Negative Regulation of Local Hepatocyte Growth Factor Expression by Angiotensin II and Transforming Growth Factor-β in Blood Vessels

Potential Role of HGF in Cardiovascular Disease

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Abstract—Because hepatocyte growth factor (HGF) is a member of the endothelium-specific growth factors, we hypothesized that HGF may play a role in cardiovascular disease. Therefore, we first examined the role of local HGF production in endothelial cell (EC) growth. Addition of anti-HGF antibody to EC resulted in a significant decrease in EC number. Moreover, coculture of vascular smooth muscle cells (VSMC) with EC resulted in an increase in EC number that was completely inhibited by anti-HGF antibody, suggesting that HGF secreted from EC and VSMC regulates EC growth in an autocrine-paracrine manner. Interestingly, transforming growth factor (TGF)-β significantly decreased HGF secretion from EC, whereas interleukin 6 stimulated immunoreactive HGF secretion. In human VSMC, TGF-β and angiotensin II suppressed local HGF production in a dose-dependent manner. Interestingly, anti–TGF-β antibody resulted in significant but not complete inhibition of the decrease in local HGF production. To further study the regulation of local HGF production, we used a coculture system. Coculture of VSMC with EC resulted in a significant decrease in local HGF secretion. The decrease in local HGF production by coculture was significantly attenuated by anti–TGF-β antibody, suggesting that inhibition of local HGF production in the coculture system was due to TGF-β activation. Moreover, a further decrease in local HGF production in the coculture system by angiotensin II was also observed. Finally, we studied the role of angiotensin II in the regulation of the local HGF system in vivo by using a balloon injury rat model. Of importance, local HGF production was significantly decreased in balloon-injured arteries compared with intact vessels, accompanied by a reduction of HGF mRNA. An angiotensin-converting enzyme inhibitor (cilazapril) or an angiotensin II type 1 receptor antagonist (E-4177) significantly stimulated local vascular HGF production associated with the inhibition of neointimal formation after balloon injury. In contrast, hydralazine did not alter local HGF production or neointimal formation despite decreasing blood pressure to a similar level as that in rats treated with cilazapril or E-4177. Overall, local HGF secretion from vascular cells was negatively regulated by TGF-β and angiotensin II. The present study also demonstrated that blockade of angiotensin II significantly inhibited neointimal formation, accompanied by a significant increase in local vascular HGF production in vivo in the balloon injury model. Given the strong mitogenic activity of HGF on endothelial cells, increased local HGF production by blockade of angiotensin II may enhance reendothelialization after balloon injury. Downregulation of the local vascular HGF system by TGF-β and vascular angiotensin may play an important role in the pathogenesis of cardiovascular diseases. (Hypertension. 1998;32:444-451.)

Key Words: endothelium ■ muscle, smooth, vascular ■ autocrine-paracrine ■ restenosis ■ remodeling

Endothelial cells are known to secrete various vasoactive substances. Recently, it has been hypothesized that endothelial cells may also modulate vascular growth because many antiproliferative factors are secreted by endothelial cells.1,2 It is apparent that dysfunction of endothelial cells 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 have previously reported that hepatocyte growth factor (HGF) has a unique characteristic to stimulate only endothelial cell growth but not VSMC growth.3,4 Moreover, the presence of HGF and its specific receptor, c-met, has been detected in vascular tissues.5 However, there are no reports as to how HGF is regulated in vascular tissues. HGF is well known as a mesenchyme-derived pleiotropic factor that
regulates cell growth, cell motility, and morphogenesis of various types of cells and is thus considered a humoral mediator of epithelial-mesenchymal interactions responsible for morphogenic tissue interactions during embryonic development and organogenesis. Therefore knowledge of HGF regulation would be important in understanding the pathophysiology of vascular diseases. In restenosis after angioplasty and atherosclerosis, the contribution of growth factors and cytokines has been reported. For example, transforming growth factor (TGF)-β, which has bifunctional effects on cell growth, is known to be upregulated in restenosis after angioplasty and atherosclerosis. In addition, activation of angiotensin II is also believed to play an important role in the pathogenesis of cardiovascular disease. Therefore we examined the regulation of local HGF secretion by TGF-β and angiotensin II in vascular cells. Moreover, the role of angiotensin II in the regulation of the local HGF system was also studied in vivo with a balloon injury rat model.

Methods

In Vitro Experiment

Cell Culture

Human aortic endothelial cells and human aortic VSMC (passage 5) were obtained from Clonetech Corp and cultured in modified MCDB131 medium with 5% fetal calf serum, 10 ng/mL epidermal growth factor, 2 ng/mL basic fibroblast growth factor (bFGF), and 1 mmol/L dexamethasone. These cells showed the specific characteristics of endothelial cells and VSMC by immunohistochemical and morphologic observation. All the cells were used within passage 5 to 6.

Cell Counting Assay

In the preparation of experiments for determination of cell count, the cells were grown to subconfluence. After cells reached 80% confluence, the medium was changed to fresh defined serum-free medium (DSF) containing antibody or control. The cells were then incubated overnight. On day 1, the medium was again changed to fresh DSF. On day 4, an index of cell proliferation was determined with a WST cell counting kit (Wako).

Coculture

(1) Coculture of quiescent, subconfluent endothelial cells with confluent VSMC (coculture 1; see Figure 2): Human aortic VSMC were seeded onto cell culture inserts (Becton Dickinson, 3.0 mm pore size) and were grown in 10% Dulbecco’s modified Eagle’s medium (DMEM) to reach confluence. Human endothelial cells were seeded onto 6-well plates (Becton Dickenson), maintained in 10% DMEM and placed in DSF for 48 hours after confluence. At 60% confluence of endothelial cells, the inserts containing VSMC were put into the wells containing the quiescent endothelial cells. Endothelial cells were cocultured for 48 hours with VSMC in 0.1% fetal calf serum with anti-HGF antibody or immunoglobulin G (IgG) control.

(2) Coculture of quiescent, confluent VSMC with confluent endothelial cells (coculture 2; see Figure 6a): Human aortic endothelial cells were seeded onto cell culture inserts (Becton Dickenson, 3.0 μm pore size) and were grown in 10% DMEM. Human VSMC were seeded onto 6-well plates (Becton Dickenson), maintained in 10% DMEM, and placed in DSF for 48 hours after confluence. At confluence of VSMC, the inserts containing endothelial cells were put into the wells containing the quiescent VSMC. VSMC were cocultured for 24 hours with endothelial cells in DSF, and the concentration of HGF in the medium was determined by enzyme immunoassay (EIA).

Effect of Neutralizing Anti-HGF Antibody

The effect of endogenously produced HGF was examined by a neutralization procedure with the use of rabbit anti-human HGF antibody. For the antibody, the IgG fraction (purified with protein A-agarose) was able to neutralize a biological activity of 10 ng/mL HGF, at a concentration of 10 μg/mL. Normal rabbit serum IgG fraction (10 μg/mL) was used as a control. Cell number in the following groups was examined for both types of the human vascular cells: Protocol 1, group 1: incubation of endothelial cells with anti-HGF antibody (final 10 μg/mL) for 48 hours; group 2: incubation of endothelial cells with normal IgG (final 10 μg/mL) for 48 hours; Protocol 2, group 1: incubation of endothelial cells cocultured with VSMC with anti-HGF antibody (10 μg/mL) for 48 hours; group 2: incubation of endothelial cells cocultured with VSMC with normal IgG (10 μg/mL); and group 3: incubation of endothelial cells without VSMC with normal IgG (10 μg/mL).

Measurement of HGF in Conditioned Medium

Human endothelial cells and VSMC were seeded on 6-well plates (Corning) at a density of 5 × 10⁴ cells/cm². After replacing the medium with fresh DSF and after culture for 24 hours, the concentration of HGF in the medium was determined by ELISA with the use of anti-human HGF antibody. This enzyme-linked immunosorbent assay (ELISA) specifically detects rat or human HGF by lack of cross-reactivity of the antibodies.

ELISA and Western Blotting of TGF-β

Conditioned medium was collected from confluent VSMC maintained in DSF 48 hours later, centrifuged at 600g for 10 minutes, and stored at −20°C. ELISA for immunoreactive TGF-β, in the supernatant was performed with an ELISA kit (Amersham). The antibody against TGF-β, cross-reacts with rat active TGF-β, but not with latent rat TGF-β, TGF-β, or TGF-β. After conversion of TGF-β from the inactive to the active form by the addition of hydrochloride, measurement of latent TGF-β was performed by ELISA. Western blotting was also performed for analysis of TGF-β protein.

Effect of Neutralizing Anti-TGF-β Antibody

Augmentation of decreased local HGF production in human endothelial cells and VSMC was characterized as TGF-β specific by a neutralization procedure, with the use of rabbit anti-human TGF-β (R&D Research). The IgG fraction (purified with protein A-agarose) was able to neutralize the biological activity of TGF-β. Normal rabbit serum IgG fraction was used as a control.

In Vivo Experiment

Experimental Design

Male Wistar-Kyoto rats (15 weeks-old; Charles River Breeding Laboratories) were anesthetized with ketamine, and the left common carotid artery was surgically exposed. A cannula was introduced into the common carotid through the external carotid artery. After balloon injury, blood flow to the common carotid was restored by release of the ligatures and the wound was then closed. Male Wistar-Kyoto rats were divided into 4 groups and treated from 1 week before injury to 2 weeks after operation as follows: vehicle (distilled water), cilazapril (10 mg/kg per day), E-4177 (30 mg/kg per day), or hydralazine (8 mg/kg per day) (n = 13). The drugs were kindly donated by Eisai Pharmaceutical Company. The animals were randomly allocated to each group, and the drug was administrated by gavage. After treatment, the rats were killed by decapitation and blood was collected. Systolic blood pressure was measured in conscious rats by the tail-cuff method with a sphygmomanometer (Softron Co Ltd).

Reverse Transcription–Polymerase Chain Reaction/Southern Blotting

RNA was extracted from intact normal vessels from sham-operated rats and balloon-injured vessels at 14 days after injury by RNAzol (Tel-Test Inc). Levels of HGF and G3PDH mRNAs were measured by reverse transcription–polymerase chain reaction (RT-PCR). The HGF 5 primer (nucleotides 1409 to 1426 of human sequence) was
Serum and tissue HGF concentration was determined by EIA with anti-rat HGF antibodies (HGF EIA kit, Tokushumeneki Research Center). The carotid arteries were promptly removed without excess fat after perfusion from the apex of the heart with saline, frozen in liquid nitrogen, and stored at −70°C until use. On the day of the extraction, the tissue was thawed at 4°C and homogenized by sonication with trace amounts of experimental RNA. Aliquots of RNA (0.5 μg) derived from cultured cells were amplified simultaneously by PCR (30 cycles) by individuals who were blinded to the identity of the samples and compared with a negative control (primers without RNA). In the preliminary experiments, the number of amplification cycles for each gene was examined by performing RT-PCR for 20, 15, 30, 35, and 40 cycles. PCR products were within the linear logarithmic phase of the amplification curve until 40 cycles were performed. To ensure that the RT-PCR–amplified product reflects transcribed HGF RNA without significant DNA contamination, RNA samples treated with RNase A or amplified without reverse transcriptase were simultaneously amplified as negative controls. These samples did not result in a visual band. Moreover, PCR products were cut by restriction enzymes, and the fragments were identical to the theoretical bands. The PCR products then were blotted by capillary transfer onto membranes for Southern blotting.

Histological Studies

For histological analyses, a segment of each artery was fixed with 4% paraformaldehyde. Medial and luminal area were measured on a digitizing tablet (South Micro Instruments, model 2200) after staining with hematoxylin. 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 the transfected arterial segments were analyzed. Animals were coded so that the analysis was performed without knowledge of which treatment each individual animal received.

Measurement of Serum and Tissue HGF Concentration

Serum and tissue HGF concentration was determined by EIA with anti-rat HGF antibodies (HGF EIA kit, Tokushumeneki Research Center). The carotid arteries were promptly removed without excess fat after perfusion from the apex of the heart with saline, frozen in liquid nitrogen, and stored at −70°C until use. On the day of the extraction, the tissue was thawed at 4°C and homogenized by Polytron in assay solution. Each specimen was centrifuged at 20 000g for 30 minutes at 4°C to remove the lysates. Tissue HGF concentration was determined by EIA with anti-rat HGF antibody.

Statistical Analysis

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

Results

In Vivo Experiments

The presence of HGF and its receptor (c-met) mRNAs was detected in endothelial cells and VSMC of rat and human by RT-PCR. The secretion of HGF was also readily detected by ELISA with specific human anti-HGF antibody in human endothelial cells and VSMC. Therefore we initially studied the role of endogenously produced HGF from vascular cells in endothelial cell growth. Addition of neutralizing antibody against human HGF to human aortic endothelial cells resulted in a significant decrease in the number of endothelial cells (P<0.01, Figure 1), whereas IgG control did not alter endothelial cell number. Moreover, we used coculture system 1 (see “Methods”; Figure 2) to clarify the role of local HGF production from VSMC in endothelial growth. As shown in Figure 2, coculture of VSMC with endothelial cells resulted in a significant increase in number of endothelial cells compared with endothelial cells alone (P<0.01), revealing that factors that stimulated endothelial cells were secreted.
from VSMC. Importantly, the increase in number of endothelial cells was significantly attenuated by coincubation with anti-HGF antibody ($P<0.01$). These results demonstrated that local HGF production from endothelial cells and VSMC regulated growth of endothelial cells in an autocrine-paracrine manner.

We therefore examined the regulation of local HGF production in vascular cells. Interestingly, addition of TGF-β significantly inhibited HGF secretion into the culture medium of endothelial cells compared with basal secretion ($P<0.01$, Figure 3), whereas bFGF and interleukin (IL)-6 stimulated it (Figure 3). Next, we examined the regulation of HGF in human aortic VSMC. In VSMC, TGF-β significantly decreased immunoreactive HGF release into the culture medium of VSMC as compared with vehicle treatment (Figure 4a), which was abolished by addition of neutralizing anti–TGF-β antibody (data not shown). The inhibitory effect of TGF-β on local HGF production was dose-dependent (Figure 4b). In contrast, IL-6 stimulated the release of HGF significantly, whereas bFGF had no effect on HGF. Moreover, we tested whether angiotensin II affects local HGF production in VSMC. Interestingly, angiotensin II also significantly decreased vascular HGF production in a dose-dependent manner (Figure 5a). As previously reported, angiotensin II stimulated TGF-β expression in VSMC. Therefore, to elucidate the participation of TGF-β stimulation by angiotensin II in

**Figure 4.** a, Effect of various cytokines on concentration of HGF released from human aortic VSMC. $n=8$ per group. DSF indicates vehicle; TGF, human recombinant TGF-β (10 ng/mL) added to VSMC; FGF, human recombinant bFGF (10 ng/mL) added to VSMC; IL-6, human recombinant IL-6 (50 ng/mL) added to VSMC. **$P<0.01$ vs DSF.** b, Effect of TGF-β on concentration of HGF released from human aortic VSMC. $n=8$ per group. DSF indicates vehicle; TGF, human recombinant TGF-β (0.1 to 10 ng/mL) added to VSMC. **$P<0.01$ vs DSF, ###$P<0.01$ vs 0.1 ng/mL.

**Figure 5.** a, Effect of angiotensin II on concentration of HGF released from human aortic VSMC. $n=8$ per group. DSF indicates vehicle; Ang II, angiotensin II (10$^{-6}$ to 10$^{-8}$ mol/L) added to VSMC. **$P<0.01$ vs DSF, #$P<0.05$ vs angiotensin II 10$^{-8}$ mol/L, +$P<0.05$ vs angiotensin II 10$^{-7}$ mol/L.** b, Effect of neutralizing anti–TGF-β antibody on angiotensin II–induced local HGF production in human VSMC. $n=8$ per group. UN indicates vehicle; +IgG, VSMC with addition of normal IgG; +Ab, VSMC with addition of neutralizing anti–TGF-β antibody; +Ang II, angiotensin II (10$^{-8}$ mol/L) added to VSMC. **$P<0.01$ vs UN.** c, Western blot of active and latent TGF-β in conditioned medium of cultured human VSMC.
decreased local HGF production, we studied the effect of neutralizing anti–TGF-β antibody on the angiotensin II–induced decrease in local HGF production in VSMC that secreted latent and active TGF-β as assessed by Western blotting (Figure 5c). Interestingly, neutralizing anti–TGF-β antibody resulted in significant but not complete inhibition of local HGF production by angiotensin II (Figure 5b). During the experimental period (24 hours after stimulation), neither angiotensin II nor TGF-β altered VSMC growth (DSF, 0.359±0.015; angiotensin II, 0.362±0.036; TGF-β, 0.357±0.011; not significant).

To evaluate the effect of activation of TGF-β in vascular tissues, we used a coculture system of VSMC with endothelial cells (see “Methods”; Figure 6a). Coculture of VSMC with endothelial cells resulted in a significant decrease in local HGF production by VSMC, probably through TGF-β activation (Figure 6a), because latent TGF-β is known to be converted to active by contact with endothelial cells. Indeed, active TGF-β could be detected in conditioned medium of cocultured VSMC with endothelial cells by the use of ELISA (coculture of VSMC with endothelial cells, 2.55±0.13 pg/mL; endothelial cells alone, 1.15±0.24 pg/mL; VSMC alone, 0.05±0.01 pg/mL). Decrease in local HGF production by coculture was almost completely attenuated by neutralizing anti–TGF-β antibody (Figure 6b), suggesting that inhibition of local HGF production by coculture was due to the activation of TGF-β. In the coculture system, angiotensin II also caused a further reduction of local HGF production compared with coculture alone (Figure 6a).

In Vivo Experiments
We further examined the effect of angiotensin II on local vascular HGF production in a balloon injury rat model because the significant contribution of angiotensin II and TGF-β is well known in the pathogenesis of neointimal formation in this model.10–13 Indeed, a marked reduction of vascular HGF mRNA was observed in injured vessels compared with normal vessels, whereas no apparent change in G3PDH mRNA was observed between normal uninjured vessels and injured vessels at 14 days after balloon injury (Figure 7). Therefore rats were treated with cilazapril (an angiotensin-converting enzyme [ACE] inhibitor), E-4177 (an angiotensin-1 receptor antagonist), hydralazine, and vehicle between 7 days and 14 days after balloon injury. Treatment of rats with cilazapril, E-4177, or hydralazine for 3 weeks significantly decreased systolic blood pressure (P<0.05) compared with rats treated with vehicle (data not shown). Heart rate did not differ among all the groups (data not shown). Nevertheless, administration of cilazapril and E-4177 but not hydralazine resulted in significant inhibition of neointimal formation after balloon injury (Figure 8, a and b, P<0.01). In contrast, no significant changes in medial areas were observed among groups (vehicle, 0.140±0.010;
hydralazine, 0.141±0.016; cilazapril, 0.139±0.013; E-4177, 0.138±0.025 mm²; not significant). Consistent with the decreased local HGF mRNA in balloon-injured vessels, local HGF production was also significantly decreased in balloon-injured vessels compared with normal intact artery (P<0.01, Figure 8c). In contrast, vascular HGF concentration was significantly increased in balloon-injured rats treated with cilazapril or E-4177 compared with vehicle (Figure 8c, P<0.01), whereas there was no significant change in rats treated with vehicle or hydralazine. On the other hand, no significant change in serum HGF concentration was observed in balloon-injured and sham-operated rats. The inhibitory effect of cilazapril and E-4177 was not accompanied by an increase of serum HGF concentration (Figure 8d, P>0.05). Similarly, the other groups also failed to show any increase in serum HGF concentration.

Discussion

Endothelial cells may have potential therapeutic actions in abnormal VSMC growth through the secretion of multiple endothelium-derived substances that have profound influences on vascular smooth muscle function. From this viewpoint, we sought an endothelium-specific growth factor that does not stimulate VSMC growth. As previously reported, HGF has the characteristics of an endothelium-specific growth factor, similar to vascular endothelial growth factor (VEGF). VEGF has been reported to be secreted from VSMC and to act on endothelial cells, whereas HGF is secreted from endothelial cells and VSMC and acts on endothelial cells, as shown in this study. The local HGF system as well as VEGF is expected to have a role in the pathogenesis of cardiovascular disease, although the regulation of VEGF and HGF has not yet been clarified in vascular tissues. Because anti-HGF antibody attenuated endothelial cell growth, local HGF production in vascular cells may have a pathophysiological role in endothelial growth in an autocrine-paracrine manner. Therefore, the regulation of local HGF production is noteworthy. In this study, we examined the effects of angiotensin II and TGF-β on HGF secretion from vascular cells to study the cytokine network around the local HGF system.

We focused on the interaction of TGF-β with the HGF system because TGF-β was increased in atherosclerotic...
lesions and restenotic lesions after angioplasty.11–13 Our present study documented a marked reduction of local HGF production by TGF-β treatment in human aortic endothelial cells and VSMC. Inhibition of local HGF production by TGF-β is also suggested by the observation that coculture of VSMC with endothelial cells resulted in a significant decrease in HGF production by VSMC, because the conversion of latent into active TGF-β is well known in coculture of VSMC with endothelial cells.16–17,26 Indeed, neutralizing anti–TGF-β antibody attenuated the inhibition of local HGF production by coculture system. Local HGF mRNA expression in VSMC was also decreased by TGF-β treatment (unpublished observation). Because the promoter region of HGF gene contains various binding sites for transcriptional factors, for example, a TGF-β–inhibitory element and a cAMP responsive element,27 TGF-β inhibits local HGF production probably through a TGF-β–inhibitory element. In experimental hypertensive and restenosis models, activation of the vascular renin-angiotensin and TGF-β was also reported in the vasculature.10–15,28,29 In vitro studies also revealed that angiotensin II stimulates TGF-β gene expression in VSMC.23 Of importance, the present study demonstrated that angiotensin II also results in a significant decrease in local HGF production in VSMC. Moreover, inhibition of local HGF production by angiotensin II was still observed in coculture of VSMC with endothelial cells. Because neutralizing anti–TGF-β antibody did not completely attenuate the decrease in local HGF production by angiotensin II, inhibition of local HGF production by angiotensin II may be mediated by angiotensin II–specific mechanisms, for example, decrease in cAMP, in addition to TGF-β induced by angiotensin II. Taken together, activation of the local TGF-β and vascular renin-angiotensin system may negatively regulate local HGF production in vascular tissues. Suppression of local HGF production might be important to maintain endothelial function because HGF prevents apoptosis of endothelial cells mediated by several conditions.30

This phenomenon provides the interesting hypothesis that disruption of the autocrine-paracrine local HGF system, which maintains endothelial cell growth by TGF-β and angiotensin II, may result in abnormal growth of VSMC because endothelial cells secrete antiproliferative substances (nitric oxide [NO], prostaglandin I₂ [PGI₂], C-type natriuretic peptide [CNP], and so on) as a biological barrier. In contrast, in balloon-injured vessels, impairment of the endothelial cells may occur through downregulation of local HGF production by TGF-β and/or angiotensin II. Therefore, proliferation of VSMC may be accelerated by loss of antiproliferative substances.

**Figure 9.** Hypothesis in the role of HGF in the maintenance of the vascular structure. In static state, HGF secreted from VSMC and endothelial cells may maintain vascular structure in an autocrine-paracrine manner, whereas endothelial cells may inhibit VSMC growth through production of antiproliferative substances (nitric oxide [NO], prostaglandin I₂ [PGI₂], C-type natriuretic peptide [CNP], and so on) as a biological barrier. In contrast, in balloon-injured vessels, impairment of the endothelial cells may occur through downregulation of local HGF production by TGF-β and/or angiotensin II. Thus an ACE inhibitor or an angiotensin-1 receptor antagonist was administered in the balloon injury model to study the role of angiotensin II in the downregulation of local vascular HGF. Expectedly, blockade of angiotensin II by cilazapril or E-4177 significantly stimulated local HGF production associated with the inhibition of neointimal formation, whereas hydralazine did not. Because HGF is an endothelium-specific growth factor,3–4 increased local HGF production probably would stimulate regeneration of endothelial cells after balloon injury. It is noteworthy that administration of an ACE inhibitor restored endothelial dysfunction after percutaneous transluminal coronary angioplasty in human subjects31 and that treatment with ACE inhibitors improved endothelial dysfunction in human hypertensive patients.10 Increased local HGF production may participate in the improvement of endothelial dysfunction observed in those cases treated by blockers of angiotensin II. Additionally, our preliminary results showed that in vivo gene transfer of HGF into balloon-injured artery resulted in significant inhibition of neointimal formation by reendothelialization.32 Increased local HGF production itself by blockade of angiotensin II may have therapeutic value against abnormal VSMC growth through the stimulation of reendothelialization, in addition to the blockade of angiotensin II–mediated VSMC growth. As angiotensin II stimulated TGF-β in VSMC, increased TGF-β may also participate in the downregulation of local HGF production.

Although the exact mechanisms of HGF regulation are not yet understood, these data demonstrate that TGF-β and angiotensin II suppressed local vascular HGF production. Moreover, we demonstrated that cilazapril and E-4177 but not hydralazine significantly inhibited neointimal formation, accompanied by a significant increase in local vascular HGF production. Given the strong mitogenic activity of HGF on endothelial cells, increased local vascular HGF production by angiotensin II blockade may have therapeutic value against abnormal VSMC growth by enhancing reendothelialization after balloon injury. Negative regulation of local HGF production by angiotensin II and TGF-β may have physiological roles in vascular disease, given the activation of the vascular TGF-β and vascular renin-angiotensin system in atherosclerosis, restenosis, and hypertension in humans and experimental models.10–15,28,29

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References


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