Exaggerated Hypotensive Effect of Vascular Endothelial Growth Factor in Spontaneously Hypertensive Rats

Renhui Yang, Annie K. Ogasawara, Thomas F. Zioncheck, Zhen Ren, Guo-Wei He, Geralyn G. DeGuzman, Nicolas Pelletier, Ben-Quan Shen, Stuart Bunting, Hongkui Jin

Abstract—Vascular endothelial growth factor (VEGF) induces hypotension in normotensive subjects, which is considered to be a major side effect for treatment of ischemic diseases. However, the hypotensive effect of VEGF has not been investigated in the setting of hypertension. This study determined effects of VEGF on hemodynamics, pharmacokinetics, and release of NO and prostaglandin I₂ (PGI₂) in vivo and on vasorelaxation of mesentery artery rings in vitro in spontaneously hypertensive rats (SHR) compared with Wistar-Kyoto rats (WKY). Intravenous infusion of VEGF for 2 hours produced a dose-related decrease in arterial pressure, which was enhanced in conscious SHR compared with WKY (P < 0.01), and an increase in heart rate in WKY but not in SHR. In response to similar doses of VEGF, compared with WKY, SHR had a higher plasma VEGF level and lower VEGF clearance (P < 0.01). Circulating NO and PGI₂ levels after VEGF administration were not increased in SHR versus WKY, and VEGF-induced vasorelaxation was blunted in SHR versus WKY in vitro, suggesting endothelial dysfunction in SHR. One-week VEGF infusion also caused greater hypotension (P < 0.05) in the absence of tachycardia in SHR compared with WKY controls. Thus, despite blunted vasorelaxation in vitro because of endothelial dysfunction, SHR exhibited exaggerated hypotension without tachycardia in response to VEGF, which was independent of NO and PGI₂. The exaggerated hypotensive response to VEGF in SHR may be owing to impaired baroreflex function and reduced VEGF clearance. The data may also suggest that more caution should be taken when VEGF is administered in patients with hypertension. (Hypertension. 2002;39:815-820.)

Key Words: growth substances ■ rats, spontaneously hypertensive ■ hemodynamics ■ hypotension ■ blood pressure

Vascular endothelial growth factor (VEGF) is a unique mitogen specific for vascular endothelial cells.1–4 As a fundamental mediator of normal and pathological angiogenesis, VEGF has been shown to induce a strong angiogenic response in vitro5–8 and in vivo.2,3,7–9 Animal studies have demonstrated that administration of VEGF produces beneficial angiogenic effects in peripheral vascular ischemia10–12 and in coronary ischemia.13–16

VEGF has been shown to induce endothelium-dependent vasorelaxation in normal animals in vitro.17–19 Because of its vasodilatory effect, VEGF induces hypotensive and tachycardic responses in vivo in normotensive animals, including rats, rabbits, and pigs,14,21 and the hypotensive effect is mediated, at least in part, by NO.20,21 In humans, intracoronary administration of VEGF caused a hypotensive effect that was dependent on VEGF infusion rate.22 Although hypotensive effects of VEGF have been characterized in normotensive subjects, these effects have not been investigated in the setting of hypertension.

Our previous studies have demonstrated that VEGF induces endothelium-dependent vasorelaxation in aortic rings in vitro, which is diminished in spontaneously hypertensive rats (SHR) compared with normotensive Wistar-Kyoto rats (WKY).23 The present study was designed to determine the effects of VEGF on mean arterial pressure (MAP), heart rate (HR), and release of NO and prostaglandin I₂ (PGI₂) in conscious SHR and to compare the levels to those in WKY controls. Furthermore, pharmacokinetic responses to VEGF were examined in SHR versus WKY. In addition, we also examined the in vitro vasorelaxant effects of VEGF in isolated mesentery arteries of SHR and WKY. Our results showed that compared with WKY, SHR exhibited an enhanced hypotensive response to VEGF in vivo, which may be caused by impaired baroreflex function and reduced VEGF clearance, despite a blunted vasorelaxant response to VEGF in vitro due to endothelial dysfunction.

Methods

Male SHR and WKY age 12 weeks were obtained from Charles River Breeding Laboratories (Wilmington, Mass). The experimental procedures were approved by the Institutional Animal Care and Use Committee of Genentech.

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Measurements of MAP and HR
After anesthesia with intraperitoneal injection of ketamine 80 mg/kg and xylazine 10 mg/kg, catheters were implanted into the right femoral artery and vein. MAP and HR were measured in conscious unrestrained SHR (n=53) and WKY (n=51) 1 day after catheterization with a pressure transducer coupled to a polygraph. VEGF (recombinant human VEGF165, Genentech Inc) was infused intravenously at 0.3, 1.5, or 4.5 μg/kg per min in normal saline (5 μL/min) for 2 hours.

Plasma NO Assessment
Blood (0.2 to 0.25 mL) was collected from the arterial catheter before and 2 hours after intravenous infusion of VEGF (1.5 μg/kg per min) in SHR and WKY (n=9 in each group). Serum nitrates were measured by capillary electrophoresis.

Pharmacokinetic Study
Blood (300 μL) was collected in 6 SHR and 6 WKY before and after intravenous infusion of VEGF at 1.5 μg/kg per min for 120 minutes. Plasma rhVEGF was measured using a dual monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA). This assay utilizes a capture antibody MAb 3.5 F8 that binds to the VEGF heparin-binding domain (amino acid Nos. 111 to 165) and a detecting antibody, MAb A 4.6.1 that binds to the KDR-binding region (amino acid Nos. 82, 84, and 86). A one-compartmental method was used to calculate VEGF pharmacokinetic parameters.

Plasma PGI2 Assay
The levels of 6-ketoprostaglandin F1α, a nonenzymatically hydrated product from PGI2, in the plasma samples were measured by using an ELISA kit from Cayman Chemicals according to the manufacturer’s instructions. The amount of 6-ketoprostaglandin F1α (pg/mL) was calculated based on a standard curve.

Chronic Administration of VEGF
An Alzet pump was implanted for infusion of saline or VEGF into the right jugular vein at the dose of 0.16, 0.32, or 0.42 μg/kg per min for 7 days in 28 SHR and 28 WKY. Six days after the continuous infusion, the arterial catheter was implanted. One day later, MAP and HR were measured in conscious rats.

Microarterial Preparation and Mounting In Vitro
The mesentery arteries were dissected and cut into cylindrical rings under a microscope. A pair of stainless steel wires (diameter, 40 μm) was guided through the lumen of each ring. One wire was fixed tightly on a jaw in a 2-channel myograph (Model 500A, J.P. Trading), and another wire was anchored to another jaw of the same chamber. The measurement was performed as described previously.

Statistical Analysis
Results are expressed as mean±SEM. One-way ANOVA was performed to assess differences in parameters under the same condition between groups and to compare changes over time within each group. Two-way ANOVA was used to compare parameters in response to chronic VEGF infusion. Significant differences were then subjected to posthoc analyses using the Newman-Keuls method. P<0.05 was considered to be statistically significant.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results
Hemodynamic Responses to Acute Infusion of VEGF
Intravenous administration of VEGF for 2 hours caused a dose-related reduction in MAP in both SHR and WKY (Figure 1A). The reduction in MAP was significantly greater in SHR than in WKY. A concomitant increase in HR, however, was observed in WKY at the 3 doses but not in SHR at any doses studied (Figure 1, bottom). For example, following 2-hour infusion at the same dose (1.5 μg/kg per min), the maximal hypotensive response was exaggerated by 2.5-fold in SHR compared with WKY controls (Figure 1A). In contrast, this dose of VEGF significantly increased HR in WKY (P<0.01) but not in SHR (Figure 1B).

Effects of VEGF on Plasma NO and PGI2
Because of a very short half-life, NO gets converted rapidly to nitrites and nitrates, and nitrites also get converted to nitrates in the presence of oxygen. Accordingly, plasma nitrate concentration was used as an indicator of NO release in vivo. After 2-hour infusion of VEGF at the same dose (1.5 μg/kg per min), the plasma level of nitrates (an indicator of NO) was elevated by 28% in WKY (P<0.01) and by 25% in SHR (P<0.068) (Figure 2A). The VEGF-induced increase in plasma nitrates was not significantly different between the 2 strains (P>0.314). There is no difference in plasma levels of 6-ketorostaglandin F1α, an indicator of PGI2, before and after VEGF administration between SHR and WKY (Figure 2B).

Pharmacokinetic Responses to VEGF
Intravenous infusion of VEGF at 1.5 μg/kg per min produced substantially higher plasma levels of VEGF in SHR than WKY (P<0.01) (Figure 2C). Areas under the curve (pg/mL...
per min) in SHR were 2-fold larger than those in WKY \( (P<0.01) \). This was associated with a 57% decrease in VEGF clearance in SHR compared with WKY controls \( (P<0.01) \) (Figure 2D).

**Hemodynamic Responses to Chronic Intravenous Infusion of VEGF**

Intravenous infusion of VEGF at 0.16 \( \mu \text{g/kg per min} \) for 7 days did not produce a significant change in MAP in conscious SHR and WKY (data not shown). VEGF at 0.32 \( \mu \text{g/kg per min} \), however, resulted in a 27% reduction in MAP in SHR \( (P<0.01) \) but only a small decrease in MAP in WKY \( (P=\text{NS}) \) (Figure 1C). In contrast, the chronically infused VEGF at this dose increased HR by 45% in WKY \( (P<0.01) \) but did not alter HR in SHR (Figure 1D). Further, VEGF at 0.42 \( \mu \text{g/kg per min} \) caused a marked fall in MAP \( (36\% \text{ in WKY and } 46\% \text{ in SHR compared with the respective vehicle-treated controls}) \), with a 50% mortality rate in each group.

**VEGF-Induced Relaxation of the Mesentery Artery In Vitro**

The mean internal diameter of the rings at an equivalent transmural pressure of 100 mm Hg (\( D_{100} \)) was 432.5 ± 1.15 \( \mu \text{m} \) as determined from the normalization procedure. When the mesentery microartery rings were set at a resting diameter of 90\% \( D_{100} \), the equivalent transmural pressure was 58.7 ± 0.6 mm Hg, and the resting force was 6.21 ± 0.31 mN. There was no significant difference in the internal diameter, resting force, and U46619-induced precontraction force between groups.

In both the WKY and SHR groups, VEGF induced relaxation in a concentration-dependent manner in the mesentery microartery rings (Figure 3, top and middle). The VEGF-induced vasorelaxation was significantly attenuated by pretreatment with \( \text{N}-\text{nitro-L-arginine} \) (a specific inhibitor of NO synthase) and completely abolished by removing endothelium \( (P<0.01) \) vs control group; \( ##P<0.01 \) vs the SHR and WKY group.

**Discussion**

The present study showed that acute and chronic administration of VEGF resulted in a dose-related reduction in MAP in both conscious SHR and WKY. The VEGF-induced reduction in MAP was significantly greater in the hypertensive SHR than in normotensive animals (Figure 1A). This is the first report documenting that systemic administration of VEGF induces exaggerated hypotension in the setting of hypertension.
Interestingly, our in vitro experiments demonstrated that VEGF induced an endothelium-dependent vasorelaxation in the mesentery microartery rings, which was significantly blunted in the SHR group compared with the WKY group (Figure 3). This finding is consistent with our previous observation that SHR exhibits a diminished vasorelaxant response to VEGF in aortic rings.23 Furthermore, the basal level of plasma NO before VEGF administration was decreased by 27% in SHR compared with WKY. Intravenous infusion of VEGF at 1.5 μg/kg per min for 2 hours significantly increased the plasma NO level in WKY (P < 0.01), whereas the same dose of VEGF tended to increase the NO level in SHR (P = 0.068). This is despite the fact that an increase in circulating VEGF concentration induced by the VEGF infusion was significantly greater in SHR than WKY (see below). Our findings are consistent with previous studies, which have shown that chronic hypertension is associated with endothelial dysfunction.25–31 SHR has been shown to exhibit impairment in endothelium-dependent vasorelaxation. Recently, Brovkovych et al32 demonstrated that in hypertensive SHR, endothelial dysfunction is associated with decreased endothelial NO release compared with normotensive rats, which is likely owing to a higher production of O2– from oxygen in SHR. Endothelial dysfunction, however, would be expected to reduce the hypotensive response to VEGF in SHR, and apparently cannot account for the exaggerated hypotension in response to VEGF observed in the hypertensive animals.

The present study suggests 2 mechanisms that may contribute to the enhanced hypotensive effect of VEGF in SHR. First is a decrease in VEGF clearance. Our pharmacokinetic study revealed that intravenous administration of VEGF at the same dose produced a significantly higher plasma level of VEGF and a significantly lower VEGF clearance in SHR than in WKY (Figure 2 C and 2 D). It has been suggested by renal transplantation studies that an intrinsic defect in the kidney plays an important role in the development and maintenance of hypertension in SHR.33 Renal dysfunction identified in SHR includes reduced renal excretory function, low renal plasma flow, reduced glomerular filtration rate, and reduced renal interstitial hydrostatic pressure.34–37 These abnormalities in renal function would result in a decrease in VEGF clearance in SHR. Although the higher plasma level of VEGF after infusion did not induce a higher circulating level of NO in SHR compared with WKY because of endothelial dysfunction, factors other than NO could mediate the VEGF-induced hypotension, because pretreatment with specific inhibitors of NO synthase only partially attenuated vasorelaxant responses to VEGF in both SHR and WKY (Figure 3). Recent in vitro studies suggest that in addition to NO, VEGF also stimulates synthesis of PGI2 that may be involved in VEGF-induced vasodilation.38,39 Our results, however, showed there was no difference in plasma PGI2 before and after VEGF infusion between SHR and WKY, suggesting that the exaggerated hypotensive effect of VEGF in SHR is not attributed to PGI2.

Second is a defect in baroreflex function. The hypotensive response to VEGF is accompanied by an increase in HR in conscious normal animals.20 VEGF, however, does not alter HR in the isolated heart preparation,20 suggesting that VEGF-induced tachycardia may be caused by a reflex response to a decrease in arterial pressure, which is primarily through the baroreflex, rather than a direct effect on the cardiac pacemaker. This is a compensatory mechanism that prevents a further decrease in arterial pressure. The present study demonstrated that both acute and chronic infusion of VEGF caused a concomitant increase in HR in WKY but not in SHR (Figure 1B and 1D). It is known that SHR exhibits damage to the baroreflex function,40–47 and the baroreflex control of HR was diminished in SHR compared with WKY. The defect of baroreflex would lead to a complete lack of the tachycardic response to a fall in arterial pressure induced by VEGF, thereby contributing to the exaggerated hypotension.

The hypotension induced by acute administration of VEGF is transient or lasts a short time, and usually can be tolerated in normal animals. The side effect of VEGF, however, could be life threatening in the setting of severe heart diseases when given by bolus injection. Hariawala et al14 have reported that intravenous administration of VEGF (2 mg) as a single bolus injection improves myocardial blood flow but produces severe hypotension, incurring a 50% chance of death (4 in 8) in pigs with chronic myocardial ischemia. We have previously demonstrated that although the hypotensive response to VEGF is attenuated when given by intravenous infusion compared with bolus injection, a dose-related reduction in arterial pressure is still observed after VEGF infusion.48 The present study shows that in response to VEGF infusion, the hypertensive animals exhibit exaggerated hypotension compared with that of normotensive controls. In addition, we also found chronic infusion of VEGF at a higher dose for 7 days caused severe hypotension with a 50% mortality rate in both SHR and WKY, suggesting that chronic, continuous infusion of VEGF at higher doses should be avoided even in normotensive subjects. In contrast to administration of the VEGF protein that induces hypotension, however, gene therapy with VEGF DNA may avoid this side effect. Clinical studies indicate that gene transfer of VEGF DNA produces beneficial effects without evidence of hypotension in patients with coronary artery disease.49–50 Further clinical trials in a large number of patients are needed to confirm the benefits of the gene therapy.

In conclusion, despite the in vitro observations that VEGF induced a blunted vasorelaxation in SHR compared with WKY because of endothelial dysfunction, both acute and chronic administration of VEGF resulted in an exaggerated hypotension with no tachycardia in conscious SHR. The exaggeration of the hypotensive response to VEGF may be owing to damaged baroreflex function and reduced VEGF clearance in SHR. Our data suggest that more precautions may be necessary when VEGF is systemically administered in patients with hypertension and/or renal dysfunction. Further studies may be needed to determine whether local administration of VEGF reduces the dose required and thus the hypotensive effect observed.

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References


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