Review

Tissue Angiotensin II Generating System by Angiotensin-Converting Enzyme and Chymase

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Abstract. It had been believed that angiotensin II (Ang II) was produced by the renin-angiotensin system (RAS), which was established in the 1950’s. After a while, people realized that the multiple functions of Ang II could not be explained by the conventional RAS. We have tried to determine the existence of the tissue Ang II generating system. At first, we found that vascular angiotensin-converting enzyme (ACE) was increased to generate local Ang II in the vessels of hypertension and was enhanced in lipid-loaded atherosclerosis, to respond to ACE inhibitor or Ang II antagonist (ARB). In both cases, Ang II production in vessels was independent from the systemic RAS that was estimated by the plasma renin activity. On the way to clarifying the roles of the vascular ACE, we noticed that vascular Ang II production was not completely suppressed by ACE inhibitor alone. This evidence led us to discover different types of chymase as a new Ang II producing enzyme. Now, we have obtained a strategy to distinguish the Ang II one by one, that is, circulating RAS derived, tissue ACE derived, and chymase derived. It is essential to understand not only the intracellular mechanisms of Ang II but also the process of Ang II productions in each disease to show accurate indications of the effectiveness of ACE inhibitor, ARB, and chymase inhibitor.

Keywords: renin-angiotensin system, angiotensin II, angiotensin-converting enzyme, chymase, angiotensin receptor blocker

Introduction

A number of articles on the biological actions of angiotensin II (Ang II), in particular those mediated by the Ang II type 1 (AT1) receptor subtype, have been reported. Many of these studies suggested the existence of local actions of Ang II that appear to differ from the pressor action induced by the conventional circulating renin-angiotensin system (RAS). On the other hand, evidence that administration of an angiotensin-converting enzyme (ACE) inhibitor or an angiotensin receptor blocker (ARB) protects against cardiovascular organ damage indirectly supports the role of Ang II at the tissue level. However, we should not necessarily connect the results obtained by direct and excessive administration of Ang II to cells or excessive amplification of Ang II by genetic manipulation to those obtained by administering drugs systemically. For this association to be made, there must be a pathophysiological mechanism connecting the two: a tissue Ang II generating system that differs from a miniature version of the conventional RAS.

Actions of Ang II in tissue

Ever since the concept of the RAS was established, Ang II has been known to mainly contract vascular smooth muscle cells by Ca2+ mobilization and to accelerate aldosterone secretion via effects on synthetic enzymes. However, it has been clarified that Ang II is also involved in organ damage, that is, pathologic tissue remodeling. In other words, Ang II induces cellular hypertrophy, proliferation and/or migration, and extracellular matrix proliferation. In this regard, detailed investigations have been conducted to examine the intra-
cellular signal transduction system downstream of the Ang II receptor. Many of the functions of Ang II have been confirmed in vitro by adding it directly to cells. However, these actions do not necessarily occur in vivo. In fact, diseases in which the level of Ang II is constantly elevated in the circulating blood are rare. Therefore, even if it were possible to force or eliminate gene expression induced by Ang II, this remains an assumption in vitro. It is therefore important to objectively determine the existence of the tissue Ang II generating system.

The existence of the tissue Ang II generating system has been suggested for some time. For example, the presence of renin in the brain was documented a long time ago (1). More recently, with advances in measurement technology, the various components of the RAS have been detected in different tissues. Based on those evidences, Dzau proposed the concept of the tissue RAS (2). While this concept is quite insightful, at that time, there was no proof for the functional existence of a miniature RAS in which renin acts as a rate-limiting factor and regulates Ang II production in tissues. We have been investigating Ang II-derived functions in vivo and the Ang II generating system for some time, and we have succeeded in identifying the existence of the Ang II generating system in tissue, particularly vascular tissue. Figure 1 is a diagram of the tissue Ang II generating system and the circulatory Ang II generating system based on the conventional RAS. In the latter system, renin originating from the kidney is a rate-limiting factor for Ang II generation in plasma, but in the former system, that is, in vascular tissue, ACE and chymase regulate Ang II generation. The role of renin in organs other than the kidney has yet to be clarified. It will accordingly be necessary to at least elucidate the roles of renin in tissue, although this will be difficult in vascular tissue.

Circulatory Ang II is thought to be mainly involved in maintaining physiologic blood pressure. Conditions in which physiologic role of Ang II is disrupted are relatively rare, and malignant renal hypertension with hyperreninemia is one such disease. This means that tissue Ang II, which is involved in essential hypertension, atherosclerosis, and even diabetes, is produced by the tissue Ang II generating system, which might not be directly controlled by renal renin. Therefore, the term “tissue RAS” is misleading because it suggest that renin plays a rate-limiting role, as occurs in the “systemic RAS”. In the tissues, ACE and/or chymase actually acts as a key factor for the production of Ang II.

**ACE-dependent tissue Ang II and related disorders**

*Ang II generation in hypertensive vessels*

Hypertension and atherosclerosis are two diseases involving the ACE-dependent Ang II generating system. First of all, how can pharmacologists interpret the evidence that ACE inhibitors are effective against essential hypertension even if plasma renin activity (PRA) is not high, suggesting that this effect is independent of the conventional RAS? This evidence can be evaluated using one of the following two assumptions: 1) that ACE inhibitors have additional hypotensive actions other than the suppression of Ang II production or 2) Ang II is produced via another unknown mechanism not depending on plasma renin. Table 1 summarizes

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**Fig. 1.** Tissue Ang II generating system.
findings from the viewpoint of the latter assumption (3–8). Spontaneous hypertensive rats (SHR) are thought to be an essential hypertensive model, and it was once thought that hypertension in SHR was mostly caused by genetic factors and therefore that the RAS was suppressed. However, once hypertension is established, even if PRA is low, ACE inhibitors effectively lower blood pressure. In SHR, studies have shown that only vascular ACE is elevated with the increase of Ang II concentration in vascular tissue, although there is no change in ACE in plasma and other tissues (4). Therefore, arteries not only respond to circulating Ang II, but also utilize vascular ACE to convert Ang I to Ang II and respond to this locally-produced Ang II. This is supported by the fact that isolated arteries in an artificial-nutrient solution contract in the presence of not only Ang II but also Ang I, an inactive peptide, and that this contraction is suppressed in the presence of an ACE inhibitor. In SHR, hypertension is maintained because ACE produces a sufficient amount of Ang II in vessels.

Furthermore, in two-kidney one-clip hypertensive rats (regarded as a model of RAS-dependent hypertension), hypertension in the acute phase is due to plasma Ang II produced by an increase in PRA, and the level of vascular ACE is low (6). In this scenario, hypertension is highly responsive to ACE inhibitors. However, in the chronic phase, PRA normalizes, but responsiveness to an ACE inhibitor is maintained with increased vascular ACE activity. In this manner, an ACE inhibitor lowers blood pressure only if vascular ACE is elevated without an increase in Ang II synthase, a chymase. When compared to ACE-related pathology, chymase-dependent pathology is even more localized, as described later. In addition, chymase inhibitors do not have a hypotensive effect (9). Indeed, ACE promotes both Ang II production and bradykinin degradation. On the other hand, ACE inhibitors enhance the hypotensive action of intravenous bradykinin, indicating that ACE-inhibitors have different hypotensive action other than the suppression of Ang II production. However, there is no strong evidence to prove that suppression of the kinin-system is the universal cause of hypertension. Moreover, animal studies have not shown marked differences in hypotensive action between ACE inhibitors and ARB (5).

**Vascular ACE-dependent Ang II as a factor in the onset and development of atherosclerosis**

ACE-dependent local generation of Ang II plays an important role in lipid deposition into the vascular wall, a trigger for atherosclerosis.

In monkeys, a high-fat diet increases the level of total cholesterol and low-density lipoprotein (LDL) cholesterol and decreases the level of high-density lipoprotein (HDL) cholesterol in plasma. In six months, atherosclerotic lesions were widely formed in the carotid and aorta. Furthermore, in these lesions, ACE activity and Ang II receptor expression were elevated. When a high-fat diet was combined with either ACE inhibitor or ARB administration, the extent of atherosclerosis decreased significantly (10–12) (Fig. 2). Fat loading did not influence PRA. Therefore, Ang II might have been involved at the tissue level. Moreover, because the monkeys were normotensive models, the effects on blood pressure were minimal; therefore, hypotension might be not involved. These two Ang II suppressors prevented progression of atherosclerosis without either lowering plasma lipid levels or blood pressure levels. Increased Ang II generation via vascular ACE in situ facilitates lipid uptake by the vascular wall, and this notion that Ang II suppression in vasculatures hinders lipid intake is totally new in the treatment of atherosclerosis. We have proved this point using monkey models but not ApoE-knock-out mice.

Considerable data exists to support the relevance of Ang II to the intra-cellular events in atherosclerosis as shown Fig. 3. Macrophages (Mφ) are one of the major players in atherosclerosis; these cells form vascular lesions when they absorb oxidized denatured LDL (oxLDL) and then facilitate foam cell formation. Such
Mφ that have invaded vascular tissue express not only ACE (13), but also angiotensinogen, renin, and AT1 receptor (14, 15). It is may be possible that such Mφ independently generate Ang II. Furthermore, Ang II activates 15-lipoxygenase in Mφ and supplies reactive oxygen to the vascular wall to facilitate conversion from LDL to oxLDL (16, 17). The reactive oxygen induces the production of intracellular adhesion molecule (ICAM)-1, thus resulting in monocyte adhesion and infiltration into the vascular wall. In vascular endothelial cells, Ang II elevates the generation of reactive oxygen and the genetic expression of various subunits of the component proteins of NAD(P)H oxidase, thus inducing endothelial dysfunction by reactive oxidase (18). From the viewpoint of endothelial injury, Mφ-derived reactive oxygen contributes to endothelial damage by suppressing endothelial nitric oxide synthase (eNOS). In atherosclerotic lesions, oxLDL uptake by endothelial cells is mediated by the lectin-like oxLDL (LOX-1), and Ang II increases the
level of this protein (19, 20). However, in patients taking ACE inhibitors, the expression of this protein is lowered. Monocyte chemoattractant protein (MCP-1) is also important for monocyte migration, activation, and Mφ differentiation, and Ang II has been shown to induce MCP-1 via nuclear factor-κB (NF-κB) (21).

The importance of neovascularization as an onset factor for atherosclerosis has also been reported. In human coronary atherosclerotic lesions, the expression of vascular endothelial growth factor (VEGF), a neovascularization accelerator, correlates to the severity of atherosclerosis (22). Ang II is not only a potent inducer of VEGF, but also activates matrix metalloproteinase (MMP), which destroys tissue structure to allow neovascularization (23). Therefore, Ang II is an essential factor for the pathogenesis of atherosclerosis and this definitely indicates the pharmacological mechanism of ACE inhibitors and ARBs.

In recent years, we have attempted to evaluate the potential of ARB not only for prevention but also for cure of atherosclerosis (24). After inducing atherosclerosis by loading monkeys with a high fat diet for six months, ARB was administered while continuing the high fat diet for additional six months. ARB administration not only ceased further development but also markedly alleviated atherosclerosis at the end of this six-month period. Hence, ACE inhibitors and ARBs are novel antiatherogenic agents that are effective without lowering plasma lipid level and therefore act in a different manner to conventional antiatherogenic agents that have lipid lowering action. However, concerning the use of these agents, it is unfortunate that no diagnostic indicators exist that are more convenient, such as plasma lipid level for lipid lowering agents, even if they do not reflect the direct effects. However, with the recent rapid advances in diagnostic imaging, it may be possible to confirm the direct effectiveness of ACE inhibitors and ARBs on tissue lesions in the very near future.

**Chymase-dependent tissue Ang II and related pathology**

While conducting experiments on ACE inhibitors using excised canine arteries, we noticed that an ACE inhibitor alone could not completely abolish the contractile reaction of Ang I. This contraction remained stronger in human vessels than canine vessels. Although the fluid in which vessels were soaked contained excessive amounts of ACE inhibitor, an Ang II peak continued to be detected by liquid chromatography. However, ARB (called Ang II analogue at the time) completely suppressed both contraction and Ang II generation, respectively. Moreover, when an ACE inhibitor was combined with chymostatin (chymotrypsin-like enzyme inhibitor), Ang I contraction, and Ang II generation were completely suppressed. This showed for the first time that an enzyme other than ACE was involved in vascular Ang II generation in situ, and we tentatively named this enzyme chymostatin sensitive angiotensin generating enzyme (CAGE); this enzyme is widely cited in many text books even now (25). While the identity of CAGE was not known at the time, it was discovered several years later that chymase extracted from human heart, an enzyme produced by mast cell, converted Ang I to Ang II (26, 27). It was later confirmed by us that vascular CAGE is also chymase (28).

As far as the function of chymase-derived Ang II is concerned, we were certain of its biological significance because we detected its ability to trigger vascular contraction, although we did not investigate in vitro enzyme activity. However, it took some time before the related disease was identified. In coronary stenosis, an ACE inhibitor was effective in the rat model (29) but was ineffective against restenosis following PTCA in humans (30). These findings prompted us to look for and subsequently find elevation of vascular chymase activity in a vascular stenosis canine model (31). Chymase is a serine protease that is synthesized in mast cell granules and released locally from mast cells that had migrated due to various inflammatory stimuli. The first documented action of human chymase is Ang II generation. Marked inter-species difference are evident and rat and rabbit chymases do not produce Ang II. Among different species, including the monkey, dog, and hamster, Ang II generating potency is highest in humans (26, 32). Therefore, the finding that there was a difference in Ang II generation between humans and rats indicated the involvement of an Ang II producing chymase. When the carotid artery of dogs was damaged using a balloon, luminal stenosis and chymase activity were specifically elevated.

ARB suppresses all AT1-Ang II receptors. Therefore, it may be possible to estimate the function of chymase from the difference between the actions of ACE inhibitor and ARB. As chymase inhibitors effective in vivo application, became available, further investigations in different experimental models were developed. Table 2 lists the diseases that have been identified so far. However, our results differ partially from results reported generally in human studies using ACE inhibitors or ARBs. The functional difference between the two drugs are still unclear clinically. Our results in Table 2 obtained from precise experiments using firstly chymase inhibitor suggested the possible differentiation
of drug actions between ACE inhibitors and ARBs.

Chymase-related pathology seems more local when compared to ACE-related models because chymase originates from mast cells. Forced expression of the chymase gene in vessels can increase blood pressure, but this does not mean that chymase is involved in blood pressure regulation. It is obvious that uncontrolled expression of Ang II producing enzyme is not different from systemic Ang II infusion. It has recently been reported that the Ang II generating chymase gene is expressed in rat vascular tissues. In this regard, we also confirmed that Ang II producing chymase is high in lung arteries of drug-induced pulmonary hypertensive rats (33).

While the pathophysiology of Ang II in the heart has been widely discussed, it has not been clarified whether Ang II suppressors, including ACE inhibitors and ARBs, directly affect the heart or indirectly influence it by improving hemodynamics. Chymase-derived Ang II is undoubtedly involved in the tissue fibrosis associated with cardiomyopathy (34, 35). In addition, in the acute myocardial infarction model, chymase inhibitor and ARB improved survival rate to a comparable degree (36). In a cardiac infarction model, arrhythmia frequently occurs soon after coronary ligation, and since chymase inhibitor drastically reduces the frequency of the onset of arrhythmia (37), it may be involved in tissue Ang II generation in the heart, particularly via chymase.

The notion that chronic tissue remodeling is a type of inflammation is accordingly gaining more support. Hence, chymase may be involved in heart failure, although there is not enough data to clarify this point.

**Conclusion**

The present article dealt with the existence and function of the tissue Ang II generating system, mainly in vascular tissue. The most important point of this article is that the tissue system is completely different from the conventionally systemic RA system and also from the postulated tissue RAS because of the involvement of chymase. Ang II administration and modification of certain protein genes do not represent the exact roles of Ang II in the living body, particularly in humans. When discussing certain pathologic functions in the experimental setting, it is essential to show objectively how Ang II can be generated physiologically in that tissue.

We have to continue to elucidate the individual role of ACE and chymase in multiple diseases to find additional and accurate indications for the use of ACE inhibitors, ARBs, and chymase inhibitor.

**Table 2. Comparison of effects of three different Ang II suppressants in animal models**

<table>
<thead>
<tr>
<th>Disease</th>
<th>ACE inhibitor</th>
<th>ARB</th>
<th>Chymase inhibitor</th>
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<tbody>
<tr>
<td>Hypertension</td>
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<td>O</td>
<td>X</td>
</tr>
<tr>
<td>Atherosclerosis</td>
<td>O</td>
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<td>X</td>
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<td>Restenosis PTCA</td>
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<tr>
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<td>Adhesion</td>
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