The heparins and cancer: review of clinical trials and biological properties

Roberto Castelli[a], **Fernando Porro**[a] **and Paolo Tarsia**[b]

Abstract: The association between cancer and thromboembolic disease is a well-known phenomenon and can contribute significantly to the morbidity and mortality of cancer patients. The spectrum of thromboembolic manifestations in cancer patients includes deep vein thrombosis, pulmonary embolism, but also intravascular disseminated coagulation and abnormalities in the clotting system in the absence of clinical manifestations. Unfractioned heparin (UFH) and particularly low molecular weight heparins (LMWHs) are widely used for the prevention and treatment of thromboembolic manifestations that commonly accompany malignancies. Malignant growth has also been linked to the activity of heparin-like glycosoaminoglycans, to neoangiogenesis, to protease activity, to immune function and gene expression. All these factors contribute in the proliferation and dissemination of malignancies. Heparins may play a role in tumour cell growth and in cancer dissemination. The aims of the study are to review the efficiency of heparins in the prevention and treatment of cancer-related thromboembolic complications, and review the biological effects of heparins. Heparins are effective in reducing the frequency of thromboembolic complications in cancer patients. Meta-analyses comparing unfractioned heparins and LMWHs for the treatment of deep vein thrombosis have shown better outcome with a reduction of major bleeding complications in patients treated with LMWHs. LMWH have antitumour effects in animal models of malignancy: heparin oligosaccharides containing less than 10 saccharide residues have been found to inhibit the biological activity of basic fibroblast growth factor (bFGF), whereas heparin fragments with less than 18 saccharide residues have been reported to inhibit the binding of vascular endothelial growth factor (VEGF) to its receptors on endothelial cells. It has been shown that LMWH, in contrast with UFH, can hinder the binding of growth factors to their high-affinity receptors as a result of its smaller size. In vitro heparin fragments of less than 18 saccharide residues reduce the activity of VEGF, and fragments of less than 10 saccharide residues inhibit the activity of bFGF. Small molecular heparin fractions have also been shown to inhibit VEGF- and bFGF-mediated angiogenesis in vivo, in contrast with UFH. Moreover, heparin may influence malignant cell growth through other different interrelated mechanisms: inhibition of (1) heparin-binding growth factors that drive malignant cell growth; (2) tumour cell heparinases that mediate tumour cell invasion and metastasis; (3) cell surface selectin-mediated tumour cell metastasis and blood coagulation. The above evidence, together with favourable pharmaco-properties and with a reduction in major bleeding complications, suggests an important role for LMWHs in thromboprophylaxis and in the therapy of venous thromboembolism in cancer patients. There is sufficient experimental data to suggest that heparins may interfere with various aspects of cancer proliferation, angiogenesis, and metastasis formation. Large-scale clinical trials are required to determine the clinical impact of the above activities on the natural history of the disease.

Key words: cancer; heparins; literature research; thromboembolic diseases; tumour cells

Introduction

Venous thromboembolism and cancer are linked by a two-way clinical correlation. On one hand, venous thromboembolism may be the first clinical presentation on an occult cancer. In fact, prospective cohort studies showed an increased incidence of cancer after an episode of idiopathic venous thromboembolism as compared with the incidence in the general population.[1] Two very large retrospective population-based studies were published in 1998, both of which demonstrated that the incidence of cancer was increased during the first year following the diagnosis of venous thromboembolism (VTE) and that the effect persisted for up to 10 years.

In the studies of Sorensen et al and Baron et al,[2,3] the authors examined data from both cancer and thromboembolic disease registries in Denmark and Sweden and calculated standardized incidence ratios (observed number of cases/expected number of cases in the same age group in the normal population). In both studies the investigators found a significantly increased risk for developing cancer,

[a]Emergency Medicine Department, IRCCS Ospedale Maggiore di Milano, Milano, Italy; [b]Institute of Respiratory Medicine, Milan University, IRCCS Ospedale Maggiore di Milano, Milano, Italy

Author for correspondence: Robe Xrto Castelli, Unità Operativa di Medicina d'Urgenza, IRCCS Ospedale Maggiore di Milano, Via F. Sforza 35, Milano 20122, Italy. Tel: +39 2 55033602; E-mail: Castelli39@interfree.it

10.1191/1358863x04vm566ra

particularly in the first year after the diagnosis of VTE. The diagnosis of cancer was much higher in patients presenting with idiopathic than in patients with secondary VTE.

On the other hand, patients with clinical overt cancer may develop venous thromboembolic complications. In fact, an increased incidence of VTE in patients with known malignancies has been convincingly demonstrated. Post mortem studies have shown a markedly increased incidence of thromboembolic disease in patients who died from cancer.[4,5] Patients with cancer are more likely to develop VTE than patients without malignancy. The risk varies with different tumour types and is thought to be highest in tumours of the ovary, pancreas, and central nervous system. Many factors are thought to contribute to the risk of VTE, including the primary tumour site, age, immobility, and type of therapeutic intervention. Chemotherapy, particularly when combined with hormone therapy, also increases the risk of VTE.

The pathogenesis of thromboembolic disease in cancer patients is complex and multifactorial: decreased levels of inhibitors of coagulation, impaired fibrinolysis, the presence of antiphospholipid antibodies and an acquired activated protein C resistance contribute to the hypercoagulable state in cancer. The activation of coagulation is also implicated in tumour proliferation through the interactions of coagulation with inflammation and increased tissue factor pathway inhibitor. Laboratory diagnosis of the thrombophilic state include elevation of clotting factors, fibrinogen/fibrin degradation products, hyperfibrinogenemia and thrombocytosis and elevation of specific markers of activation of coagulation: fibrinopeptide A, fragment $1+2$, thrombin-antithrombin complexes and D-dimers. However, none of the tests has any predictive value for the occurrence of thrombotic events in one individual patient. Clinically silent haemostatic abnormalities are found in a vast majority of cancer patients. Clinically relevant abnormalities are present in a limited number of cases and include VTE, pulmonary embolism, or disseminated intravascular coagulation (DIC).[6,7]

In the present review, we systematically evaluated the efficacy of standard heparins and low molecular weight heparins (LMWHs) in the prevention and therapy of VTE and pulmonary embolism in cancer patients; moreover, because experimental studies support the hypothesis that cancer progression can be influenced by heparins, we critically evaluated studies in which heparins have been tested as anticancer drugs.

Pro-thrombotic mechanisms in cancer

Malignant cells interact with monocytes and macrophages releasing tumour necrosis factor, interleukin 1 (IL-1) and interleukin 6 (IL-6), causing endothelial damage, and converting the vascular lining into a thrombogenic surface. The interaction between tumour cells and macrophages also activates platelets, factor XII (FXII) and factor X (FX), leading to the generation of thrombin and thrombosis.

Moreover, substances in tumour cells such as cysteine proteases and tissue factor have procoagulant or thromboplastin-like activity leading to clot activation. Tissue factor (TF), a transmembrane receptor protein, is the primary initiator of blood coagulation via its interaction with factor VIIa (FVIIa), a serine-protease expressed by most tumour leukaemia cells. The effect of tumour cell TF production is activation of the coagulation cascade resulting in production of fibrin and in platelet activation.[8–10]

In addition, recent evidence has implicated TF in the regulation of the synthesis of the pro-angiogenic factor vascular endothelial growth factor (VEGF) by tumour cells. Thrombin-catalysed, cross-linked fibrin (XLF) formation is a characteristic histopathological finding in many human and experimental tumours and is thought to be of importance in local host defences responses, in the production of tumour blood vessels and the production of metastasis.

Recently, a new procoagulant factor has been described: CP, a cysteine-protease derived from a broad spectrum of malignant and embryonic tissues.[11,12] This procoagulant factor exerts vitamin K-dependent activity and directly activates FX in the absence of FVII. Increased levels of CP have been reported in different types of advanced tumours and in acute promyelocytic leukaemia. The sialic acid moieties of mucin from adenocarcinomas lead to a non-enzymatic activation of FX.[13–17]

Antitumour agents such as platinum compounds, high doses of fluorouracyl, mitomycin, tamoxifen, and growth factors (granulocyte colony stimulating factors, granulocyte–monocyte colony stimulating factors and erythropoietin) increase the risk of thrombosis.[18] Recently, an increase in thromboembolic manifestations has also been seen in myeloma or myelodysplastic patients treated with thalidomide.[19–21]

Central venous catheters can also be a risk factor for thrombosis in cancer. The thrombogenic surface of these catheters can activate platelets, and serine protease, such as FXII and FX.[22,23] Infected central venous catheters can activate coagulation via the release of endotoxin in gram-negative infections, inducing the release of tissue factors, tissue necrosis factor, and IL-1. Gram-positive organisms can release bacterial mucopolysaccharides that directly activate FXII.

Figure 1 summarizes the mechanisms of thrombosis in cancer.

Thromboprophylaxis in cancer patients

The main objective of thromboprophylaxis in cancer patients is to reduce the risk of fatal pulmonary embolism. The risk of pulmonary embolism is markedly increased after surgery. In general surgery, the risk for VTE is doubled in cancer patients as compared to non-cancer patients, but the risk of pulmonary embolism increases seven-fold in cancer patients undergoing general surgery.[24]

Data from a number of large autopsy studies have revealed pulmonary embolism as the primary cause of death in 8–35% of cancer patients and a contributing cause in a further 43%. A study of 21 530 Swedish

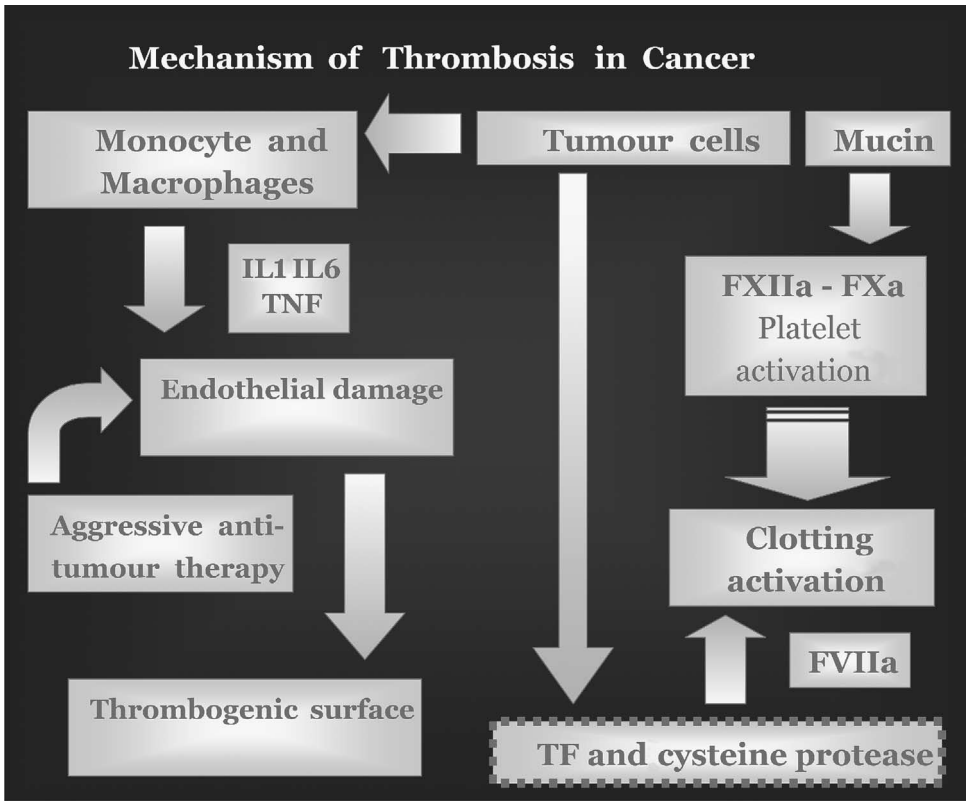

Figure 1 Malignant cells interact with monocytes and macrophages releasing tumour necrosis factor, interleukin 1 (IL-1), interleukin 6 (IL-6), causing endothelial damage, and converting the vascular lining into a thrombogenic surface. The interaction between tumour cells and macrophages also activates platelets, factor XII (FXII) and factor X (FX), leading to the generation of thrombin and thrombosis. Cysteine proteases and tissue factor have procoagulant or thromboplastin-like activity leading to clot activation. A new procoagulant factor has been described: CP, a cysteine-protease derived from a broad spectrum of malignant and embryonic tissues. This procoagulant factor exerts vitamin K-dependent activity and directly activates FX in the absence of FVII.

autopsies over a 24-year period showed the highest prevalence of pulmonary embolism was in patients with ovarian cancer, cancer of the extra-hepatic bile duct system and cancer of the stomach.[25] Similar figures emerged from an Italian autopsy study of 27 410 patients.[26]

There are significant variations in VTE incidence according to the site of the cancer, whereas associations with disease extension are less convincing. Clarke-Pearson et al, in a study on 281 women with cervical cancer undergoing surgery, found that the advanced clinical stage is a risk factor for VTE.[27] Johnson et al[28] found a 52% prevalence of deep vein thrombosis (DVT) in patients with advanced cancer but this may be influenced by confounding factors, as all were hospitalized patients receiving various adjuvant treatments. The Swedish Cancer Registry study demonstrated that VTE can complicate occult cancer with a lead time of as much as 10 years, well before any metastatic disease. Among 61 998 VTE patients, 2509 had cancer diagnosed at the time of admission or within 1 year.[3]

The American College of Chest Physicians analysed the incidence of venous thrombosis and pulmonary embolism in high-risk patients with several risk factors. The incidence of distal DVT (40–80%) or proximal DVT (10–20%) was particularly elevated among patients not undergoing thromboprophylaxis. Fatal pulmonary embolism occurred in 1–5% of the patients.[29]

Unfractioned heparins

Clagget and Reisch[30] in their meta-analysis on 29 controlled studies in patients undergoing abdominal surgery (non-gynaecological), identified 10 studies in which the results for cancer patients were separately analysed. The incidence of DVT, in patients undergoing prophylaxis with unfractioned heparin (UFH) was reduced by 50%.

The optimal dose of UFH is still unclear. In fact, many studies indicate that UFH dosage and the frequency of administration requirements are greater in cancer patients compared with non-cancer patients undergoing surgery. In one such study, 4121 patients undergoing surgery were randomized to receive UFH or placebo administered as a 5000 unit dose 2 hours prior to surgery, and continued subcutaneously every 8 hours for 7–10 days following surgery.[31] The authors demonstrated a significant reduction in the number of deaths due to pulmonary embolism following active treatment. The incidence of pulmonary embolism was 1.6% in controls and 0.4% in patients treated with UFH.

Low molecular weight heparin clinical trials

LMWHs are commonly used in the prevention of DVT in general surgery patients. Individual studies comparing the

effects of UFH and LMWH on DVT rates in cancer patients indicate similar prophylactic effects for the two agents, with secondary safety profiles.[32–36] A study compared 2500 IU with 5000 IU of LMWH in 2070 patients, 65% of whom underwent laparotomy for cancer. DVT rates fell from 14.9% in those receiving 2500 IU units to 8.5% in patients receiving 5000 IU.[37] This study was the first to demonstrate that increased doses of LMWHs can improve thrombo-prophylactic efficacy in cancer patients without increasing the risk of bleeding complications.

Recently, the CLOT study provided evidence of an effective and safe approach of LMWHs to thromboembolic disease in patients with cancer. A total of 672 patients with cancer and symptomatic proximal DVT, pulmonary embolism, or both, were randomly assigned to receive the LMWH dalteparin at the therapeutic dose of 200 IU/kg of body weight given subcutaneously once daily either for 5 or 7 days, followed by 6 months of therapy with standard dose warfarin. Alternatively, patients received full dose dalteparin for 1 month followed by reduced doses of dalteparin (approximately 150 IU/kg daily). The incidence of recurrent thromboembolism in the dalteparin group was half that in the warfarin group. The probability of recurrent thromboembolism at 6 months was 9% in the dalteparin group and 17% in the warfarin group. The incidence of major bleeding in the two groups was not significantly different.[38]

In the recent FAMOUS study, Kakkar et al studied 385 patients with advanced malignancy.[39] The patients were randomized to receive either a once daily subcutaneous injection of dalteparin (5000 IU) or placebo for 1 year. The Kaplan–Meier survival estimates at 1, 2, and 3 years after randomization for patients receiving dalteparin were 46%, 27%, and 21%, respectively, compared with 41%, 18%, and 12%, respectively, for patients receiving placebo ($p = 0.19$). In an analysis not specified a priori, survival was examined in a subgroup of patients (dalteparin, $n = 55$; placebo, $n = 47$) who had a better prognosis and who were alive 17 months after randomization. In these patients, Kaplan–Meier survival estimates at 2 and 3 years from randomization were significantly improved for patients receiving dalteparin versus placebo (78% vs 55% and 60% vs 36%, respectively, $p = 0.03$). The rates of symptomatic venous thromboembolism were 2.4% and 3.3% for dalteparin and placebo, respectively, with bleeding rates of 4.7% and 2.7%, respectively. The results of this study showed that dalteparin administration did not significantly improve 1-year survival rates in patients with advanced malignancy. However, the observed improved survival in a subgroup of patients with a better prognosis suggests a potential modifying effect of dalteparin on tumour biology.[39]

Antitumour effects of heparins

It is a matter of debate whether heparins and anticoagulant agents interfere with cancer progression and alter the prognosis of patients with malignancies. There is evidence that, outside thrombosis management in patients with cancer, coagulation proteases play a significant role in tumour biology.

Heparins are members of a family of polysaccarides, the glycosaminoglycans, together with other compounds, such as heparan sulphate, dermatan sulphate, and chondroitin 4 sulphate. UFH and LMWH exert their anticoagulant effects by activating the physiological coagulation inhibitor antithrombin, which neutralizes many of the serine proteases involved in the coagulation system, particularly thrombin and activated factor X (FXa).

Over the last 50 years, the effects of heparins on experimentally induced metastasis have been investigated. In animal studies, cancer cells were injected in the tail or portal vein and the number of metastases was evaluated.[40–44] Several of these studies showed that heparin treatment inhibits metastasis. Hagmar and Norrby[45] suggested that heparins alter the distribution pattern of cancer cells in experimental animals by their strong negative charge rather than through their anticoagulant effects. As a result of the binding of anionic heparins to cancer cells, adherence to the negatively charged endothelium would be inhibited.

The effects of the heparins on primary tumour growth and metastasis were also studied. In most studies, heparin treatment did not affect local growth of subcutaneously or intramuscular transplanted tumours.[46] In some studies, the incidence of spontaneous metastases was increased in heparin-treated animals.[47] On the other hand, heparin treatment significantly reduced metastasis from subcutaneously implanted fibrosarcomas, and lung, prostate, and mammary carcinomas.[48,49]

Interference of heparins with proliferation of cancer cells

Heparins can inhibit the proliferation of various cell types including vascular smooth muscle cells, fibroblasts and epithelial cells.

The antiproliferative effects of heparins are related to the inhibition of expression of proto-oncogenes, such as c-*fos* and c-*myc*, via alterations in the protein kinase C-dependent signal transduction pathway.[50–52] Recent studies have shown that heparins selectively inhibit the phosphorylation of the mitogen protein kinase C signalling cascade.[53,54]

Some authors have evaluated the effects of the heparins on cancer cells. Bertolesi et al have evaluated the effect in vitro of the heparin and heparin-like molecules on murine mammary adenocarcinoma. They found that heparin inhibited the proliferation of M3 cells with or without fetal calf serum.[40] Zvibel and colleagues showed that soluble heparin, similar in its chemistry to liver heparin proteoglycan, regulates the growth of colon cancer cells via the modulation of Erb-B2 gene expression.[41] Lapierre et al observed that heparin and chemically modified heparin have angiostatic, antitumour and antimetastatic properties on tumour growth of a subcutaneous human pancreatic adenocarcinoma in nude mice and in experimental melanoma lung metastasis.[42]

Interference of heparins with the immune system

Heparins can interfere with immune reactions by affecting the adhesion of leukocytes to endothelium at the sites of

inflammation or tumour invasion. In addition, heparins may inhibit leukocyte activation and affect complement activation. Leukocyte recruitment from the vasculature to sites of inflammation or tumours is a dynamic multi-step process that starts with complex interactions between inflammatory cells and the endothelium. First, leukocytes tether and roll on the endothelium due to interactions between selectins and their counter ligands, sialyl-Lewis^{x} and sialyl-Lewis^{a} (They are special oligosaccharide structures expressed on epithelia, blood vessels and leucocytes. The sialyl Lewis^{x} determinant on leucocytes serves as a ligand for selectin family cell adhesion molecules, and selectin carbohydrate interaction is considered to play an important role in the process of leucocyte extravasation during inflammation, in the formation of glands and blood vessels, in immune regulation reactions and in tumour growth.). Selectins are expressed on leukocytes (L-selectin), activated endothelium (E- and P-selectin), and platelets (P-selectin), and serve to slow down leukocytes, a critical first step in their recruitment.[55] Heparins and heparin oligosaccharides can interfere with the binding of selectins to their carbohydrate ligand and have been found to inhibit the adhesion of leukocytes to endothelium during acute inflammation.[56]

After the initial adhesion of leukocytes to the endothelium, rolling is triggered by direct interaction with surface molecules on the endothelium or chemokines and other chemotactic molecules that are secreted by either leukocytes or cancer cells.

These chemoattractants include C5a, leukotriene-B4, and various chemokines such as interleukin-8 (IL-8), and macrophage inflammatory protein-1. Heparins have also been found to affect the second more tightly integrin-dependent adhesion of leukocytes to endothelium Mac adhesion molecule-1 (ICAM-1) that can be expressed by activated endothelium.[57] In vitro, heparins can act on multiple steps in the complement cascade of both the classical and alternative pathway, including inhibition of C3b, factor H, and C4b.[58–60]

In addition to the direct effects of heparins on the immune system, Gorelik and colleagues have suggested that heparins inhibit metastasis by rendering cancer cells more vulnerable to the cytotoxic effects of natural killer (NK) cells.[61] Figure 2 summarizes the interference of the heparins with immune system.

Interference of heparins with angiogenesis

Angiogenesis is a complex multistep process involving endothelial cell activation, controlled proteolytic degradation of the extracellular matrix (ECM), proliferation and migration of endothelial cells, and formation of capillary vessel lumina. Both animal and in vitro experiments have shown that heparins interfere with the angiogenic process and that these effects are not exclusively related to the anticoagulant function of heparins.

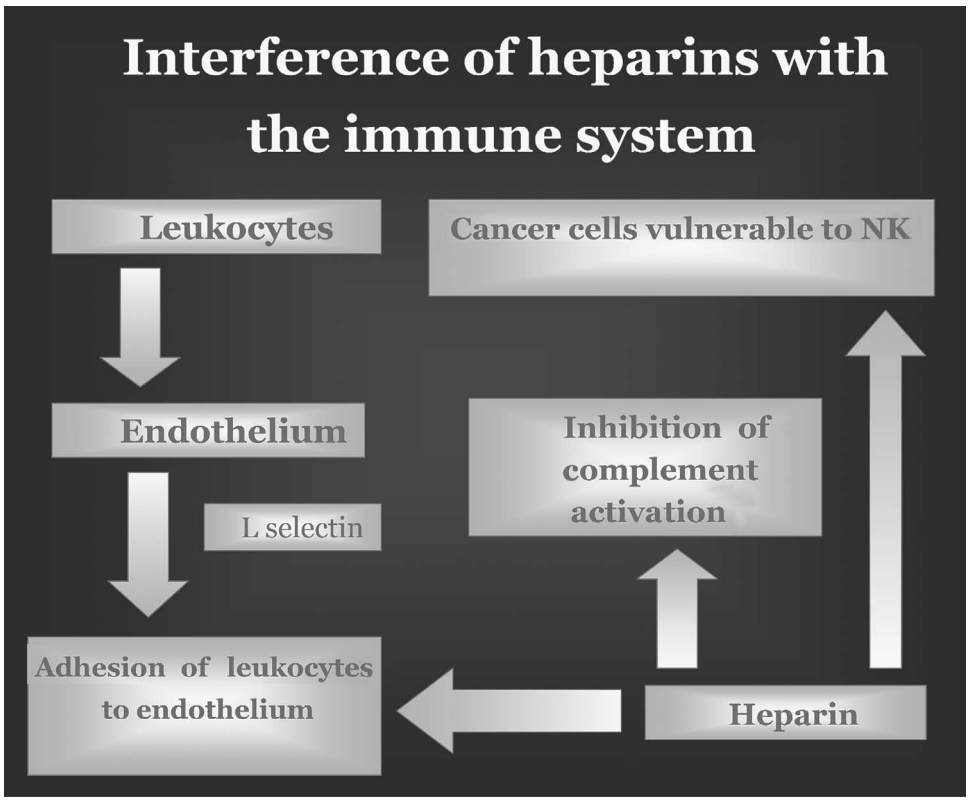

Figure 2 Heparins can interfere with immune reactions by affecting the adhesion of leukocytes to endothelium at the sites of inflammation or tumour invasion. In addition, heparins may inhibit leukocyte activation and affect complement activation. Leukocyte recruitment from the vasculature to sites of inflammation or tumours is also involved. In vitro, heparins can act on multiple steps in the complement cascade of both the classical and alternative pathway, including inhibition of C3b, factor H, and C4b. In addition to the direct effects of heparins on the immune system, there is evidence that heparins inhibit metastasis by rendering cancer cells more vulnerable to the cytotoxic effects of natural killer (NK) cells.

Heparins and angiogenic growth factors

Tumours release a number of angiogenic growth factors, including VEGF, basic fibroblast growth factor (bFGF), and scatter factor.[62,63] In concert with other cytokines, these growth factors stimulate angiogenesis via interactions with their high-affinity receptors on endothelial cells, which possess intracellular intrinsic tyrosine kinase activity. The angiogenic growth factors can bind to heparan sulfate proteoglycans that are present on the endothelial cell surface and in the ECM. Soluble heparins compete with heparan sulfates for the binding of growth factors and other proteins, and may cause release of these proteins from the ECM.[64] In man, therapeutic dosages of UFH can indeed cause an increase in plasma levels of growth factors, such as scatter factor and bFGF.[65,66]

Interestingly, it has been shown that LMWH, in contrast with UFH, can hinder the binding of growth factors to their high-affinity receptors as a result of its smaller size.[67,68] In vitro heparin fragments of less than 18 saccharide residues reduce the activity of VEGF, and fragments of less than 10 saccharide residues inhibit the activity of bFGF. Small molecular heparin fractions have also been shown to inhibit VEGF- and bFGF-mediated angiogenesis in vivo, in contrast with UFH.[69] Nevertheless, treatment with either UFH or LMWH had no effect on tumour-associated angiogenesis in an experimental model of colon cancer metastasis in rat liver.[70]

Heparins can also interfere with the activity of growth factors other than VEGF and bFGF that are involved in angiogenesis and tumour development.[71,72] Transforming growth factor (TGF) is a potent immunosuppressor and an important regulator of growth, differentiation, and adhesion of a wide variety of cells. TGF is expressed by cancer cells and its overproduction correlates with a poor prognosis.[73] In cooperation with VEGF and bFGF, TGF induces tumour-associated angiogenesis. The relationship between heparin and TGF is explained by Lyon et al in a recent paper.[74] The authors undertook a comparative study of the interaction of the three mammalian transforming growth factors (TGF-β) with heparin and heparan sulphate. They concluded that TGF-β_1 and -β_2, but not -β_3, bind to heparin and the highly sulphated liver heparan sulphate. These polysaccharides potentiate the biological activity of TGF-β_1 (but not the other isoforms), whereas a low sulphated mucosal heparan sulphate fails to do so. Potentiation is due to antagonism of the binding and inactivation of TGF-β_1 by α_2-macroglobulin, rather than by modulation of growth factor-receptor interactions. TGF-$\beta_2\alpha_2$-macroglobulin complexes are more refractory to heparin/heparan sulphate, and those involving TGF-β_3 cannot be affected. The effects of heparins on angiogenesis have been explained mainly by their interference with activity of angiogenic growth factors, but heparins also modulate angiogenesis through their anticoagulant function, interference with the activity of proteolytic enzymes, binding to ECM components, or by their potential effects on pericytes.

Effects on angiogenesis via the anticoagulant function of heparins are mainly inhibitory. Cancer cells express tissue factor (TF)-like protein, vitamin K-dependent procoagulants or direct activators of factor X, which contribute to thrombin and fibrin formation.[75,76] TF appears to have an important regulatory role in tumour-associated angiogenesis. VEGF is up-regulated by overexpression of TF, whereas expression of thrombospondin, an angiogenesis suppressor, is down-regulated. Heparins induce elevated levels of TF pathway inhibitor in plasma and have been shown to inhibit TF production in stimulated human monocytes.

In addition to TF, other coagulation proteins, including thrombin and fibrin, are necessary for the formation of new capillaries in tumours.[77,78] Heparins inhibit the function of thrombin by potentiation of antithrombin, resulting in suppression of fibrin formation. Besides coagulation activation, activation of proteolytic enzymes is necessary for angiogenesis to enable endothelial cells to invade into the ECM.[79] Three classes of proteases have been associated with angiogenesis: serine proteases, especially plasminogen activators (PAs), matrix metalloproteinases (MMPs), and cathepsin. Stimulatory as well as inhibitory effects of heparins on the expression of PAs and MMPs have been reported, but not for cathepsins. Endothelial cells need binding to adhesive proteins in the ECM for invasion and migration. Heparins can bind to various adhesive proteins such as fibronectin, vitronectin, and laminin and thus affect invasion of endothelial cells.[80]

Finally, various experimental studies have reported that angiogenesis can be inhibited by treatment with combinations of UFH and corticosteroids, whereas treatment with corticosteroids alone has little or no effect. Although the mechanism by which this combination inhibits angiogenesis is unknown, it has been postulated that heparins concentrate steroid molecules on the surface of vascular endothelial cells by hydrophilic binding to sulfated polyanions. The steroid then suppresses endothelial cell proliferation.[81]

In conclusion, heparins may affect angiogenesis by modulating expression and function of angiogenic growth factors and inhibitors. Whereas UFH and high molecular weight heparins appear to enhance binding of these growth factors to their receptors, LMWH and small heparin fractions inhibit this binding. In addition, heparins can affect other steps in the process of angiogenesis, including fibrin formation, migration of endothelial cells and degradation of the ECM. However, it is still unknown whether and how heparin treatment affects tumour-associated angiogenesis in man because of the complex and often opposite effects of heparins. The antiangiogenetic effects of the heparins are summarized in Figure 3.

Interference of heparins with migration of cancer cells and endothelial cells

Migration of cells is an important process in both metastasis and angiogenesis. After detachment from their original site, cancer cells and vascular endothelial cells migrate into surrounding ECM. Both cancer cells and endothelial cells adhere by the presence of adhesive proteins. Integrins bind to specific components of the ECM, such as collagen, laminin, fibrinogen, fibronectin, and vitronectin. Interactions between heparin-like molecules on the cell surface and

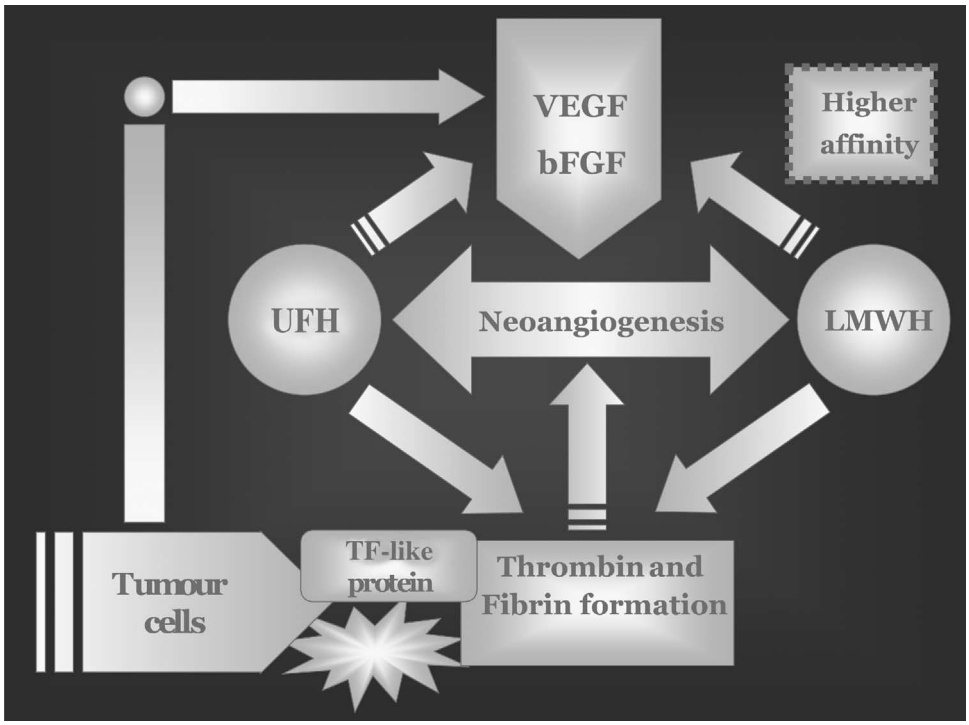

Figure 3 Tumours release a number of angiogenic growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and scatter factor. In concert with other cytokines, these growth factors stimulate angiogenesis. Small molecular heparin fractions have also been shown to inhibit VEGF- and bFGF-mediated angiogenesis in vivo, in contrast with unfractionated heparin (UFH). In vitro heparin fragments of less than 18 saccharide residues reduce the activity of VEGF, and fragments of less than 10 saccharide residues inhibit the activity of bFGF. Cancer cells express tissue factor (TF)-like protein, vitamin K-dependent procoagulants or direct activators of factor X, which contribute to thrombin and fibrin formation.

heparin-binding domains on fibronectin, vitronectin, or laminin can enhance cell migration.[82]

In conclusion, heparins may restrain the migration of cells by inhibiting the adhesion of cells to ECM proteins. Moreover, heparins can either stimulate or inhibit synthesis of ECM proteins, which may indirectly modulate the migration of cells. However, the net effects of heparins on the in vivo migration of cells are not yet well established. Moreover, heparin can inhibit the adhesion of cancer cells to the endothelium reducing the activity of integrins that regulate both white blood cell and cancer cell adhesion activity.

Conclusions

Cancer patients have an increased risk of thromboembolic disease. For this reason, many patients are treated with anticoagulants, including heparins, to reduce the risk of recurrent thromboembolic disease or to treat their thromboembolic complications. Unfractioned heparin has been the standard treatment for thromboembolic disease for many years, but recent randomized trials have demonstrated that LMWHs are as effective and as safe as UFH.[83] The results of these trials have also demonstrated that treatment with heparins may affect the survival of patients with malignancy. Cancer patients who have been treated with LMWHs for their thrombosis had 3 months of survival improvement as compared with patients who received UFH.[84]

Many experimental studies, reviewed in this paper, support the hypothesis that heparin can affect cancer progression in many of the different steps of cancer biology. First of all, due to their anticoagulant effect, heparins may interfere with thrombin generation and with fibrin formation induced by cancer cells, thus inhibiting the mechanism of metastasis. Studies in tumour-bearing experimental animals have shown that drugs modifying the coagulation cascade limit tumour growth and metastasis. This evidence suggests a novel form of experimental cancer therapy with anticoagulants, and limited data on their effects are promising. Nevertheless, these concepts remained largely untested clinically. In addition to their anticoagulant effects, heparins bind to growth factors and extracellular matrix proteins affecting proliferation and migration of cancer cells. Moreover, heparins can affect angiogenesis, cancer cell oncogene expression, and interact with the immune system. Some of the effects of LMWHs differ from the effects of UFH, particularly regarding angiogenesis. In fact, in contrast with UFH, small molecular heparin fractions have been shown to inhibit VEGF- and bFGF-mediated angiogenesis in vivo.

LMWH may be the first candidates for testing anticancer properties of heparins in clinical trials. In fact, information available from experimental studies provides a strong rationale in terms of efficacy and favourable pharmacological profile.

Some clinical trials, originally not targeted at assessing the anticancer properties of heparins, have shown an improvement in survival in patients with malignancies as compared to patients treated with placebo or not receiving

any treatment. Recently, the FAMOUS study showed that Dalteparin administration improves survival in a subgroup of cancer patients with a better prognosis, suggesting a potential modifying effect of dalteparin on tumour biology.[39] Heparins appear to improve survival rates in human malignancies, but more extensive trials are needed to confirm this hypothesis.

Currently, in our opinion, since definitive trials demonstrating the safety and significant advantages are lacking, there is no evidence to treat cancer patients with heparins or anticoagulants outside prophylaxis or treatment of venous thromboembolism or within the framework of clinically controlled trials.

Different studies have examined chemically modified heparins in animal models.[42,85] These heparin derivatives show diminished anticoagulant activity, but preserved angiostatic, antitumour and antimetastatic properties. If the antineoplastic effects of these agents are confirmed by clinical trials in the future, they might be used in association with anticancer agents to treat human malignancies.

Large-scale clinical trials are required to determine the clinical impact of the above activities on the natural history of the disease. Further definition is also needed regarding the efficacy at different time points during disease progression: cancer prevention in high-risk patients, as an addition to standard antitumoural treatment, prevention or limitation of metastasis formation.

Acknowledgement

The authors wish to thank M Terzi for his technical support with the photographic images.

References

1 Agnelli G. Venous thromboembolism and cancer: a two way clinical association. *Thromb Haemost* 1997; **78**: 117–20.
2 Sorensen HT, Mellemkjer L, Steffensen FH, Olsen JH, Nielsen GL. The risk of a diagnosis of cancer after primary deep venous thrombosis or pulmonary embolism. *N Engl J Med* 1998; **338**: 1169–73.
3 Baron JA, Gridley G, Weiderpass E, Nyren O, Linet M. Venous thromboembolism and cancer. *Lancet* 1998; **35**: 1077–80.
4 Sproul EE. Carcinoma and venous thrombosis. The frequency of association of carcinoma in the body or tail of the pancreas with multiple venous thrombosis. *Am J Cancer* 1938; **34**: 556–85.
5 Ambrus Jl, Ambrus CM, Mink IB, Picckern JW. Causes of death in cancer patients. *J Med* 1975; **6**: 61–64.
6 Gouin-Thibault L, Samama MM. Laboratory diagnosis of the thrombophilic state in cancer. *Semin Thromb Hemost* 1999; **25**: 167–72.
7 Gouin-Thibault L, Achakar A, Samama MM. The thrombophilic state in cancer patients. *Acta Hematol* 2001; **106**: 33–42.
8 Falanga A, Alessio MG, Donati MB, Barbui T. A new procoagulant in acute leukemia. *Blood* 1998; **71**: 870–75.
9 Falanga A, Consonni R, Marchetti M et al. Cancer procoagulant in the human promyelocytic cell line NB4 and its modulation by all-transretinoic acid. *Leukemia* 1994; **8**: 156–59.
10 Donati MB, Gambacorti Passerini C et al. Cancer procoagulant in human tumour cells: evidence from melanoma patients. *Cancer Res* 1986; **46**: 6471–74.
11 Delfini F, Colucci M, de Bellis Vitti G et al. Cancer cell procoagulant: a novel vitamin K dependent activity. *Thromb Res* 1981; **24**: 263–66.
12 Grignani G, Falanga A, Pacchiarini L et al. Human breast and colon carcinomas express cysteine proteinase activities with pro-aggregating and pro-coagulant properties. *Int J Cancer* 1988; **42**: 554–57.
13 Celi A, Lorenzet R, Furie B, Furie BC. Platelet–leukocyte–endothelial cell interaction on the blood vessel wall. *Semin Hematol* 1997; **34**: 327–35.
14 Napoleone M, Di Santo A, Lorenzet R. Monocytes upregulate endothelial cell interaction expression of tissue factor: a role for cell–cell contact and cross talk. *Blood* 1997; **89**: 541–49.
15 Contrino J, Hair G, Kreutzer DL, Rickles FR. In situ detection of tissue factor in vascular endothelial cells. Correlation with the malignant phenotype of human breast disease. *Nat Med* 1996; **2:** 209–15.
16 Poggi A, Stella M, Donati MB. The importance of blood cell–vessel wall interactions in tumour metastasis. *Baillieres Clin Haematol* 1993; **6**: 731–52.
17 Falanga A. Mechanisms of hypercoagulation in malignancy and during chemotherapy. *Haemostasis* 1998; **28** (suppl S3): 50–60.
18 Barbui T, Finazzi G, Grassi A. Thrombosis in cancer patients treated with haemopoietic growth factors – a metaanalysis. *Thromb Haemost* 1996; **75**: 368–71.
19 Barbui T, Falanga A. Thalidomide and thrombosis in multiple myeloma. *J Thromb Haemost* 2003; **3**: 421–22.
20 Camba L, Peccatori J, Pescorollo A, Tresoldi M, Corradini P, Bregni M. Thalidomide and thrombosis. *Haematologica* 2001; **10**: 1108–109.
21 Staurer M, Sudmeier I, Stauder R, Gastl G. Thromboembolic events in patients with myelodisplastic syndrome receiving thalidomide with darbepoietin. *Br J Haematol* 2003; **4**: 101–103.
22 Verso M, Agnelli G. Venous thrombosis associated with long term use of central venous catheters in cancer patients. *J Clin Oncol* 2003; **19**: 3655–75.
23 Bona RD. Central line thrombosis in patients with cancer. *Curr Op in Pulm Med* 2003; **5**: 362–65.
24 Clagett GP, Reisch JS. Prevention of thromboembolism in general surgical patients. Results of a meta analysis. *Am Surg* 1988; **288**: 227–40.
25 Svedenssen E, Karwinski B. Prevalence of pulmonary embolism at necroscopy in patients with cancer. *J Clin Pathol* 1989; **42**: 805–809.
26 Bussani R, Cosatti C. Pulmonary embolism: epidemiologic analysis of 27 410 autopsies during a 10 years period. *Medicina (Firenze)* 1990; **10**: 40–43.
27 Clarke-Pearson DL, Jelovsek FR, Creasman WT. Thromboembolism complicating surgery for cervical and uterine malignancy: incidence, risk factors, and prophylaxis. *Obstet Gynecol* 1983; **61**: 87–94.
28 Johnson MJ, Sproule MW, Paul J. The prevalence and associate variables of deep venous thrombosis in patients with advanced cancer. *Clin Oncol* 1999; **11**: 105–10.
29 Clagget GP, Anderson FA, Heit J et al. Prevention of thromboembolism. *Chest* 1995; **34**: 312–34.
30 Clagget GP, Reisch JS. Prevention of venous thromboembolism in general surgical patients. Results of a meta-analysis. *Ann Surg* 1998; **208**: 227–40.
31 The International Multicentre Trial. Prevention of fatal postoperative pulmonary embolism by low doses of heparin. *Lancet* 1975; **ii**: 45–51.
32 Deitcher SR. Cancer and thrombosis: mechanism and treatment. *J Thromb Thrombolysis* 2003; **16**: 21–31.
33 Mousa SA. Low molecular weight heparin in thrombosis and cancer. *Thromb Hemost* 2004; **1**: 25–30.
34 Petralia P, Kakkar AK. Antithrombotic therapy with low molecular weight heparin in cancer patients. *Eur J Med Res* 2004; **3**: 119–24.
35 Mousa SA. Low molecular weight heparin in thrombosis and cancer: emerging links. *Cardiovasc Drug Rev* 2004; **22**: 121–34.
36 Holzheimer RG. Low-molecular-weight heparin (LMWH) in the treatment of thrombosis. *Eur J Med Res* 2004; **4**: 225–39.
37 Bergqvist D, Burmark US, Flordal PA et al. Low molecular weight heparin started before surgery as prophylaxis against deep vein thrombosis: 2500 versus 5000 anti Xa units in 2070 patients. *Br J Surg* 1995; **82**: 496–501.
38 Lee AYY, Levine MN, Baker RI et al. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. *N Engl J Med* 2003; **349**: 146–53.
39 Kakkar AK, Levine M, Kadziola Z et al. Low molecular weight heparin, therapy with delta-heparin, and survival in advanced cancer:

the fragmin advanced malignancy outcome study (FAMOUS). *Clin Oncol* 2004; **22**: 1944–48.
40 Bertolesi GE, Lauria de Cidre L, Eryann AM. Growth inhibition in vitro of murine mammary adenocarcinoma cells by heparin and chemically modified heparins. *Tumour Biol* 1994; **15**: 275–83.
41 Zvibel I, Halpern Z, Papa M. Extracellular matrix modulates expression of growth-factor receptors in liver-colonizing colon cancer cells lines. *Int J Cancer* 1998; **77**: 295–301.
42 Lapierre F, Holme K, Lam L et al. Chemical modifications of heparins that diminish its anticoagulant but preserve its heparanase-inhibitory, angiostatic, antitumor and antimetastatic properties. *Glycobiology* 1996; **6**: 355–66.
43 Retik AB, Arons MS, Ketchmann AS, Mantel N. The effects of heparins on primary tumors and metastases. *J Surg Res* 1962; **11**: 49–53.
44 Hagmar B, Boyerid B. Disseminating effect of heparin on experimental tumour metastases. *Pathol Eur* 1969; **4**: 274–82.
45 Hagmar B, Norrby K. Evidence for effects of heparin on cell surfaces influencing experimental metastases. *Int J Cancer* 1970; **5**: 72–84.
46 Drago JR, Weed P, Fralisch A. The evaluation of heparin in control of metastasis of Nb rat androgen insensitive prostate carcinoma. *Anticancer Res* 1984; **4**: 171–72.
47 Wood S Jr, Holyoke ED, Yardley JH. Mechanism of metastasis production by blood born cancer cells. *Can Cancer Conf* 1961; **4**: 167–233.
48 Lee AE, Rogers LA, Longcroft JM, Jeffery RE. Reduction of metastasis in a murine mammary tumour model by heparin and polyinosinic-polycytidylic acid. *Clin Exp Metastasis* 1990; **8**: 165–71.
49 Lee AE, Rogers LA, Jeffery RE, Longcroft JM. Comparison of metastatic cell lines derived from a murine mammary tumour, and reduction of metastasis by heparin. *Clin Exp Metastasis* 1988; **6**: 463–71.
50 Castellot JJ Jr, Pukac LA, Caleb BL, Wright TC Jr, Karnovsky MJ. Heparin selectively inhibits a protein kinase C-dependent mechanism of cell cycle progression in calf aortic smooth muscle cells. *J Cell Biol* 1989; **109**: 3147–55.
51 Pukac LA, Ottlinger ME, Karnovsky MJ. Heparin suppresses specific second messenger pathways for protooncogene expression in rat vascular smooth muscle cells. *J Biol Chem* 1992; **267**: 3707–11.
52 Ottlinger ME, Pukac LA, Karnovsky MJ. Heparin inhibits mitogen-activated protein kinase activation in intact rat vascular smooth muscle cells. *J Biol Chem* 1993; **268**: 19173–76.
53 Mishra-Gorur K, Castellot JJ Jr. Heparin rapidly and selectively regulates protein tyrosine phosphorylation in vascular smooth muscle cells. *J Cell Physiol* 1999; **178**: 205–15.
54 Imai T, Hirata Y, Marumo F. Heparin inhibits endothelin-1 and proto-oncogene c-fos gene expression in cultured bovine endothelial cells. *J Cardiovasc Pharmacol* 1993; **22** (suppl 8): S49–S52.
55 Nelson RM, Cecconi O, Roberts WG, Aruffo A, Linhardt RJ, Bevilacqua MP. Heparin oligosaccharides bind L- and P-selectin and inhibit acute inflammation. *Blood* 1993; **82**: 3253–58.
56 Koenig A, Norgard-Sumnicht K, Linhardt R, Varki A. Differential interactions of heparin and heparan sulfate glycosaminoglycans with the selectins. Implications for the use of unfractionated and low molecular weight heparins as therapeutic agents. *J Clin Invest* 1998; **101**: 877–89.
57 Diamond MS, Alon R, Parkos CA, Quinn MT, Springer TA. Heparin is an adhesive ligand for the leukocyte integrin Mac-1 (CD11b/CD1). *J Cell Biol* 1995; **130**: 1473–82.
58 Weiler JM, Edens RE, Linhardt RJ, Kapelanski DP. Heparin and modified heparin inhibit complement activation in vivo. *J Immunol* 1992: **148**: 3210–15.
59 Linhardt RJ, Rice KG, Kim YS, Engelken JD, Weiler JM. Homogeneous, structurally defined heparin-oligosaccharides with low anticoagulant activity inhibit the generation of the amplification pathway C3 convertase in vitro. *J Biol Chem* 1988; **263**: 13 090–96.
60 Pangburn MK, Atkinson MA, Meri S. Localization of the heparin-binding site on complement factor H. *J Biol Chem* 1991; **266**: 16 847–53.
61 Gorelik E, Bere WW, Herberman RB. Role of NK cells in the antimetastatic effect of anticoagulant drugs. *Int J Cancer* 1984; **33**: 87–94.
62 Senger DR, Perruzzi CA, Feder J, Dvorak HF. A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. *Cancer Res* 1986; **46**: 5629–32.
63 Kumar R, Kuniyasu H, Bucana CD, Wilson MR, Fidler IJ. Spatial and temporal expression of angiogenic molecules during tumor growth and progression. *Oncol Res* 1998; **10**: 301–11.
64 Colin S, Jeanny JC, Mascarelli F et al. In vivo involvement of heparan sulfate proteoglycan in the bioavailability, internalization, and catabolism of exogenous basic fibroblast growth factor. *Mol Pharmacol* 1999; **55**: 74–82.
65 D'Amore PA. Capillary growth: a two-cell system. *Semin Cancer Biol* 1992; **3**: 49–56.
66 Folkman J, Weisz PB, Joullie MM, Li WW, Ewing WR. Control of angiogenesis with synthetic heparin substitutes. *Science* 1989; **243**: 1490–93.
67 Soker S, Goldstaub D, Svahn CM, Vlodavsky I, Levi BZ, Neufeld G. Variations in the size and sulfation of heparin modulate the effect of heparin on the binding of VEGF165 to its receptors. *Biochem Biophys Res Comm* 1994; **203**: 1339–47.
68 Lepri A, Benelli U, Bernardini N et al. Effect of low molecular weight heparan sulphate on angiogenesis in the rat cornea after chemical cauterization. *J Ocul Pharmacol* 1994; **10**: 273–80.
69 Jayson GC, Gallagher JT. Heparin oligosaccharides: inhibitors of the biological activity of bFGF on Caco-2 cells. *Br J Cancer* 1997; **75**: 9–16.
70 Smorenburg SM, Vink R, Te Lintelo M et al. In vivo treatment of rats with unfractionated heparin (UFH) or low molecular weight heparin (LMWH) does not affect experimentally induced colon carcinoma metastasis. *Clin Exp Metastasis* 1999; **17**: 451–56.
71 Rosen EM, Goldberg ID. Regulation of angiogenesis by scatter factor. *EXS* 1997; **79**: 193–208.
72 Pepper MS. Transforming growth factor-beta: vasculogenesis, angiogenesis, and vessel wall integrity. *Cytokine Growth Factor Rev* 1997; **8**: 21–43.
73 Saito H, Tsujitani S et al. The expression of transforming growth factor-beta1 is significantly correlated with the expression of vascular endothelial growth factor and poor prognosis of patients with advanced gastric carcinoma. *Cancer* 1999; **86**: 1455–62.
74 Lyon M, Rushton G, Gallagher JT. The interaction of the transforming growth factor-betas with heparin/heparan sulfate is isoform-specific. *J Biol Chem* 1997; **272**: 18 000–8006.
75 Costantini V, Zacharski LR. Fibrin and cancer. *Thromb Haemostasis* 1993; **69**: 406–14.
76 Van Hinsbergh VW, Koolwijk P, Hanemaaijer R. Role of fibrin and plasminogen activators in repair-associated angiogenesis: in vitro studies with human endothelial cells. *EXS* 1997; **79**: 391–411.
77 Collen A, Smorenburg SM, Peters E. The effects of unfractionated and low molecular weight heparins on microvascular endothelial cell proliferation and formation of capillary-like tubular structures in a fibrin matrix. *Cancer Res* 2000; **60**: 6196–200.
78 Gordon SG, Mielicki WP. Cancer procoagulant: a factor X activator, tumor marker and growth factor from malignant tissue. *Blood Coagul Fibrinolysis* 1997; **8**: 73–86.
79 Rabbani SA. Metalloproteases and urokinase in angiogenesis and tumor progression. *In Vivo* 1998; **12**: 135–42.
80 Au YP, Kenagy RD, Clowes MM, Clowes AW. Mechanisms of inhibition by heparin of vascular smooth muscle cell proliferation and migration. *Haemostasis* 1993; **23** (suppl 1): 177–82.
81 Folkman J, Langer R, Linhardt RJ, Haudenschild C, Taylor S. Angiogenesis inhibition and tumor regression caused by heparin or a heparin fragment in the presence of cortisone. *Science* 1983; **221**: 719–25.
82 Van Noorden CJF, Meade-Tollin LM, Bosman FT. Metastasis. *Am Sci* 1998; **86**: 130–41.
83 Smorenburg SM, Hettiarachchi RJK, Vink R, Büller HR. The effects of unfractionated heparin on survival in patients with malignancy–a systematic review. *Thromb Haemostasis* 1999; **82**: 1600–604.
84 Hettiarachchi RJ, Smorenburg SM, Ginsberg J, Levine M, Prins MH, Büller HR. Do heparins do more than just treat thrombosis? The influence of heparins on cancer spread. *Thromb Haemostasis* 1999; **82**: 947–52.
85 Yoshitomi Y, Nakanishi H, Kusano Y et al. Inhibition of experimental lung metastases of Lewis lung carcinoma cells by chemically modified heparin with reduced anticoagulant activity. *Cancer Lett* 2004; **207**: 165–74.