Marked Species-Difference in the Vascular Angiotensin II-Forming Pathways: Humans versus Rodents

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ABSTRACT—Using isolated arteries, we demonstrated a marked difference in the angiotensin II-forming systems between human and rodent vessels. In human arteries, only 30–40% of the conversion of angiotensin I to angiotensin II depended on the angiotensin-converting enzyme (ACE), and the rest of the angiotensin II formation was ascribed to chymostatin-sensitive angiotensin II-generating enzyme (CAGE). On the contrary, angiotensin II formation in rodent arteries totally depended upon ACE, without any sign of CAGE involvement. Such a marked species-difference can be relevant to the reported difference between humans and rodents in the ACE inhibitor effects on the myointimal hyperplasia after intimal balloon injury.

Keywords: Captopril, Chymostatin, Chymase

In blood vessels of humans, monkeys, and dogs, we demonstrated the presence of a unique enzyme that converts angiotensin (ANG) I to ANG II but is different from angiotensin-converting enzyme (ACE, EC. 3.4.15.1) (1–3). The newly found enzyme, designated as chymostatin-sensitive angiotensin II-generating enzyme (CAGE), is a mast cell chymase (EC. 3.4.21.39)-like protease (1, 2), located in the adventitia of the above-mentioned species (2), and it is inhibited by chymostatin, but not inhibited by captopril or any other ACE inhibitor (1). Therefore, the combination of captopril and chymostatin is essentially required to totally block the ANG II formation in primates and canine blood vessels (1). Despite our findings, other investigators failed to show any evidence for the presence of non-ACE ANG II-forming enzyme in the vasculature, since they observed that the responses to ANG I were completely abolished by captopril alone (4–6). Carefully assessing the possible causes for these conflicting results, we realized that all of those studies in which no alternate converting enzyme was detected were performed using rat or rabbit blood vessels. Such contradictory results between primates and rodents led us to suspect a potential species difference in the vascular ANG II-forming systems.

In addition, there is another contradiction that suggests the species difference in ANG II-forming systems: it was clearly shown that an ACE inhibitor prevented the myointimal proliferative response to an intimal injury in the rodent vessels (7–9), whereas the agent failed to show any beneficial effect in humans (10) and baboons (11). These results from primates are consistent with our previous data for primate vessels (1, 2) showing that ACE inhibitors could not totally block the vascular formation of ANG II, which is a known stimulator of migration and proliferation of vascular smooth muscle cells (12). Such inconsistent results between rodents and primates led us to postulate that ACE inhibitors would entirely abolish the ANG II formation in the rodent vessels, unlike primate vessels.

In this study, we determined the vascular conversion of ANG I to ANG II by a bioassay technique using isolated arteries from humans, rabbits, and rats. The assay is based on the following facts: 1) ANG I is converted in situ to ANG II which contracts the arteries and 2) ANG I per se has no intrinsic vasoactivity. These facts were clearly proven by our previous studies following not only bioassay procedures but also analytical chemical ones (1). It was also shown in human arteries that the ACE activity was completely abolished by 1 μM captopril and the CAGE activity was totally blocked by 25 μM chymostatin (1, 2). By comparing the susceptibilities of ANG II-formation to captopril and/or chymostatin among these species, the present study reveals a striking difference between humans and rodents in the vascular ANG II-form-
ing pathways.

The following arteries from humans, rabbits, and rats were used for the study according to the method as previously published (1, 3). Special care was taken to avoid any involuntary damage to the endothelium. Animals were used in line with institutional guidelines.

Human gastroepiploic arteries were isolated from the omenta attached to the surgically resected stomachs of 32 patients (17 males and 15 females) aged 34–76 years (average age 58.5 years) who underwent total or partial gastrectomy because of gastric cancer or ulcer but had no apparent vascular complications such as hypertension, atherosclerosis, diabetic vasculopathy, and autoimmune vasculitis. The superior rectal and ileocolic branches of human inferior mesenteric arteries were isolated from the surgically resected colons from 8 patients with colon cancers (3 males and 5 females) aged 19–77 years (average age of 63.5 years). These human arteries were isolated from normal-looking tissues free from cancer infiltrations and were cut into helical strips, 20 mm long and 2 mm wide.

Eight male Japanese White rabbits (Keari Co., Osaka), weighing 3–3.5 kg, were killed by bleeding from the common carotid arteries under anesthesia with intravenous 50 mg/kg sodium pentobarbital. The gastroepiploic and mesenteric arteries, which were the equivalent portion to the above-mentioned human arteries, were isolated and used as helically cut strips, 20 mm in length and 1–2 mm in width, according to their outer diameters (0.3–1.3 mm).

The common carotid arteries and thoracic aortae were isolated from six 13-week-old male Wistar rats (Japan SLC, Inc., Hamamatsu), weighing 350–390 g, under diethyl ether anesthesia. The helical strips cut from the carotid arteries were 20 mm in length and 1.5-mm-wide, and those cut from the thoracic aortae were 20 mm in length and 2-mm-wide.

The arterial strips were set on a myograph (Nihon Kohden, Tokyo) under appropriate resting tensions: 1.5 g for human arteries, 0.8–1.5 g for rabbit strips according to their width, 1.0 g for rat carotid arteries, and 2.0 g for rat thoracic aortae. The bathing medium consisted of 145 mM sodium, 5.4 mM potassium, 2.2 mM calcium, 1 mM magnesium, 131.8 mM chloride, 25.0 mM bicarbonate and 5.6 mM dextrose (1). The medium was continuously aerated with O₂/CO₂ (95:5), which maintained the pH of the medium at 7.3 at 37°C. To avoid possible interference with responses to ANGs by endogenous prostanoids of which biosynthesis and release are induced by ANG II (13), 1 pM indomethacin was added to the medium throughout the experiments. Isometrically developed tension signals were amplified and recorded on analogue recorders (Nippon Denshi Kagaku, Kyoto). As previously reported (1), responses to ANG I and II were stabilized with sufficient reproducibility after the third or fourth repeated doses. Therefore, the fourth response was regarded as the control response for the respective strips. The ACE inhibitor captopril (a gift from Sankyo Co., Ltd., Tokyo) and/or the CAGE inhibitor chymostatin (Peptide Institute, Minoh) were added to the tissue bath 20 min before the addition of ANGs.

Fig. 1. A series of representative tracings showing the effects of 10 μM captopril and 50 μM chymostatin, alone or in combination, on the responses to 100 nM ANG I in a human gastroepiploic artery strip. Arrowheads indicate the time when ANG I was applied. Captopril and/or chymostatin were added 20 min prior to the addition of ANG I.
The working solution of chymostatin (100 mM) was made with neat dimethylsulfoxide (1). Thus, dimethylsulfoxide was added to the bathing media as a solvent blank for the control (no inhibitors) and captopril-alone experiments so that the final concentration of dimethylsulfoxide was 0.05% throughout the experiments.

Based on the respective dose-response curves for human and rabbit arteries (6 individuals each), 30 nM ANG I (ca. ED₅₀) and 100 nM ANG I (ca. ED₉₀) were chosen for the experiments hereafter. On the other hand, the half-maximal dose for ANG II was 10 nM for both human and rabbit arteries, so this dose was employed in the following studies.

In human gastroepiploic arteries, 10 μM captopril suppressed the responses to half-maximal and submaximal doses of ANG I by at most 30–40% (Figs. 1 and 2A). A higher concentration (30 μM) of captopril failed to exert any further suppression of the contractile responses (data not shown), which indicated that ACE inhibition was at its maximum with 10 μM captopril. On the other hand, 50 μM chymostatin suppressed the responses to ANG I by 60–70% (Figs. 1 and 2A). As shown in Figs. 1 and 2A, the combination of captopril and chymostatin, which is known to totally inhibit the in situ formation of ANG II (1), almost completely abolished the responses to ANG I, but showed no effect on the responses to the half-maximal dose of ANG II. These results indicated that the combined treatment shut-off the ANG II formation, while it did not block the ANG II receptors.

In human mesenteric arteries, contractile responses to 30 nM ANG I were attenuated with 10 μM captopril by 44.1±11.8% (n=6); the percent inhibition was 71.1±3.9% (n=11) with 50 μM chymostatin and 90.1±2.6% (n=14) with the combination of captopril and chymostatin. Similar results were obtained for the responses to 100 nM ANG I.

The results obtained for rabbit arteries surprisingly contrasted with those for human arteries. In rabbit gastroepiploic arteries, captopril alone succeeded in the total blocking of the ANG I response, which, however, chymostatin alone did not affect at all (Fig. 2B). Chymostatin in combination with captopril showed no additive influence upon the inhibitory effect of captopril alone (Fig. 2B). In addition, the responses to ANG I in rabbit mesenteric arteries and rat blood vessels were also totally blocked by captopril alone, while they were not affected by chymostatin alone. In all the specimens from the rabbits or rats, ANG II responses were not affected by captopril or chymostatin either in combination or each alone.

As mentioned earlier, special care was taken to conserve the integrity of the endothelium, which was confirmed as follows: Full lining of luminal surface with endothelial cells was confirmed in each preparation with hematoxylin-eosin staining. In addition, the relaxation response to 1 μM acetylcholine (ca. 30–40% relaxation upon 1 μM phenylephrine-induced contraction) was evident in all preparations examined. This indicates the functional integrity of the endothelium in all the vascular strips used, because the intentionally denuded strips did not relax at all in response to acetylcholine (data not shown).

Fig. 2. Effects of 50 μM chymostatin and 10 μM captopril, alone or in combination, on ANG I responses in (A) human and (B) rabbit gastroepiploic arteries. Solid columns and hatched columns represent the responses to 30 nM and 100 nM ANG I, respectively. Means±S.E.M. are shown for vessel specimens from different individuals; the number of specimens are shown above each column. Tensions developed in the absence of inhibitors (control responses) were 2034±230 mg with 30 nM ANG I and 4244±321 mg with 100 nM ANG I in human arteries, and they were 197±71 with 30 nM ANG I and 351±90 mg with 100 nM ANG I in rabbit arteries.
The present study has revealed the marked species-difference in vascular ANG II-forming systems between humans and rodents. In human arteries, as much as 60–70% of the vascular ANG II formation depends upon CAGE, while only 30–40% depends upon ACE. In contrast, rabbit and rat arteries possess only ACE, the classical pathway, but no alternative pathway for ANG II formation. Thus, so far as the rodent vessels were concerned, our data were consistent with those of other investigators (4–6).

The smaller contribution of the ACE-dependent pathway as contrasted with that of the CAGE-dependent one in human vessels is unlikely to be an artifact resulting from the loss of endothelium, since the integrity of the endothelium was confirmed by both morphological and functional examinations. Accordingly, the dominance of CAGE over ACE may be inherent to human vessels. Our present findings imply that a substantial amount of ANG II formation in human arterial tissues in vivo would persist even after the maximal ACE inhibition. Thus, it is not surprising for us to know that ACE inhibitors failed to prevent the intimal hyperplasia after balloon injury in humans (10) or baboons (11) despite the ability of the inhibitors to prevent neointimal hyperplasia in rat injury models (7–9) and rabbit atherosclerosis (14). Accordingly, the present results are by no means merely representing a new finding regarding the comparative physiology between humans and rodents, but are also critically relevant to the current therapeutic problems in human arterial diseases.

Our present findings in human vessels would raise a question as to why ACE inhibitors alone can be useful antihypertensives for humans if the major portion of ANG II is formed by CAGE, instead of ACE, in humans. Possible answers to this question are that ACE inhibitors suppress not only the vascular ACE but also the systemic ACE activities of the brain, heart, kidney and other blood pressure-regulating organs (15), whereas CAGE is a local enzyme that generates ANG II mainly in the adventitia (2) and thus unlikely to be involved in systemic blood pressure regulation.

In conclusion, we revealed a marked difference between humans and rodents in their vascular ANG II-forming systems. Accordingly, in order to explore the pathophysiology of human vascular diseases, we should employ those species that have ANG II forming systems similar to those in humans.

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REFERENCES