sFlt-1, a Potential Antagonist for Exogenous VEGF

To the Editor:

Results from the VEGF in Ischemia for Vascular Angiogenesis (VIVA) clinical trial, which demonstrated the therapeutic use of vascular endothelial growth factor (VEGF) in angina, were presented at the 48th Scientific Sessions of the American College of Cardiology. These results indicated that no significant difference existed in the symptoms or clinical events of patients infused with VEGF and those of the placebo group. Although this may be due in part to inadequate dosage or an inappropriate delivery method, this unexpected result could also be attributed to the presence of the soluble VEGF receptor Flt-1 (sFlt-1). An analysis of this receptor reveals that it, like the membrane-bound receptor, binds VEGF with high affinity and inhibits VEGF-mediated events in vitro.2,3

Our laboratory developed a specific and sensitive enzyme-linked immunosorbent assay using commercial antibodies and recombinant standards (R&D Systems) to measure the plasma levels of free sFlt-1, which can bind VEGF, in vitro. This assay was developed for an ongoing study testing the hypothesis that patients with atherosclerotic vascular disease will have abnormal levels of VEGF and sFlt-1 when compared with healthy controls. Our pilot study of 60 patients (45 men; mean age, 58 ± 10 years) with coronary artery disease (all with a previous myocardial infarction; samples collected 6 weeks post–myocardial infarction) were compared with an equal number of age- and sex-matched healthy controls. The median level of VEGF (as determined by ELISA, R&D Systems) in the citrated plasma from patients was 430 pg/mL (interquartile range, 136 to 1004 pg/mL); the median sFlt-1 level was 14 ng/mL (range, 1 to 45 ng/mL). In the controls, the median levels of VEGF and sFlt-1 were 75 pg/mL (interquartile range, 20 to 125 pg/mL) and 23 ng/mL (range, 15–37 ng/mL), respectively. An analysis of the data using the Mann–Whitney U test confirmed that VEGF levels in patients with coronary artery disease were significantly higher than those of the control group (P < 0.001) but that levels of sFlt-1 were lower in patients compared with controls (P < 0.05). Nevertheless, levels of sFlt-1 in both patients and controls were 33-fold and 306-fold greater than those of VEGF, respectively.

Although early clinical trials and studies of animal models indicated that the therapeutic use of VEGF could improve collateral circulation, the high plasma levels of sFlt-1 may act as a potential antagonist for VEGF. Thus, any exogenously administered VEGF would have to completely neutralize its soluble receptor (sFlt-1) before adequate levels would reach target organ(s). This may have contributed to the lack of effect of VEGF that was experienced by the VIVA investigators.

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Response

Angiogenesis is a complex process with a relative balance between proangiogenic factors and inhibitors of angiogenesis.3 Therapeutic angiogenesis is an attempt to enhance this natural process by the use of exogenous angiogenic growth factors or genes encoding for those growth factors. Inhibitors of angiogenesis, such as angiostatin, endostatin, thrombospondin-1, asymmetric dimethylarginine, metalloproteinase inhibitors, soluble receptors, and others, may well influence this attempt. In addition, clinical variables such as age, diabetes, or use of medications such as angiotensin-converting enzyme inhibitors, nitrates, or heparin may influence the response to exogenous angiogenesis growth factors.

The letter from Belgore et al raises the question of whether the presence of soluble Flt-1 receptors (sFlt-1) for vascular endothelial growth factor (VEGF) could account for the lack of efficacy seen at 60 days in the VEGF in Ischemia for Vascular Angiogenesis (VIVA) trial. The VIVA trial is currently the largest randomized, placebo-controlled trial in the field of therapeutic angiogenesis, with 178 patients randomized to receive placebo or 17 or 50 ng · kg⁻¹ · min⁻¹ of intracoronary plus intravenous VEGF-165. At 60 days, all 3 groups had improvements in chest pain, quality of life, and exercise time compared with baseline, but no significant difference existed between groups, in large part because of a prominent placebo effect.2 The final 120-day results demonstrated a decrease in the benefits of placebo and ongoing improvement in patients receiving high-dose VEGF, which resulted in a statistically significant improvement in angina class (P = 0.04) and a trend for improvement in exercise time (14 versus 47 seconds; P = 0.17).3 In addition, the trial had an excellent safety profile, with all adverse events (2 deaths, 3 cancers, and 1 retinopathy) occurring in the placebo group.

The VEGF monoclonal ELISA used in the analysis of VIVA patient plasma samples demonstrated >89% recovery of VEGF-165 when exogenous VEGF-165 was spiked into human plasma.4 The excellent recovery of VEGF immunoreactivity in our assay suggests that little or no sFlt-1 is found in human plasma, because sFlt-1 is known to inhibit the detection of VEGF-165 in this assay. We also repeated and confirmed the work of others who reported the presence of sFlt-1 in gravid serum but not in nongravid serum. It is difficult to resolve the apparent discrepancies between our observations (suggesting little or no sFlt-1 in normal human plasma) and those of Belgore et al without a knowledge of their ELISA qualification data. It will also be important to rule out the possibility of a sFlt-1 cross-reactive substance being detected in their polyclonal assay format.

Further analysis of the VIVA data, including that on responders and nonresponders, is essential to provide insight into the questions raised by Belgore et al. Elevated levels of sFlt-1 or other VEGF antagonists may well influence the response to exogenously administered VEGF. Our search for the ideal therapeutic regimen will be successful only when we advance our understanding of the complex process of angiogenesis. We
hope that the results gleaned from the VIVA trial will contribute to this search.

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