Anticoagulants in Thrombosis and Cancer: The Missing Link

Shaker A. Mousa, Ph.D., MBA, FACC, FACB

ABSTRACT

Many cancer patients reportedly have a hypercoagulable state, with recurrent thrombosis due to the impact of cancer cells and chemotherapy on the coagulation cascade. Studies have demonstrated that unfractionated heparin (UFH) or low–molecular-weight heparin (LMWH) interferes with various processes involved in tumor growth and metastasis. These processes might include fibrin formation, binding of heparin to angiogenic growth factors such as basic fibroblast growth factor and vascular endothelial growth factor, modulation of tissue factor, and other mechanisms. Clinical trials have indicated a clinically relevant effect of LMWH as compared with UFH on the survival of cancer patients with deep vein thrombosis. Similarly, the impact of warfarin on the survival of cancer patients with thromboembolic disorders was demonstrated. Recent studies from our laboratory defined the role of an LMWH (tinzaparin), warfarin, anti–factor VIIa, and recombinant tissue factor pathway inhibitor in the modulation of angiogenesis, tumor growth, and tumor metastasis.

KEYWORDS: Heparin, LMWH, warfarin, TF/VIIa, TFPI, FGF2, VEGF, cancer, coagulation, platelet, angiogenesis, venous thrombosis

Objectives: Upon completion of this article, the reader should be able to (1) list therapeutic agents that might be effective in reducing the thrombotic risk of cancer patients, and (2) explain some of the effects that these compounds may have on tumor evolution.

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HEPARIN AND LOW-MOLECULAR-WEIGHT HEPARIN

Despite the research and development efforts in newer anticoagulants, unfractionated heparin (UFH) and low–molecular-weight heparin (LMWH) will continue to play a pivotal role in the management of thrombotic disorders. Although bleeding and heparin-induced thrombocytopenia are major side effects of this drug, it has remained the anticoagulant of choice for the prophylaxis and treatment of arterial and venous thrombotic disorders, surgical anticoagulation, and interventional use. It is the understanding of the structure of heparin that led to the development of LMWHs, synthetic heparinomimetics, antithrombin, and anti–factor Xa (anti-Xa) agents.1–4

As heparin was discovered over a half-century ago, our knowledge of the chemical structure and molecular interactions of this fascinating polycomponent
was limited at the early stages of its development. Through the efforts of major multidisciplinary groups of researchers and clinicians, it is now well recognized that heparin has multiple sites of action and can be used in multiple indications. We may witness the impact of these drugs on the management of various diseases in the not too distant future.

Tinzaparin sodium is an LMWH produced by controlled enzymatic depolymerization of conventional, unfractionated porcine heparin.\textsuperscript{3,5,6} In clinical trials, tinzaparin was more effective than UFH as treatment for deep vein thrombosis (DVT),\textsuperscript{7} was effective in the treatment of pulmonary embolism\textsuperscript{8} and the prevention of DVT in abdominal surgery patients,\textsuperscript{9} and was superior to warfarin as thromboembolism prophylaxis in subjects undergoing orthopedic joint (hip or knee) replacement surgery.\textsuperscript{10} It is also an effective anticoagulant for hemodialysis extracorporeal circuits.\textsuperscript{11}

Anti-Xa activity has served as the primary biomarker for assessing the exposure of tinzaparin and other LMWHs. It is used to define the in vitro potency and to monitor therapeutic response.\textsuperscript{12} Given that LMWHs are polycomponent moieties with multiple biological actions and distinct time courses, the true pharmacokinetic behavior of these agents cannot be assessed with assays developed for a single pharmacological activity. The absolute bioavailability is approximately 90% based on anti-Xa activity\textsuperscript{13} and 93% based on plasma tissue factor pathway inhibitor (TFPI) (data not shown).

Clinical trials in which LMWHs with distinct in vitro potency (anti-Xa/anti-IIa ratio) and ex vivo anti-Xa and anti-IIa activities were tested in patients with DVT following hip replacement found no difference in efficacy or safety measures as compared with UFH\textsuperscript{14,15} despite distinct differences in biomarker activity profiles. However, anti-Xa activity is sensitive as an indicator of molecular weight distribution differences with various heparin fractions.\textsuperscript{16} LMWHs vary in their affinity for antithrombin (AT), presumably as a result of production method.\textsuperscript{17} Such differences have been cited as explaining, in part, the differences in LMWH pharmacodynamics as assessed by anti-Xa activity and one reason why they cannot be used interchangeably. In contrast, TFPI, a vascular endothelial biomarker, might represent a greater potential for the role of LMWH in various diseases.\textsuperscript{18,19}

**THROMBOSIS AND CANCER**

The etiology of thrombosis in malignancy is multifactorial, and mechanisms include release of procoagulants by tumor cells plus other predisposing hypercoagulable states mediated by chemotherapeutic and radiotherapeutic agents.\textsuperscript{20–25} Unexplained thromboembolism may be an early indicator of the presence of a malignant tumor before signs and symptoms of the tumor itself become obvious.\textsuperscript{20}

Hemostatic abnormalities are present in a majority of patients with metastatic cancer. These abnormalities can be categorized as (1) increased platelet aggregation and activation, (2) abnormal activation of the coagulation cascade, (3) release of plasminogen activator inhibitor 1 (PAI-1), and (4) decreased hepatic synthesis of anticoagulant proteins such as protein C and AT. The abnormal activation of the coagulation cascade is mediated through release of tissue factor and other procoagulants from the plasma membrane vesicles of tumor cells\textsuperscript{22,25} (Fig. 1).

Increasing evidence suggests that thrombotic episodes may also precede the diagnosis of cancer by months or years, thus representing a potential marker for occult malignancy.\textsuperscript{20} Emphasis has been given to the potential risk of cancer therapy (both surgery and chemotherapy) in enhancing the risk for thromboembolic disease.\textsuperscript{22,25} Postoperative DVT is indeed more frequent in patients operated for malignant diseases than for other disorders. On the other hand, both chemotherapy and hormone therapy are associated with an increased thrombotic risk, which can be prevented by low-dose oral anticoagulation.\textsuperscript{26,27} In particular, procoagulant activities of tumor cells have been extensively studied; one of these specific cancer procoagulants could represent a novel marker of malignancy.

**TREATMENT OF VENOUS THROMBOEMBOLISM IN CANCER PATIENTS**

The management of DVT and pulmonary embolism (PE) in patients with cancer can be a clinical dilemma. Comorbid conditions, warfarin failure, difficult venous access, and a high bleeding risk are some of the factors that often complicate anticoagulant therapy in these patients. In addition, the use of central venous access devices is increasing but the optimal treatment of catheter-related thrombosis remains controversial. UFH is the traditional standard for the initial treatment of venous thromboembolism (VTE), but LMWHs have been shown to be equally safe and effective in hemodynamically stable patients. For long-term treatment or secondary prophylaxis, vitamin K antagonists remain the mainstay of treatment. However, the inconvenience and narrow therapeutic window of oral anticoagulants make extended therapy unattractive and problematic. As a result, LMWHs are being evaluated as an alternative for long-term therapy.\textsuperscript{26,27} The role of inferior vena cava filters in cancer patients remains ill defined, but these devices remain the treatment of choice in patients with contraindications for anticoagulant therapy.

A growing body of evidence has provided a convincing demonstration of a strong association between
cancer and VTE (Table 1). Patients with cancer are at a remarkably higher risk of VTE than patients free from malignant disorders during prolonged immobilization for any cause and following surgical interventions. Standard heparin in adjusted doses or a LMWH in doses commonly recommended for high-risk surgical patients represents the prophylactic treatment of choice for cancer patients undergoing an extensive abdominal or pelvic intervention. In cancer patients affected by DVT, the treatment with LMWH has been reported to reduce mortality to a greater extent than the standard heparin therapy. Such an observation suggests that these agents might modify tumor growth progression directly or indirectly.

Studies have provided convincing evidence for increased incidences of newly diagnosed malignancy among patients with unexplained VTE during the first 6–12 months after the thromboembolic event (see Table 1 for tumors associated with VTE). Figure 2 illustrates the positive feedback loop between tumor and clot in magnifying each other.

Tumor fibrin is a consistent feature of tumor stroma and is deposited shortly after tumor cell inoculation. As there are several ways in which fibrin may be beneficial to tumor growth, it is possible that the ability of normal or malignant tissue to generate fibrin may influence metastasis. Many normal tissues and tumor cells possess a procoagulant activity that is due to a complex of tissue factor and factor VII.

**ANGIOGENESIS**

Angiogenesis is a process that is dependent upon coordinated production of angiogenesis stimulatory and inhibitory (angiostatic) molecules, and any imbalance in this regulatory circuit might lead to the development of a number of angiogenesis-mediated diseases (Table 2). Angiogenesis is a multistep process including activation, adhesion, migration, proliferation, and transmigration of endothelial cells across cell matrices to or from new capillaries and from existing vessels. Angiogenesis is a process involved in the formation of new vessels by sprouting from preexisting vessels. In contrast, vessel rudiments may organize in place, a process termed vasculogenesis. Endothelial heterogeneity and organ specificity might contribute to differences in the response to different antiangiogenic mechanisms (cultured endothelial cells vs. microvascular endothelial cells isolated from different tissues). Under normal physiological conditions, in a mature organism endothelial cell turnover is extremely slow (months to years); this is in contrast to the fast turnover of endothelial cells under pathological angiogenesis.

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**Table 1  Tumor Type Associated with Venous Thrombosis**

<table>
<thead>
<tr>
<th>Tumor Type</th>
</tr>
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<tbody>
<tr>
<td>Pancreatic tumors</td>
</tr>
<tr>
<td>Mucin-secreting adenocarcinoma</td>
</tr>
<tr>
<td>from the gastrointestinal system</td>
</tr>
<tr>
<td>Lung carcinoma</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
</tr>
<tr>
<td>Intracranial tumors</td>
</tr>
<tr>
<td>Acute promyelocytic leukemia</td>
</tr>
<tr>
<td>Myeloproliferative disorders</td>
</tr>
</tbody>
</table>
Figure 2  Interplay between tumor and clot illustrating a positive feedback loop. Tumor promotes hypercoagulable state and clot (platelet-fibrin clot) induces tumor growth and tumor angiogenesis.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Prevalence (or Incidence*)</th>
</tr>
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<tbody>
<tr>
<td>Solid tumor cancer</td>
<td>&gt;600,000</td>
</tr>
<tr>
<td>Lung, breast, prostate, colon, renal, bladder, pancreatic, glioblastomas, neuroblastomas, and others</td>
<td></td>
</tr>
<tr>
<td>Ocular ailments</td>
<td></td>
</tr>
<tr>
<td>Macular degeneration</td>
<td>650,000</td>
</tr>
<tr>
<td>Diabetic retinopathy</td>
<td>300,000</td>
</tr>
<tr>
<td>Corneal transplant</td>
<td>100,000</td>
</tr>
<tr>
<td>Myopic degeneration</td>
<td>200,000</td>
</tr>
<tr>
<td>Inflammatory diseases</td>
<td></td>
</tr>
<tr>
<td>Arthritis</td>
<td>2.1 million</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>3.0 million</td>
</tr>
<tr>
<td>IBD/other chronic IDs†</td>
<td>&gt;2.0 million</td>
</tr>
</tbody>
</table>

*New cases per year.
†IBD, inflammatory bowel disease; ID, inflammatory diseases.

conditions. However, angiogenesis can be activated for a limited time in certain situations such as wound healing and ovulation.

In certain pathological states, such as human metastasis and ocular neovascularization disorders including diabetic retinopathy and age-related macular degeneration, there is excessive and sustained angiogenesis. Hence, understanding the mechanisms involved in the regulation of angiogenesis could have a major impact in the prevention and treatment of pathological angiogenesis processes. In addition, endothelial cells play a major role in the modeling of blood vessels. The interplay of growth factors, cell adhesion molecules, and specific signal transduction pathways either in the maintenance of the quiescent state or in the reactivation of endothelium is critical in physiological and pathological angiogenesis processes. A combined defect in the overproduction of positive regulators of angiogenesis and a deficiency in endogenous angiostatic mediators are a feature documented in tumor angiogenesis, psoriasis, rheumatoid arthritis, and other neovascularization-mediated disorders. Hence,
understanding the mechanisms involved in the regulation of angiogenesis could have a major impact in the treatment of pathological angiogenesis.34

**Angiogenesis-Modulating Mechanisms and Agents**

The origin of the mechanisms and agents involved in angiogenesis modulation35–40 includes the following:

1. Extracellular matrix (ECM): collagen (endostatin), fibronectin, or vitronectin (RGD analogues).
2. Cell adhesion receptors: αvβ3, α5β1, and α1β1.
3. Fibrinolytic system: kininogen domain 5 and plasminogen-driven angiostatin.
4. Coagulation system: heparin-like glycosaminoglycans (GAGS) and fibrin.
5. Platelet derived: platelet-derived growth factor (PDGF), platelet factor 4 (PF4), thrombospondin, and thrombomodulin.

**ACTIVATION OF COAGULATION AND ANGIOGENESIS IN CANCER**

Tissue factor has been implicated in the upregulation of proangiogenic factors such as vascular endothelial growth factor (VEGF) by tumor cells. This is due to a complex interaction between tumor cells, macrophages, and endothelial cells leading to tissue factor expression, fibrin formation, and tumor angiogenesis.41,42

**ANTICOAGULANTS IN THE MODULATION OF ANGIOGENESIS**

The effects of the LMWH tinzaparin, warfarin, anti-VIIa, and recombinant TFPI (r-TFPI) on the modulation of angiogenesis-related processes, including in vitro endothelial tube formation and in vivo angiogenesis mediated by angiogenic factors and cancer cells, were demonstrated. The in vivo effects of those different anticoagulants on angiogenesis in the chick chorioallantoic membrane (CAM) model were determined. Twenty-four hours after stimulating angiogenesis on the CAM with basic fibroblast growth factor (FGF2), lipopolysaccharide (LPS), or colon carcinoma (HCT-116), tinzaparin, warfarin, anti-VIIa, or r-TFPI were directly applied to the growth factor–saturated filter disk or injected intravenously into the embryonic circulation. Data demonstrated significant and comparable inhibitory effects of the LMWH tinzaparin, anti-VIIa, and r-TFPI in a concentration-dependent manner on endothelial cell tube formation. Tinzaparin, warfarin, anti-VIIa, and r-TFPI blocked FGF2-induced angiogenesis in the CAM model by 80–100%. In addition, significant inhibition of colon or lung carcinoma–induced angiogenesis (Fig. 3), tumor growth (Fig. 4), and regression was demonstrated with tinzaparin, warfarin, anti-VIIa, and r-TFPI. These studies demonstrated a significant role for tinzaparin, warfarin, anti-VIIa, and tinzaparin-releasable TFPI in the regulation of angiogenesis and tumor growth.37,41 Thus, modulation of tissue factor/VIIa noncoagulant activities by those different agents might be a useful therapeutic approach for the inhibition of angiogenesis associated with human tumor growth and inflammatory diseases. See Table 2 for diseases associated with pathological angiogenesis.

**LMWH, TFPI, and Tumor Dissemination**

The significance of tissue factor in cancer biology is suggested by studies reporting its involvement in metastasis and angiogenesis. Tinzaparin is an LMWH that is produced by heparinase depolymerization of UFH allowing for its relatively high sulfate-to-carboxylate ratio. Beyond its potent plasmatic effects on AT-inhibitable coagula-

![Figure 3](image-url)  
**Figure 3** Antiangiogenesis efficacy of tinzaparin on tumor-induced angiogenesis.
tion factors, tinzaparin is a very effective LMWH in causing the release of TFPI from the endothelial cells, the natural inhibitor of tissue factor procoagulant and noncoagulant effects. The present study was undertaken to investigate the effect of the LMWH tinzaparin as well as recombinant TFPI on experimental lung metastasis.

Using the B16 melanoma injectable model of metastasis, we found that subcutaneous injection of tinzaparin (10 mg/kg) 4 hours before intravenous injection of 2.5 x 10^5 melanoma cells reduced lung tumor formation in experimental mice by 89% (31 ± 23 vs. 3 ± 2, \( P < 0.001 \)). In a second experimental group, in addition to the initial (pre-tumor cell) dose, subcutaneous tinzaparin (10 mg/kg) was administered daily for 14 days, at which time lung seeding was assessed. In the latter group, lung tumor formation was reduced by 96% (\( P < 0.001 \)). No bleeding problems were observed in any of the heparinized animals. In order to determine the anticoagulant activity of tinzaparin, 4 hours after a single subcutaneous dose, whole blood recalcification was measured using a Sonoclot® analyzer. Tinzaparin (10 mg/kg subcutaneously) prolonged the clotting time fourfold. Furthermore, measuring the platelet count (a sensitive marker of intravascular coagulation) before and 15 minutes after determined the effect of tinzaparin on tumor cell–induced clotting activation in vivo after tumor cell injection in control and tinzaparin–treated animals. Following intravenous injection of 2 x 10^6 tumor cells, a rapid and significant fall in platelet count was observed (from 939 ± 37 x 10^6/mL to 498 ± 94 x 10^6/mL, \( P < 0.01 \)). In tinzaparin–treated animals, a significant reversal to normal platelet count was achieved (921 ± 104 x 10^6/mL). Intravenous injection of TFPI (700 ng) 5 minutes prior to tumor cell injection also reduced B16 lung metastasis (85%, \( P < 0.01 \)) and abolished tumor cell–induced thrombocytopenia. Our results support the potential role of the LMWH tinzaparin and its releasable TFPI in tumor metastasis.\(^{44}\)

**Hemostasis and Tumor Dissemination**

Platelets and fibrin are key catalysts for tumor adhesion, survival, and metastasis. Tumor cell metastasis and thrombosis are the major causes of death in cancer patients.\(^{31,46,47}\)

**SELECTED ANGIOGENESIS INHIBITOR STRATEGIES UNDER INVESTIGATION**

Recent evidence suggests that, in spite of the redundancy of angiogenic factors potentially involved in pathological angiogenesis, strategies aimed at antagonizing one specific endothelial cell mitogen at its release or receptor levels may form the basis for an effective and safe treatment of various angiogenesis-mediated disease processes (Table 3).\(^{48,49}\)

Tumors are dynamic, complex, living tissues undergoing the varied processes of tissue growth under the guidance of aberrant malignant cells. Cytotoxic anti-

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**Table 3 Examples of Different Antiangiogenesis Strategies under Investigation**

<table>
<thead>
<tr>
<th>VEGF receptor–tyrosine kinase antagonists, Tie1, Tie2 (SU-5416)</th>
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</thead>
<tbody>
<tr>
<td>Anti-VEGF antibody</td>
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<tr>
<td>Humanized form of LM609 (Vitaxin)</td>
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<tr>
<td>Small molecule αvβ3 antagonists</td>
</tr>
<tr>
<td>TNP470</td>
</tr>
<tr>
<td>2-Methoxyestradiol</td>
</tr>
<tr>
<td>CM-101</td>
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<tr>
<td>Shark cartilage extract (AE-941)</td>
</tr>
<tr>
<td>Thalidomide and thalidomide analogues</td>
</tr>
<tr>
<td>Endostatin and angiostatin</td>
</tr>
<tr>
<td>High-molecular-weight kininogen domain 5</td>
</tr>
<tr>
<td>Matrix metalloproteinase inhibitors (AG3340)</td>
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<tr>
<td>Urokinase receptor antagonist</td>
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</table>
cancer therapies have focused solely on the eradication of the malignant cell, which is an absolute necessity; however, even the most heroic therapeutic strategies rarely achieve cure of many tumor types. The recognition that the growth processes of tumors are normal processes, that the invasion processes of tumors are normal processes, and that it is inappropriate activation of the processes that results in the morbidity of malignant disease allows the elucidation of a broad spectrum of new therapeutic targets in cancer. The integration of antiangiogenic agents into existing cancer chemotherapy regimens might lead to improved efficacy and safety for many standard cytotoxic therapies.

### Adjuvant use of 5-Fluorouracil and LMWH in Proliferative Vitreoretinopathy

A recent investigation assessed the safety and efficacy of adjuvant combination therapy using 5-fluorouracil (5-FU) and LMWH for prevention of proliferative vitreoretinopathy (PVR). After vitrectomy and retinal reattachment surgery, the study was a prospective randomized, double-masked, placebo-controlled trial. The participants were 174 high-risk patients randomized to receive either 5-FU and LMWH therapy or placebo. Patients were selected from all patients undergoing primary vitrectomy for rhegmatogenous retinal detachment. Standard surgery with 5-FU and LMWH therapy or placebo was compared at the 6-month follow-up. The main outcome measures were development of postoperative PVR, retinal reattachment at 6 months after surgery, single operation reattachment rate, number of reoperations, and best corrected visual acuity. There were 87 patients in the 5-FU and LMWH therapy group and 87 in the placebo group. The incidence of postoperative PVR was significantly lower ($P = 0.02$) in the 5-FU and LMWH therapy group compared with the placebo group. There was a significant reduction in the incidence of postoperative PVR in patients receiving the 5-FU and LMWH therapy and in the reoperation rate resulting from PVR. This trial shows that the incidence of PVR can be reduced with 5-FU and LMWH.50

### ANTIANGIOGENESIS AGENTS IN MALIGNANCY AND HEMOSTASIS

Recent evidence suggests enhanced thrombogenicity and fibrinolytic deficit with different angiogenesis inhibitor agents.50,51 The exact mechanism for the interaction between angiogenesis modulation mechanisms and hemostasis is unclear at this point.

The following preclinical and clinical investigations should provide information needed to optimize the benefit of anticoagulants in oncology and to better understand the mechanisms of the anticoagulant effect:

1. Subcutaneous LMWH plus low-dose oral warfarin might provide improved efficacy and safety. The addition of a daily aspirin might provide additional benefits in some tumor types.
2. Long-term (6 months) versus short-term (6 weeks) use of the preceding regimen.
3. Efficacy of LMWH versus warfarin on different tumor types and at different stages of tumor progression.
4. Interactions of anticoagulant with chemotherapeutic agents and with other antiangiogenesis strategies.

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