

Review Article

Anti-cancer properties of low-molecular-weight heparin: Preclinical evidence

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Summary

Malignant conditions are frequently associated with a hypercoagulable state, with recurrent thrombosis due to the impact of cancer cells and chemotherapy or radiotherapy on the coagulation cascade. Heparin and, its pharmacokinetically improved versions, low-molecular-weight heparins (LMWH) are effective in the prevention and treatment of thromboembolic events in cancer patients. There are several lines of preclinical evidence suggesting potential benefits of LMWH in hypercoagulation and thrombosis as well as in various processes involved in tumour growth and metastasis. Tinzaparin is a LMWH produced by controlled enzymatic depolymerisation of unfractionated heparin. The efficacy of tinzaparin has been documented in several clinical trials across various conditions and in special patient populations. The main objective of this review is to present the existing knowledge on the preclinical anti-cancer properties of tinza-

parin and other LMWH. The evidence for tinzaparin, as well as other LMWH, regarding interference with cancer-induced hypercoagulation, cancer cell proliferation, degradation of extracellular matrix, angiogenesis, selectin-mediated binding of platelet and cancer cells, chemokine signalling, tumour progression, and metastasis are reviewed. Certain clinical trials suggest improved survival of cancer patients with deep venous thrombosis treated with LMWH versus unfractionated heparin and when added to the promising preclinical anti-cancer properties of LMWH this warrants further investigations in prospective, randomised, controlled clinical trials in cancer patients. The benefits of LMWH in cancer might at least in part, be independent from its anti-coagulant activities, but may still be partially dependent on its anti-coagulant activities.

Keywords

Heparin, low-molecular-weight heparin, thrombosis, cancer, metastasis, tissue factor, tissue factor pathway inhibitor, selectin, angiogenesis, cancer-associated thrombosis, deep venous thrombosis, prophylaxis, treatment

Thromb Haemost 2009; 102: 258–267

Introduction

The association between cancer and venous thromboembolism (VTE) has been acknowledged for more than a century, and VTE represents a major challenge in the management of cancer patients (1, 2). In comparison to non-cancer patients, cancer patients experience more episodes of VTE and greater morbidity and mortality secondary to VTE (3, 4).

Unfractionated heparin (UFH), used for decades in the prophylaxis and treatment of VTE, has largely been replaced by low-molecular-weight heparins (LMWH) and, according to recent international guidelines, LMWH is the recommended anticoagulant regimen in cancer patients (5–8). Heparin is a glycosami-

noglycan containing various polymeric units and therefore has widely different molecular weight components. LMWH is synthesised by partial hydrolysis or enzymatic degradation of standard heparin and has a narrower spectrum of smaller polymeric units. In recent years, several studies have shown that heparin and LMWH have effective anti-inflammatory and anti-angiogenesis activities in addition to their traditional anticoagulant activities (Table 1).

Several lines of preclinical research suggests that heparin and its pharmacokinetically improved version LMWH possess an array of potential anti-cancer properties (9, 10). The objective of this review is to highlight the existing preclinical evidence for the anti-cancer properties of the LMWH, tinzaparin and other LMWH.

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Received: December 20, 2008
Accepted after major revision: May 14, 2009

Prepublished online: July 3, 2009
doi:10.1160/TH08-12-0832

Cancer-associated hypercoagulation

The link between cancer and thrombosis has been extensively studied demonstrating that there are positive feedback loops between tumour and the coagulation system and vice versa (11, 12). The increased frequency of VTE in cancer patients may, to some extent, be related to prolonged bed rest, chemotherapy, use of central venous catheters, and other risk factors (1, 2, 13). However, the tumour itself appears to cause a state of hypercoagulation, and components of the coagulation and fibrinolytic system contribute directly or indirectly to cancer progression and mortality. Malignant cells can activate blood coagulation in several ways primarily involving pro-coagulant factors; activation of a multitude of tumour-derived cytokines or direct interference with vascular components (Fig. 1) and a multitude of cells, mediators, and components from different biochemical pathways that are involved in the hypercoagulation state (11, 12, 14–16).

Hypercoagulation can be detected in the early stages of malignant disease as low-grade intravascular coagulation (17). Strong evidence indicates tumour cells to be the source or the promoter of pro-coagulants, among those tissue factor (TF) and cancer pro-coagulant, a hypothesised protein, which is most likely a cysteine protease enzyme (12, 13). In health, pro-inflammatory stimuli can induce expression of TF on endothelial cells, monocytes/macrophages, and neutrophils, but TF is constitutively

Table 1: Variable poly-component and poly-pharmacological effects of LMWH as a function of their structural heterogeneity.

Site of actions	Pharmacological effects
AT-dependent plasmatic effects	Anti-Xa, Anti-IIa, and modulation of other AT-dependent coagulation factors
AT-independent vascular effects	Modulation of vascular TPFI, NO, and vWF
Cell adhesion molecules	Modulation of Selectins (P-, L-, and E-selectins) and Immunoglobulins (soluble ICAM-I, VCAM-I)
Fibrinolytic system	Modulation of t-PA and PAI-I
Inflammatory mediators	Modulation of TNF-alpha, IL-6
Matrix-degrading enzymes	Modulation of various carbohydrate dependent matrix degrading enzymes such as aggrecanases, and heparanases

expressed in cancer cells (12, 15). TF can induce angiogenesis and promote tumour progression by coagulation-dependent as well as coagulation-independent mechanisms (18).

Tumour cells can also synthesise and release various cytokines and chemokines of importance in thromboembolic-mediated complications, including vascular endothelial growth factor (VEGF), tumour necrosis factor α (TNF- α), interleukin-6

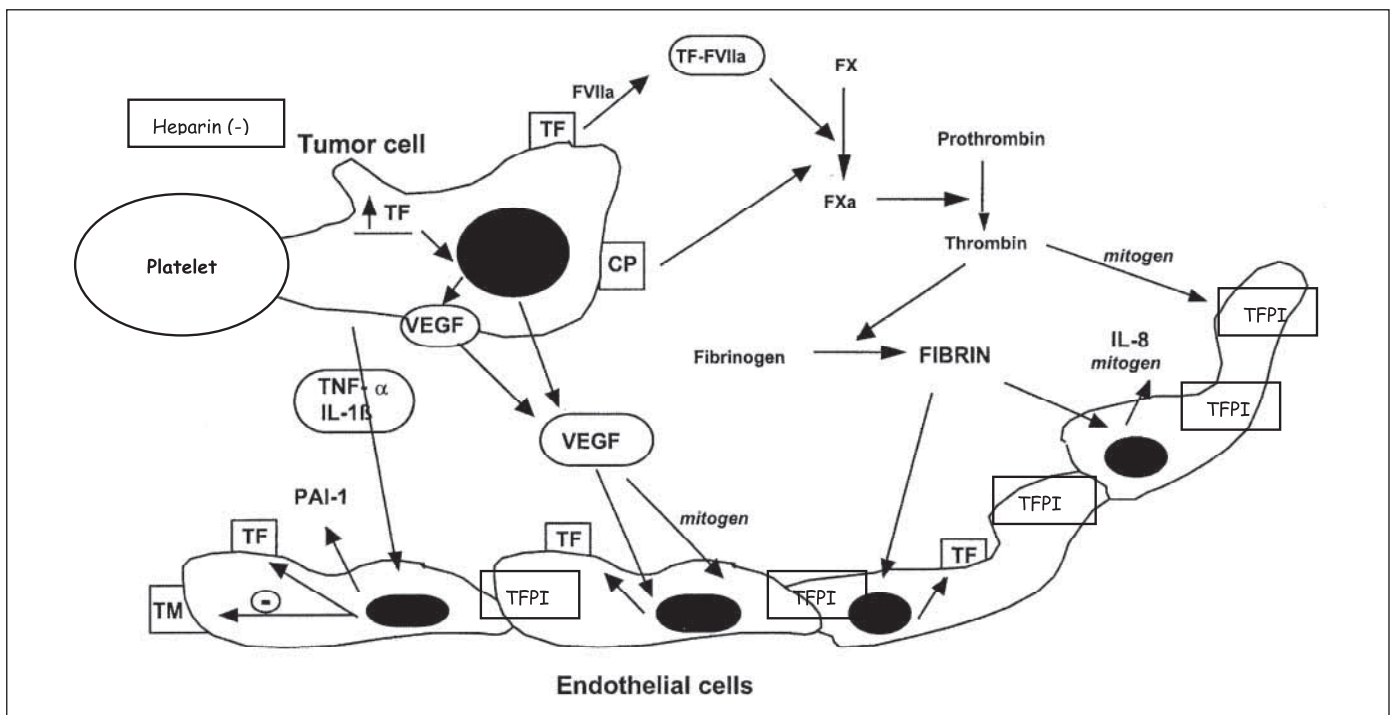


Figure 1: Regulation of cancer cell and endothelial cell pro-coagulant functions in the pathogenesis of thrombosis in cancer. Tissue factor (TF) and cancer procoagulant (CP) are synthesised and expressed on the surface of tumour cells. The effects of these tumour cell pro-coagulants are enhanced by the local production of the important pro-angiogenesis cytokines IL-8 (from the endothelial cells), and vascular endothelium growth factor (VEGF) and the inflammatory cytokines TNF- α , and IL-1 β from tumour cells. These cytokines convert the normal anticoagulant endothelium to a procoagulant endothelium as

follows: 1) Down-regulation of thrombomodulin (TM) expression, and 2) increased synthesis of TF and plasminogen activator inhibitor-1 (PAI-1). Fibrin, produced in response to activation of clotting by TF and CP, increases both TF and IL-8 production by the endothelium, further enhancing thrombogenesis and angiogenesis. TF also increases angiogenesis by the tumour cell by increasing the synthesis of VEGF. TFPI (1 and 2) are differentially expressed in endothelial cells but not cancer cells and are released by heparin or LMWH. Platelet-cancer adhesion is effectively blocked by heparin or LMWH.

(IL-6), and IL-1 β (12, 19). Cytokines from tumour cells and tumour-associated macrophages are involved in the expression of TF, regulation of thrombin receptor, promotion of plasminogen-activator-1 activity, and expression of adhesion molecules implicated in leukocyte homing, rolling, migration, and transmigration (20, 21). Release of TF from tumour-associated macrophages, as well as circulating monocytes has been reported to be higher in cancer patients than in controls (22). In addition, tumour-derived cytokines also attract and activate neutrophils capable of releasing reactive oxygen species, vascular growth factors, and proteases (23, 24).

Finally, tumour cells express adhesion molecules on their surface that allow them to interact with endothelial cells, platelets, and leukocytes (25–27). Tumour cells adherent to the endothelium also induce clotting, promote platelet and leukocyte trafficking, and adherence, to the vessel wall. These cells in turn, release tumour growth factors which assist transmigration of tumour cells through the vessel wall. In addition, tumour cells interact with tumour-associated macrophages, natural killer cells, myeloid-derived suppressor cells, and dendritic cells (24–26).

Disorders of haemostasis in cancer patients may also result from a shift in the activity of coagulation and fibrinolysis inhibitors. Some of the most important inhibitors of blood coagulation are tissue factor pathway inhibitor (TFPI) (18, 28), TFPI-2 (29), protein Z (or protein Z-dependent proteinase inhibitor), antithrombin (AT) (30), the protein C system (31), heparin cofactor II, and thrombomodulin (32). There is evidence that these inhibitors of coagulation may contribute not only to VTE and bleeding complications, but also to cancer progression (33). For example, TFPI released by heparin or LMWH act effectively to block the coagulant, as well as the non-coagulant (angiogenesis, and inflammation), effects mediated by TF/factor VIIa (Fig. 2).

Anti-cancer properties of heparin

Heparin is a polydisperse sulphated glycosaminoglycan consisting of repeating disaccharide units, with a mean molecular weight in the range of 12,000 to 14,000 Daltons (total range of 2,000 to 40,000 Daltons). It is extracted from porcine intestinal mast cells and used for various clinical purposes. Heparins can

bind to a wide range of molecules via electrostatic interactions with the glycosaminoglycan chains, and possess numerous biological properties beyond their anti-coagulant effects, including anti-cancer properties.

Effect on tumour growth and metastasis

The anti-cancer properties of heparin have been a focus of interest for decades (3, 10, 34, 35). A predominant proportion of animal in-vivo studies of tumour growth have shown no inhibitory effect of heparin on growth of subcutaneously or intramuscularly implanted sarcomas, carcinomas or melanomas (34), but due to methodological differences it is difficult to make firm conclusions across studies (10). It may well be that the anti-proliferative properties of heparin are dose-related, since intra-peritoneal administration of heparin has resulted in tumour regression in most studies of colon cancer in animals. Moreover, substantial preclinical evidence indicates that heparin is a potent inhibitor of metastasis. A number of studies have shown heparin to reduce the number of lung metastases following injection of tumour cells into the tail vein in animal models of haematogenous spread of melanoma, sarcoma, breast and colonic carcinoma. Some studies also indicate that heparin can inhibit liver metastasis in colon cancer model. There are conflicting data regarding the ability of heparin to inhibit metastases of tumours implanted subcutaneously or intramuscularly (10, 34).

Anti-cancer mechanisms exhibited by heparin

There is extensive documentation of the potential anti-cancer properties of heparin as presented in recent reviews (3, 10, 14, 34). Interference with cellular proliferation can occur through interference with proto-oncogenes, protein kinase-C activity, and mitogen-activated protein kinase phosphorylation. There is substantial documentation on the effect of heparin on angiogenesis and metastasis. Heparins inhibit many steps in the coagulation cascade, but ultimately antagonise thrombin activity and thereby the formation of fibrin, which might have an impact on tumour progression. Additionally, part of heparin's anti-cancer properties may be related to release of the coagulation inhibitor TFPI from vascular endothelium. Heparin-released TFPI in turn inhibits TF signalling, which appears to suppress tumour growth (36). In addition to TFPI release, heparin also interacts with a variety of vascular growth factors released from tumour cells or the endothelium, including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) (37, 38). Heparin inhibits fibrin and platelet deposition around tumour cells rendering them more vulnerable to the cytotoxic effects of natural-killer cells (39).

Additionally, there is substantial evidence that heparin may interfere with the adhesion of leukocytes to sites of inflammation or tumour invasion (25, 26), largely due to the ability of heparin to block selectin- or integrin-mediated adhesion of tumour cells, platelets, and leukocytes to the endothelium. Furthermore, chemotaxis of tumour cells, regulated by chemokine receptors and their organ specific ligands relevant for metastatic spread, can also be inhibited by heparins (40–42).

Invasion and metastasis are also dependent on specific proteolytic enzymes. Heparanase is an endo-glycosidase which cleaves heparan sulphate and hence participates in degradation

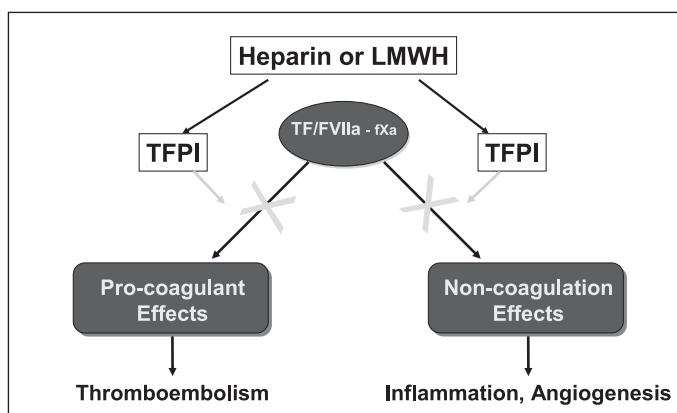


Figure 2: Key role of LMWH-induced endothelial cell releasable TFPI in the regulation of TF/FVIIa-mediated pro-coagulant and non-coagulant (inflammation and angiogenesis) effects.

and remodelling of the extracellular matrix and probably tumour invasion. Heparanase is also involved in the release of growth factors bound to heparan sulphate proteoglycans and the expression of tissue factor on vascular endothelial and tumour cells (43). Heparins potently inhibit heparanase, and this effect may contribute significantly to the anti-cancer and anti-inflammatory properties of heparin (44, 45). On the contrary, heparanase cleaves, and partly neutralises, the anticoagulation effects of unfractionated heparin (UFH) and LMWH (46).

Low-molecular-weight heparins (LMWH)

LMWH have replaced UFH in clinical practice in most indications. LMWH exhibit much higher and consistent subcutaneous bioavailability, a convenient dosing schedule, opportunity for outpatient therapy for acute VTE, and no need for therapeutic monitoring (47).

Several meta-analyses have shown a favourable efficacy and tolerability profile of LMWH versus UFH in treatment of VTE (48–51). Interestingly, several meta-analyses indicate LMWH to confer a survival benefit versus UFH, particularly in cancer patients (48–50). A recent Cochrane review demonstrated a significant, 29% mortality reduction in cancer patients receiving LMWH for initial treatment of VTE versus those receiving UFH (52). Furthermore, several studies with anticoagulants in cancer patients with no indication for antithrombotic therapy have indicated that heparin and LMWH improve survival in cancer patients (53–55). The latest prospective randomised trials have shown that use of LMWH is associated with significant clinically relevant differences in overall survival in cancer patients compared to UFH (56–59). However, conflicting data have been presented, and at present there is no approved use of heparins for survival gain in cancer patients without a need for VTE prophylaxis or treatment (5–7).

Tinzaparin

Tinzaparin sodium is a LMWH produced by controlled, enzymatic, depolymerisation of unfractionated, porcine heparin (60). The efficacy and safety of tinzaparin for prophylaxis and treatment of VTE has been substantially documented (61–65). In one study, use of tinzaparin showed a low rate of recurrent VTE compared to UFH (2.8% vs. 6.9%) and a significantly lower rate of major bleedings (0.5% vs. 5.0%) for treatment of deep venous thrombosis (DVT) (63). Also, tinzaparin was superior to warfarin for prevention of DVT in orthopaedic knee joint replacement surgery (66). Several additional trials have confirmed clinical efficacy and tolerability of tinzaparin, including in cancer patients where tinzaparin was found more effective and as safe as warfarin for long-term treatment (67–73). Studies have also been performed in special populations, e.g. elderly, obese patients, patients with renal insufficiency, and pregnant women (60, 74–79).

In comparison to UFH, fondaparinux and some LMWH, tinzaparin exhibits a strong vascular distribution in addition to its plasmatic distribution, with a relatively long residence time. Indications of high vascular distribution is evident by the binding kinetics to vascular endothelium and the releasing capacity of endothelial TFPI (80, 81). This may explain why tinzaparin, in comparison to fondaparinux and some LMWH having relatively

low-molecular-weight distributions, does not exhibit significant accumulation in patients with renal failure, and it can be used without dose adjustment (75–78). The use of anti-factor Xa activity to standardise the biological actions of LMWH may be inappropriate, since it does not address the other AT-dependent and the AT-independent actions of LMWH (Table 1) (13, 82).

Anti-cancer properties of tinzaparin and other LMWH

A series of coordinated steps are pivotal for cancer development and metastasis, including proliferation of cancer cells, invasion into surrounding tissue, avoiding attack from the immune system, angiogenesis, and metastatic spread (34). The published literature reporting the pre-clinical studies of the anti-cancer properties of tinzaparin in key processes in cancer development is the main focus of this review. We have included only studies, where commercially available LMWH has been used. Several studies have investigated heparin fragments with molecular size similar to marketed LMWH, e.g. tinzaparin (37). However, chemically modified heparin products may differ from marketed products in physicochemical properties and may therefore exhibit different pharmacokinetic or pharmacodynamic properties. Most pre-clinical studies of LMWH have used chemical modified heparin, not marketed LMWH (10).

Effect on cancer-associated hypercoagulation

Cancer cell-induced release of TF is considered to play a pivotal role in the induction of the coagulation cascade and fibrin production (12, 15). TF may not be the principal or sole mediator of hypercoagulation as shown in haematological malignancies (83). The inhibitory effect of different LMWH, alone or in combination with platelet glycoprotein IIb/IIIa antagonists, on TF- or cancer-activated thrombosis has been determined in human blood using thrombelastography (84). In this study, tinzaparin demonstrated five-fold greater potency in inhibiting either TF or cancer-mediated hypercoagulation as compared to enoxaparin. An overall antithrombotic additive to synergistic effect occurred when each LMWH was combined with the glycoprotein IIb/IIIa antagonist (84, 85). These data have been confirmed using prostate cancer cells as inducers of clot formation (86). Whether these differences among LMWH on cancer-induced hypercoagulation turn out to show clinical relevant advantages remain to be clarified.

Preclinical data indicate platelets to have a direct, or indirect, role in many aspects of tumour progression (11, 27). Platelet counts are very labile in cancer where anti-cancer treatment and bone marrow invasion tend to lower platelet counts. The phenomenon of tumour cell induced platelet aggregation is, in part, caused by increased expression of platelet adhesion receptors (e.g. CD62, CD63, and P-selectin). Platelets can also be trapped by clots and fibrin deposits at the site of leukocyte and tumour cell adhesion during metastasis. This may lead to a secondary consumption of platelets and development of thrombocytopenia. Such phenomenon can be observed in animal studies with injection of a notable amount of tumour cells. Abolition of cancer-cell induced thrombocytopenia has been demonstrated with UFH, LMWH (enoxaparin), and warfarin but not non-anticoagulant LMWH (87, 88). Preclinical data have also indicated that tinzaparin inhibits cancer cell-induced thrombocytopenia. In an ex-

perimental melanoma model in mice, intravenous injection of tumour cells into the tail vein induced rapid reduction in the platelet count of approximately 50%. The cancer cell-induced thrombocytopenia was fully abolished by subcutaneous administration of tinzaparin (87).

Effect of LMWH on tumour cell proliferation and local tumour growth

Generally, preclinical studies have shown no effect of UFH on the growth of cancer cells *in vitro* or the growth of the primary tumour in animal models (10, 34). The LMWH dalteparin, enoxaparin, and tinzaparin have been shown to inhibit downstream phosphorylation of the ERK kinase pathway in tumour-derived endothelial cells, and thus potentially the ability to interfere with cell proliferation (89). Inhibition of the ERK kinase pathway has been shown to be pivotal for several anti-cancer drugs, e.g. epidermal growth factor inhibitors and multi-target tyrosine kinase inhibitors (90, 91). Preliminary data indicate dalteparin to be most potent in this assay followed by tinzaparin and enoxaparin. Despite that LMWH may interfere with pivotal cell proliferation pathways, most studies have shown no effect of LMWH on cancer cell proliferation *in vitro*. Neither UFH nor dalteparin influenced *in vitro* or *in vivo* growth of human melanoma cells (92), dalteparin or UFH did not reduce proliferation of lung carcinoma cells *in vitro* (93), and tinzaparin did not inhibit cellular proliferation or induce apoptosis in human breast cancer cells *in vitro* (42). However, in a study with primary cell cultures of human brain cancer cells, enoxaparin showed a modest (approximately 20%), but significant, inhibitory effect (94).

A number of *in-vivo* animal studies have examined the effect of UFH (34), chemically modified heparin as LMWH (10), and non-coagulant LMWH (88, 95) on tumour growth and metastases, but most studies have failed to show any effect on local tumour growth. Conflicting data have been shown with marketed LMWH. In a study with subcutaneous inoculated lung carcinoma cells, tumour growth was significantly reduced by dalteparin, and to a lesser degree by UFH (93). On the other hand, neither UFH nor dalteparin influenced *in vivo* growth of human melanoma cells (92). Some studies have shown LMWH to inhibit metastasis from peritoneal administration of tumour cells (96, 97). Pross et al. conducted a series of studies with the LMWH reviparin. A combination of intraperitoneal and subcutaneous administration of reviparin most potently reduced tumour growth. Preliminary data indicate tinzaparin to possess some inhibitory effects on the growth of primary tumours (98). In a chick embryo chorioallantoic membrane (CAM) angiogenesis model, tinzaparin abolished platelet/fibrin clot-induced growth of fibrosarcoma and colon tumours.

Effect on angiogenesis

Heparin-induced inhibition of angiogenesis, and the mechanism by which heparins may interfere with angiogenesis, has been an area of great focus (12, 24, 34, 37). Data from *in-vivo* growth of tumours have indicated that heparin-induced inhibition is associated with reduced angiogenesis. Reduction of growth was associated with reduced micro-vessel density (93). Angiogenesis may be pivotal for both local tumour growth and metastasis.

UFH and chemically modified LMWH have demonstrated impairment of proliferation of endothelial cells (34). UFH and LMWH, but not fondaparinux, were shown to inhibit proliferation of endothelial cells induced by FGF or VEGF (93, 99, 100). The greatest effect was observed with a 6-kDa fraction of LMWH. The ability of endothelial cells to form blood vessels has been studied using the matrigel assay. LMWH, but not UFH or fondaparinux, inhibited FGF-induced tube formation in this assay (99, 100). LMWH-induced inhibition of endothelial capillary tube formation was independent of the type of stimulation (FGF, TNF- α , VEGF or medium from cultured malignant cells). The inhibitory effect was comparable across LMWH (enoxaparin, dalteparin, and tinzaparin) (99). In some studies, LMWH (dalteparin) and UFH have shown similar degree of inhibition of VEGF-induced endothelial tube formation in matrigel studies (93).

The ability of LMWH to interfere with the generation of new tumour blood vessels has also been studied using the CAM angiogenesis model (37, 81). Tinzaparin and recombinant-TFPI blocked angiogenesis induced by a variety of angiogenic factors (13, 101) (Fig. 3A). The inhibitory effect of tinzaparin was blocked by a TFPI monoclonal antibody (81). These studies demonstrated a significant role of the interaction of LMWH and TFPI on the regulation of angiogenesis (13, 101). Furthermore, LMWH inhibition of TFPI inhibits VEGF expression, a potent inducer of tumour vasculature (18, 102).

A series of studies have been conducted to explore the effect of LMWH on endothelial cell tube formation, predominantly using the model of human umbilical vein endothelial cells (HUVEC), an index of tumour vascularity (37, 101). Whereas UFH stimulated tube formation, LMWH (reviparin) has been shown to inhibit FGF- or TNF- α induced tube formation (103). The differential effect was likely due to effects on the structure of the fibrin matrix. Inhibition of tube formation has also been shown with tinzaparin, which elicited a dose-dependent abolition of FGF-induced HUVEC tube formation (81, 101). High doses of tinzaparin completely reversed the effect of FGF (Fig. 3B).

Effect on metastasis

The ability of UFH and chemically modified heparin to inhibit metastasis in animal models is substantially documented (10, 34). In an experimental melanoma model of metastasis, the LMWH tinzaparin, dalteparin, nadroparin, and enoxaparin have shown potent inhibition of lung and liver metastases and colony formation (87, 92, 104, 105), whereas fondaparinux has only modest if any effect in these experiments. For example, administration of tinzaparin prior to intravenous injection of melanoma cells reduced lung tumour formation by 89% by day 15 (87). Tumour formation was almost completely blocked if the animals received a pre-tumour cell dose followed by daily dosing for 14 days. Similar findings were shown by several groups. Stevenson et al. found tinzaparin and UFH to have comparable efficacy regarding inhibition of lung metastases after intravenous injection of colon carcinoma cells as well as melanoma cells (106). The anti-metastatic properties of tinzaparin have also been shown in an *in-vivo* model of metastasis using severe combined immunodeficiency mice inoculated with human breast cancer cells (42).

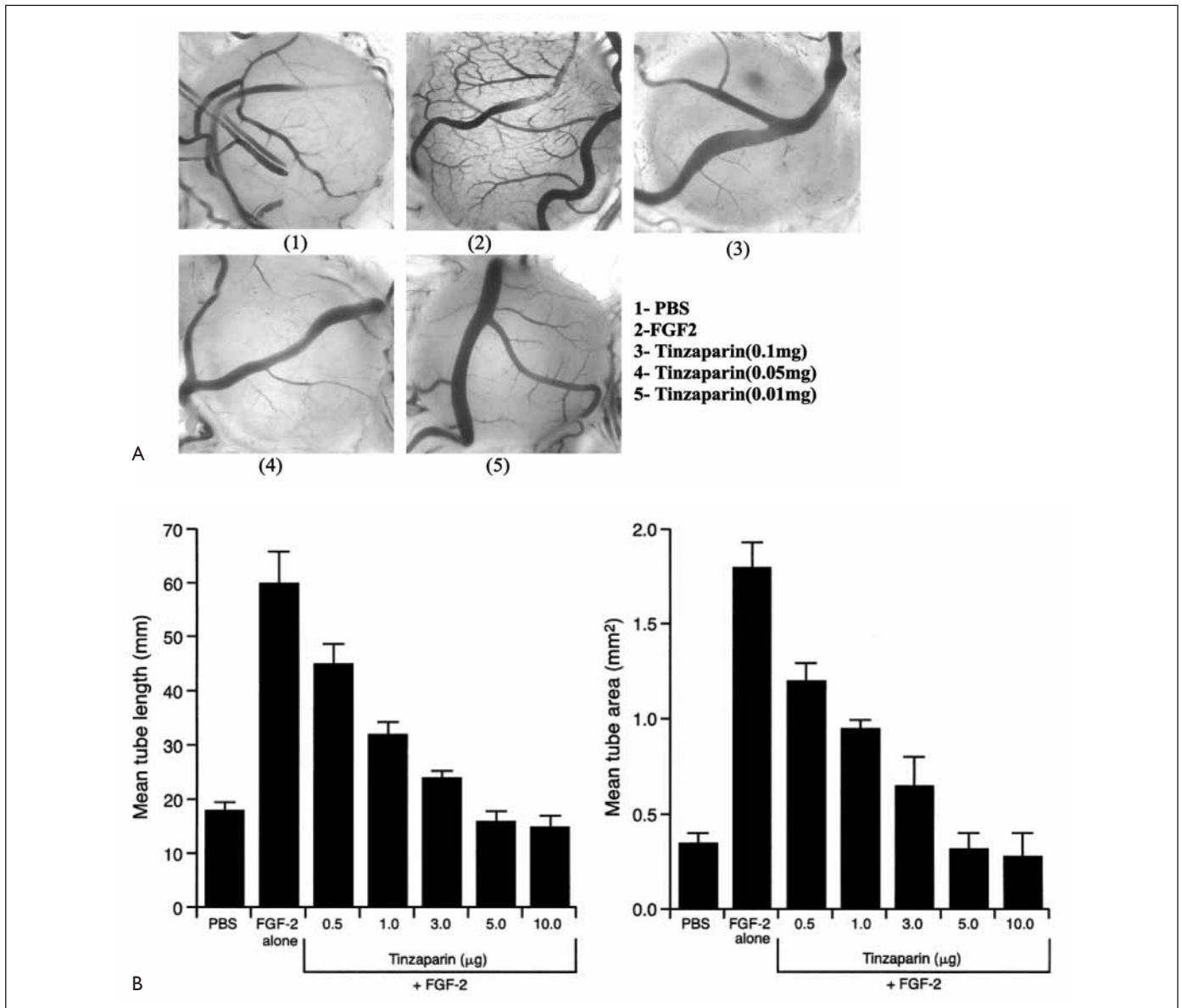


Figure 3: Tinzaparin inhibits FGF2-stimulated tube new vessel formation in the CAM model (A) and displayed a dose-dependent reduction in tube length and tube area in a HUVEC model of angiogenesis (B). Figure 3A and B reproduced with permission from (Mousa and Mohamed, Oncol Report 2004) and (Mousa and Mohamed, Thromb Haemost 2004), respectively.

UFH and tinzaparin caused a significant reduction in metastatic sites and metastatic nodule volume, effects likely related to CXCR4 signalling (42).

Most studies of metastasis have used intravenous administration of tumour cells. Some studies have shown LMWH to inhibit metastasis from peritoneal administration of tumour cells (96, 97). Pross et al. conducted a series of studies with the LMWH reviparin and showed reduced metastasis. Anti-metastatic effects of LMWH have been documented also for non-anti-coagulant LMWH indicating that such effects are not directly related to effects on anti-Xa or anti-IIa activity (88, 107, 108).

Anti-metastatic properties of LMWH are likely due to interference with endothelial cell adhesion. The ability of heparins to interfere with selectin binding appears to be a major pathway for

their anti-metastatic properties (10, 44, 45, 87, 106, 109, 110). The possible mechanism may also involve interaction with VLA-4/VCAM-1 (111). The importance of selectins is emphasised by findings that anti-metastatic effect of heparins cannot be demonstrated in animals deficient of P- or L-selectin (44, 112). A broad selection of licensed heparin formulations have been evaluated for their ability to inhibit P-selectin and L-selectin binding to human carcinoma cells *in vitro* (106). This study showed tinzaparin to be more potent than enoxaparin and dalteparin; UFH was more potent than any LMWH. Superior affinity of UFH over LMWH (with comparable results for enoxaparin and nadroparin) for binding to P- and L-selectin has also been shown using a quartz crystal microbalance biosensor (109). While in another study nadroparin was found comparable to UFH but superior to

enoxaparin for interference with selectin binding (105). Fondaparinux did not inhibit selectin binding (105, 106). Finally, when formation of P-selectin dependent platelet-leukocytes aggregates from healthy donors was studied by Maugheri et al. parnaparin was superior to enoxaparin and UFH (113). There seems to be a strong correlation between inhibition of selectin binding and inhibition of metastatic spread (105). The inhibitory effect of LMWH on P-selectin seems to be related to a relatively small, and non-coagulant, fraction of the high-molecular-weight components (106, 110).

In conclusion, these data confirm that tinzaparin and other LMWH possess potent anti-metastatic properties *in vivo*, probably via interaction with selectins.

Effect on multi-system pathways

Some pathways cannot easily be described as being involved solely in tumour cell proliferation, tumour growth, invasion, angiogenesis, or metastasis. This section describes effects of tinzaparin and other LMWH on mechanisms considered involved in several anti-cancer properties.

Effect on tissue factor pathway inhibitor (TFPI)

The role of TF in cancer growth, angiogenesis, and metastasis is well known (10, 18, 28, 34, 101). A potentially important role of heparin and LMWH is the release of TFPI, a natural inhibitor of TF. TFPI is released from endothelial cells and acts a major down-regulator of pro-coagulant activity of TF-factor VIIa complex (18, 28, 29). LMWH, e.g. dalteparin, and bemiparin, have been shown superiority versus UFH for up-regulation of TFPI mRNA and release of soluble TFPI *in vitro* (114, 115). Tinzaparin has been shown to induce TFPI release in a time-dependent fashion using HUVEC *in vitro* (101). TFPI release was increased by more than 50-fold from baseline to 8 hours. Tinzaparin released TFPI more potently than UFH and enoxaparin in the presence of plasma proteins (116), and it appears that TFPI release is dependent on the molecular size of the heparin used. Tinzaparin has a mean molecular mass of 6.5 kDa and heparin chains with a molecular weight of 6–8 kDa have been shown to induce maximum TFPI release, whereas minimal TFPI release was observed at fractions below 4 kDa (117).

TFPI release has been shown in humans with most LMWH, such as enoxaparin, dalteparin, bemiparin, ardeparin, and reviparin (118–122). Injection of a single therapeutic dose of tinzaparin in healthy volunteers demonstrated rapid and sustained TFPI release (82). Repeated administration of tinzaparin seven days later showed identical TFPI release, indicating no depletion of TFPI or tachyphylaxis to tinzaparin. Tachyphylaxis of TFPI release has been shown by UFH, but not with LMWH enoxaparin and dalteparin (123). In contrast, the pentasaccharide anticoagulant, fondaparinux, has no effect on TFPI release (101). Significant differences have been shown for LMWH-induced TFPI release *in vitro*, but it is difficult to draw conclusions about the clinical relevance. Only one study has compared TFPI release among several LMWH (124). Women undergoing caesarian procedure were randomised to receive enoxaparin, dalteparin, and tinzaparin and all groups showed similar profiles of TFPI release.

Effect on chemokine signalling and chemotaxis

Chemokines are implicated in a variety of biological processes, including cell proliferation and angiogenesis. Cancer cells express chemokines and chemokine receptors, which play a key role in leukocyte recruitment and migration (125). Heparins may inhibit chemokine synthesis and chemokine function (26). The impact of tinzaparin on CXCL12/CXCR4 signalling, which has an important role in metastatic breast cancer, has been investigated (42). Following transfection of wild-type Chinese hamster ovary cells with the chemokine receptor CRXR4, UFH and clinically relevant doses of tinzaparin elicited a dose-related inhibition of CXCL12 (the sole ligand to CXCR4) binding to the CXCR4-transfected cells. The inhibitory capacity was stronger for UFH than for tinzaparin, but tinzaparin caused a significantly stronger inhibition of CXCR4-mediated chemotaxis. Tinzaparin abolished CXCL12-induced chemotaxis at a concentration that was 10-fold lower than UFH. In addition to being able to inhibit chemotaxis in transfected cells, tinzaparin also significantly and dose-dependently impaired chemotaxis in breast cancer cells. The authors concluded that tinzaparin was a potent inhibitor of chemokine activation in human breast cancer cells. Moreover, in direct comparison, tinzaparin inhibited chemotaxis significantly better than UFH.

Effect on extracellular matrix proteases

As described earlier, heparanase, a proteolytic enzyme involved in modelling of extracellular matrix, may facilitate cell invasiveness in cancer (43, 126) and the potent inhibitory effect of heparin on heparanase could be important for the anti-cancer properties of heparin (43, 127). UFH and dalteparin have been shown to inhibit mRNA and heparanase activity in lung carcinoma cells *in vivo* in rodents (93). No studies have yet explored the capability of tinzaparin for inhibition of heparanase. However, LMWH have also been found to inhibit aggrecanase, another proteolytic enzyme, found primarily in bone interfaces where it is responsible for breakdown of cartilage. In comparative studies with commercially available LMWH, tinzaparin (which has the highest mean molecular weight of all LMWH) was shown to be the most potent inhibitor of aggrecanase followed by nadroparin and enoxaparin (128). Enoxaparin was ineffective in inhibition of invasion of primary brain cancer cell in a matrigel assay (94).

All LMWH are not alike

Recommendations for anticoagulant therapy for prophylaxis and treatment of VTE generally do not differentiate much between individual LMWH (7, 8). Most LMWH have mean molecular masses in the range of 4–8 kDa, but different pharmacological properties may be related to distinct fractions of heparin within a narrow range (47, 117). The anti-metastatic effects of heparin and LMWH in animal models have been extensively documented, and it is evident that the anti-metastatic and anti-angiogenic properties are not directly related to their anti-coagulant activity (34, 99, 106, 107, 110). In addition to differences in mean molecular sizes, LMWH differ in the range of molecular-weight distribution, sulphation modifications, and ring openings of uronic acid residues due to chemical or enzymatic processing (80, 117). The ability of heparins to induce TFPI release may be

of clinical relevance; silencing of TF appears to suppress tumour growth (36). TFPI release has been shown to be determined by LMWH molecular size and the degree of sulphation (117). Still, several LMWH have been compared for TFPI release in patients with very comparable results (124). Tinzaparin has been shown to be more potent than any other LMWH in regards to inhibition of selectins (44, 45, 105, 106, 109, 129), molecules of pivotal importance for metastasis (44, 45, 105, 106, 109, 129). The observation that LMWH are not all alike is supported by recent studies showing that interference with P- and L-selectins as well the inhibition of lung metastasis were notably lower for nadroparin than enoxaparin (105, 109). The clinical importance of such findings remains to be confirmed.

However, these differences in physicochemical properties result in different biological actions and sometimes varying results in clinical trials performed at optimised doses for individual products. Regulatory authorities as well as scientific societies consider each LMWH to be a distinct pharmacological entity that cannot be interchanged with another LMWH (130–132).

Prospects for LMWH in cancer therapy

Tinzaparin has a favorable molecular weight composition and has demonstrated anti-neoplastic properties *in vitro* and *in vivo* in animal models, in particular regarding inhibition of angiogenesis and metastasis. Inhibition of angiogenesis has been demonstrated to be of great clinical importance in management of cancer as demonstrated by the VEGF monoclonal antibody bevacizumab (133–135). The preclinical data with tinzaparin and other LMWH are promising, but not truly predictive for the clinical benefit in cancer patients. Prospective clinical trials are required to demonstrate therapeutic benefit of LMWH alone or

in combination with other anti-cancer therapies. The expectations of such results are not unreasonable since several meta-analyses have shown notable survival benefit of LMWH over UFH in cancer patients (53–55).

There are indications that patients eligible for clinical trials of LMWH in cancer should be restricted to those with localised disease with a high risk of metastasis. The satisfactory safety and tolerability profile of LMWH and opportunity for subcutaneous administration at home is advantageous for long-term anti-cancer therapy, e.g. as an adjuvant therapy. Conditions such as renal cell cancer, non-small cell lung cancer, pancreatic cancer, and melanoma are possible indications, also characterised with a high frequency of VTE. There are currently a number of ongoing studies with LMWH alone or in combination with standard chemotherapy in various cancer indications. Among those trials are the TILT study, a randomized study of tinzaparin on the overall survival of patients with localized non small cell lung cancer after complete surgical resection, and the FRAGMATIC and PROSPECT-CONKO 004 studies evaluating dalteparin and enoxaparin with chemotherapy on VTE prophylaxis and survival in lung cancer and pancreatic cancer, respectively (136).

Conclusion

In-vitro and in-vivo studies have shown that tinzaparin and other LMWH have potent anti-cancer properties. These promising preclinical findings warrant further investigations in prospective, randomised, controlled clinical trials in cancer patients.

Conflicts of interest

Lars J. Petersen has received research grant and consulting fees from LEO Pharma, Ballerup, Denmark.

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