Contributions of ACE and mast cell chymase to endogenous angiotensin II generation and leucocyte recruitment in vivo

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Aims

In vitro studies suggest that mast cell chymase (MCP) is more important than angiotensin-converting enzyme (ACE) for generating angiotensin II (Ang II) within the cardiovascular system. We investigated in vivo the relative contributions of ACE and MCP to leucocyte recruitment induced by endogenously generated Ang II.

Methods and results

Exposure of the murine cremasteric microcirculation of C57BL/6 mice to Ang I (100 nM for 4 h) induced leucocyte–endothelium interactions. Either losartan (an Ang II receptor-1 antagonist, AT1) or enalapril (an ACE inhibitor), but not chymostatin (a chymase inhibitor), inhibited Ang I-induced responses. Mast cell degranulation with compound 48/80 (CMP48/80, 1 μg/mL) also induced leucocyte adhesion but this was only weakly affected by the inhibitors.

When Ang I and CMP48/80 were co-administered, AT1 receptor expression was increased, MCP-4 was found surrounding the vessel wall, and ACE was detected in the endothelium. Ang I + CMP48/80 induced enhanced leucocyte adhesion that was attenuated by losartan, enalapril, enalapril + chymostatin, and cromolyn (a mast cell stabilizer). The use of male mast cell-deficient WBB6F1/J-Kit+/Kitw− mice (C57BL/6 background) confirmed these findings.

Conclusion

In vivo, Ang II is primarily generated by ACE under basal conditions, but in inflammatory conditions, the release of MCP amplifies local Ang II concentrations and the associated inflammatory process. Thus, AT1 receptor antagonists may be more effective than ACE inhibitors for treating ongoing Ang II-mediated vascular inflammation.

Keywords

Angiotensin-I • ACE • Mast cells • Chymase • Leucocytes • Vascular inflammation

1. Introduction

Endothelial dysfunction leads to a proinflammatory and prothrombotic phenotype of the endothelium1 and provokes the attachment and the subsequent migration of leucocytes. Angiotensin II (Ang II), the main effector peptide of the renin–angiotensin system, is implicated in endothelial dysfunction beyond its haemodynamic effects.2 In this context, we have demonstrated that 4 h exposure to Ang II in vivo causes arteriolar mononuclear leucocyte adhesion in the rat mesenteric microcirculation3 and this event is partly mediated through the generation and release of tumour necrosis factor-α and different CXC and CC chemokines.4–6

Angiotensin-converting enzyme (ACE) is well known to catalyse the conversion of Ang I to Ang II. However, previous in vitro studies have suggested the existence of ACE-dependent and -independent pathways in the formation of Ang II. The ACE-independent production of Ang II is mainly catalysed by serine proteases, the major contributor being chymase. These observations were made by virtue of the fact that chymostatin, a relatively specific inhibitor of chymase, attenuates the functional response to Ang I.7–13 In vitro, this enzyme can account for as much as 80–90% of Ang I conversion to Ang II in the heart10,11 and in human arteries, whereas ACE-inhibitors are relatively ineffective.14–17

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In human vascular tissues, ACE is expressed mainly in endothelial cells, whereas chymase is stored in a macromolecular complex in secretory granules of mast cells. Total Ang II-forming activity is significantly higher in atherosclerotic and aneurysmal lesions than in normal human aortas. In this context, ACE is found in macrophages of atheromatous neointima and both the number of activated mast cells and the chymase activity are increased in atherosclerotic lesions. In animal models, a chymase inhibitor was shown to significantly suppress the raised blood pressure following myocardial infarction. Chymase-generated Ang II has also been implicated in the acute constriction of resistance arterioles in response to lipopolysaccharide. Therefore, although ACE seems to be the main enzyme involved in Ang II generation in both basal and hypertensive states, different studies have indicated that mast cell chymase (MCP) may be another relevant enzyme implicated in the synthesis of this peptide hormone within the injured vasculature.

Because in vitro studies using chymostatin in tissue homogenates and isolated blood vessels have pointed out the involvement of MCP in Ang II generation, these methodological approaches may detect increased activity that actually happens in vivo. Indeed, these experimental approaches have led to cast doubt on the functional significance of the MCP. In vivo, MCP is normally not secreted unless mast cells are stimulated. Although chymase is partially sensitive to its natural inhibitors present in the extracellular fluid, heparin binding can protect the enzyme from this inhibitory activity. Hence, the real contribution of chymase activity in vivo systems requires to be established.

The present study is the first report, using an in vivo pharmacological approach, that has systematically characterized the relative contribution of ACE vs. chymase to the proinflammatory activity of endogenously generated Ang II. Intravital microscopy within the murine cremaster microcirculation was used to determine leukocyte-endothelial cell interactions induced by the conversion of Ang I to Ang II. Additionally, since chymase activity is tightly controlled in normal tissues, its activity being much more apparent in diseased vascular tissues, compound 48/80 (CMP48/80) was used to induce mast cell degranulation and chymase activation.

2. Methods

2.1 Animals

The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health and was approved by the ethics review board of the Faculty of Medicine, University of Valencia. C57BL/6 mice were from Charles River Laboratories (Barcelona, Spain). Male mast cell-deficient WBB6F1/J-Kit−/−Kit+ mice (background, C57BL/6) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Animal colonies were bred and maintained under specific pathogen-free conditions. For all the experimental period, the mice were fed with autoclaved balanced diet and water. All the protocols used in this study were approved by the Animal Care and Use Committee, Faculty of Medicine, University of Valencia. The animals used were 22–30 g weight.

2.2 Intravital microscopy

The mouse cremaster preparation used in this study was similar to that described previously. Mice were anaesthetized by ip injection with a mixture of xylazine hydrochloride (10 mg/kg) and ketamine hydrochloride (200 mg/kg). Additional anaesthetic (30 μL, iv) was administered every 20 min to maintain profound anaesthesia. A polyethylene catheter was placed in the jugular vein to permit the iv administration of additional anaesthetic which was administered every 2 h until the end of the experiment (4.5 h), and then animals were humanely euthanized by anaesthetic overdose. The cremaster muscle was dissected free of tissues and exteriorized onto an optical clear viewing pedestal. Further details of the technique can be found in the Supplementary material online.

2.3 Experimental protocol

In initial experiments, a range of doses of Ang I (1, 10, and 100 nM), Ang II 100 nM, or CMP48/80 (1 μg/mL) in 200 μL sterile saline was injected under the scrotal skin adjacent to the cremaster muscle. Intracrotal injection localizes the stimulus solution beneath the scrotal skin directly adjacent to the cremaster muscle and results in the stimulus solution directly bathing the cremaster tissue. Leucocyte accumulation was assessed using intravital microscopy 4 h later. Significant arteriolar leucocyte accumulation was observed at 100 nM of Ang I, so all additional experiments were performed using this dose. For these and all subsequent experiments, including those using gene-deficient mice, control animals were injected with the same volume of sterile saline and examined 4 h later.

In other experiments, mice were pre-treated with an Ang II AT1 receptor antagonist, losartan (10 mg/kg, ip), with an ACE-inhibitor, enalapril (30 mg/kg, po), or with a chymase inhibitor, chymostatin (10 mg/kg, ip) 30 min before Ang I (100 nM) or CMP48/80 (1 μg/mL) intracrotal administration. Mepyramine (3 mg/kg), an H3 histamine receptor antagonist, was also administered 30 min before intracrotal CMP48/80 (1 μg/mL). Leucocyte responses were evaluated 4 h after stimulus administration. The doses of losartan, enalapril, and chymostatin were proved in previous in vivo studies to inhibit Ang II-induced responses, ACE, and chymase activity, respectively. Another group of animals were intracrotally injected with a combination of both Ang I (100 nM) and CMP48/80 (1 μg/mL) and the responses compared with those obtained with the administration of either stimulus alone. In subsequent experiments, animals were pre-treated with losartan, enalapril, chymostatin, or with a combination of enalapril and chymostatin 30 min before the intracrotal administration of both stimuli.

In another set of experiments, some animals were pre-treated with cromolyn, a mast cell stabilizer agent. It was given at 100 mg/kg, ip 30 min before and 3.5 h after the intracrotal stimuli administration. Additionally, 0.33 mg/mL was added to the intravital superfusate as described previously. To extend the investigation of a mast cell contribution to leucocyte adhesion responses, saline, Ang I, CMP48/80, or the combination of Ang I + CMP48/80 was administered to mast cell-deficient WBB6F1/J-Kit−/−Kit+ mice and responses evaluated 4 h later. Some of these animals were also pre-treated with cromolyn before intracrotal administration of the stimulus.

2.4 Histology, immunohistochemistry, and immunofluorescence

After the completion of the intravital microscopy measurements, the cremaster muscle was isolated, fixed in 4% paraformaldehyde, dehydrated using graded acetone washes at 4°C, and embedded in paraffin wax for detecting degranulated mast cells and the immunolocalization of ACE and MCP-4 (mMCP-4). The details can be found in the Supplementary material online.

2.5 Expression of AT1A and AT1B receptors and ACE by real-time PCR

For AT1A and AT1B receptors and ACE expression in mouse cremaster, total mRNA was isolated using TRIzol reagent. AT1A and AT1B receptors
and ACE mRNA levels were measured using the primers and the protocol described in the Supplementary material online.

2.6 Measurement of ACE- and chymase-dependent Ang II-forming activities in the murine cremaster and peritoneal macrophages

Ang II-forming activity in the cremaster homogenate and peritoneal macrophages were determined as described previously.\(^{30,31}\) The details can be found in the Supplementary material online.

2.7 Statistical analysis

All values are reported as mean $\pm$ SEM. Data within groups were compared using an analysis of variance (one-way ANOVA) with a Newman–Keuls post hoc correction for multiple comparisons. Statistical significance was set at $P < 0.05$.

2.8 Materials

Ang I, Ang II, CMP48/80, captopril, chymostatin, cromolyn, enalapril, ethidium bromide, and mepyramine were purchased from Sigma Química (Madrid, Spain). Losartan was kindly donated by Merck Sharp and Dohme, Madrid, Spain. The rabbit anti-mouse mMCP-4 antibody was a generous gift of Dr Guo-Ping Shi (Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA, USA). Other materials used are listed in the Supplementary material online.

3. Results

We used intravital microscopy to examine leucocyte–endothelial cell interactions within the mouse cremasteric microcirculation. In initial experiments, exposure to Ang I (100 nM for 4 h) induced a significant enhancement of arteriolar leucocyte adhesion ($\text{Figure 1A}$), venular leucocyte adhesion ($\text{Figure 1B}$), and a concomitant decrease in leucocyte rolling velocity ($\text{Figure 1C}$), whereas lesser responses were elicited by 1–10 nM Ang I. Leucocyte emigration followed a similar pattern to that observed for leucocyte adhesion (data not shown). For comparison, Ang II (100 nM) induced responses of similar magnitude to an equal dose of Ang I ($\text{Figure 1}$). The mast cell degranulating agent, CMP48/80 (1 $\mu$g/mL), also induced significant leucocyte–endothelial cell interactions in both arterioles and venules. None of these stimuli at the doses tested caused any effect on arteriolar or venular shear rate (see Supplementary material online, Table S1).

In order to investigate the possible involvement of ACE and MCP in the leucocyte responses elicited by Ang I, we first established that the effects induced by this peptide were due to its endogenous conversion to Ang II. Pre-treatment with the Ang II AT$_1$ receptor antagonist, losartan, reduced the Ang I-induced leucocyte adhesion to arterioles and post-capillary venules to basal levels ($\text{Figure 2A and B}$). These responses were also inhibited, by 88 and 73%, respectively, in animals pre-treated with the ACE-inhibitor enalapril but not with the MCP inhibitor, chymostatin ($\text{Figure 2A and B}$). Arteriolar and venular leucocyte adhesion responses to CMP48/80 were not significantly inhibited by losartan, enalapril, chymostatin, or mepyramine ($\text{Figure 2D and E}$). Thus, ACE appears to be responsible for the majority of the Ang II-induced leucocyte adherence in vivo, whereas leucocyte responses to mast cell degranulation appear to be independent of Ang II generation and histamine release. However, losartan and chymostatin did attenuate the decrease in leucocyte rolling velocity induced by CMP48/80 ($\text{Figure 2F}$).

In light of the latter findings, a small contribution of mast cell proteases to the endogenous generation of Ang II cannot be discarded. To test this, we co-administered Ang I $+$ CMP48/80 which significantly increased the arteriolar and venular leucocyte adhesion when compared with the effects elicited by either stimulus alone ($\text{Figure 3A}$), although no further reductions in leucocyte rolling velocity were detected ($\text{Figure 3Ac}$). We also examined the effect of ACE or chymase inhibition on the Ang II-forming activity of cremasteric homogenates and mouse peritoneal macrophages. In these in

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**Figure 1** Effects of Ang II, Ang I, and CMP48/80 on arteriolar leucocyte adhesion ($A$), venular leucocyte adhesion ($B$), and venular leucocyte rolling velocity ($C$) within the cremasteric microcirculation. Results are mean $\pm$ SEM of $n = 5–6$ animals per group: $**P < 0.01$ relative to the saline group.
in vitro analyses, the contribution of chymase to Ang II generation was greater than that of ACE in both the cremasteric tissue and mouse peritoneal macrophages (Figure 3B). Ang II AT_{1A} receptor and ACE expression were unchanged in different experimental groups; however, increased AT_{1B} receptor expression was observed in the groups injected with Ang II or with the combination Ang I + CMP48/80, but not with Ang I alone, indicating that Ang II and Ang I + CMP48/80 have similar actions in this particular effect (Figure 3C). We next performed an immunohistochemical analysis of the cremasteric microcirculation of different experimental groups. All groups showed clear expression of ACE within the vascular lumen (Figure 4). Ang I did not induce mast cell degranulation and did not affect the number of mast cells degranulated in response to CMP 48/80 (Supplementary material online, Figure S1A and Figure 4 where degranulated mast cells are indicated by red arrows). Whether mast cell degranulation is partial or total is difficult to distinguish by the technique employed. In the animals exposed to CMP48/80, the expression of murine MCP-4 (mMCP-4) was located surrounding the vessel wall (Figure 4).

In a separate set of experiments, we examined the possible contributions of ACE and mast cell proteases to the responses to the combination of Ang I + CMP48/80. The AT_{1} receptor antagonist losartan inhibited leucocyte adhesion to arterioles and post-capillary venules, by 74 and 81%, respectively, and reversed the decrease in leucocyte rolling velocity (Figure 5). The ACE-inhibitor enalapril also inhibited leucocyte adhesion and reversed the changes in leucocyte rolling velocity, but to a lesser extent than in the losartan-treated group (Figure 5). In contrast, chymostatin did not significantly affect the leucocyte adhesion provoked by Ang I + CMP48/80 but reversed the decrease in leucocyte rolling velocity (Figure 5). Interestingly, the combination of enalapril and chymostatin was more effective than either inhibitor alone; indeed, the inhibitory effects were similar to those

Figure 2 Effect of losartan, enalapril, and chymostatin on arteriolar leucocyte adhesion (A and D), venular leucocyte adhesion (B and E), and venular leucocyte rolling velocity (C and F) induced by Ang I or CMP48/80. Results are mean ± SEM of n = 5–10 animals per group: *P < 0.05 or **P < 0.01 relative to the saline group; †P < 0.05 or ‡P < 0.01 relative to the Ang I or CMP48/80 group.
seen in the losartan-treated group (Figure 5). These results again suggest that both ACE and mast cell proteases contribute to the endogenous conversion of Ang I to Ang II.

These results led us to test the effects of the mast cell-stabilizing agent, cromolyn, on the leucocyte responses induced by the combination of Ang I + CMP48/80. We first established that cromolyn clearly decreased the number of degranulated mast cells induced by Ang I + CMP48/80 (see Supplementary material online, Figure S1B). Cromolyn inhibited the arteriolar and venular leucocyte adhesion responses to Ang I + CMP48/80 by 88 and 38%, respectively (Figure 6A and B). However, cromolyn had no effect on the reduction in leucocyte rolling velocity induced by Ang I + CMP48/80 (Figure 6C). Ang I + CMP48/80-induced leucocyte adhesion to both arterioles and venules was significantly reduced in mast cell-deficient WBB6F1/J-Kitw/Kitw-v mice compared with wild-type mice from the same background. Interestingly, although cromolyn had no effect in the post-capillary venules of mast cell-deficient mice, as expected, it further reduced the arteriolar leucocyte adhesion elicited by Ang I + CMP48/80 (Figure 6). Finally, the immunohistochemical analysis of the cremasteric microcirculation of the mast cell-deficient mice revealed the expression of ACE within the vascular lumen of all experimental groups (see Supplementary material online, Figure S2).

No mast cells were encountered in the cremasteric microcirculation of mast cell-deficient animals (see Supplementary material online, Figure S2) and no mMCP-4 expression was detected when these animals were injected with CMP48/80.

4. Discussion

Previous in vitro studies have suggested the existence of ACE-dependent and -independent pathways in the conversion of Ang I to Ang II within the cardiovascular system. As previously described, some in vitro studies have raised some concerns due to the potential mast cell activation that may occur manipulating the vessels in organ baths for vascular reactivity determinations leading to increased chymase activity. In addition, there are controversial data regarding the functional role of MCP in vivo.16,19,23,30,32 Until now, the significance of these pathways in vivo has not been fully established.

In the present study, we demonstrate that intrascrotal injection of Ang I induced leucocyte–endothelial cell interactions that were mediated by endogenously generated Ang II since losartan, a specific AT1 receptor antagonist, almost completely inhibited the responses. In the absence of mast cell degranulation, the endogenous generation of Ang II was mainly due to the action of ACE, because it was inhibited
Figure 4 Effects of Ang I, CMP48/80, and Ang I + CMP48/80 on ACE expression, mast cell degranulation, and mMCP-4 expression within the cremasteric microcirculation. Brown reaction product indicates positive ACE localization. Red arrows show degranulated mast cells. Green fluorescence surrounding the vessel indicates mMCP-4 expression (white arrows). Black arrows show the vessel under investigation.

Figure 5 Effects of losartan, enalapril, chymostatin, and the combination of enalapril + chymostatin on arteriolar leucocyte adhesion (A), venular leucocyte adhesion (B), and venular leucocyte rolling velocity (C) induced by Ang I + CMP48/80 within the cremasteric microcirculation. Results are mean ± SEM of n = 8–10 animals per group. *P < 0.05 or **P < 0.01 relative to the saline group; †P < 0.05 or ‡P < 0.01 relative to the Ang I + CMP48/80 group. &P < 0.05 or &&P < 0.01 relative to the enalapril-treated group; ∆P < 0.05 or ∆∆P < 0.01 relative to the chymostatin-treated group.
by enalapril, rather than to chymase or other mast cell proteases that are inhibited by chymostatin. These results are in agreement with those found in a recent study in which ACE is the key Ang II-forming enzyme in the left ventricular interstitial fluid of conscious mice.29

Chymase is stored as an inactive macromolecular complex in the secretory granules of mast cells and exhibits enzymatic activity immediately following its release into the interstitial tissues after mast cell stimulation (e.g., vascular injury by a balloon catheter or in grafted veins).19,20,23 Mast cell degranulation with CMP48/80 induced leucocyte–endothelial interactions that were largely independent of Ang II and may be explained by mediators such as platelet-activating factor, cytokines, and chemokines, which are released from mast cells by CMP48/80.29,33,34 Although histamine contributes to CMP48/80-induced leucocyte rolling flux in the rat mesenteric microcirculation,29 due to a rapid P-selectin expression, we did not detect any inhibitory effect of mepyramine (an H1 histamine receptor antagonist) in the mouse cremasteric microcirculation where P-selectin is constitutively expressed. We suggest that the effects of losartan and chymostatin on CMP48/80-induced leucocyte rolling velocity are probably due to their effects on Ang II-induced E-selectin and vascular cell adhesion molecule-1 expression.33,35,36 In this regard, Yusof et al.21 suggested that Ang II, formed by the enzymatic activity of both ACE and chymase, played an important role in inducing leucocytic rolling and adhesion in post-ischaemic intestinal venules. However, although chymostatin administration inhibited ischaemia–reperfusion-induced leucocyte adhesion in their study, we could only detect a moderated role for chymostatin at reversing the decrease in the leucocyte rolling velocity after intrascrotal administration of CMP48/80. On the other hand, co-administration of Ang I with CMP48/80 induced enhanced leucocyte–endothelial cell interactions, compared with responses to either stimulus alone. In this situation, both chymase and ACE contributed to the responses, which were predominantly Ang II-mediated.

Although a previous study has found the lack of effect of arterial-delivered Ang I on coronary reversibility in rats where mast cell degranulation was induced by iv administration of CMP48/80,37 we found increased leucocyte–endothelial cell interactions when both stimuli were co-administered. These discrepancies might be explained by species differences. In this context, although in humans, α-chymase is the major non-ACE Ang II generator, in rats, β-chymases destroy Ang II by cleaving at Tyr(4)–Ile(5). In contrast to rat MCP-1, mMCP-4, a β-chymase, readily generates Ang II from Ang I.30,38,39

Ang II stimulates two main receptors, AT1 and AT2, virtually all of its physiological actions being mediated by AT1. Rodents have two AT1 receptor subtypes, AT1A and AT1B, which are 95% identical in their amino acid sequence.40 Ang II, acting at AT1, can induce further AT1 receptor expression.41 We found that both Ang II and the combination of Ang I + CMP48/80, but not Ang I alone, significantly increased mRNA expression of the AT1B receptor. Taking into consideration the known AT1B receptor-inducing effect of Ang II, further support is added to the contribution of MCP to the increased endogenous generation of Ang II in this model. The predominant chymases expressed in the mouse, at least in the aorta, are those for types 4 and 5 isoforms and both are efficient Ang II-forming enzymes.30,38,39 Expression of mMCP-4 was detected surrounding the vessel in those animals injected or co-injected with CMP48/80. In contrast, ACE expression was restricted to the endothelium and increased ACE mRNA expression was not observed in different experimental groups, albeit in some in vitro studies, different stimuli can increase ACE expression in different cell types.42 Both ACE- and chymase-dependent Ang II-forming activities were detected in cremaster homogenates in vitro. However, in contrast to the

Figure 6 Effects of cromolyn on arteriolar leucocyte adhesion (A), venular leucocyte adhesion (B), and venular leucocyte rolling velocity (C) induced by Ang I + CMP48/80 within the cremasteric microcirculation of wild-type and mast cell-deficient mice. Results are mean ± SEM of n = 5–12 wild-type mice per group and n = 6 of mast-deficient mice per group. *P < 0.05 or **P < 0.01 relative to the respective treatment in wild-type mice.

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important role of ACE in vivo, particularly under basal conditions, the potential contribution of chymase released from mast cells was clearly demonstrated by its predominance in vitro.

When animals were pre-treated with cromolyn, which inhibited mast cell degranulation, leucocyte adhesion in response to Ang I + CMP48/80 was reduced. Interestingly, this effect was more dramatic in the arterioles (88% inhibition) than in the post-capillary venules (38% inhibition). This may be a result of the periarteriolar abundance of mast cells which has been reported in a number of tissues and species, including the mouse.13 Since a similar effect to just Ang I injection was expected, this result led us to test responses to Ang I + CMP48/80 in mast cell-deficient WBB6F1/J-Kit+/Kit− mice in which the mutation does not affect the number or function of blood leucocytes.20 Although ACE was present in the microvasculature, no mast cells or chymase expression was detected in these animals. There was a significant reduction in the leucocyte responses compared with that in their wild-type littermates. Indeed, the responses in the mast cell-deficient mice were similar to those to Ang I alone. It is noteworthy that in the absence of mast cells, pre-treatment with cromolyn did not further reduce the Ang I + CMP48/80-induced leucocyte adhesion to the post-capillary venules, whereas arteriolar adhesion was severely blunted. Therefore, it seems that the effects of cromolyn in arterioles are beyond its mast cell-stabilizing activity, showing a potential use in the prevention of vascular inflammation at the arterial level.

We conclude that in the absence of mast cell activation, endothelial ACE is the major Ang II-forming enzyme in the microcirculation in vivo. However, when mast cells are activated which may occur under pathological conditions, ACE still plays a role but the release of extracellular MCP can enhance the production of Ang II. The Ang II induces inflammatory leucocyte recruitment, including that of mononuclear cells in the arteriolar wall, and as such may contribute to the onset and progression of the endothelial dysfunction that precedes the atherogenic process. Indeed, in contrast to in vitro studies, neither ACE inhibition nor chymase inhibition alone is particularly effective at blocking Ang I-mediated inflammatory responses during mast cell degranulation in vivo. However, the combined inhibition of ACE and chymase inhibits leucocyte—endothelial cell interactions to the same extent as when using an antagonist of the Ang II AT1 receptor. Although this combination was found to improve several cardiac functions after myocardial infarction in hamsters compared with ACE inhibition alone,22 the potential beneficial effect of Ang II AT1 receptor antagonist administration has not been investigated. Since some clinical trials have revealed significant benefit of a pharmacological regimen using AT1 receptor antagonist compared with treatment with ACE-inhibitors in disorders that specially have an inflammatory component,44–46 it is tempting to speculate that these effects are due to incomplete blockade of Ang II-induced responses, regardless of the enzymatic pathway involved in its generation. Thus, we suggest that Ang II AT1 receptor antagonists may be more effective than ACE-inhibitors at controlling ongoing vascular inflammation when Ang II-dependent components of atherogenesis are involved.

Supplementary material
Supplementary material is available at Cardiovascular Research online.

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