Identification and Localization of Heparin-Binding Region of Snake Venom VEGF and Its Blocking of VEGF-A165

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Key Words

Vascular endothelial growth factor · Snake venom · Heparin-binding protein

Abstract

Vascular endothelial growth factor (VEGF-A165) displays diverse effects through binding to its receptor, KDR (kinase domain-containing receptor). Heparan sulfate/heparin-like molecules greatly contribute to their interaction. Indeed, the mitogenic potency of VEGF-A165 lacking the C-terminal heparin-binding region is less than 1% compared with intact VEGF-A165. We previously found novel heparin-binding VEGFs, designated VEGF-F that specifically recognizes KDR in snake venoms. VEGF-Fs almost completely lack the C-terminal heparin-binding region compared with VEGF-A165, despite their heparin-binding potential. In this study, we attempted to identify the heparin-binding region of VEGF-F using synthetic peptides. We have demonstrated that the heparin-binding site of VEGF-F is located in its C-terminal region, particularly localized on the N-terminal portion of this region. Furthermore, a synthetic peptide of this region blocks the biological activity of VEGF-A165 in vitro and in vivo.

Vascular Endothelial Growth Factor (VEGF) and Heparin-Binding Potential

Vascular endothelial growth factor (VEGF-A165) displays multiple effects, such as promoting endothelial growth and vascular permeability and hypotension, through binding to its receptor, KDR (kinase domain-containing receptor) [1–3]. Heparan sulfate/heparin-like molecules are known to greatly modulate their interaction (fig. 1). In fact, the mitogenic potency of VEGF-A165 lacking the C-terminal heparin-binding region (55-amino acid residues) is less than 1% compared with that of intact VEGF-A165 [4].

We previously reported the isolation and characterization of two novel heparin-binding VEGF, designated vamin and VR-1, from two distinct viper venoms [5]. Vamin and VR-1 exhibit more potent biological activity when compared to VEGF-A165 and selectively recognize KDR rather than other VEGF receptors [5]. Due to their unique receptor selectivity and biological properties, we proposed a new subtype, VEGF-F, for snake venom-derived VEGFs that specifically recognize KDR.

VEGF-A165 is an ~45-kDa homodimeric glycoprotein possessing a 55-residue basic heparin-binding region in its C-terminus. A C-terminal-deficient form of VEGF-A165 (110 residues) generated by plasmin digestion was

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Fig. 1. Schematic model of the interaction between VEGF-A165, KDR, and heparin. VEGF-A165 is composed of two domains, an N-terminal receptor-binding domain (grey) and a C-terminal heparin-binding domain (dark grey). Heparin/heparan sulfate-like molecules modulate the interaction between VEGF-A165 and KDR by binding via C-terminal heparin-binding domain.

not retained from a heparin affinity column and displayed markedly reduced (greater than 100-fold) potency of growth factor activity when compared with intact VEGF-A165 [4]. VEGF-Fs are ~25-kDa homodimeric heparin-binding proteins. The primary structure of VEGF-Fs possesses ~50% identity with that of VEGF-A165, and they have a markedly short C-terminal portion (16–17 amino acid residues) when compared with VEGF-A165. This region does not include cysteine bridges, and does not show any significant homology with other proteins or domains, including the C-terminal heparin-binding region of other VEGF subtypes.

We herein report the C-terminal region of VEGF-F fully mediates its heparin-binding activity and inhibits the biological activity of VEGF-A165 *in vitro* and *in vivo*. These results suggest that the C-terminal peptide of VEGF-F inhibits the biological activity of VEGF-A165 via binding to heparin-like molecules.

Identification of Heparin-Binding Region of Snake Venom VEGF and VEGF-Blocking Activity

We first synthesized the C-terminal region of vammin (corresponding to residues 94–110; 17 residues), designated peptide 1. Peptide 1 was purified by two steps column chromatography; heparin affinity chromatography followed by reversed-phase HPLC. Peptide 1 and intact vammin were tested for heparin-binding ability using heparin affinity chromatography. Heparin-binding potential of vammin and peptide 1 were virtually equivalent as determined by the NaCl concentrations required for elution (0.33 and 0.36 *M*, respectively), indicating that the heparin-binding activity is completely mediated by the C-terminal region.

We speculated that if the C-terminal heparin-binding region of vammin recognizes similar heparin structures, it competes with VEGF-A165 on the endothelial surface and inhibits the biological activities of VEGF-A165. The effect of peptide 1 on endothelial cell proliferation activity of vammin or VEGF-A165 was thus investigated. Peptide 1 completely inhibited both vammin- and VEGF-A165-stimulated endothelial proliferation with similar IC_{50} values (320 and 280 μM , respectively). We also examined the effect of peptide 1 on rat arterial blood pressure *in vivo*. Administration of peptide 1 (3 $\mu g/g$, 5 min before following vammin administration) via femoral vein completely blocked the vammin (0.1 $\mu g/g$)-induced hypotensive effect. In contrast, a higher dose of peptide 1 (30 $\mu g/g$) was required to completely inhibit VEGF-A165-induced hypotension. These results indicate that peptide 1 is able to block the biological activity of VEGF-A165 even *in vivo*.

Further Localization of Heparin-Binding Site in Peptide 1 Sequence

To further investigate localization of heparin-binding site of peptide 1, we synthesized two additional peptides that correspond to N- or C-terminal part of peptide 1, designated peptide 2 (corresponding to residues 94–101; 9 residues) and peptide 3 (corresponding to residues 102–110; 11 residues), respectively. The heparin-binding potential of synthetic peptides was assessed by heparin affinity chromatography. Peptide 2 was eluted at 0.34 *M* NaCl, showing almost identical activity to vammin and peptide 1 for heparin binding. In contrast, peptide 3 was not retained after washing the column with NaCl-free buffer, indicating substantially lower affinity to the heparin column. These data indicate that the basic residues

clustered in the N-terminal portion of peptide 1 primarily mediate the heparin-binding function.

We next evaluated the effect of synthetic peptides on VEGF-A165 activity using cultured human umbilical vein endothelial cells. Relatively weak inhibition was observed in peptide 2-treated cells when compared with peptide 1, while peptide 3 had an even smaller effect, thus showing good agreement with the heparin-binding affinity data. These results strongly suggest that the basic amino acid residues in peptide 2 primarily mediate the heparin-binding activity of VEGF-Fs, but are not sufficient for binding and that the C-terminal portion (peptide 3 region) is also involved in the interaction. In addition, we also estimated the effect of synthetic peptides on VEGF-A165-induced hypotension. When pre-treatment of peptide 2, VEGF-A165-induced hypotension completely

blocked as well as pre-treatment of peptide 1. These in vivo data show good agreement with the in vitro results obtained using cultured endothelial cells.

Conclusion

In conclusion, we have demonstrated that the heparin-binding site of VEGF-F is located in its C-terminal short region, particularly localized in the N-terminal portion of peptide 1 region. Furthermore, a synthetic peptide of this region blocks the biological activity of VEGF-A165 in vitro and in vivo. Because this heparin-binding region has a relatively small structure, it may be a valuable tool for designing new anticancer drugs that target VEGF-heparin interaction.

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