Antithrombotics in thrombosis and cancer
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Keywords
Asetylsalicylic acid, GPIIb/IIIa antagonists, heparin, LMWH, TF/VIIa, TFPI, FGF2, VEGF, cancer, angiogenesis, thrombosis

Summary
Many cancer patients have a hypercoagulable state, with recurrent thrombosis due to the impact of cancer cells and chemotherapy or radiotherapy on the coagulation cascade. Studies have demonstrated that unfractionated heparin (UFH) or its low molecular weight fractions interfere with various processes involved in tumour growth and metastasis. These include fibrin formation, binding of heparin to angiogenic growth factors, such as basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF); modulation of tissue factor; and perhaps other more important modulatory mechanisms, such as enhanced tissue factor pathway inhibitor (TFPI) release and inhibition of various matrix-degrading enzymes. Clinical trials have suggested a clinically relevant effect of low molecular weight heparin (LMWH), as compared to 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. Studies from our laboratory demonstrated a significant role for LMWH, warfarin, anti-VIIa, and LMWH-releasable TFPI on the regulation of angiogenesis, tumour growth, and tumour metastasis. Thus, modulation of tissue factor/VIIa non-coagulant activities by LMWH, warfarin, anti-VIIa, or TFPI might be a useful therapeutic method for the inhibition of angiogenesis associated with human tumour growth and metastasis. Additionally, antithrombotic drugs could have an impact on tumour metastasis, and the combination of anti-platelets and anticoagulants at adjusted doses might provide greater benefits to cancer patients.

Antithrombotika bei Thrombose und Krebs
Hämostaseologie 2005; 25: 380–6

Coagulation and cancer
The association between coagulation system activation and systemic thrombosis in human cancers has been recognized for over a century since Trousseau’s original description of migratory thrombophlebitis complicating gastrointestinal malignancy (53). An improved appreciation in recent years of the interdependency of the coagulation system and malignant behavior has led to an understanding of how an activated coagulation system in turn may enhance cancer cell growth (48). While this does not establish causality or even a biologic association, it is of interest that a recent Danish study showed that patients with cancer who developed venous thrombosis during the course of their disease had significantly shorter cancer-related survival than similar patients who remained thrombosis-free (50). Resting on greater strength of evidence, several studies, including randomized clinical tri-
als, have documented improved cancer-related survival in patients treated with anticoagulants compared to those not receiving anticoagulants (5, 6, 26, 51).

A recent study has indicated that thromboembolic events are important predictors of cancer (47). Cancer screening in patients without identifiable risk factors for thrombosis could be helpful for early detection, diagnosis, and management of cancer.

Thrombin generation and fibrin formation are constantly detectable in patients with malignancy, who are at increased risk of thromboembolic complications. Most importantly, fibrin formation is also involved in the processes of tumour spread and metastasis. Activation of blood coagulation in cancer is a complex phenomenon, involving many different pathways of the haemostatic system and numerous interactions of the tumour cell with other blood cells, including platelets, monocytes, and endothelial cells. Tumour cells possess the capacity to interact with all parts of the haemostatic system. They can directly activate the coagulation cascade by producing their own procoagulant factors or they can stimulate the prothrombotic properties of other blood cell components.

The aetiology of thrombosis in malignancy is multifactorial; mechanisms include release of procoagulants by tumour cells plus other predisposing factors leading to a hypercoagulable state that is amplified by chemotherapeutic and radiotherapeutic agents (3, 14, 16, 20, 28, 44). Unexplained thromboembolism may be an early indicator of the presence of a malignant tumour before signs and symptoms of the tumour itself become obvious.

Haemostatic abnormalities are present in a majority of patients with metastatic cancer. These abnormalities can be categorized as increased platelet aggregation and activation, abnormal activation of coagulation cascade, release of plasminogen activator inhibitor type 1 (PAI-1), and decreased hepatic synthesis of anticoagulant proteins like protein C and antithrombin (AT) III.

Activation of the coagulation cascade is mediated through release of tissue factor (TF) and other procoagulants from the plasma membrane vesicles of tumour cells (20, 44).

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 (3). Recently, emphasis has been given to the potential risk of cancer therapy (both surgery and chemotherapy) in enhancing the risk for thromboembolic disease (20, 44). Postoperative deep-vein thrombosis (DVT) is indeed more frequent in patients who have undergone surgery 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 (25, 49).

In particular, procoagulant activities of tumour cells have been extensively studied. These specific tumour procoagulants could represent novel markers of malignancy.

Platelets and cancer

Activated platelets release angiogenic growth factors, and therefore it has been proposed that they contribute to tumour angiogenesis (30, 43, 60). Growth factors in platelets might include the following:

- vascular endothelial growth factor (VEGF),
- basic fibroblast growth factor (bFGF),
- platelet-derived growth factor (PDGF).

The role of platelets in tumour biology has been suggested (58). Serum levels of VEGF have been shown to correlate with platelet counts during chemotherapy (57). Platelet-tumour cell interactions are believed to be important in tumour metastasis. Tumour cell TF expression enhances metastasis and angiogenesis, and it is primarily responsible for tumour-induced thrombin generation and the formation of tumour cell-platelet aggregates. Activated platelets express and release CD40 ligand (CD40L), which induces endothelial TF expression by ligation to CD40.

Recent data led to the conclusion, that, in malignancy, the increase in cellular TF activity via interaction with CD40 (tumour cell) and CD40L (platelet) may possibly enhance intravascular coagulation and haematogenous metastasis (1). Inhibition of experimental metastasis and tumour growth was demonstrated in animals by thrombocyteptosis and antithrombocytopenia.

Cancer disturbs those cellular activities that maintain multicellular organisms, namely, growth, differentiation, apoptosis, and tissue integrity. There are numerous clinical and experimental observations showing that invasion results from the cross-talk between cancer cells and host cells (i.e., platelets, myofibroblasts, endothelial cells, and leukocytes, all of which are themselves invasive). In bone metastases, host osteoclasts serve as targets for therapy. The molecular analysis of invasion-associated cellular activities (namely, homotypic and heterotypic cell-to-cell adhesion, cell-to-matrix interactions and ectopic survival, migration, and proteolysis) reveal branching signal transduction pathways with extensive networks between individual pathways. The role of proteolysis in invasion is not limited to breakdown of extracellular matrix but also causes cleavage of proinvasive fragments from cell-surface glycoproteins (GPs).

In vivo tumour cells interact with a variety of host cells, such as endothelial cells and platelets, and these interactions are mediated by integrins GPIIb/IIIa and αvβ3. In the xenograft model, m7E3 Fab2 binds to both human tumour and host platelet GPIIb/IIIa and endothelial αvβ3 integrins, thus participating as an antiangiogenic and an antitumour agent. Data have suggested that combined blockade of GPIIb/IIIa and αvβ3 affords significant antiangiogenic and antitumour benefit (52).

Classic studies have indicated that the formation of tumour cell-platelet complexes in the bloodstream is important in facilitating the metastatic process. Metastasis in animal models can be inhibited by heparin, and retrospective analyses of heparin use in human cancer have turned out promising (56).
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
- high bleeding risk.

are some of the factors that often complicate anticoagulant therapy. In addition, the use of central venous access devices is increasing, but the optimal treatment of catheter-related thrombosis remains controversial.

Unfractionated heparin (UFH) is the traditional standard for the initial treatment of venous thromboembolism (VTE), but low molecular weight heparins (LMWHs) have been shown to be equally safe and effective in haemodynamically stable patients. For long-term treatment or secondary prophylaxis, vitamin K antagonists remain the mainstay treatment. However, the inconvenience and narrow therapeutic window of these anticoagulants make extended therapy unattractive and problematic.

As a result, LMWHs are being evaluated as an alternative for long-term therapy (21, 63). 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.

Several clinical investigations with various LMWHs — including enoxaparin, dalteparin, certoparin, and tinzaparin — demonstrated survival benefits as compared to UFH in cancer patients, with certain tumour types and at early stages (32, 59, 64). Additionally, the efficacy and safety profile for LMWH was also shown to be superior as compared to UFH or other anticoagulants such as warfarin (32).

A growing body of evidence has provided convincing demonstration of tumour-mediated hypercoagulation state (Tab. 1) and a strong association between cancer and VTE (Tab. 2). Patients with cancer are at a remarkably higher risk of VTE than are patients who are free from malignant disorders and who experience prolonged immobilisation from any cause or following surgical interventions. In cancer patients affected by DVT, the treatment with LMWH has been reported to lower mortality to a greater extent as compared to standard heparin therapy. Such an observation suggests that these agents might modify tumour growth progression directly or indirectly.

Studies have provided convincing evidence for increased incidence of newly diagnosed malignancy among patients with unexplained VTE during the first 6-12 months after the thromboembolic event (Tab. 2) (10, 17, 22, 54). A positive feedback loop between tumour and clot in magnifying each other has been demonstrated (36). Tumour-fibrin is a consistent feature of tumour stroma and is deposited shortly after tumour cell inoculation (9, 36). Since fibrin may be beneficial to tumour growth, it is possible that the ability of normal or malignant tissue to generate fibrin may influence metastasis (9).

Heparin and LMWH

Heparin and its fractionated derivative, LMWH, are glycosaminoglycans (18). Each residue is heavily polysulfated, thus giving the biopolymer a highly negative charge (12, 40). This anionic property is responsible for heparin’s inhibitory effect on malignant processes (including angiogenesis and tumour cell adhesion) and malignant cell transformations. The antithrombotic effect of heparin is another effective countermeasure against thrombosis induced by malignancy, chemotherapy, radiation, catheter, and surgery.

Because heparin was discovered over a half century ago, our knowledge of the chemical structure and molecular interactions of this fascinating poly-component 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. The impact of heparin derivatives or LMWH in the management of various diseases may be witnessed soon.

LMWHs vary in their affinity for ATIII, presumably as a result of the production method (29). 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 that is ATIII-independent, might represent a greater potential for the role of LMWH in various diseases (33).

Tinzaparin sodium is an LMWH produced by controlled enzymatic depolymerization of conventional porcine UFH (29). In clinical trials, tinzaparin is more effective than UFH for treatment of DVT and is effective in the treatment of PE (33, 49). Meta-analysis of LMWH versus UFH treatment in DVT with or without cancer demonstrated equivalent-to-superior profiles in terms of efficacy and safety.

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Tab. 1 Mechanisms of tumour-mediated hypercoagulable states (36)

<table>
<thead>
<tr>
<th>malignant tumour promotes</th>
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<tr>
<td>tumour cell surface tissue factor</td>
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<tr>
<td>macrophage tissue factor</td>
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<tr>
<td>expression of cell surface phospholipids that support coagulation activation</td>
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<tr>
<td>other tumour-mediated platelet activation and aggregation</td>
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<tr>
<td>tumour-induced endothelial cell activation</td>
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Tab. 2 Rates of venous thromboembolism in malignancies in comparison to 57 cases in patients without cancer according to Rickles and Levine (45).

<table>
<thead>
<tr>
<th>cancer location</th>
<th>rate of VTE per 10 000 patients</th>
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<tbody>
<tr>
<td>head/neck</td>
<td>16</td>
</tr>
<tr>
<td>breast</td>
<td>22</td>
</tr>
<tr>
<td>uterus</td>
<td>44</td>
</tr>
<tr>
<td>prostate</td>
<td>55</td>
</tr>
<tr>
<td>lung</td>
<td>61</td>
</tr>
<tr>
<td>liver</td>
<td>69</td>
</tr>
<tr>
<td>colon</td>
<td>76</td>
</tr>
<tr>
<td>leukemia</td>
<td>81</td>
</tr>
<tr>
<td>renal</td>
<td>84</td>
</tr>
<tr>
<td>stomach</td>
<td>85</td>
</tr>
<tr>
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<td>98</td>
</tr>
<tr>
<td>pancreas</td>
<td>110</td>
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<td>brain</td>
<td>117</td>
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<tr>
<td>ovary</td>
<td>120</td>
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Tumour factors predicting sensitivity to anticoagulants

The various tumour types differ in the nature of their interactions with the coagulation system. In this regard there are two types of tumours. They either
- activate the coagulation system directly or
- mediate coagulation activation indirectly via a paracrine mechanism.

Tumours in the first group include renal cell cancer (RCC), melanoma, and ovarian and small cell lung cancers. These tumours over-express procoagulant molecules such as TF, cancer procoagulant, or, in the case of RCC, hepsin on cell surfaces. The entire coagulation pathway is assembled on the surface of these tumor cells, leading to fibrin formation in close proximity to the tumours. This, at least partly, explains the occasional finding in RCC of clot emanating from the tumour and extending into the renal vein and inferior vena cava.

Tumours in the second group, on the other hand, tend to activate systemic coagulation by releasing cytokines, e.g., tumour necrosis factor-alpha, interleukin (IL)-1-beta, that, in turn, stimulate the production procoagulant molecules on the surface of circulating monocytes. Examples of these tumour types include breast, colorectal, and non-small cell lung cancers.

Based on this difference in the biology of coagulation activation, one would predict that tumours in the first group might be more likely to respond to anticoagulation that interferes with TF/VIIa than would tumours in the second group. In support of this hypothesis, anticoagulants have had more significant activity in melanoma and small cell lung cancer but not in breast, colorectal, and non-small cell lung cancers in prospective trials (5, 6, 19, 16, 26, 51).

Angiogenesis

The coagulation system, which is activated in most cancer patients, has an important role in tumour biology. It may make a substantial contribution to tumour angiogenesis, which represents an imbalance in the normal mechanisms that allow organized healing after injury. The recently recognized, but steadily growing, knowledge of the relationship between the coagulation and angiogenesis pathways has research and clinical implications. Manipulation of these systems may minimize both the neoangiogenesis essential for tumour growth and associated thromboembolic complications.

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. Angiogenesis is a multistep process that includes 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 that forms new vessels by sprouting from preexisting vessels. A combined defect in the overproduction of positive regulators of angiogenesis and a deficiency in endogenous angiostatic mediators are documented in tumor angiogenesis, psoriasis, rheumatoid arthritis, and other neovascularization-mediated disorders (27, 38).

<table>
<thead>
<tr>
<th>Tab. 3 Heparin effects in cancer (36)</th>
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<tr>
<td>Inhibition of</td>
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<tr>
<td>angiogenesis</td>
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<tr>
<td>proteases</td>
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<tr>
<td>growth factors</td>
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<td>coagulation factors</td>
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<td>oncogene expression</td>
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Heparin/LMWH antineoplastic effects

Reports from animal studies have indicated that metastasis can be inhibited by UFH. There have also been clinical reports suggesting survival benefits from UFH and LMWH that go beyond their antithrombotic effects. Evidence from several experiments has suggested various possible antineoplastic mechanisms for heparin and heparin derivatives (Tab. 3). However, the primary antineoplastic mechanism for heparin still remains to be clinically defined.

Activation of coagulation and angiogenesis in cancer

Many cancer patients have haemostatic abnormalities that predispose them to develop platelet activation and fibrin formation leading to clinical or subclinical thrombosis (13, 31). Thus, cancer leads to thrombosis, which, in turn, enhances the metastatic spread of tumour cells. Heparin therapy is effective and safe for thromboprophylaxis, and LMWH works just as well or better compared to UFH. Its antithrombotic action is another method by which heparin exhibits an inhibitory effect on malignant processes.

TF has been implicated in the upregulation of proangiogenic factors such as VEGF by tumour cells. This is due to a complex interaction between tumour cells, macrophages, and endothelial cells, leading to TF expression, fibrin formation, and tumour angiogenesis (46). A recent study has suggested that thrombin generation occurs via the extrinsic (TF-dependent) coagulation pathway on cell surfaces and that some chemotherapeutic agents are able to upregulate TF mRNA and protein expression in cancer cells (41).

The role of the coagulation system in angiogenesis

The processes of blood coagulation and the generation of new blood vessels both play crucial roles in wound healing. Platelets, for example, are the first line of defense during vascular injury and contain at least a dozen promoters of angiogenesis, which may be induced to secrete into the surrounding vasculature upon activation by thrombin (23). It follows that these pathways are also intricately linked within human tumours. Targeting both the coagulation and angiogenesis pathways may provide more potent antitumor effect than targeting either pathway alone. Elucidation of the TF signaling path-
way using tumour cells as a model system should provide new insights into the cellular biology of TF that might be applied to signaling in endothelial cells, smooth muscle cells, and fibroblasts. Also, because new classes of anticoagulant molecules have been recently developed that selectively target TF and/or TF-VIIa complex (42, 52, 56), an understanding of this pathway might provide the rational basis for the development of new agents to prevent and/or reduce angiogenesis-related disorders, tumour-associated thrombosis, and the positive feedback loop between thrombosis and cancer (7).

Activation of the blood coagulation system stimulates the growth and dissemination of cancer cells through multiple mechanisms, and anticoagulant drugs inhibit the progression of certain cancers. Laboratory data on the effects of anticoagulants in various tumours suggest that this treatment approach has considerable potential in some cancers but not others. For example, RCC is one of a small number of human tumour types in which the tumour cell contains an intact coagulation pathway leading to thrombin generation and conversion of fibrinogen to fibrin immediately adjacent to viable tumour cells (61). Similar observations have been made in melanoma, ovarian cancer, and small cell lung cancer but not in breast, colorectal, and non-small cell lung cancers (62). This is of considerable relevance to the finding that growth of melanoma and small cell lung cancer is inhibited by anticoagulants, but that no such effect has been observed in those other tumour types (5).

Based on the relatively unique features of the interaction of RCC with the coagulation system, RCC might respond to anticoagulation therapy in a similar manner as small cell lung cancer and melanoma. Hence, an anticoagulant that inhibits at the TF/VIIa level might have improved efficacy and safety in inhibiting tumour-associated thrombosis, angiogenesis, and metastasis.

Anticoagulants in the modulation of angiogenesis
TF, FGF2, VEGF, and IL-8, a chemokine, are also proangiogenic (65). Heparin counters these factors, although the inhibitory effect occurs through different actions. The natural inhibitor of TF is known as TFPI. In the presence of heparin, Zhang et al. (65) showed that TFPI activity is enhanced and the stimulatory effects of TF on angiogenesis are reduced.

Chemokines have positively charged domains (11). Heparin might exhibit its inhibitory effect on IL-8 by binding these positive domains. In addition to angiogenesis, another key component of metastasis is the adhesion of cells to areas away from the primary tumour growth. Selectins and integrins are families of cellular components that mediate cell adhesion and are involved in a complex cascade of events following endothelial cell activation. Tumour cells act as a ligand for the activation of these cellular elements. Studies have shown that heparin inhibited selectin and integrin-mediated interactions with tumour cells (39).

The effects of LMWH tinzaparin, anti-VIIa, and r-TFPI on the modulation of angiogenesis-related processes including in vitro endothelial tube formation and in vivo angiogenesis that is mediated either by angiogenic factors and/or by cancer cells were demonstrated (35). Data demonstrated significant and comparable inhibitory effects of the LMWH tinzaparin, anti-VIIa, or r-TFPI in a concentration-dependent manner on endothelial cell tube formation. Tinzaparin, anti-VIIa, or r-TFPI blocked FGF2-induced angiogenesis in the chick chorioallantoic membrane (CAM) model. Additionally, a significant inhibition of colon or lung carcinoma-induced angiogenesis, tumour growth, and regression was demonstrated with tinzaparin, anti-VIIa, and r-TFPI (35). These studies demonstrated a significant role for tinzaparin, anti-VIIa, and tinzaparin-releasable TFPI in the regulation of angiogenesis and tumour growth (35).

LMWH, TFPI and tumour dissemination
Using the experimental metastasis B16 melanoma injectable model in mice, subcutaneous injection of tinzaparin (10 mg/kg body weight) 4 hours before intravenous injection of $2.5 \times 10^5$ melanoma cells reduced lung tumour formation in experimental mice (2). Similarly, intravenous injection of TFPI (700 ng) 5 minutes prior to tumour cell injection also reduced B16 lung metastasis and abolished tumour cell-induced thrombocytopenia. These results support the potential role of the LMWH and its releasable TFPI in tumour growth and metastasis (37).

Conclusion
Many cancer patients reportedly have a hypercoagulable state, with recurrent thrombosis due to the impact of cancer cells and chemotherapy, radiation, immobility, and catheter on further activation of coagulation cascade. Several experimental studies have demonstrated that UFH or LMWH interfere with various processes involved in tumour growth and metastasis that still need to be clinically documented. These processes might include fibrin formation; binding of heparin to angiogenic growth factors such as FGF2 and VEGF; modulation of TF; TFPI release; inhibition of matrix-degrading enzymes; and other mechanisms. Clinical trials have suggested a clinically relevant effect of LMWH, as compared to UFH, on the survival of cancer patients with DVT that needs to be further documented in a large multicenter trial in cancer patients with defined tumor types and tumor stage. Recent studies from our laboratory defined the role of the LMWH, anti-factor VIIa, and r-TFPI in the modulation of angiogenesis, tumor growth, and tumour metastasis. Furthermore, antiplatelet drugs could provide any additional benefit in reducing tumour metastasis.

References


[385] Antithrombotics
Hämostaseologie 4/2005


