, evidence of interactions will be provided in humans and animals, followed by a discussion of potential mechanisms by which RAS and aberrant regulation of cholesterol metabolism lead to augmented vascular diseases. The specific diseases that will be discussed are hypertension, atherosclerosis and abdominal aortic aneurysms.

Hypertension

Human observations

Hypertension is a common cardiovascular disease that is defined as systolic and diastolic blood pressures that exceed 140 and 90 mmHg, respectively. However, the risk from hypertension is a continuum leading to a definition of prehypertension (120–139 and 80–89 mmHg for systolic and diastolic blood pressure, respectively) [5]. Hypertension is associated with a range of end-organ damage, including blood vessels, predisposing to atherosclerotic diseases, such as stroke and coronary artery disease. While many different systems contribute to primary hypertension, elevated blood pressure is commonly associated with excessive activity of vasoconstrictors, such as Ang II.

Although RAS has been a focus for mechanisms of hypertension, there is evolving evidence that dyslipidemias also affect blood pressure, potentially through RAS activation. Epidemiological studies have demonstrated that hypercholesterolemia is associated with increased blood pressure [6]. Conversely, pharmacologically-induced reductions in plasma

cholesterol concentrations lead to modest lowering of blood pressure. There have been a number of studies in which reduced plasma cholesterol concentrations during statin administration were associated with reductions in both systolic and diastolic blood pressures. These include studies using pravastatin [7,8] and simvastatin [8,9]. However, statins generally exert no effects on blood pressure in normotensive subjects [10]. Statins lower LDL-C plasma concentrations through inhibition of HMG-CoA reductase. This enzyme is one of the initial steps of the multienzyme pathway of cholesterol biosynthesis that also yields many other biological products [11]. Therefore, statins have the potential to exert these effects by reductions in intermediate products other than cholesterol. Some evidence for an effect of statins on blood pressure independent of cholesterol lowering was derived from a study comparing simvastatin with the bile acid sequestrant, cholestyramine. These drugs were administered at doses that lowered plasma cholesterol concentrations to a similar extent, but only simvastatin reduced diastolic blood pressure [9]. Although Ang II is a well established regulator of blood pressure, none of the above studies have defined the role of the RAS in the hypotensive effects of statins.

Animal observations

Few studies have been performed in animals to investigate the role of hypercholesterolemia on blood pressure. Similar to humans, there is an increase in systolic blood pressure in hypercholesterolemic mice [12–14]. In LDLR^{-/-} mice fed a high-fat diet, the systolic blood pressure is approximately 20 mmHg higher than strain-matched LDLR wild-type mice fed a normal diet [14]. Fat feeding of LDLR-deficient mice caused a profound increase in plasma cholesterol concentrations to approximately 1000 mg/dl, which probably accounts for the large changes in blood pressure seen in mice relative to the associations noted for hypercholesterolemia in humans.

The role of the RAS has been directly demonstrated in hypercholesterolemia-induced hypertension by the administration of the renin inhibitor, aliskiren [14]. The higher dose of this drug led to a sustained decrease in systolic blood pressure to values obtained in normocholesterolemic mice, whereas the maximal dose of aliskiren had no effect on systolic blood pressure in normocholesterolemic mice.

Mechanistic studies

Medial vascular smooth muscle cells and endothelial cells are important regulators of arterial blood pressure. There have been several *in vitro* and *in vivo* studies in which changes in cholesterol metabolism have been demonstrated to regulate the response of the RAS, primarily through effects on Ang II (Table 1).

Cell culture studies have provided evidence that alterations of cholesterol metabolism regulate responses to Ang II. One of the earliest findings was that incubation of cultured rat vascular smooth muscle cells with LDL increased the expression of AT1-receptor mRNA and ¹²⁵I-Ang II binding [15]. The smooth muscle cells in this study were incubated with up to 200 µg protein/ml of LDL, which corresponds to approximately 50 mg cholesterol/dl. This would be below the usual values for LDL-C in plasma. However, *in vivo*, smooth muscle cells are in direct contact with LDL in the interstitial fluid rather than plasma. LDL-C concentrations in interstitial fluid may account for approximately 20% of the concentrations in plasma [16]. Therefore, it is likely that the LDL-C concentrations used in this study mimic those normally present in interstitial fluid of arterial medial layers.

Decreasing the cholesterol content of cultured smooth muscle cells also has effects on the RAS. This has been achieved by incubation with statins that, as described above, are inhibitors of the rate-limiting enzyme in the complex multienzyme synthesis of cholesterol. Incubation of

vascular smooth muscle cells with cerivastatin or fluvastatin promoted a downregulation of AT1-receptor mRNA and the number of ^{125}I -Ang II membrane binding sites [17]. Further studies are needed to determine the basis for changes in cellular cholesterol homeostasis on transcriptional regulation of AT1 receptors. It should be noted that statin-induced inhibition of HMG-CoA reductase leads to reductions in many bioactive intermediates in this pathway. Hence, the contribution of inhibition of cholesterol synthesis versus development of other intermediates also needs to be determined.

Increased cholesterol also changes Ang II responses in cultured endothelial cells, with incubation of LDL (100 $\mu\text{g/ml}$) transiently increasing AT1-receptor mRNA [18]. Unlike smooth muscle cells, endothelial cells are in direct contact with plasma. Therefore, it is likely that endothelial cells *in vivo* would normally be exposed to much higher LDL-C concentrations than in this study. Oxidized forms of LDL also increase AT1-receptor mRNA in cultured human umbilical vein endothelial cells [18]. Endothelial cells express ACE to convert Ang I to Ang II. This enzyme is upregulated by either native or oxidized LDL, which could increase blood pressure [18,19].

Cholesterol-induced regulation of the RAS in hypertension-associated vascular responses has also been noted in *ex vivo* studies. For example, increased AT1-receptor mRNA and enhanced ^{125}I -Ang II binding to membranes occur in aortic tissue extracted from diet-induced and LDLR-deficient hypercholesterolemic rabbits [20–22]. These increases in AT1-receptor expression are consistent with enhancement of Ang II-induced contractile responses in aortic rings from hypercholesterolemic rabbits [21,23]. The enhanced Ang II-induced contractions noted in aortic rings from hypercholesterolemic rabbits are not due to generalized changes, since norepinephrine and phenylephrine contractions are not affected by hypercholesterolemia [21,22]. Endothelial-dependent dilation of internal thoracic artery segments isolated from humans undergoing coronary artery bypass was improved dose-dependently by a 4-week treatment with atorvastatin, and was accompanied by a decreased vascular response to Ang II [24]. Similar effects were noted in patients administered pravastatin, with more pronounced effects on combined administration of a statin and AT1-receptor antagonist to improve endothelial function [25].

The interaction of hypercholesterolemia on Ang II-induced vascular responses has also been noted in human studies *in vivo*. A common measurement of endothelial function is based on hyperemic responses following transient ischemia of the arm. In hypercholesterolemic patients (LDL-C >160 mg/dl), a role for Ang II in this response was provided by administration of an AT1-receptor antagonist, candesartan, which greatly enhanced the hyperemic response [26]. An indication of selectivity for Ang II responses was provided by the lack of effect of the calcium-channel antagonist felodipine. Nitroglycerin dilates arteries by direct delivery of the vasodilator nitric oxide to smooth muscle cells and, hence, provides an approach to distinguish endothelial-dependent versus independent effects on arterial relaxation. The lack of effect of candesartan on nitroglycerin-induced dilations in hypercholesterolemic patients provides an indication that the hyperemic dysfunction was endothelial specific.

Further mechanistic insight of the interaction of cholesterol metabolism and the RAS on blood pressure was obtained *in vivo* by intravenous Ang II infusion into normo- (mean 181 mg/dl) versus hypercholesterolemic (mean 294 mg/dl) males [27]. Neither systolic nor diastolic blood pressure was different at baseline between the normo- and hypercholesterolemic subjects. However, Ang II infusion in an escalating dose of 0.5–20 ng/kg/min over a 30-min interval promoted a much greater increase in systolic blood pressure in the hypercholesterolemic males, with no significant difference on diastolic blood pressure between the groups. This increase was not due to a generalized increase in vascular responses, since infusion of norepinephrine caused the same increase in systolic blood pressure in both normo- and hypercholesterolemic

subjects. Some of these patients were subsequently treated with either atorvastatin or simvastatin to reduce plasma cholesterol concentrations. Subsequently, reinfusion with Ang II attenuated the increased systolic blood pressure compared with that obtained in these patients prior to statin administration [27]. Administration of the HMG-CoA reductase inhibitor fluvastatin in patients with familial hypercholesterolemia also led to an attenuation of Ang II-induced increases in systolic blood pressure [28].

All of the mechanisms discussed above have focused on processes in which cholesterol metabolism influences the arterial responses to the RAS. A further mode in which these two systems may interact in hypertension is through the enhancement of angiotensin peptide availability. In this regard, hypercholesterolemia in mice is associated with a large increase in plasma concentrations of angiotensinogen and angiotensin peptides, particularly Ang II [29]. It would be of interest to determine the association of plasma concentrations of cholesterol and angiotensin peptides in humans. However, the difficulty of obtaining authentic angiotensin peptide measurements in plasma provides an impediment to these studies [30]. Rather than measure angiotensin peptides, a relationship between plasma–renin activity and cholesterol concentrations was demonstrated in 66 patients over the long-term (15 years) development of hypertension [31]. The authors concluded that the presence of hypercholesterolemia can promote the development of stable hypertension through its interaction with the circulating RAS in patients with prehypertension. Finally, while a strict role for cholesterol has not been defined, several studies demonstrate that hypercholesterolemia of obesity is associated with elevated systemic concentrations and activities of RAS components [32–35].

Overall, there is consistent evidence, both *in vitro* and *in vivo*, that regulation of cholesterol metabolism will influence the activity and responsiveness of the RAS in pivotal cell types involved in blood pressure regulation, particularly for the effects of Ang II.

Atherosclerosis

Human observations

Atherosclerosis is the underlying cause of ischemic heart disease, which is the most common basis of morbidity and mortality in developed countries. Epidemiological, genetic and clinical trial evidence has demonstrated that hypercholesterolemia is consistently associated with the development of atherosclerotic diseases. As a consequence, measurement of plasma cholesterol concentrations is the primary diagnostic test for determining therapeutic strategies. The pathology of atherosclerotic diseases also demonstrates a major role of cholesterol, with the formative stages of lesions being characterized by a predominance of cholesterol-laden macrophages [36,37]. This cell type persists throughout lesion evolution. Therefore, there is abundant evidence that aberrant cholesterol metabolism is the basis for the initiation and progression of atherosclerosis, although the specific mechanisms by which this sterol causes the disease have not been unequivocally defined.

Since hypercholesterolemia is a well-validated risk factor for the development of atherosclerotic diseases, the major therapeutic efforts have been directed at lowering plasma concentrations of total cholesterol and LDL-C. Inhibition of HMG-CoA reductase with statins promotes profound reductions in plasma concentrations of LDL-C associated with consistent reductions in cardiovascular events [38].

Although lowering plasma cholesterol concentrations has been the most common approach to reducing atherosclerotic-based diseases, more recently, large, multinational clinical trials have been performed to determine whether inhibition of the RAS provides a complementary approach to further reduce cardiovascular risk in atherosclerotic diseases. The Heart Outcomes Prevention Evaluation (HOPE) trial was the first to determine the effect of ACE inhibition on

cardiovascular diseases in patients without overt heart failure [39]. The HOPE trial studied 9297 high-risk patients over 55 years of age. Participants were assigned to either a placebo or the ACE inhibitor ramipril (10 mg once daily). The dose of ramipril caused a modest reduction in systolic blood pressure, which was 3 mmHg lower than the placebo group at the end of the trial. This study was terminated prematurely as the safety monitoring board determined that a clear benefit of ramipril had already been established. A substudy of HOPE was the Study to Evaluate Carotid Ultrasound Changes in patients treated with Ramipril and vitamin E (SECURE) in which 732 patients were subjected to B-mode carotid ultrasounds to determine atherosclerosis progression [40]. This study provided evidence that ACE inhibition reduced the physical dimensions of human atherosclerotic lesions. These data were confirmed and enhanced by the European trial on Reduction of cardiac events with Perindopril in stable coronary Artery disease (EUROPA) [41]. However, contradictory data were generated in the Prevention of Events with Angiotensin Converting Enzyme inhibition (PEACE) trial, which failed to demonstrate an effect of tran-dolapril on any of the primary end points of cardiovascular disease [42]. It is difficult to reconcile the contrasting results of these clinical trials. However, compared with the PEACE trial, participants in the HOPE trial were older, had more severe cardiovascular disease and a lower prevalence of taking lipid-lowering drugs. Currently, patients with atherosclerotic diseases are still recommended to take RAS inhibitors [43,44].

A recent study has compared the effects of ACE inhibition to AT1-receptor antagonism and the combination of these drugs in a large multinational study of patients with atherosclerotic diseases or diabetes that do not have overt heart failure [45]. The ACE inhibitor used was ramipril, which was demonstrated to effectively decrease atherosclerotic cardiovascular diseases in the HOPE trial. Ramipril administration was compared with the AT1-receptor antagonist telmisartan. This study of 25,620 patients, who had been followed for a median of 56 months, showed no difference between the drug treatments, alone or combined, in the composite primary end points of death from cardiovascular causes, myocardial infarction, stroke or hospitalization for heart failure. Therefore, this study demonstrated an equivalence of effect of ACE inhibition and AT1-receptor antagonism in regard to their benefit in reducing atherosclerotic disease.

Animal observations

There are large numbers of studies demonstrating inhibition of the RAS at different steps on the development of hypercholesterolemia-induced atherosclerosis. The literature on ACE inhibition is particularly impressive and consistent. Investigators have used many different members of this class of drugs and reported reduced lesion size in several different species. These animal species include cynomolgus monkeys, mini-pigs, cholesterol-fed rabbits, Watanabe heritable hyperlipidemic rabbits, hamsters and ApoE^{-/-} mice [46–50]. The uniformity of the antiatherosclerotic effects of many structurally diverse ACE inhibitors is consistent with this being a class effect of this group of drugs.

Initial studies on AT1-receptor antagonism had either no effect or reductions in atherosclerosis in cholesterol-fed rabbits [51,52] and ApoE^{-/-} mice [50,53]. The reason for the range of effects of AT1-receptor antagonism in this earlier literature is unknown. More recent studies have consistently demonstrated reductions in lesion size by administration of AT1-receptor antagonists to either ApoE^{-/-} mice or hyperlipidemic cynomolgus monkeys [54–56]. In addition, AT1a-receptor deficiency has been shown to greatly decrease atherosclerosis in both ApoE^{-/-} [57,58] and LDLR^{-/-} mice [29]. These recent studies have demonstrated that AT1-receptor antagonism has an equivalent effect to ACE inhibition in reducing experimental atherosclerosis.

For many decades, there has been a concept of renin inhibition being the most effective approach to reducing RAS activity, since renin is the initial and ratelimiting enzyme in the reaction to generate angiotensin peptides from the cleavage of angiotensinogen. Despite the theoretical attraction of this target, it proved to be difficult to develop a pharmacological inhibitor of this enzyme. However, there is now a renin inhibitor – aliskiren – that is approved for the treatment of hypertension in patients. Aliskiren, which also effectively inhibits mouse renin, dose-dependently reduced atherosclerotic lesion size in hypercholesterolemic LDLR^{-/-} mice [14]. This effect was unrelated to the change in blood pressure *per se*, as is the case generally in murine atherosclerosis [59]. In ApoE^{-/-} mice with 2-kidney, 1-clip (rennin-dependent) or 1-kidney, 1-clip (rennin-independent) hypertension, aliskiren also reduced atherosclerotic lesion size [60]. Furthermore, renin inhibition with aliskiren had a profound effect in reducing atherosclerosis in Watanabe heritable hyperlipidemic rabbits [61]. The effectiveness of ACE inhibitors, angiotensin-receptor blockers and a renin inhibitor to exert profound effects in reducing hypercholesterolemia-induced atherosclerosis, occur without significant alterations in plasma lipid concentrations.

In addition to RAS inhibition reducing atherosclerotic lesion size [59], the converse has also been routinely noted; that chronic subcutaneous infusion of Ang II into hypercholesterolemic mice greatly augments atherosclerosis [62]. Ang II promotion of atherosclerosis has been noted in LDLR^{-/-} [63] and ApoE^{-/-} mice [64–69].

Mechanistic studies

The major mechanisms that have been studied relating the RAS to atherosclerosis involve the mode in which cholesterol is deposited in the arterial wall and its role in the cellular changes of lesion progression. Development of atherosclerotic lesions involves complex interactions of resident cells of the arterial wall and infiltrating leukocytes. The principal arterial cell types involved in atherosclerosis are endothelial and smooth muscle cells. Although there are a large number of different leukocyte types infiltrating atherosclerotic lesions, the most abundant are macrophages and T lymphocytes [70].

One of the approaches to defining mechanisms of Ang II-induced atherosclerosis has made use of the consistent observation that atherosclerosis is markedly attenuated in AT1a-receptor-deficient hypercholesterolemic mice [29,57]. This finding has been used in bone marrow transplantation studies to generate chimeric mice that have AT1a-receptor deficiency in either resident or infiltrating leukocytes. Although macrophages express AT1a receptors [14], the initial study failed to demonstrate any effect of AT1a-receptor genotype on infiltrating leukocytes in Ang I-augmented atherosclerosis in mice with compound deficiencies for LDLRs and AT1a receptors [71]. A subsequent study demonstrated similar data, with the exception that AT1a-receptor expression in infiltrating leukocytes promotes a minor increase in atherosclerosis of mice with compound deficiencies of LDLRs and AT1a receptors [72]. Bone marrow transplantation studies have also failed to demonstrate a significant effect of AT1a-receptor expression in infiltrating leukocytes on the development of hypercholesterolemia-induced atherosclerosis in LDLR^{-/-} mice [14]. Therefore, these studies are consistent with the predominant atherosclerotic effect of Ang II being due to AT1a-receptor stimulation of resident cells of the arterial wall, such as endothelial and smooth muscle cells.

Angiotensin II has many actions on resident cells of the arterial wall that would augment hypercholesterolemia-induced atherosclerosis. A primary effect of Ang II on endothelial cells in the atherogenic process is the augmentation of leukocyte recruitment and adhesion. Ang II consistently increases leukocyte adhesion in cultured endothelium [14,73,74], which is considered to be one of the primary events in the atherogenic process. The adhesion molecule responsible for the Ang II-induced monocyte adhesion has not been as consistent. VCAM-1 is proposed as a pivotal adhesion molecule. Ang II increases VCAM-1 mRNA and protein in

cultured rat aortic endothelial cells [75], but has no effect in cells isolated from human aortas, coronary arteries, umbilical veins and rabbit aortas [14,73,74]. E-selectin has been proposed to mediate Ang II-induced monocyte adhesion, although the literature has contradictory reports [73,74].

The potential atherogenic effects of Ang II have also been demonstrated with cultured smooth muscle cells. Smooth muscle cells are relatively sparse in many of the mouse models that have been used in recent studies to define the effects of RAS on atherosclerosis. Therefore, some of the major effects in experimental atherosclerosis may relate to Ang II stimulating smooth muscle cells and subsequently promoting leukocyte infiltrations. In this regard, one effect of Ang II is the enhanced secretion of monocyte chemoattractant protein-1 (MCP-1) from cultured vascular smooth muscle cells [76]. MCP-1 has been demonstrated to have a role in the development of experimental atherosclerosis [77]. Deficiency of the major receptor for MCP-1 (CC chemokine receptor [CCR]2) decreased aortic macrophage recruitment and atherosclerosis in Ang II-infused mice [66,78]. Ang II can also augment the release of other atherogenic cytokines, including IL-1 β , -6 and -18 [79–81]. In more advanced stages of human atherosclerosis, there is a substantial presence of smooth muscle cells. These are likely to have phenotypes and responses that differ from smooth muscle cells present in the medial aspect of the artery. The response of these intimal smooth muscle cells to Ang II has not been defined.

In addition to Ang II interacting with hypercholesterolemia to generate atherosclerosis, as mentioned previously, high plasma cholesterol concentrations also increase plasma concentrations of angiotensin peptides [29]. Similarly, elevated cholesterol concentrations may also result in local activation of the RAS within atherosclerotic lesions. Cultured macrophages secrete angiotensin peptides [14,82] and all the components of the RAS are present in atherosclerotic lesions [29,83,84]. There is a major contribution of local angiotensin peptide secretion to atherogenesis based on the large reductions in lesion size in LDLR^{-/-} mice that have been irradiated and repopulated with bone marrow-derived stem cells harvested from renin^{-/-} mice [14].

Overall, animal models of atherosclerosis have consistently shown that hypercholesterolemia stimulates the RAS and inhibitors of this system have a profound effect on atherosclerosis without overt effects on plasma cholesterol concentrations. Bone marrow transplantation studies are consistent with the major atherogenic effect of Ang II being exerted on resident cells of the artery. The use of cell-specific conditional deficient mice will be needed to determine whether a single cell type is involved. Identification of a specific cell type will enable further refinement of mechanisms by which Ang II interacts with hypercholesterolemia to initiate and promote atherogenesis.

In addition to hypercholesterolemia augmenting RAS responses, there are also a few studies of the converse in which Ang II regulates cholesterol metabolism. For example, Ang II regulates macrophage cholesterol synthesis [85], the lipid transporter ABCA1 [86] and a receptor that transports oxidized LDL into cells, LOX-1 [87]. In addition, there is limited evidence that Ang II can stimulate lipoprotein oxidation through a lipoxygenase [88].

Abdominal aortic aneurysms

Human observations

Abdominal aortic aneurysms (AAAs) are progressive dilations of the infrarenal aorta that can rupture, commonly leading to death. Unlike the other vascular maladies discussed in this review, AAAs are less prevalent, being primarily present in males over 60 years of age. Contrary to atherosclerotic-based diseases, there is an increasing prevalence of AAAs. In part, this may be due to enhanced efforts to identify individuals with the disease through

implementation of screening. This increase is also related to the demographics of many industrialized nations that have aging populations. Unlike the other vascular diseases discussed in this review, there is a paucity of information on the provoking factors and there is no proven medical strategy to attenuate disease progression. The only treatment option to prevent rupture is surgical, either by open repair or endovascular approaches [89].

No studies have been published on interventions for AAAs that target hypercholesterolemia or the RAS [90]. The only indication of benefit for inhibiting these systems comes from a retrospective analysis of databases containing information on drug use of patients admitted to hospital in the Ontario healthcare system that were diagnosed with intact or ruptured abdominal aortas. Patients who were receiving ACE inhibitors at the time of admission were significantly less likely to present with a ruptured aorta [91].

Animal observations

Most contemporary animal studies of AAAs are performed with mice [92]. AAAs have been observed in mice that have chronic and severe hypercholesterolemia [93]. In these studies, there has not been a link to the RAS. Acute development of AAAs is induced by subcutaneous infusion of subpressor or pressor doses of Ang II [94]. The initial studies of Ang II-induced AAAs were performed in hypercholesterolemic ApoE^{-/-} and LDLR^{-/-} female mice [62,63]. No AAAs developed in normocholesterolemic female mice [62]. Later studies demonstrated the greater susceptibility of male mice to develop Ang II-induced AAAs compared with female mice [68,69,95]. Subsequent studies have demonstrated that Ang II infusion induces AAAs in normocholesterolemic male mice, but with a much lesser incidence than in hypercholesterolemic mice [96,97]. Therefore, hypercholesterolemia augments Ang II-induced AAAs. However, the propensity for AAAs does not appear to be a direct function of increasing plasma cholesterol concentrations, since modestly hypercholesterolemic ApoE^{-/-} mice fed a normal diet have a similar incidence and severity of Ang II-induced AAAs. The current data are more consistent with a threshold effect for plasma cholesterol concentrations in facilitating Ang II-induced AAA formation.

Mechanistic studies

Angiotensin II infusions generate AAAs in hypercholesterolemic mice that have been localized to the same region of the abdominal aorta in all of the published studies [92]. This indicates some unique property of this aortic region that is influenced by hypercholesterolemia and the RAS. Prior to the initiation of the disease, endothelial and smooth muscle cells are the predominant cell types in this region. Results from bone marrow transplantation studies using AT1a-receptor deficient mice have been consistent with cells of the aorta being the determinant in the generation of Ang II-induced AAAs [71]. As previously mentioned, interactions of hypercholesterolemia and the RAS with vascular wall cells may contribute to enhanced AAA formation in hypercholesterolemic mice. There are several specific pathways that have been inferred in the development of Ang II-induced AAAs, including mediators of leukocyte infiltration, such as the MCP-1 receptor, CCR2 [98], leukotrienes [99] and prostanoids [97]. Since development of large luminal dilations, which are associated with AAAs, requires the destruction of elastin and collagen fibers, it is likely that there is an augmentation of secretion and activation of enzymes that degrade the extracellular matrix [100].

Conclusion

The interactions of hypercholesterolemia and the RAS have been demonstrated in both humans and animals for hypertension and atherosclerosis, as well as in animals for AAAs. These are relatively mild in hypertension, with lipid-lowering therapy having a modest impact on blood pressure in human studies. The interactions in atherosclerosis are more profound with both

animal and human studies, demonstrating impressive reductions in lesion size and clinical end points, respectively. The data are more limited for AAAs, although increased plasma cholesterol concentrations clearly increase the ability of Ang II infusions to generate experimental aneurysms in mice.

Several potential mechanisms have been advanced to explain some of these interactions of hypercholesterolemia and the RAS. The most common observation is hypercholesterolemia augmenting RAS responses. In addition, there are a few studies of the converse, in which Ang II regulates cholesterol metabolism. For example, Ang II regulates macrophage cholesterol synthesis [85], the lipid transporter ABCA1 [86] and a receptor that transports oxidized-LDL into cells, LOX-1 [87]. The lack of substantiated mechanisms to fully explain how hypercholesterolemia interacts with RAS activation provides fertile ground for future studies.

Future perspective

Hypercholesterolemia on angiotensin peptide responses

Several studies have demonstrated that the cholesterol content of smooth muscle cells regulates the expression of AT1 receptors. Cell culture studies have demonstrated that LDL increases AT1-receptor mRNA. However, the mechanism of this increase is undefined. For example, no studies have addressed whether there are direct or indirect sterol effects that lead to increased gene transcription.

Lowering plasma cholesterol has been demonstrated to decrease blood pressure in hypertensive patients and mice [10,14]. In addition, lowering plasma cholesterol concentrations reduces blood pressure responses during Ang II infusion. Human studies have generally been performed using statins as the mode to reduce plasma cholesterol concentrations. However, it remains to be defined whether the reductions in blood pressure and Ang II responses are due to reductions in plasma cholesterol concentration *per se*, or one of the many other effects that statins exert as inhibitors of one of the initial steps in a multienzymatic pathway that generates many bioactive intermediates.

The mechanism of augmentation of the RAS responses by hypercholesterolemia may vary in different vascular diseases. For example, cholesterol and RAS interactions are likely related to smooth muscle cell response in hypertension. For atherosclerosis and AAAs, it is not clear whether the most important interaction involves endothelial cells, smooth muscle cells or another cell type. The development of mice in which angiotensin receptors can be depleted in selective cell types will provide mechanistic insight on this issue.

Hypercholesterolemia on angiotensin peptide synthesis

Hypercholesterolemia is associated with increases in plasma concentrations of angiotensin peptides in experimental studies [29]. The mechanism of this effect is unknown, with no evidence that cholesterol has a direct effect on any of the proteins in the angiotensin peptide biosynthesis pathway. It would be of great interest to determine whether hypercholesterolemic states and administration of lipid-lowering drugs are associated with regulation of systemic RAS in humans. One need for the completion of these studies is an accurate and specific assay for the routine determination of plasma concentrations of angiotensin peptides. Despite the intense interest in the biological properties of these peptides, validated measurements for clinical studies have been difficult to achieve.

Executive summary

Hypertension

- Hypercholesterolemia is associated with hypertension and promotion of Angiotensin II (Ang-II)-induced increases in blood pressure. Treatment of hypercholesterolemia with statins lowers blood pressure, although the contribution of cholesterol lowering versus the many other effects of statins has not been defined.

Atherosclerosis

- Inhibition of the renin–angiotensin system (RAS) is a highly effective approach to reducing hypercholesterolemia-induced atherosclerosis.

Abdominal aortic aneurysms

- Ang II-induced experimental abdominal aortic aneurysm formation is augmented by hypercholesterolemia in animals. Evidence for an interaction in the human disease is lacking.

Conclusion

- Definition of interactions of hypercholesterolemia and RAS will provide insight for the more effective treatment of a range of vascular diseases.

Acknowledgments

Financial & competing interests disclosure

Alan Daugherty has received grant funding from Novartis and Merck. Lisa Cassis has received grant funding from Novartis. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

1. Raizada MK, Ferreira AJ. ACE2: a new target for cardiovascular disease therapeutics. *J. Cardiovasc. Pharmacol* 2007;50:112–119. [PubMed: 17703127]
2. Carey RM, Siragy HM. Newly recognized components of the renin–angiotensin system: potential roles in cardiovascular and renal regulation. *Endocrin. Rev* 2003;24:261–271.
3. Goldstein JL, Brown MS. Binding and degradation of low density lipoproteins by cultured human fibroblasts. Comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J. Biol. Chem* 1974;249:5153–5162.5162 [PubMed: 4368448] ▪▪
Groundbreaking discovery that formed the basis for a Nobel prize.
4. Krieger M, Herz J. Structures and functions of multiligand lipoprotein receptors: macrophage scavenger receptors and LDL receptor-related protein (LRP). *Ann. Rev. Biochem* 1994;63:601–637. [PubMed: 7979249]
5. Chobanian AV, Bakris GL, Black HR, et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. *Hypertension* 2003;42:1206–1252. [PubMed: 14656957]

6. Lloyd-Jones DM, Evans JC, Larson MG, O'Donnell CJ, Wilson PW, Levy D. Cross-classification of JNC VI blood pressure stages and risk groups in the Framingham Heart Study. *Arch. Intern. Med* 1999;159:2206–2212. [PubMed: 10527298]
7. Glorioso N, Troffa C, Filigheddu F, et al. Effect of the HMG-CoA reductase inhibitors on blood pressure in patients with essential hypertension and primary hypercholesterolemia. *Hypertension* 1999;34:1281–1286. [PubMed: 10601131]
8. Golomb BA, Dimsdale JE, White HL, Ritchie JB, Criqui MH. Reduction in blood pressure with statins: results from the UCSD Statin Study, a randomized trial. *Arch. Intern. Med* 2008;168:721–727. [PubMed: 18413554]
9. Tonolo G, Melis MG, Formato M, et al. Additive effects of Simvastatin beyond its effects on LDL cholesterol in hypertensive Type 2 diabetic patients. *Eur. J. Clin. Invest* 2000;30:980–987. [PubMed: 11114960]
10. Borghi C, Veronesi M, Prandin MG, Dormi A, Ambrosioni E. Statins and blood pressure regulation. *Curr. Hypertens. Rep* 2001;3:281–288. [PubMed: 11470010]
11. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. *Nature* 1990;343:425–430. [PubMed: 1967820]
12. Yang R, Powell-Braxton L, Ogaoawara AK, et al. Hypertension and endothelial dysfunction in apolipoprotein E knockout mice. *Arterioscler. Thromb. Vasc. Biol* 1999;19:2762–2768. [PubMed: 10559023]
13. Trieu VN, Uckun FM. Male-associated hypertension in LDL-R deficient mice. *Biochem. Biophys. Res. Comm* 1998;247:277–279. [PubMed: 9642116]
14. Lu H, Rateri DL, Feldman DL, et al. Renin inhibition reduces hypercholesterolemia-induced atherosclerosis in mice. *J. Clin. Invest* 2008;118:984–993. [PubMed: 18274671]
15. Nickenig G, Sachinidis A, Michaelsen F, Bohm M, Seewald S, Vetter H. Upregulation of vascular angiotensin II receptor gene expression by low-density lipoprotein in vascular smooth muscle cells. *Circulation* 1997;95:473–478. [PubMed: 9008466]
16. Parini P, Johansson L, Broijersens A, Angelin B, Rudling M. Lipoprotein profiles in plasma and interstitial fluid analyzed with an automated gel-filtration system. *Eur. J. Clin. Invest* 2006;36:98–104. [PubMed: 16436091]
17. Ichiki T, Takeda K, Tokunou T, et al. Downregulation of angiotensin II type 1 receptor by hydrophobic 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in vascular smooth muscle cells. *Arterioscler. Thromb. Vasc. Biol* 2001;21:1896–1901. [PubMed: 11742861]
18. Catar RA, Muller G, Heidler J, Schmitz G, Bornstein SR, Morawietz H. Low-density lipoproteins induce the renin–angiotensin system and their receptors in human endothelial cells. *Horm. Metab. Res* 2007;39:801–805. [PubMed: 17992634]
19. Li DY, Singh RM, Liu L, et al. Oxidized-LDL through LOX-1 increases the expression of angiotensin converting enzyme in human coronary artery endothelial cells. *Cardiovasc. Res* 2003;57:238–243. [PubMed: 12504834]
20. Nickenig G, Jung O, Strehlow K, et al. Hypercholesterolemia is associated with enhanced angiotensin AT1-receptor expression. *Am. J. Physiol* 1997;272:H2701–H2707. [PubMed: 9227549]
21. Yang BC, Phillips MI, Mohuczy D, et al. Increased angiotensin II type 1 receptor expression in hypercholesterolemic atherosclerosis in rabbits. *Arterioscler. Thromb. Vasc. Biol* 1998;18:1433–1439. [PubMed: 9743232]
22. Warnholtz A, Nickenig G, Schulz E, et al. Increased NADH-oxidase-mediated superoxide production in the early stages of atherosclerosis – evidence for involvement of the renin-angiotensin system. *Circulation* 1999;99:2027–2033. [PubMed: 10209008]
23. Dam JPM, Vleeming W, Riezebos J, Post MJ, Porsius AJ, Wemer J. Effects of hypercholesterolemia on the contractions to angiotensin II in the isolated aorta and iliac artery of the rabbit: role of arachidonic acid metabolites. *J. Cardiovasc. Pharmacol* 1997;30:118–123. [PubMed: 9268230]
24. van der Harst P, Wagenaar LJ, Buikema H, et al. Effect of intensive versus moderate lipid lowering on endothelial function and vascular responsiveness to angiotensin II in stable coronary artery disease. *Am. J. Cardiol* 2005;96:1361–1364. [PubMed: 16275178]
25. Morawietz H, Erbs S, Holtz J, et al. Endothelial protection, AT1 blockade and cholesterol-dependent oxidative stress: the EPAS trial. *Circulation* 2006;114:I296–I301. [PubMed: 16820589]

26. Wassmann S, Hilgers S, Laufs U, Bohm M, Nickenig G. Angiotensin II type 1 receptor antagonism improves hypercholesterolemia-associated endothelial dysfunction. *Arterioscler. Thromb. Vasc. Biol* 2002;22:1208–1212. [PubMed: 12117739]
27. Nickenig G, Baumer AT, Temur Y, Kebben D, Jockenhovel F, Bohm M. Statin-sensitive dysregulated AT1 receptor function and density in hypercholesterolemic men. *Circulation* 1999;100:2131–2134. [PubMed: 10571970]
28. van der Linde NA, Sijbrands EJ, Boomsma F, van den Meiracker AH. Effect of low-density lipoprotein cholesterol on angiotensin II sensitivity: a randomized trial with fluvastatin. *Hypertension* 2006;47:1125–1130. [PubMed: 16618834]
29. Daugherty A, Rateri DL, Lu H, Inagami T, Cassis LA. Hypercholesterolemia stimulates angiotensin peptide synthesis and contributes to atherosclerosis through the AT1A receptor. *Circulation* 2004;110:3849–3857. [PubMed: 15596561]
30. Cassis LA, Huang J, Gong MC, Daugherty A. Role of metabolism and receptor responsiveness in the attenuated responses to Angiotensin II in mice compared with rats. *Regul. Pept* 2004;117:107–116. [PubMed: 14700746]
31. Borghi C, Veronesi M, Cosentino E, et al. Interaction between serum cholesterol levels and the renin–angiotensin system on the new onset of arterial hypertension in subjects with high-normal blood pressure. *J. Hypertens* 2007;25:2051–2057. [PubMed: 17885547]
32. Boustany CM, Bharadwaj K, Daugherty A, Brown DR, Randall DC, Cassis LA. Activation of the systemic and adipose renin–angiotensin system in rats with diet-induced obesity and hypertension. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 2004;287:R943–R949. [PubMed: 15191907]
33. Boustany CM, Brown DR, Randall DC, Cassis LA. AT1-receptor antagonism reverses the blood pressure elevation associated with diet-induced obesity. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 2005;289:R181–R186. [PubMed: 15774768]
34. Gupte MN, Boustany-Kari CM, Bharadwaj K, et al. ACE2 is expressed in mouse adipocytes and regulated by a high fat diet. *Am. J. Physiol. Regul. Integr. Comp. Physiol* 2008;295:R781–R788. [PubMed: 18650320]
35. Engeli S, Bohnke J, Gorzelniak K, et al. Weight loss and the renin–angiotensin–aldosterone system. *Hypertension* 2005;45:356–362. [PubMed: 15630041]
36. Stary HC, Chandler AB, Glagov S, et al. A definition of initial, fatty streak and intermediate lesions of atherosclerosis – a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Arterioscler. Thromb* 1994;14:840–856.856 [PubMed: 8172861] • With [37], these are major reviews that form the basis for most discussions on the evolution of atherosclerotic lesions.
37. Stary HC, Chandler AB, Dinsmore RE, et al. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis: a report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. *Circulation* 1995;92:1355–1374.1374 [PubMed: 7648691] • With 36], these are major reviews that form the basis for most discussions on the evolution of atherosclerotic lesions.
38. Rader DJ, Daugherty A. Translating molecular discoveries into new therapies for atherosclerosis. *Nature* 2008;451:904–913. [PubMed: 18288179]
39. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. The Heart Outcomes Prevention Evaluation Study Investigators. *N. Engl. J. Med* 2000;342:145–153.153 [PubMed: 10639539] • First evidence that suppression of the renin–angiotensin system by administration of the angiotensin-converting enzyme (ACE) inhibitor ramipril led to large decreases in atherosclerotic diseases.
40. Lonn EM, Yusuf S, Dzavik V, et al. Effects of ramipril and vitamin E on atherosclerosis – The Study to Evaluate Carotid Ultrasound Changes in patients treated with Ramipril and vitamin E (SECURE). *Circulation* 2001;103:919–925. [PubMed: 11181464]
41. Fox KM. Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). *Lancet* 2003;362:782–788. [PubMed: 13678872]

42. Braunwald E, Domanski MJ, Fowler SE, et al. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. *N. Engl. J. Med* 2004;351:2058–2068. [PubMed: 15531767]
43. Dagenais GR, Pogue J, Fox K, Simoons ML, Yusuf S. Angiotensin-converting-enzyme inhibitors in stable vascular disease without left ventricular systolic dysfunction or heart failure: a combined analysis of three trials. *Lancet* 2006;368:581–588. [PubMed: 16905022]
44. Smith SC Jr, Allen J, Blair SN, et al. AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update: endorsed by the National Heart, Lung, and Blood Institute. *Circulation* 2006;113:2363–2372. [PubMed: 16702489]
45. Yusuf S, Teo KK, Pogue J, et al. ONTARGET Investigators: Telmisartan, ramipril, or both in patients at high risk for vascular events. *N. Engl. J. Med* 2008;358:1547–1559. [PubMed: 18378520]
46. Aberg G, Ferrer P. Effects of captopril on atherosclerosis in cynomolgus monkeys. *J. Cardiovasc. Pharmacol* 1990;15:S65–S72. [PubMed: 1694934]
47. Charpiot P, Rolland PH, Friggi A, et al. ACE inhibition with prindopril and atherogenesis-induced structural and functional changes in minipig arteries. *Arterio. Thromb* 1993;13:1125–1138.
48. Campbell JH, Fennessy P, Campbell GR. Effect of perindopril on the development of atherosclerosis in the cholesterol-fed rabbit. *Clin. Exp. Pharmacol. Physiol* 1992;19:13–17.
49. Kowala MC, Grove RL, Aberg G. Inhibitors of angiotensin converting enzyme decrease early atherosclerosis in hyperlipidemic hamsters. Fosinopril reduces plasma cholesterol and captopril inhibits macrophage-foam cell accumulation independently of blood pressure and plasma lipids. *Atherosclerosis* 1994;108(1):61–72. [PubMed: 7980708]
50. Hayek T, Attias J, Coleman R, et al. The angiotensin-converting enzyme inhibitor, fosinopril, and the angiotensin II receptor antagonist, losartan, inhibit LDL oxidation and attenuate atherosclerosis independent of lowering blood pressure in apolipoprotein E deficient mice. *Cardiovasc. Res* 1999;44:579–587. [PubMed: 10690290]
51. Schuh JR, Blehm DJ, Friedrich GE, McMahon EG, Blaine EH. Differential effects of renin–angiotensin system blockade on atherogenesis in cholesterol-fed rabbits. *J. Clin. Invest* 1993;91:1453–1458. [PubMed: 8473494]
52. Li J, Hirose N, Kawamura M, Arai Y. Antiatherogenic effect of angiotensin converting enzyme inhibitor (benazepril) and angiotensin II receptor antagonist (valsartan) in the cholesterolfed rabbits. *Atherosclerosis* 1999;143:315–326. [PubMed: 10217360]
53. Makaritsis KP, Gavras H, Du Y, Chobanian AV, Brecher P. $\alpha(1)$ -adrenergic plus angiotensin receptor blockade reduces atherosclerosis in apolipoprotein E deficient mice. *Hypertension* 1998;32:1044–1048. [PubMed: 9856971]
54. Suzuki J, Iwai M, Li Z, et al. Effect of combination of calcium antagonist, azelnidipine, and AT1 receptor blocker, olmesartan, on atherosclerosis in apolipoprotein E-deficient mice. *J. Hypertens* 2005;23:1383–1389. [PubMed: 15942461]
55. Doran DE, Weiss D, Zhang Y, Griendling KK, Taylor WR. Differential effects of AT1 receptor and Ca^{2+} channel blockade on atherosclerosis, inflammatory gene expression, and production of reactive oxygen species. *Atherosclerosis* 2007;195:39–47. [PubMed: 17224157]
56. Strawn WB, Chappell MC, Dean RH, Kivlighn S, Ferrario CM. Inhibition of early atherogenesis by losartan in monkeys with dietinduced hypercholesterolemia. *Circulation* 2000;101:1586–1593. [PubMed: 10747353]
57. Wassmann S, Czech T, Van Eickels M, Fleming I, Bohm M, Nickenig G. Inhibition of diet-induced atherosclerosis and endothelial dysfunction in apolipoprotein E/Angiotensin II type 1A receptor double-knockout mice. *Circulation* 2004;110:3062–3067. [PubMed: 15277329]
58. Li Z, Iwai M, Wu L, et al. Fluvastatin enhances the inhibitory effects of a selective AT1 receptor blocker, valsartan, on atherosclerosis. *Hypertension* 2004;44:758–763. [PubMed: 15452025]
59. Lu H, Cassis LA, Daugherty A. Atherosclerosis and arterial blood pressure in mice. *Curr. Drug Targets* 2007;8:1181–1189. [PubMed: 18045096]
60. Nussberger J, Aubert JF, Bouzourene K, Pellegrin M, Hayoz D, Mazzolai L. Renin inhibition by aliskiren prevents atherosclerosis progression: comparison with irbesartan, atenolol, and amlodipine. *Hypertension* 2008;51:1306–1311. [PubMed: 18391092]

61. Imanishi T, Tsujioka H, Ikejima H, et al. Renin inhibitor aliskiren improves impaired nitric oxide bioavailability and protects against atherosclerotic changes. *Hypertension* 2008;52:563–572. [PubMed: 18645051]
62. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J. Clin. Invest* 2000;105:1605–1612. [PubMed: 10841519]
63. Daugherty A, Cassis L. Chronic angiotensin II infusion promotes atherogenesis in low density lipoprotein receptor^{-/-} mice. *Ann. NY Acad. Sci* 1999;892:108–118. [PubMed: 10842656]
64. Weiss D, Kools JJ, Taylor WR. Angiotensin II-induced hypertension accelerates the development of atherosclerosis in ApoE-deficient mice. *Circulation* 2001;103:448–454. [PubMed: 11157699]
65. Bruemmer D, Collins AR, Noh G, et al. Angiotensin II accelerated atherosclerosis and aneurysm formation is attenuated in osteopontin deficient mice. *J. Clin. Invest* 2003;112:1318–1331. [PubMed: 14597759]
66. Ishibashi M, Egashira K, Zhao Q, et al. Bone marrow-derived monocyte chemoattractant protein-1 receptor CCR2 is critical in angiotensin II-induced acceleration of atherosclerosis and aneurysm formation in hypercholesterolemic mice. *Arterioscler. Thromb. Vasc. Biol* 2004;24:E174–E178. [PubMed: 15331433]
67. Ayabe N, Babaev VR, Tang Y, et al. Transiently heightened angiotensin II has distinct effects on atherosclerosis and aneurysm formation in hyperlipidemic mice. *Atherosclerosis* 2005;184:312–321. [PubMed: 16023123]
68. Henriques TA, Huang J, D'Souza SS, Daugherty A, Cassis LA. Orchiectomy, but not ovariectomy, regulates angiotensin II-induced vascular diseases in apolipoprotein E deficient mice. *Endocrinology* 2004;145:3866–3872. [PubMed: 15105380]
69. Henriques T, Zhang X, Yiannikouris FB, Daugherty A, Cassis LA. Androgen increases AT1a receptor expression in abdominal aortas to promote angiotensin II-induced AAAs in apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol* 2008;28:1251–1256. [PubMed: 18451329]
70. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N. Engl. J. Med* 2005;352:1685–1695. [PubMed: 15843671] ■■ Authoritative review from an individual who has forged many of the research efforts in immune function and atherosclerosis.
71. Cassis LA, Rateri DL, Lu H, Daugherty A. Bone marrow transplantation reveals that recipient AT1a receptors are required to initiate angiotensin II-induced atherosclerosis and aneurysms. *Arterioscler. Thromb. Vasc. Biol* 2007;27:380–386. [PubMed: 17158350]
72. Fukuda D, Sata M, Ishizaka N, Nagai R. critical role of bone marrow angiotensin II type 1 receptor in the pathogenesis of atherosclerosis in apolipoprotein E deficient mice. *Arterioscler. Thromb. Vasc. Biol* 2007;28:90–96. [PubMed: 17962627]
73. Kim JA, Berliner JA, Nadler JL. Angiotensin II increases monocyte binding to endothelial cells. *Biochem. Biophys. Res. Comm* 1996;226:862–868. [PubMed: 8831702]
74. Grafe M, Auch-Schwelk W, Zakrzewicz A, et al. Angiotensin II-induced leukocyte adhesion on human coronary endothelial cells is mediated by E-selectin. *Circ. Res* 1997;81:804–811. [PubMed: 9351454]
75. Pueyo ME, Gonzalez W, Nicoletti A, Savoie F, Arnal JF, Michel JB. Angiotensin II stimulates endothelial vascular cell adhesion molecule-1 via nuclear factor-κB activation induced by intracellular oxidative stress. *Arterioscler. Thromb. Vasc. Biol* 2000;20:645–651. [PubMed: 10712386]
76. Chen XL, Tummala PE, Olbrych MT, Alexander RW, Medford RM. Angiotensin II induces monocyte chemoattractant protein-1 gene expression in rat vascular smooth muscle cells. *Circ. Res* 1998;83:952–995. [PubMed: 9797345]
77. Gu L, Okada Y, Clinton SK, et al. Absence of monocyte chemoattractant protein-1 reduces atherosclerosis in low density lipoprotein receptor-deficient mice. *Mol. Cell* 1998;2:275–281. [PubMed: 9734366]
78. Bush E, Maeda N, Kuziel WA, et al. CC chemokine receptor 2 is required for macrophage infiltration and vascular hypertrophy in angiotensin II-induced hypertension. *Hypertension* 2000;36:360–363. [PubMed: 10988265]

79. Kunieda T, Minamino T, Nishi JJ, et al. Angiotensin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a p21-dependent pathway. *Circulation* 2006;114:953–960. [PubMed: 16908765]
80. Cui R, Tieu B, Recinos A, Tilton RG, Brasier AR. RhoA mediates angiotensin II-induced phospho-Ser536 nuclear factor κ B/RelA subunit exchange on the interleukin-6 promoter in VSMCs. *Circ. Res* 2006;99:723–730. [PubMed: 16960103]
81. Sahar S, Dwarakanath RS, Reddy MA, Lanting L, Todorov I, Natarajan R. Angiotensin II enhances interleukin-18 mediated inflammatory gene expression in vascular smooth muscle cells: a novel cross-talk in the pathogenesis of atherosclerosis. *Circ. Res* 2005;96:1064–1071. [PubMed: 15860756]
82. Okamura A, Rakugi H, Ohishi M, et al. Upregulation of renin–angiotensin system during differentiation of monocytes to macrophages. *J. Hypertens* 1999;17:537–545. [PubMed: 10404956]
83. Diet F, Pratt RE, Berry GJ, Momose N, Gibbons GH, Dzau VJ. Increased accumulation of tissue ACE in human atherosclerotic coronary artery disease. *Circulation* 1996;94:2756–2767. [PubMed: 8941100]
84. Potter DD, Sobey CG, Tompkins PK, Rossen JD, Heistad DD. Evidence that macrophages in atherosclerotic lesions contain angiotensin II. *Circulation* 1998;98:800–807. [PubMed: 9727551]
85. Keidar S, Attias J, Heinrich R, Coleman R, Aviram M. Angiotensin II atherogenicity in apolipoprotein E deficient mice is associated with increased cellular cholesterol biosynthesis. *Atherosclerosis* 1999;146:249–257. [PubMed: 10532681]
86. Takata Y, Chu V, Collins AR, et al. Transcriptional repression of ATP-binding cassette transporter A1 gene in macrophages: a novel atherosclerotic effect of angiotensin II. *Circ. Res* 2005;97:E88–E96. [PubMed: 16224068]
87. Li DY, Zhang YC, Philips MI, Sawamura T, Mehta JL. Upregulation of endothelial receptor for oxidized low-density lipoprotein (LOX-1) in cultured human coronary artery endothelial cells by angiotensin II type 1 receptor activation. *Circ. Res* 1999;84:1043–1049. [PubMed: 10325241]
88. Scheidegger KJ, Butler S, Witztum JL. Angiotensin II increases macrophage-mediated modification of low density lipoprotein via a lipoxygenase-dependent pathway. *J. Biol. Chem* 1997;272:21609–21615. [PubMed: 9261183]
89. Thompson MM, Bell PR. ABC of arterial and venous disease. Arterial aneurysms. *B. Med. J* 2000;320:1193–1196.
90. Baxter BT, Terrin MC, Dalman RL. Medical management of small abdominal aortic aneurysms. *Circulation* 2008;117:1883–1889. [PubMed: 18391122]
91. Hackam DG, Thiruchelvam D, Redelmeier DA. Angiotensin-converting enzyme inhibitors and aortic rupture: a population-based case–control study. *Lancet* 2006;368:659–665.665 [PubMed: 16920471]
 - Provocative report of a retrospective n analysis that demonstrated an association between ACE inhibition and resistance to rupture of abdominal aortic aneurysms.
92. Daugherty A, Cassis LA. Mouse models of abdominal aortic aneurysms. *Arterioscler. Thromb. Vasc. Biol* 2004;24:429–434. [PubMed: 14739119]
93. Tangirala RK, Rubin EM, Palinski W. Quantitation of atherosclerosis in murine models: correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor-deficient and apolipoprotein E-deficient mice. *J. Lipid Res* 1995;36:2320–2328. [PubMed: 8656070]
94. Lu H, Rateri DL, Cassis LA, Daugherty A. The role of the renin–angiotensin system in aortic aneurysmal diseases. *Curr. Hypertens. Rep* 2008;10:99–106. [PubMed: 18474175]
95. Manning MW, Cassis LA, Huang J, Szilvassy SJ, Daugherty A. Abdominal aortic aneurysms: fresh insights from a novel animal model of the disease. *Vasc. Med* 2002;7:45–54. [PubMed: 12083734]
96. Deng GG, Martin-McNulty B, Sukovich DA, et al. Urokinase-type plasminogen activator plays a critical role in angiotensin II-induced abdominal aortic aneurysm. *Circ. Res* 2003;92:510–517. [PubMed: 12600880]
97. King VL, Trivedi D, Gitlin JM, Loftin CD. Selective cyclooxygenase-2 inhibition with celecoxib decreases angiotensin II-induced abdominal aortic aneurysm formation in mice. *Arterioscler. Thromb. Vasc. Biol* 2006;26:1137–1143. [PubMed: 16514081]

98. Ni W, Kitamoto S, Ishibashi M, et al. Monocyte chemoattractant protein-1 is an essential inflammatory mediator in angiotensin II-induced progression of established atherosclerosis in hypercholesterolemic mice. *Arterioscler. Thromb. Vasc. Biol* 2004;24:534–539. [PubMed: 14739122]
99. Ahluwalia N, Lin AY, Tager AM, et al. Inhibited aortic aneurysm formation in BLT1-deficient mice. *J. Immunol* 2007;179:691–697. [PubMed: 17579092]
100. Saraff K, Babamusta F, Cassis LA, Daugherty A. Aortic dissection precedes formation of aneurysms and atherosclerosis in angiotensin II-infused, apolipoprotein E-deficient mice. *Arterioscler. Thromb. Vasc. Biol* 2003;23:1621–1626. [PubMed: 12855482]

Figure 1. Synthesis of angiotensin peptides

Enzymes in the system are represented in boxes and receptors are represented in ovals. ACE: Angiotensin-converting enzyme; Ang: Angiotensin; AT1R: Ang II type 1 receptor; AT2R: Ang II type 2 receptor.

Table 1

Potential mechanisms by which hypercholesterolemia impacts the activity of the renin–angiotensin system in selected vascular diseases.

Disease	Potential mechanisms
Hypertension	<ul style="list-style-type: none"> ↑ AT1-receptor mRNA in vascular smooth muscle and endothelial cells ↑ Membrane location of AT1 receptor in signaling domains ↑ Arterial contractility ↑ Impaired arterial dilation ↑ Response on systolic blood pressure to Ang II infusion ↑ Plasma concentrations of angiotensin peptides
Atherosclerosis	<ul style="list-style-type: none"> ↑ Adhesion molecular expression ↑ Leukocyte-endothelial adhesion ↑ Chemokine secretion ↑ Plasma concentrations of angiotensin peptides
Abdominal aortic aneurysms	Unknown