Inhibition of Neointima by Angiotensin-Converting Enzyme Inhibitor in Porcine Coronary Artery Balloon-Injury Model

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Abstract—Because hepatocyte growth factor (HGF) stimulates growth of endothelial cells exclusively without replication of vascular smooth muscle cells, we hypothesized that HGF may play a role in cardiovascular disease. In human vascular smooth muscle cells, angiotensin II suppressed local vascular HGF production in a dose-dependent manner. Using a rat balloon-injury carotid artery model, we demonstrated that blockade of angiotensin II inhibited neointimal formation, accompanied by a significant increase in local HGF production. However, the relation of vascular HGF to endothelial function was not clarified. Moreover, it is important to test the hypothesis in animal models that are more similar to human restenosis. Thus, in the present study, we used a porcine coronary artery balloon-injury model to study the role of angiotensin II in regulation of the local HGF system in vivo. Expression of HGF mRNA was significantly decreased in balloon-injured coronary arteries versus intact vessels. An angiotensin-converting enzyme (ACE) inhibitor (perindopril) significantly inhibited neointimal formation after balloon injury compared with vehicle (P<0.05). In addition, vasodilator response of balloon-injured coronary arteries to bradykinin was restored by perindopril treatment, whereas no vasodilator response was observed in balloon-injured vessels treated with vehicle. Vasodilator response of balloon-injured arteries induced by perindopril was completely abolished by Nω-nitro-L-arginine methyl ester. Of particular interest, vascular HGF mRNA was significantly increased in balloon-injured vessels treated with perindopril as compared with vehicle. Overall, the present study demonstrated that ACE inhibitor significantly inhibited neointimal formation, accompanied by significant improvement of endothelial dysfunction and a significant increase in local vascular HGF mRNA in vivo in a porcine coronary artery balloon-injury model. Given the strong mitogenic activity of HGF on endothelial cells, improvement of endothelial dysfunction by perindopril might be due to increased local HGF expression through enhancement of reendothelialization after balloon injury, in addition to its direct effect, ACE inhibition. Downregulation of the local vascular HGF system may play an important role in the pathogenesis of cardiovascular disease. (Hypertension. 2001;37:270-274.)

Key Words: endothelium ■ muscle, smooth, vascular ■ angiotensin ■ angiotensin-converting enzyme inhibitors ■ remodeling

Recent studies hypothesized that endothelial cells may modulate vascular growth, because many antiproliferative factors are secreted by endothelial cells.1,2 Dysfunction of endothelial cells apparently may promote abnormal vascular growth, such as in atherosclerosis. Given the importance of endothelial cells, we hypothesize that rapid regeneration of endothelial cells, not accompanied by growth of vascular smooth muscle cells (VSMC), may have therapeutic potential in abnormal vascular growth, such as neointimal formation after angioplasty. From this viewpoint, we previously reported that hepatocyte growth factor (HGF) has the unique characteristic of stimulating endothelial cell growth exclusively without stimulating VSMC growth,3,4 although HGF is well known to be a mesenchyme-derived pleiotropic factor that regulates cell growth and motility and morphogenesis of various types of cells.5,6 Moreover, the presence of HGF and its specific receptor, c-met, has been detected in vascular tissues.7 Our previous reports documented that angiotensin (Ang) II significantly inhibited local HGF production through transforming growth factor (TGF)–β activation and a non–TGF–β pathway.8,9 In addition, an angiotensin-converting enzyme (ACE) inhibitor or Ang II receptor antagonist attenuated the decrease in local vascular HGF production in a rat carotid artery balloon-injury model, accompanied by inhibition of neointimal formation.8 Therefore, we speculate that increased local vascular HGF production by ACE inhibition may have therapeutic value against abnormal VSMC growth because it enhances reendothelialization after balloon injury.
However, none of the reports has documented the relationship among ACE inhibition, vascular HGF expression, and endothelial function. To address this issue is extremely important, given that improvement of endothelial dysfunction after angioplasty by an ACE inhibitor has been reported in human restenosis.\textsuperscript{10–12} Therefore, we further examined regulation of local HGF secretion by Ang II in blood vessels. In the present study, we used the porcine coronary artery balloon-injury model instead of the rat carotid artery to test the hypothesis, because the porcine model more closely resembles human restenosis. Thus, we addressed the following questions in the porcine coronary artery balloon-injury model: (1) how an ACE inhibitor influences endothelial dysfunction and (2) how an ACE inhibitor affects vascular HGF expression.

Method

Experimental Design

Ten male Gottingen miniature pigs (Niseiken, Ibaragi, Japan) weighing 18 to 23 kg were housed individually under controlled room temperature and were fed laboratory chow. Each pig was lightly anesthetized with ketamine hydrochloride 12.5 mg/kg IM followed by pentobarbital sodium 20 mg/kg IV. The pig was intubated and ventilated with a positive pressure respirator. The femoral artery was surgically exposed, and a cannula was introduced into the orifice of the left coronary artery. Before insertion of catheter, heparin 5000 IU was injected intravenously. A balloon catheter (length, 2 cm; balloon size, 2.5 or 3.0 mm; Boston Scientific Co) was placed in the left ascending branch of the coronary artery through the guidewire, and the endothelium was mechanically denuded under angiographic guidance. Balloon inflation was performed 3 times at 10 atm. Pigs were divided into 2 groups and treated from 1 week before injury to 4 weeks after operation with vehicle (distilled water) or perindopril 2 mg·kg\textsuperscript{-1} ·d\textsuperscript{-1} (n = 5 per group). Drugs were donated by Dai-ichi Pharmaceutical Company (Tokyo, Japan). Other drugs, such as heparin or antiplatelet drugs, were not used after balloon injury. The animals were randomly allocated to each group, and the drug was administrated with food. After treatment, the animals were killed.

Histological Studies

For histological analyses, a segment of each artery was perfusion-fixed with 4% paraformaldehyde. Medial and luminal areas were measured on a digitizing tablet (model 2200, South Micro Instruments) after the segment was stained with hematoxylin.\textsuperscript{13} The medial area was readily demarcated as the vessel area between the internal and external elastic laminae. At least 3 individual sections from the middle of each arterial segment were analyzed. Animals were coded so that analysis was performed without knowledge of which treatment each animal received.

Evaluation of Vasodilator Properties in Response to Bradykinin

Freshly harvested vessels were cleaned of fat and connective tissue, cut into helical strips, and mounted in 30-mL organ baths that contained Krebs-Henseleit buffer (KNB; in mmol/L: NaCl 120, KCl 4.7, CaCl\textsubscript{2} 2.5, MgSO\textsubscript{4} 1.2, KH\textsubscript{2}PO\textsubscript{4} 1.2, NaHCO\textsubscript{3} 25, glucose 5.5; pH 7.4) maintained at 37°C and oxygenated with 95% O\textsubscript{2}/5% CO\textsubscript{2}. Vessels were equilibrated for 60 minutes and bathing fluid was changed every 15 minutes. Isometric tension studies were performed from the polygraph chart recorded on a Grass model 7D polygraph. Optimal resting tension was determined every 15 minutes. Isometric tension studies were performed with a Grass model 7D polygraph. Optimal resting tension was determined in baseline studies, and response to vasoactive drugs was measured by determination of vascular relaxation to bradykinin (10\textsuperscript{-8} to 10\textsuperscript{-5} mol/L) in the presence of indomethacin.

Results

As previously reported, presence of HGF was detected in endothelial cells and VSMC of rat and human by reverse transcriptase—polymerase chain reaction and ELISA by use of specific anti-HGF antibody.\textsuperscript{7} Therefore, we initially studied the presence of HGF expression in porcine coronary artery. Consistent with rat and human vessels,\textsuperscript{8,9,16} vascular HGF mRNA could be readily detected in porcine coronary arteries 1 month after balloon injury. For Northern blot analysis, 20 μg of total RNA was subjected to electrophoresis on a 1.5% agarose-formaldehyde denaturing gel and transferred to a nitrocellulose membrane (Amer sham International plc). The filter was baked, prehybridized, and hybridized. Full-length complementary DNA (cDNA) for HGF labeled with random-primer kit (Amersham) was used as a probe for Northern blotting. The filter then was washed and exposed to X-ray film.

Northern Blot Analysis

RNA was extracted by use of RNazol (Tel-Test Inc), from balloon-injured coronary arteries 1 month after balloon injury. For Northern blot analysis, 20 μg of total RNA was subjected to electrophoresis on a 1.5% agarose-formaldehyde denaturing gel and transferred to a nitrocellulose membrane (Amersham International plc). The filter was baked, prehybridized, and hybridized. Full-length complementary DNA (cDNA) for HGF labeled with random-primer kit (Amersham) was used as a probe for Northern blotting. The filter then was washed and exposed to X-ray film.

Statistical Analysis

All values are expressed as mean±SEM. ANOVA with a subsequent Bonferroni or Dunnett test was used to determine significance of differences in multiple comparisons. Values of P<0.05 were considered statistically significant.

Figure 1. Expression of HGF assessed by Northern blotting in balloon-injured vessels and intact uninjured vessels. a, Typical example of Northern blotting of HGF mRNA; b, Effect of perindopril on vascular HGF mRNA. 1 indicates intact uninjured vessels in sham-operated rats; 2, balloon-injured vessels treated with vehicle 28 days after injury; and 3, balloon-injured vessels treated with perindopril 28 days after injury (n = 3 per group).

(3×10\textsuperscript{-6} mol/L). In the present study, we evaluated the vasodilator response to bradykinin but not acetylecholine, because response of porcine coronary artery to acetylecholine was vasospastic rather than vasodilatory in the presence of normal endothelium.\textsuperscript{15} Contractile responses were measured from the polygraph chart and expressed as a percentage of the maximal relaxation induced by papaverine (10\textsuperscript{-6} mol/L). Nitrergic oxide mediated relaxation of bradykinin responses was confirmed by blocking bradykinin-induced relaxation with N\textsuperscript{-}methy l-\textsuperscript{l}-arginine methyl ester (L-NAME) was administered 20 minutes before, vessels were contracted with KCl (50 mmol/L), and endothelial function was evaluated by determining vascular relaxation to bradykinin.
uninjured and injured vessels 28 days after balloon injury (Figure 1).

Our previous studies demonstrated negative regulation of local HGF expression by Ang II in various cells, including VSMC. Thus, we further examined the effect of Ang II on local vascular HGF production in a balloon-injury porcine coronary artery model, because the significant contribution of Ang II is well known in the pathogenesis of neointimal formation in this model. Porcine coronary artery was treated with perindopril (an ACE inhibitor) and vehicle from 7 days before to 28 days after balloon injury. Administration of perindopril resulted in a significant reduction of neointimal to medial area after balloon injury (Figure 2a and b; P < 0.01). In contrast, no significant changes in medial area were observed in each group (vehicle, 1.07 ± 0.27; perindopril, 0.86 ± 0.06 mm²; not significant). Lumen area of vessels treated with perindopril was significantly increased compared with vehicle (vehicle, 0.66 ± 0.16 mm²; perindopril, 1.10 ± 0.11 mm²; P < 0.05), whereas medial area was slightly reduced but not to a level of significance (vehicle, 1.07 ± 0.27 mm²; perindopril, 0.86 ± 0.06 mm²; not significant). These data suggest that treatment with perindopril may affect negative remodeling, although further studies with intravascular ultrasound are necessary. Vascular HGF mRNA was significantly increased in balloon-injured coronary artery treated with perindopril versus vehicle (Figure 1; P < 0.01).

Given the restoration of vascular HGF mRNA by perindopril, we hypothesized that increase in vascular HGF might enhance reendothelialization in balloon-injured vessels. Indeed, overexpression of human HGF vector in rat balloon-injured vessels resulted in a significant increase in reendothelialized area as assessed by Evans’ blue dye staining. If so, enhanced reendothelialization by increased vascular HGF would result in restoration of anatomical integrity and recovery of physiological function. Therefore, we examined vasoconstrictor response to an endothelium-dependent agonist. As shown in Figure 3, vehicle-treated arteries demonstrated a lack of vasodilator response to bradykinin administration, whereas normal arteries demonstrated nice vasodilative response to bradykinin. Of importance, administration of bradykinin into precontracted vessels treated with perindopril resulted in significant dilatation compared with injured vessels treated with vehicle (Figure 4 and 5; P < 0.01). Endothelium-dependent dilatation in arteries treated with perindopril is also supported by the observation that the increase in dilatation was completely abolished by administration of L-NAME.
Discussion

Endothelial cells may have potential therapeutic actions in abnormal VSMC growth through secretion of multiple endothelium-derived substances that have profound influences on vascular smooth muscle function.\(^1,2\) Indeed, rapid generation of endothelial cells by endothelium-specific growth factors such as vascular endothelial growth factor inhibited neointimal formation after balloon injury in a rat model and in human restenosis.\(^21,22\) From this viewpoint, HGF is interesting, given that it can stimulate growth of endothelial cells without replication of VSMC\(^3,4\) in a manner similar to vascular endothelial growth factor. Thus, the local HGF system is expected to play a role in the pathogenesis of cardiovascular disease. Our previous studies documented that local HGF production in vascular cells plays a pathophysiological role in endothelial growth in an autocrine-paracrine manner.\(^8\) In addition, regulation of local HGF production in blood vessels is determined by Ang II.\(^8\) These findings are important to understanding of the pathogenesis of endothelial dysfunction observed in cardiovascular disease, because vascular Ang II and ACE are increased in atherosclerotic lesions and restenotic lesions after angioplasty.\(^18,19,23–25\) The present study demonstrated a significant decrease in vascular HGF mRNA in porcine coronary balloon-injured artery, consistent with our previous report.\(^8\)

This phenomenon provides the interesting hypothesis that disruption of the autocrine-paracrine local HGF system, which maintains endothelial cell growth, by Ang II may result in abnormal growth of VSMC and endothelial dysfunction, given that endothelial cells secrete antiproliferative substances.\(^1,2\) Therefore, we further examined the pathophysiological roles of the vascular HGF system in cardiovascular disease. As was expected, administration of perindopril significantly increased local HGF expression associated with the inhibition of neointimal formation. Because HGF is an endothelium-specific growth factor and thus stimulates growth of endothelial cells,\(^3,4\) increased local HGF production would probably stimulate regeneration of endothelial cells after balloon injury. Of particular interest, vasodilator response of balloon-injured coronary arteries was restored by treatment with perindopril versus vehicle. This restoration of vasodilation was completely abolished by administration of L-NAME, which suggests that rapid regeneration or stabilization of endothelial cells might be related to recovery of vasodilator response to bradykinin. Administration of an ACE inhibitor restored endothelial dysfunction after percutaneous transluminal coronary angioplasty in human subjects.\(^10\) Increased local HGF production may participate in the improvement of endothelial dysfunction observed in those cases treated with ACE inhibitors. Additionally, our preliminary results showed that in vivo gene transfer of HGF into balloon-injured artery resulted in significant inhibition of neointimal formation and restoration of endothelial dysfunction by reendothelialization.\(^19\) Increased local HGF production by ACE inhibition may have therapeutic value against abnormal VSMC growth through stimulation of reendothelialization in addition to blockade of Ang II–mediated VSMC growth. The present studies in porcine coronary arteries may provide information more applicable to humans, although studies in rat carotid artery models have been reported.\(^8\) Our hypothesis is supported by the previous observation that an ACE inhibitor, perindopril, stimulated endothelial regrowth after arterial injury in a rabbit model.\(^26\) The contribution of increase in local HGF expression by ACE inhibition to endothelial function may also be more general, given that treatment with ACE inhibitors is well known to improve endothelial dysfunction in human hypertensive patients.\(^27,28\) However, the present study has limitations. Recent studies demonstrated the lack of chymase, an alternative Ang II–generating enzyme, in a porcine model.\(^29\) In human restenosis, presence of chymase may diminish the utility of ACE inhibitors, although further studies are necessary to determine this matter.

In the present study, we demonstrated that treatment with perindopril significantly inhibited neointimal formation and improved endothelial dysfunction in porcine balloon-injured coronary artery, accompanied by an increase in local vascular HGF production. Given the strong mitogenic activity of HGF on endothelial cells, increased local vascular HGF production by ACE inhibition may have therapeutic value against abnormal VSMC growth and endothelial dysfunction by enhancing reendothelialization after balloon injury. Negative regulation of local HGF production, probably by Ang II, may play a physiological role in vascular disease, given the activation of the vascular renin angiotensin system in atherosclerosis, restenosis, and hypertension in humans and in experimental models.\(^18,19,23,24\)

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