Hypothesis Regarding the Pathophysiological Role of Alternative Pathways of Angiotensin II Formation in Atherosclerosis

Kikuo Arakawa, Hidenori Urata

Abstract—The renin-angiotensin system has been studied and recognized as one of the major blood pressure–regulating systems for the past century. In the last quarter century, however, many alternative pathways of angiotensin II formation have been found, and among them, chymase has been a focus of interest because of its specificity and potency in the human cardiovascular system. Chymase evidently is not involved in functional regulation of blood pressure at least in the short term, but evidence is accumulating that it may be involved in structural remodeling of the cardiovascular system. We found increased vascular chymase activity in atherosclerotic lesions of the human aorta as well as in cardiac remodeling after myocardial infarction. We found a significant positive correlation between serum total or LDL cholesterol levels and arterial chymase-dependent angiotensin II–forming activity in patients who were undergoing coronary artery bypass operation, suggesting that high serum cholesterol may trigger upregulation of vascular chymase and facilitate the development of atherosclerosis. This hypothesis was tested in Syrian hamsters fed a high cholesterol diet containing 0.5% cholesterol: A marked lipid deposition in the aortic cusp developed and the plasma cholesterol levels were positively correlated with aortic chymase activity. An orally active nonpeptide chymase inhibitor almost canceled this lipid deposition. These clinical and experimental data indicated an association between cholesterol and vascular chymase upregulation that may facilitate the development of atherosclerosis. (Hypertension. 2000;36:638-641.)

Key Words: kallikrein ■ angiotensin-converting enzyme ■ atherosclerosis ■ cardiovascular diseases

Locally formed angiotensin (Ang) II is thought to play some pathological roles in the development of hypertensive heart disease, congestive heart failure, and acute myocardial infarction. This is substantiated by the fact that the introduction of ACE inhibitors improved the morbidity and mortality rates of patients with various cardiovascular diseases. In the last quarter century, however, many other Ang II–forming enzymes have been found.1 We have found that trypsin2 as well as kallikrein3 generates Ang II not only in test tubes but in ischemic dog heart4 and ischemic human leg5 and even normal healthy individuals when exercised.6 Among these Ang II–forming enzymes, chymase is most potent in human tissues including heart, aorta, lung, liver, and so forth.7 It is now evident, however, that chymase is not involved in blood pressure regulation,8 but evidence has been accumulating that it may be involved in cardiovascular remodeling. Two recent reports suggested that in the heart and vasculature, ACE-independent Ang II formation takes place in vivo.9,10 We further investigated how human chymase is involved in cardiovascular diseases.

Clinical Evidence for Involvement of Chymase in Atherogenesis

We have found an increased activity of arterial chymase in atherosclerotic lesions of the human aorta.11 We determined aortic Ang II–forming activity (AIIFA) and the histochemical localization of each Ang II–forming enzyme in the atheromatous human aorta. Specimens of normal (n=9), atherosclerotic (n=8), and aneurysmal (n=6) human aortas were obtained at autopsy or cardiovascular surgery. Total AIIFA was significantly higher in atherosclerotic and aneurysmal lesions than in normal aorta (Figure 1). Most (80% to 90%) AIIFA in the human aorta in vitro was chymase dependent in normal as well as in atherosclerotic aortas (Figure 1). Immunocytochemical staining of the corresponding aortic sections with anti-chymase, anti-trypsin, or anti-ACE antibodies showed that chymase-positive mast cells were located in the tunica adventitia of normal and atheromatous aortas, whereas ACE-positive cells were localized in endothelial cells of normal aorta and in macrophages and smooth muscle cells of atheromatous neo-intima. The number of activated mast...
cells in the aneurysmal lesions (18%) was significantly higher than in normal aorta. Chymase dAlIFA in atherosclerotic and aneurysmal lesions was significantly greater than in normal aorta. *P<0.01 vs total AlIFA in normal aorta; †P<0.01 vs chymase dAlIFA in normal aorta. This figure is modified from Reference 11.

Figure 1. Comparison of chymase- and ACE-dependent Ang II–forming activity (dAlIFA) in human aorta. Total AlIFA in atherosclerotic and aneurysmal lesions was significantly higher than in normal aorta. Chymase dAlIFA in atherosclerotic and aneurysmal lesions was significantly greater than in normal aorta. *P<0.01 vs total AlIFA in normal aorta; †P<0.01 vs chymase dAlIFA in normal aorta. This figure is modified from Reference 11.

Positive Correlation of Arterial Chymase Activity and Plasma Total or LDL Cholesterol Levels
The association between vascular AlIFA in the human internal thoracic artery (ITA) and various clinical parameters was studied with ITA obtained from 32 patients who underwent coronary artery bypass grafting. Only serum total and LDL cholesterol levels but no other risk factors significantly correlated with chymase-dependent (r=0.60, P<0.001) (Figure 2a) and ACE-dependent (r=0.35, P<0.05) AlIFA, respectively. The LDL cholesterol level was also correlated with chymase-dependent AlIFA (r=0.47, P<0.05) (Figure 2b). These data led us to hypothesize that an increased plasma LDL cholesterol level induces increased arterial chymase as well as ACE activity, which may in turn lead to arterial remodeling such as atherogenic changes.

Prevention of Aortic Lipid Deposit With an Orally Active Nonpeptidic Chymase Inhibitor
We examined in a hamster model what we found in human ITA, that is, the association between cholesterol and arterial chymase activity. When Syrian hamsters were fed a high cholesterol diet containing 0.5% cholesterol, a marked lipid deposition in the aortic cusp developed and the plasma cholesterol levels were positively correlated with aortic chymase activity. When an orally active nonpeptide chymase inhibitor (SUN-C8257) was given for 12 weeks together with a high cholesterol diet in this model, the aortic lipid deposition visualized by oil red O stain was almost canceled. On the other hand, the levels of systolic blood pressure and serum total and LDL cholesterol of these chymase inhibitor–treated hamsters did not change significantly. These facts indicated that the chymase inhibitor SUN-C8257 did not affect cholesterol absorption or blood pressure. Further study is necessary.
to elucidate the mechanism for the elimination of lipid deposit by a chymase inhibitor.

Pathological Involvement of Human Chymase in Atherogenesis

Our clinical and experimental data indicated a close association between cholesterol level and arterial chymase upregulation together with development of atherogenesis. Our current hypothesis is as follows (Figure 3): Hypercholesterolemia may activate adventitial mast cells containing chymase, which in turn release chymase and increase adventitial Ang II and interleukin-1β concentration. These cytokines are known to be proatherogenic and probably may facilitate intimal lipid deposition observed in our experimental model. In addition, chymase by itself is known to have a number of proatherogenic direct actions by degrading the extracellular matrix, modifying apolipoprotein AI or B, and producing endothelin (1-31). These direct actions of chymase may also cooperate, at least in part, in the atherogenic process.

Despite the considerable amount of evidence regarding pathophysiological roles of chymase, several investigators have suggested that unlike ACE, chymase contribution in the local Ang II formation in vivo is limited because of the existence of endogenous serine proteinase inhibitors in the interstitial fluid. It is obvious that chymase activity in plasma is completely inhibited by circulating endogenous serine proteinase inhibitors such as α2-macroglobulin and α1-anti-trypsin. On the other hand, a majority of tissue serine proteinases (chymase, kallikrein, and cathepsin G) are resistant to endogenous serine proteinase inhibitors, tissue plasminogen activator (t-PA), and α2-antiplasmin. Therefore, only a small portion of tissue serine proteinase activity is available to participate in blood pressure regulation, their main role appears to be associated with the development of structural tissue remodeling including post-myocardial infarction, atherosclerosis, and local inflammation.

Figure 3. Hypothesized schematic illustrations of cross-sectional arteries in physiological and atherosclerotic conditions. In hypercholesterolemia, arterial adventitial mast cell is activated and releases chymase. Chymase may be directly or indirectly involved in intimal lipid deposition, mediated through degradation of extracellular matrix, activation of IL-1β, or production of Ang II and endothelin (1-31). IL-1β indicates interleukin 1β; IEL, internal elastic lamina; EEL, external elastic lamina; and SMCs, smooth muscle cells.

References

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