Substantially Attenuated Hemodynamic Responses to 
Escherichia coli-Derived Vascular Endothelial Growth Factor 
Given by Intravenous Infusion Compared with Bolus Injection

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ABSTRACT
Vascular endothelial growth factor (VEGF) produces beneficial angiogenesis in animal models of coronary and peripheral ischemia. However, intravenous bolus injection of Chinese hamster ovary cell (CHO)-derived VEGF produces adverse effects on hemodynamics. The present study examined pharmacokinetic and hemodynamic responses to Escherichia coli-derived VEGF, which will be used in clinical patients, compared with responses to CHO-derived VEGF, and tested whether intravenous infusion of E. coli-derived VEGF attenuates the hemodynamic responses compared with the responses observed with intravenous bolus injection. Hemodynamic parameters were measured before and after administration of VEGF in conscious, instrumented rats. Intravenous injection of both CHO- and E. coli-derived VEGF produced a similar maximal reduction in arterial pressure, although E. coli-derived VEGF exhibited less of a depressor effect in the initial phase after injection. Either infusion or injection of E. coli-derived VEGF caused hypotension, tachycardia and reduced cardiac output and stroke volume, which were significantly attenuated when given by infusion compared with injection. The maximal hypotensive and tachycardic responses to infusion were decreased by 50 to 60% compared with those responses observed after injection. Cardiac output was maximally reduced by 34% after injection but only 18% after infusion. A sustained elevation in systemic vascular resistance observed after injection was avoided after infusion. Thus, the hemodynamic side effects of VEGF administration can be substantially attenuated by controlling the rate of VEGF infusion. The data indicate that infusion, instead of bolus injection, is a more appropriate regimen for VEGF administration.

Received for publication May 28, 1997

ABBREVIATIONS: VEGF, vascular endothelial growth factor; CHO, Chinese hamster ovary cell; MAP, mean arterial pressure; HR, heart rate; HSPG, heparan sulfate proteoglycans; ELISA, enzyme-linked immunosorbent assay.
used in a rabbit hindlimb ischemic model to stimulate angiogenesis produces a significant reduction in cardiac output and stroke volume (Yang et al., 1996). Furthermore, it has been reported that intracoronary administration of CHO-derived VEGF as a single bolus improves myocardial blood flow but produces severe hypotension incurring a 50% death in the pig with chronic myocardial ischemia (Hariyawala et al., 1996). These preclinical studies suggest that the adverse effects of VEGF on hemodynamics, including hypotension, tachycardia and reductions in cardiac output and stroke volume, may limit clinical use of VEGF when given by bolus injection. However, the effects of E. coli-derived VEGF, the molecule that will be used for clinical patients, on hemodynamics and cardiac function has not been investigated.

The first purpose of the present study was to compare the pharmacokinetic and hemodynamic effects of E. coli-derived VEGF versus CHO-derived VEGF. The results demonstrated that intravenous injection of both molecules at the same dose induced a similar maximal hypotensive response. Because of the hemodynamic effects of VEGF given by bolus injection, alternate regimens of therapy, including systemic infusion or local application at lower doses, should be considered. The second purpose of the present study was to examine the effects of E. coli-derived VEGF given as intravenous infusion on pharmacokinetics, hemodynamics and cardiac function, and to compare these effects with the effects of E. coli-derived VEGF given as a bolus at the same doses in conscious animals. We show that the hemodynamic responses to VEGF are substantially attenuated when given by intravenous infusion compared with injection.

**Methods**

Male Sprague-Dawley rats aged 8 weeks were obtained from Charles River Breeding Laboratories, Wilmington, MA. One week after arrival, implantations of catheters and flowprobes were performed for measurements of hemodynamic parameters. The experimental procedures, which were approved by Genentech’s Institutional Animal Care and Use Committee, conform to the guiding principles of the American Physiological Society.

**VEGF.** As described previously (Ferrara and Henzel, 1989; Leung et al., 1989; Walter et al., 1996; Ferrara et al. 1991), a nonglycosylated form of the recombinant human VEGF165 (E. coli-derived VEGF) was purified and refolded from E. coli. The glycosylated form of VEGF165 (CHO-derived VEGF) was purified from media conditioned by CHO cells. The volume was 5 μl/min for intravenous infusion and 150 to 200 μl for intravenous injection. In a pilot study, the intravenous infusion (5 μl/min for 4 hr) or injection (200 μl) of vehicle alone did not affect MAP, HR and cardiac output. Each animal received only one administration of VEGF.

**Measurements of arterial pressure and heart rate.** MAP and HR were measured by catherization as described previously (Yang et al., 1996). After anesthesia with intraperitoneal injection of 80 mg/kg ketamine (Aveco Co., Inc., Fort Dodge, IA) and 10 mg/kg xylazine (Rugby Laboratories, Inc., Rockville Center, NY), catherets (PE-10 fused with PE-50) filled with heparin-saline (50 U/ml) were implanted into the abdominal aorta via the right femoral artery for measurement of MAP and HR, and into the right femoral vein for VEGF administration.

One day after catheterization, MAP and HR were measured in conscious, unrestrained rats with a model CP-10 pressure transducer (Century Technology Company, Inglewood, CA) coupled to a Grass model 7 polygraph (Grass Instruments, Quincy, MA). After a 45-min stabilization period, E. coli-derived or CHO-derived VEGF was injected intravenously at the same dose (300 μg/kg), and MAP and HR were monitored for 1 hr after injection. The dose of 250 to 300 μg/kg is an effective dose as a single bolus for stimulation of angiogenesis in an animal model of limb ischemia (Takeshita et al., 1994; Walter et al., 1996).

To examine the responses of MAP and HR to intravenous infusion of E. coli-derived VEGF in the separate groups, rats received intravenous infusion of VEGF at 0.50, 1.04, 2.75 and 5.50 μg/kg/min in normal saline (5 μl/min) for 4 hr. The total dose was 120, 250, 660 or 1320 μg/kg, and the total volume infused was 1.2 ml. To compare the responses to E. coli-derived VEGF given by infusion versus injection, an intravenous 150- to 200-μl bolus of E. coli-derived VEGF was given at the same doses (120, 250, 660 or 1320 μg/kg). MAP and HR were monitored before and after administration of VEGF.

**Assessment of cardiac function.** Cardiac output was measured by an ultrasonic probe as described previously (Yang et al., 1996). After anesthesia and cannulation of the femoral artery and vein as described above, rats were intubated via a tracheotomy and ventilated with a respirator (Harvard Apparatus model 683, South Natick, MA). Through a right-sided thoracotomy, the ascending aorta was exposed and gently separated from the pulmonary artery. The ultrasonic perivascular flowprobe (no. 2S165, Transonic Systems Inc., Ithaca, NY) was placed around the ascending aorta and a sterile K-Y jelly injected into the space between the vessel and the flowprobe. The flowprobe cable was exteriorized at the back of the neck, and the cable connector sutured and fixed in place. The chest was closed and the tracheal incision sutured after extubation.

One day after surgery, the arterial catheter was connected to a model T 201 flowmeter (Transonic Systems Inc., Ithaca, NY). The mean blood flow curve of the ascending aorta was recorded on a chart recorder (Kipp and Zonen, Rotterdam, Holland), and cardiac output, as shown by the mean flow, was also obtained digitally by the flowmeter. Stroke volume was calculated as cardiac output divided by HR, and systemic vascular resistance as MAP divided by cardiac output. After hemodynamic stabilization, rats received an intravenous infusion of E. coli-derived VEGF (0.50, 1.04 or 5.50 μg/kg/min for 4 hr) or an intravenous injection of E. coli-derived VEGF (250 or 1320 μg/kg) as indicated above. The hemodynamic parameters were continuously recorded before and after the infusion or injection.

**Blood collection for pharmacokinetics.** To avoid influence of blood loss on hemorrhagics, blood collections were performed in separate groups of rats. Samples were collected at predose and at 0.5, 1, 3, 5, 10, 20, 40, 60, 90, 120, 180 and 240 min after the VEGF bolus injection. Blood (0.2–0.25 ml) was collected from the arterial catheter into siliconized polypropylene tubes containing ethylenediaminetetraacetic acid (pH = 8) on ice before and after intravenous injection of CHO-derived or E. coli-derived VEGF at the same dose (220 μg/kg), and before and after intravenous infusion of E. coli-derived VEGF at the four doses. Samples were centrifuged at 13,000 rpm, and plasma was stored at −70°C for measurement of VEGF levels by an ELISA assay specific for VEGF.

**Pharmacokinetic analyses.** After intravenous bolus injection, CHO- and E. coli-derived VEGF plasma concentration versus time data for individual animals were analyzed by a noncompartmental analysis according to the following relation: clearance = dose/area under the curve (AUC). For comparison, nonlinear regression analysis was also examined by use of a two-compartment model (PC-NONLIN, Statistical Consultants, Lexington, KY). The initial (α) and terminal (β) half-lives and plasma clearance were calculated by standard pharmacokinetic methods (Gibaldi and Perrier, 1982). After intravenous infusion of VEGF, a noncompartmental method was used to estimate the plasma clearance. The plasma clearance was estimated as the ratio of the infusion rate versus the steady-state VEGF plasma concentration.

**ELISA assay.** A dual monoclonal ELISA was modified from that described previously (Krey et al., 1996) for the quantitation of VEGF.
The coated antibody was an anti-VEGF165 monoclonal antibody 5F8. A VEGF165 standard curve ranging from 0.1 to 10 ng/ml was used. A neutralizing anti-VEGF murine monoclonal antibody A4.6.1 was used for capture (Kim et al., 1992); signal was generated with horseradish peroxidase-conjugated goat IgG specific for murine IgG and developed with ortho-phenylenediamine. Signal was detected via the absorbance measured at 492 nm on a microplate reader (Molecular Devices, Sunnyvale, CA). The concentration of VEGF165 was quantitated by interpolation of a standard curve with nonlinear regression analysis.

**Statistical analysis.** Results are expressed as mean ± S.E.M. One-way analysis of variance was performed to assess differences in parameters at the same time point between groups and to compare changes over time within each group. P < .05 was considered to be statistically significant.

**Results**

**Pharmacokinetic and hemodynamic responses to intravenous bolus of CHO- and E. coli-derived VEGF.** Intravenous administration of either CHO- or E. coli-derived VEGF at 220 µg/kg resulted in high plasma concentrations of 2.4 ± 0.2 µg/ml at the initial time point, 30 sec after injection (fig. 1). The initial volume of distribution for both CHO- and E. coli-derived VEGF (91 ml/kg), estimated by the ratio of dose versus initial plasma concentration, approximated plasma volume. Clearance was evaluated by the ratio of dose versus the area under the curve derived from the concentration versus time data in figure 1. Both forms of VEGF were rapidly cleared from plasma with similar rates for CHO- and E. coli-derived VEGF (6.3 ± 0.8 and 6.6 ± 0.2 ml/min/kg, respectively). Bi-exponential equations were fitted to the data for plasma concentration versus time profiles for both forms of VEGF. Clearance of CHO-derived VEGF exhibited an α- and β-phase with calculated half-lives of 1.1 ± 0.3 and 32 ± 3 min, respectively. E. coli-derived VEGF exhibited an initial phase of less than 0.5 min and a terminal phase of 37 ± 4 min. In the initial distribution phase, E. coli-derived VEGF exhibited significantly reduced plasma levels during the first 30 min compared with those observed with CHO-derived VEGF. At later times (1–4 hr), E. coli-derived VEGF appeared to achieve slightly greater plasma concentrations than CHO-derived VEGF (fig. 1).

Intravenous injection of either E. coli-derived or CHO-derived VEGF produced a significant decrease in MAP and increase in HR (fig. 2). Both molecules resulted in a similar maximal reduction in MAP, although there was a difference in the depressor response of the two forms of VEGF during the initial phase after injection. The reduction in MAP induced by E. coli-derived VEGF was significantly less than that induced by CHO-derived VEGF during the initial time period (1–7 min) after injection. There was no difference in the tachycardic response to either form of VEGF (fig. 2, bottom).

During the initial phase of clearance, CHO-VEGF exhibited 2-fold higher plasma concentrations than those of E. coli-derived VEGF. Correspondingly, the decrease in MAP observed with CHO-VEGF achieved a lower nadir at an earlier time (by approximately 2 min) than that induced with E. coli-derived VEGF (fig. 2, top).

![Fig. 1. Plasma levels of VEGF after intravenous injection of CHO-derived and E. coli-derived VEGF at the same dose (220 µg/kg). Data are presented as the mean ± S.E.M. of three animals. (The number in parentheses is the number of animals in each group.) There was a significant difference (P < .05) between the two groups in plasma levels of VEGF at the initial phase (0.5–10 min) after injection. The basal levels of VEGF were undetectable before VEGF administration.](image1)

![Fig. 2. Responses of MAP (top panel) and HR (bottom panel) to intravenous injection of vehicle, CHO-derived and E. coli-derived VEGF at the same dose (300 µg/kg) in conscious rats. Data are presented as the mean ± S.E.M. of five to nine animals. (The number in parentheses is the number of animals in each group.) There was a significant difference (P < .05) in the decrease in MAP at the initial phase (1–7 min) after injection between the CHO-derived VEGF group and the E. coli-derived VEGF group. ## P < .01, comparison between the vehicle group and other two groups.](image2)
Pharmacokinetic and hemodynamic responses to intravenous infusions of E. coli-derived VEGF. E. coli-derived VEGF was administered to rats as an intravenous infusion for 4 hr at rates which varied from 0.5 to 5.5 μg/min/kg. A steady elevation in plasma VEGF was observed during the first 3 hr of infusion at the lower dose groups (fig. 3, top). Steady-state levels of plasma VEGF were achieved with all dosing regimens during the 4-hr infusion. Clearance was calculated as the ratio of the infusion rate versus the plasma VEGF concentration at steady state. A dose-dependent saturation of clearance appeared with increasing infusion rates (fig. 3, bottom). At 0.5 μg/min/kg infusion of VEGF, the clearance was 18.6 ml/min/kg. However, at 5.5 μg/min/kg, the rate of clearance decreased approximately 3.5-fold to a value of 5.2 ml/min/kg. Saturation of plasma clearance was half-maximal at 2 μg/min/kg in the rat. These data indicate that the bolus doses of VEGF (300 μg/kg) were in fact saturating the VEGF clearance process in vivo.

Intravenous infusion of E. coli-derived VEGF at 0.5 to 5.5 μg/kg/min for 4 hr caused a gradual reduction in MAP which reached a maximal decline at 3.5 to 4 hr infusion (fig. 4, top). A dose-related depressor response was observed at the doses of 0.5 to 1.04 μg/kg/min. At 1.04 μg/kg/min the decrease in MAP was −13 mmHg at 4 hr. However, no decreases in MAP greater than this level were seen with doses up to 5.5 μg/kg/min during 4 hr (fig. 4, top).

Infusion of E. coli-derived VEGF was associated with a dose-dependent increase in HR which also reached a peak level at 3.5 to 4 hr (fig. 4, bottom). The dose-dependent tachycardic response was observed through all of the doses tested.

Effects of intravenous infusion of E. coli-derived VEGF on cardiac function. Intravenous infusion of E. coli-derived VEGF resulted in a dose-related reduction in cardiac output and stroke volume, which was significant between the dose of 0.5 and 1.04 μg/kg/min or between 0.5 and 5.5 μg/kg/min, but not between 1.04 and 5.5 μg/kg/min (fig. 5, top and middle). Actually, a transient elevation in cardiac output and stroke volume, which was not statistically significant, was seen almost immediately after infusion. A significant reduction (P < .05) in cardiac output in response to the VEGF began at 30 to 40 min after infusion, reached a nadir at 50 to 60 min and remained at this plateau during the subsequent 4 hr. The reduction in cardiac output was paralleled by the decrease in stroke volume.
Effects of intravenous infusion of E. coli-derived VEGF on cardiac output (CO, top panel), stroke volume (SV, middle panel) and systemic vascular resistance (SVR, bottom panel) in conscious rats. Data are presented as the mean ± S.E.M. of seven to eight animals. (The number in parentheses is the number of animals in each group.) * P < .05, ** P < .01, compared with the lowest dose group.

The VEGF given by infusion caused a significant decrease (P < .05) in systemic vascular resistance at the higher doses. Systemic vascular resistance began to fall almost immediately after infusion, reached a nadir at 10 to 15 min and returned to the pretreatment level at 40 min (fig. 5, bottom). Thereafter, systemic vascular resistance was slightly increased at the higher doses, but the increase was not statistically significant.

Comparison of hypotensive and tachycardic responses to infusion versus injection of E. coli-derived VEGF. There were no significant differences in basal levels of MAP and HR before administration of the VEGF between the infusion and injection groups (table 1).

Intravenous injection of E. coli-derived VEGF at 120 to 1320 μg/kg induced a dose-dependent decrease in MAP (fig. 6, top). MAP began to decrease almost immediately after injection, reached a nadir at 5 to 10 min and returned close to base line at 20 min. Concomitant with the decrease in MAP, the injection of the VEGF caused a tachycardia which reached a peak level at 3 to 7 min, and lasted 20 min at the higher doses and more than 60 min at the higher doses. The tachycardic responses, however, were not dose-dependent through all the four doses (fig. 6, bottom). This was different from the response after infusion, where the reduction in MAP was not dose-dependent at the total doses of 250 to 1320 μg/kg, but the increase in HR was dose-dependent (fig. 6).

There were marked differences in the maximal decrease in MAP and increase in HR at the same doses between the infusion and injection groups (fig. 6). The maximal hypotensive and tachycardic responses to infusion were decreased by approximately 50 to 60% compared with the responses to injection.

### Comparison of responses of cardiac function to infusion versus injection of E. coli-derived VEGF

There were no significant differences in the pretreatment levels of cardiac output, stroke volume and systemic vascular resistance were found between the infusion and injection groups (table 1).

Intravenous injection of E. coli-derived VEGF at the doses of 250 or 1320 μg/kg resulted in a transient increase in cardiac output and stroke volume, which was not statistically significant, almost immediately after injection. The cardiac output and stroke volume returned to the basal line at 3 to 5 min, began to decline at 5 to 7 min, reached a nadir at 15 min and remained decreased during the observed 60 min. The decline in stroke volume appeared to be dose-dependent when given by injection but not by infusion (fig. 7, bottom). Cardiac output was maximally reduced by 34% after injection, but only 18% after infusion at the same dose (250 μg/kg).

After injection of the VEGF, systemic vascular resistance was immediately reduced for 3 min, returned to the basal line at 5 min and then remained elevated for >60 min. The elevation in systemic vascular resistance appeared to be

### Table 1

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<tr>
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<th>Injection</th>
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<tr>
<td>MAP (mm Hg)</td>
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<tr>
<td>120 μg/kg</td>
<td>97.3 ± 2.4 (10)</td>
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<td>250 μg/kg</td>
<td>99.5 ± 1.3 (10)</td>
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<td>660 μg/kg</td>
<td>104.6 ± 3.3 (8)</td>
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<td>1320 μg/kg</td>
<td>103.7 ± 2.1 (8)</td>
<td>99.2 ± 2.3 (8)</td>
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<td>HR (bpm)</td>
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<tr>
<td>120 μg/kg</td>
<td>375.0 ± 9.3 (10)</td>
<td>377.5 ± 9.8 (10)</td>
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<td>250 μg/kg</td>
<td>377.5 ± 7.5 (10)</td>
<td>382.7 ± 7.2 (9)</td>
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<td>660 μg/kg</td>
<td>385.7 ± 8.6 (8)</td>
<td>365.0 ± 10.2 (9)</td>
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<td>1320 μg/kg</td>
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<td>250 μg/kg</td>
<td>92.7 ± 7.2 (8)</td>
<td>80.5 ± 6.0 (6)</td>
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<td>1320 μg/kg</td>
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<td>1320 μg/kg</td>
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<td>1320 μg/kg</td>
<td>1.053 ± 0.068 (10)</td>
<td>1.068 ± 0.068 (6)</td>
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* Data are presented as the mean ± S.E.M. (The number in parentheses is the number of animals in each group.) No significant difference occurred between the two groups at the same dose in the hemodynamic parameters before treatment.

MAP, mean arterial pressure; HR, heart rate; CO, cardiac output; SV, stroke volume; SVR, systemic vascular resistance.
dose-dependent (fig. 8). In contrast, infusion of the VEGF did not produce a significant increase in systemic vascular resistance. This indicates that the sustained elevation in systemic vascular resistance observed after injection was avoided after infusion.

Discussion

Native VEGF is a glycoprotein because of the presence of an N-linked glycosylation site at Asn-75 (Leung et al., 1989). Two recombinant forms of human VEGF have been purified: CHO-derived VEGF, which is glycosylated, and E. coli-derived VEGF, which is nonglycosylated (Walter et al., 1996). Previous studies have shown that bolus injection of CHO-derived VEGF induces beneficial angiogenesis in animal models of myocardial and peripheral ischemia, but may be associated with adverse effects on hemodynamics and cardiac function because of nitric oxide-mediated vasodilation and vascular hyperpermeability. Because VEGF expressed in E. coli is currently being used for clinical trials, and because deglycosylation may influence the bioactivity of VEGF, it is important to investigate the biological effect of E. coli-derived VEGF, including the angiogenic and hemodynamic effects.

The present study demonstrated that there was no difference in the maximal hypotensive and tachycardic responses to E. coli-derived versus CHO-derived VEGF in conscious animals. This finding is consistent with the observation of Walter and colleagues (1996), who showed that the angiogenic effect is similar for E. coli-derived and CHO-derived VEGF in vitro and in vivo. The data suggest that the potential for VEGF to induce both angiogenic and maximal hemodynamic responses persists unaltered in the nonglycosylated state.

Although the maximal hypotensive response to both forms of VEGF was similar, E. coli-derived VEGF had a smaller depressor effect than CHO-derived VEGF in the initial phase after injection at the same dose. In conjunction with the transient difference in the depressor effect, the plasma level of VEGF was also lower in the initial phase after injection of E. coli-derived VEGF than CHO-derived VEGF. In vitro studies suggest that E. coli-derived VEGF binds heparin with higher affinity than CHO-derived VEGF. Thus, it has been postulated that the difference in pharmacokinetic behavior between the VEGF produced in different host cells is related to differences in interactions with endogenous HSPG (DeGuzman et al., 1997). It is likely that the smaller depressor effects of E. coli-derived

Fig. 6. Comparison of maximal responses of MAP (top panel) and HR (bottom panel) to E. coli-derived VEGF or vehicle given by intravenous infusion versus injection at the same doses. Data are presented as the mean ± S.E.M. of 8 to 10 animals in each VEGF-treated group and 5 animals in each vehicle group. There was a significant difference (P < .01) in MAP or HR between the vehicle group and the respective VEGF-treated groups. * P < .05, ** P < .01, compared with the dose of 120 µg/kg, + P < .05, compared with the dose of 250 µg/kg by the same route of administration. # P < .05, ## P < .01, comparison between injection versus infusion at the same dose.

Fig. 7. Comparison of maximal responses of cardiac output (CO, top panel) and stroke volume (SV, bottom panel) to E. coli-derived VEGF or vehicle given by intravenous infusion versus injection at the same doses. Data are presented as the mean ± S.E.M. of 6 to 8 animals in each VEGF-treated group and 5 animals in each vehicle group. There was a significant difference (P < .01) in CO or SV between the vehicle group and the respective VEGF-treated groups. * P < .05, compared with the dose of 250 µg/kg by the same route of administration. # P < .05, ## P < .01, comparison between injection versus infusion at the same dose.
VEGF is also known as vascular permeability factor because of its ability to promote extravasation (Connelly et al., 1989; Keck et al., 1989) or to increase microvascular permeability (Sengel et al., 1983, 1986). We have previously shown that VEGF given as a bolus causes reductions in cardiac output and stroke volume probably because of a decline in venous return caused by vascular hyperpermeability rather than a direct effect on myocardial contractility (Yang et al., 1996). The present study demonstrated that the E. coli-derived VEGF-induced reduction in stroke volume and cardiac output was substantially attenuated when given by intravenous infusion compared with injection. Cardiac output was maximally reduced by 34% after injection, but only 18% after infusion at the same dose (250 μg/kg). In addition, a sustained elevation in systemic vascular resistance observed in the later phase after injection, which may be secondary to a reflex response to a remarkable decline in cardiac output, was avoided after infusion.

In summary, the hemodynamic effects of E. coli-derived VEGF are basically similar to those of CHO-derived VEGF. The side effects of E. coli-derived VEGF given as a bolus or hemodynamics and cardiac function can be substantially attenuated by infusion, which indicates that infusion, instead of bolus injection, is an appropriate regimen for VEGF administration.

Acknowledgments

We are grateful to Lea Berleau for her technical assistance and to Stephen Eppler for pharmacokinetic analysis.

References


Conn G, Bayne M, Soderman L, Kwok PW, Sullivan KA, Paliis TM, Hope DA and Thompson EF (1996) The vascular endothelial growth factor (VEGF) is associated with its low-affinity HSPG binding sites rather than a direct action on the cardiac pacemaker, because VEGF did not alter HR in the isolated heart preparation (Yang et al., 1996). This reflex response to a decrease in arterial pressure is primarily through the baroreflex which is actually a compensatory mechanism preventing a further decrease in arterial pressure by tachycardia and vasoconstriction. It is likely that after infusion of VEGF, a gradual, smaller decrease in MAP could initiate the dose-dependent reflex response, thereby inhibiting the further decrease in MAP at the higher doses. After injection of VEGF, however, a maximal reflex response to a rapid, larger reduction in MAP was already reached at the lower dose, so that the depressor response to the higher doses of VEGF would be increased when the reflex mechanism was limited.


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