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

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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 n angiotensin-converting enzyme n atherosclerosis n 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 atheromatus 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 antichymase, anti-trypase, or anti-ACE antibodies showed that chymase-positive mast cells were located in the tunica adventitia of normal and atheromatus aortas, whereas ACE-positive cells were localized in endothelial cells of normal aorta and in macrophages and smooth muscle cells of atheromatos neointima. The number of activated mast
cells in the aneurysmal lesions (18%) was significantly higher than in normal aorta. Chymase dAIIFA in atherosclerotic and aneurysmal lesions was significantly greater than in normal aorta. *P<0.01 vs total AIIFA in normal aorta; †P<0.01 vs chymase dAIIFA in normal aorta. This figure is modified from Reference 11.

**Development of Human Chymase ELISA and Arterial Chymase Levels**

We have produced a monoclonal antibody by immunizing Balb/c mice with recombinant human chymase purified from culture medium of Chinese hamster ovarian cells transfected by human chymase cDNA. Anti-human chymase polyclonal antibody was also produced by immunizing the same antigen to rabbits. With the use of these antibodies, a sandwich ELISA was developed. The detectable range of chymase concentration by this ELISA was 0.27 to 200 ng/mL. Because a significant correlation was found between the ELISA data and the enzymatic activity of chymase in homogenates of human artery, it appears that our monoclonal antibody against recombinant human chymase specifically recognizes native human chymase. Using this chymase ELISA, we analyzed arterial samples from many patients and found that regional differences in chymase levels of aortic branch arteries were not significantly varied, but they apparently differed by individual patients. Aortic chymase immunoreactivity was significantly higher in patients with macroscopic atherosclerotic lesions than without such lesions. These results indicate that high chymase levels in the arterial wall may be associated with the development of atherogenesis.12

**Positive Correlation of Arterial Chymase Activity and Plasma Total or LDL Cholesterol Levels**

The association between vascular AIIFA 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) AIIFA, respectively. The LDL cholesterol level was also correlated with chymase-dependent AIIFA (r=0.47, P<0.05) (Figure 2b).13 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.14 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.

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

**Figure 2.** Relation between serum total (a) and LDL (b) cholesterol concentration and chymase-dependent Ang II--forming activity in human internal thoracic artery. There was positive correlation between chymase-dependent AIIFA and total (n=32, r=0.60, P<0.001) and LDL (n=21, r=0.47, P<0.05) cholesterol concentration. ●, Pravastatin-treated patients. This figure is modified from Reference 13.
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 protease inhibitors such as α2-macroglobulin and α1–anti-trypsin. On the other hand, a majority of tissue chymase exists as a bound form by its basic ionic charge to the extracellular matrixes including proteoglycan or heparan sulfate. Since the bound-form chymase appears to be resistant to endogenous serine proteinase inhibitors, tissue chymase is likely to be enzymatically active to produce Ang II. Our recent observation supported this concept because treatment with an orally active chymase inhibitor decreased Ang II immunoreactivity in the adventitia of the hamster aorta, whereas those in the intima and media remained unchanged (Uehara and Urata, et al, unpublished observation, 2000). This result suggests that aortic chymase contributes adventitial Ang II formation in vivo.

In conclusion, several alternative Ang II–forming serine proteinases (chymase, kallikrein, and cathepsin G) are activated in several pathological conditions such as ischemia, hypercholesterolemia, hypertension, and local noninfectious inflammation. Since these alternative Ang II–forming enzymes appear not to play a major role 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.

References

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