Deciphering the cryptic macromolecular messages that regulate the state of the vasculature is challenging. One of the ongoing questions is the role of heparan sulfate proteoglycans (HSPG) in such processes as hemostasis, vascular remodeling, inflammation, and angiogenesis.1 This is important because heparin preparations, the medicinal counterparts of HSPG, are cornerstones for prevention and treatment of thrombosis. Heparin preparations have evolved in recent years with the introduction of heparin fractions with reduced molecular weight. Low-molecular-weight heparin (LMWH) has a longer half-life than unfractionated heparin (UFH) and produces a more predictable anticoagulant response, properties that explain why LMWH is gradually replacing UFH for most clinical indications. What is unknown, however, is how refinements designed to improve the anticoagulant properties of heparin affect other heparin activities, such as its interaction with heparin-binding proangiogenic growth factors and their receptors.

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In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Khorana and colleagues2 provide insight into this question. This study builds on the observations that (a) meta-analyses of clinical trials comparing heparin with LMWH for treatment of venous thromboembolism indicate improved survival in cancer patients given LMWH, and (b) proangiogenic growth factors, such as basic fibroblast growth factor (bFGF) and certain isoforms of vascular endothelial growth factor (VEGF), bind heparin, a property that can modulate their bioavailability and their interactions with high-affinity receptors.1–3 Using in vitro assays of bFGF-induced angiogenesis, Khorana et al2 examined the effects of an array of heparin fractions with mean molecular weights ranging from 1.7 kDa to 14 kDa. Also tested in this system were several commercial LMWH preparations and fondaparinux, a synthetic analog of the pentasaccharide sequence in heparin and LMWH that mediates their interaction with antithrombin.

Under the conditions of the assays used in this study, only the 3- and 6-kDa heparin fractions attenuated bFGF-induced angiogenesis in a dose-dependent fashion; larger and smaller heparin fractions had little or no activity. Thus, there is dissociation between the antiangiogenic and anticoagulant activities of these heparin fractions because the specific anti-factor Xa activity decreases as the molecular size of heparin is reduced, and 3- to 6-kDa heparin fractions had greater antiangiogenic activity than fractions of higher molecular weight.

Because commercial LMWH preparations contain heparin chains that range in molecular weight from 1 to 10 kDa, they also had antiangiogenic activity in the assays described by Khorana and colleagues.2 However, their activity was less than that of heparin fractions whose molecular weights were more narrowly restricted in the 3- to 6-kDa range, likely because LMWH preparations contain larger and smaller heparin chains that dilute the activity of more optimally sized material. This “shadow” of antiangiogenic activity could explain the potential antitumor effect of LMWH observed in clinical trials.

The concept that heparin can be used as an antiangiogenic agent in cancer is not new. In 1983, Folkman et al4 demonstrated that, in combination with corticosteroids, heparin had both antiangiogenic and antitumor effects in laboratory animals. A potential explanation for this effect came a year later with the isolation of the first tumor-derived angiogenic factor. This factor was subsequently identified as bFGF, a heparin-binding protein whose ability to modulate the growth of cultured endothelial and mesenchymal cells was influenced by heparin.5 The antitumor effects of heparin in cancer-bearing animals have been variable. Based on the findings of Khorana and colleagues2 these discrepant results may reflect, at least in part, heterogeneity in the molecular size of the heparin preparations that were tested.

Although the study by Khorana and colleagues2 is an important step forward, we still do not know how heparin derivatives influence the angiogenic response to bFGF and other proangiogenic factors. There are several possibilities (Figure). Biological activity requires assembly of a ternary complex that includes bFGF, HSPG, and the FGF receptor (FGFR). There is evidence that cell surface HSPG, particularly syndecan 4, an abundant transmembrane HSPG, serves as a low-affinity bFGF receptor to which bFGF must first bind before it activates FGFR, a process that involves interaction with phosphatidylinositol 4,5-bisphosphate and activation of protein kinase C-alpha.6 The crystal structure of this ternary complex has been elucidated using heparin as a surrogate for HSPG. This structure reveals the intimate interconnections between heparin and both bFGF and its receptor.7 Interestingly, such complexes are formed with heparin-derived decasaccharides, but not with smaller species; this phenomenon may explain why heparin fractions with a molecular weight less than 3 kDa had little or no

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FGF-induced angiogenesis. Expression of FGF biologic activity requires formation of a ternary complex that includes FGF, HSPG, and FGFR. Although FGF binds to FGFR, there is evidence that this interaction occurs in a two-step fashion; FGF first binds to HSPG, and HSPG-bound FGF then activates FGFR to trigger angiogenesis. Heparin derivatives can interfere with this process at several levels. They may compete with HSPG for FGF and/or FGFR binding. Alternatively, they may bind to FGF and/or FGFR, thereby disrupting the ternary complex.

antiangiogenic activity in the studies of Khorana and colleagues.²

When present in excess, optimally sized heparin derivatives may directly attenuate the interaction of bFGF and its receptor by binding to bFGF, FGFR, or to both bFGF and its receptor, thereby disrupting the ternary complex. Alternatively, these heparin derivatives may act indirectly by competing with HSPG for bFGF and/or FGFR binding.

Where do we go next? The “angiogenic switch” in cancer is a highly complex event that not only involves tumor-derived proangiogenic factors but also components of the hemostatic system. Platelets have proangiogenic activities, as do tissue factor and thrombin. It is possible, therefore, that the anticoagulant properties of LMWH also contribute to their antiangiogenic effects in vivo. Adding to the complexity is the likelihood that various types of tumors will respond differently to heparin depending on their repertoire of proangiogenic factors and receptors. For example, tumors demonstrate variable expression of the three major VEGF isoforms. Whereas VEGF₁₈₉ and VEGF₁₆₅ bind heparin with high affinity, VEGF₁₂₁ does not. Consequently, tumors that predominantly express VEGF₁₂₁, such as breast cancer and melanoma, are likely to be less responsive to heparin than astrocytomas, tumors that preferentially express the heparin-binding VEGF₁₆₅ isoform.⁸ Clearly, much more work needs to be done.

Although inhibition of angiogenesis may be beneficial for treatment of cancer, it may be detrimental in disease processes in which revascularization is desirable. For example, the antiangiogenic effect of LMWH observed by Khorana et al.² might render LMWH suboptimal for treatment of patients with acute coronary syndromes. In fact, the opposite is true; for short-term therapy, LMWH is at least as effective as unfractionated heparin.⁹⁻¹¹ However, when used long-term, LMWH is of less clear benefit,⁹⁻¹¹ despite evidence that these patients have ongoing activation of coagulation.¹² Could the limited long-term benefits of LMWH in acute coronary syndromes reflect, at least in part, suppression of angiogenesis? This question needs to be addressed.

In summary, the study by Khorana et al.² provides evidence that the antiangiogenic effect of heparin in vitro is size dependent. If their findings also apply in vivo, further refinements of heparin size may be necessary to optimize its antitumor effects. Because inhibition of angiogenesis has the potential to suppress processes such as wound healing or vascular remodeling, the benefits and risks of these novel heparin fractions require careful assessment.

References
Heparin and Angiogenesis: Size Matters!
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