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The Complex Effects of Heparins on Cancer Progression and Metastasis in Experimental Studies

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***Abstract*——Patients with cancer are frequently treated with anticoagulants, including heparins, to treat or to prevent thrombosis. Recent randomized trials that compared low molecular weight heparin to unfractionated heparin for the treatment of deep vein thrombosis have indicated that heparins affect survival of patients with cancer. Experimental studies support the hypothesis that cancer progression can be influenced by heparins, but results of these studies are not conclusive. Heparins are negatively charged polysaccharides that can bind to a wide range of proteins and molecules and affect their activity. As a consequence, heparins have a wide variety of biological activities other than their anticoagulant effects, which may interfere with the malignant process. In the present systematic review, we critically evaluate experimental studies in which heparins have been tested as anti-cancer drugs. All animal studies, published between 1960 and 1999, that report effects of heparins on growth of subcutaneously implanted tumors, spontaneous metastasis or experimentally induced metastasis are reviewed. In addition, we discuss mechanisms by which heparins potentially exert their activity on various steps in cancer progression and malignancy related processes. It is shown that heparins can affect proliferation, migration, and invasion of cancer cells in various ways and that heparins can interfere with adherence of cancer cells to vascular endothelium. Moreover, heparins can affect the immune system and have both inhibitory and stimulatory effects on angiogenesis. Because of the wide variety of activities of heparins, it is concluded that the ultimate effect of heparin treatment on cancer progression is uncertain.**

I. Introduction

Patients with cancer have an increased risk of venous thromboembolic complications (Smorenburg et al., 1999b). Consequently, numerous cancer patients are treated with anticoagulants, including heparins, to reduce the risk of (recurrent) thrombosis. For many years, unfractionated heparin (UFH[2]) has been the standard initial treatment for venous thromboembolism, but re-

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[2] Abbreviations: UFH, unfractionated heparin; LMWH, low molecular weight heparin; ECM, extracellular matrix; MIP-1β, macrophage inflammatory protein-1β; RANTES, regulated on activation, normal T cell-expressed and secreted; ICAM-1, intercellular adhesion molecule-1; NK, natural killer; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; TGFβ, transforming growth factor-β; TF, tissue factor; PA, plasminogen activator; tPA, tissue-type PA; uPA, urokinase-type PA; MMP, matrix metalloproteinase.

cent randomized trials have shown that low molecular weight heparin (LMWH) is at least as safe and effective as UFH (Bijsterveld et al., 1999). Interestingly, the results of these trials have also indicated that treatment with heparins may affect survival of patients with malignancy. Cancer patients who had been treated with LMWH for their thrombosis had a significantly improved 3 month survival as compared to UFH recipients with cancer, whereas this difference in mortality was not observed in patients without malignant disease (Hettiarachchi et al., 1999). The incidence of thrombotic and bleeding complications was similar in both treatment groups, suggesting a direct effect of UFH or LMWH on the malignant process.

The hypothesis that heparins affect cancer progression is supported by numerous experimental studies. These studies have shown that heparins do not solely affect cancer by their interaction with the coagulation cascade but also by various other ways. Heparins are members of a family of polysaccharides, the glycosaminoglycans. Additional members of this family include heparan sulfate, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, and hyaluronic acid. Glycosaminoglycans are linear carbohydrate polymers, which are composed of alternating uronate and hexosamine saccharides that are linked by glycosidic linkages. UFH is a mixture of glycosaminoglycan chains, each containing 200 to 300 saccharide units. LMWH consists of low molecular weight fragments of UFH produced by controlled enzymatic or chemical depolymerization, which yields chains that are less than 18 saccharide units long with a mean molecular mass of approximately 5000 Da. 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 (Xa). Heparins bind to antithrombin via a specific high-affinity pentasaccharide sequence that is only present in a minor portion of the heparin chains. Binding of the pentasaccharide to antithrombin causes a conformational change in antithrombin that accelerates by a factor of approximately one thousand its interaction with thrombin and Xa (Hirsh et al., 1998). Besides binding to antithrombin, UFH and to a lesser extent LMWH bind to a wide range of other proteins and molecules via electrostatic interactions with the polyanionic groups of the glycosaminoglycan chains. These interactions are mediated by physicochemical properties of heparin polymers such as sequence composition, sulfation pattern, charge distribution, overall charge density, and molecular size. As a consequence, UFH and LMWH have a wide variety of biological activities other than their anticoagulant effects. Thus far, numerous mechanisms by which heparins potentially affect tumor development and/or metastasis have been described, but the ultimate effects of either UFH or LMWH on cancer progression are still unknown.

In the present review, we systematically evaluate animal studies in which heparins have been tested as anticancer drugs. To our knowledge, all reports published between 1960 and 1999 on the effects of heparins on either development of experimentally induced metastasis, primary tumors, or spontaneous metastasis are included in this review. These reports are listed in Tables 1 and 2. In addition, we discuss potential mechanisms by which heparins exert their activity on various steps in cancer progression and malignancy related processes.

II. Effects of Heparins on Experimental Primary Tumor Growth and Metastasis

For about 5 decades, the effects of heparins on experimentally induced metastasis have been investigated in various models. In most animal studies, cancer cells were administered in the tail vein or portal vein, and the number of metastases in lung or liver were evaluated (Table 1). Several of these experiments showed that heparin treatment inhibits metastasis. Fisher and Fisher (1961) found fewer and smaller hepatic metastases of intraportally administered Walker carcinoma cells in heparin-treated rats in comparison with untreated animals, particularly when treatment was started before cancer cell injection. Clifton and Agostino (1962) reported that heparins reduce the incidence of lung tumors in rats that were injected with Walker sarcoma cells. Similar results were obtained in other studies using various types of cancer cells (Table 1). In contrast, other early studies have reported that heparins induce the spread of cancer cells to organs other than those to which they were targeted (Boeryd, 1965, 1966; Hagmar and Boeryd, 1969a; Hagmar and Norrby, 1970; Maat, 1978). Hagmar and Norrby (1970) suggested that heparins alter distribution patterns of cancer cells in experimental animals by their strong negative charges rather than by their anticoagulant effects. As a result of binding of anionic heparins to cancer cells, adherence to the negatively charged endothelium would be prohibited. This hypothesis was supported by observations that anionic chondroitin sulfate, which is structurally identical to heparin but lacks its anticoagulant properties, had similar effects as heparins, whereas cationic protamine, a heparin-antagonist, had opposite effects (Hagmar and Norrby, 1970).

Effects of heparins on primary tumor growth and metastasis from spontaneously metastasizing transplanted tumors have been studied as well, albeit less extensively (Table 2). In most studies, heparin treatment did not affect local growth of subcutaneously or intramuscularly transplanted tumors (Wood et al., 1961; Retik et al., 1962; Hagmar, 1968, 1969, 1970; Maat and Hilgard, 1981; Owen, 1982; Drago et al., 1984; Milas et al., 1985; Lee et al., 1988, 1990; Antachopoulos et al., 1996). Formation of spontaneous metastases was not affected in

TABLE 1

Effects of UFH on experimentally induced metastasis to lung or liver, as well as spread to other organs after intravenous injection of cancer cells; only those studies are included that report effects of UFH alone as compared with placebo or no treatment

Reference	Tumor Type	Metastases in Primary Affected Organ	Metastases in Other Organs
Beuth et al., 1987	Sarcoma i.v.	↓ Lung	
Boeryd, 1965	Rhabdomyosarcoma i.v.	↓ Lung	↑ Liver
Boeryd, 1966	Rhabdomyosarcoma i.p.	= Liver	↑ Lung
	Rhabdomyosarcoma i.v.	= Lung	= Liver
Clifton and Agostino, 1962	Walker sarcoma i.v.	↓ Lung	
Clifton and Agostino, 1963	V2 carcinoma i.v.	↓ Lung	
Coombe et al., 1987	Mammary carcinoma i.v.	↓ Lung	=
Fisher and Fisher, 1961	Walker sarcoma i.p.	↓ Liver	
Gorelik et al., 1984	Melanoma i.v.	↓ Lung	= Liver
Gorelik, 1987	Melanoma i.v.	↓ Lung	
Hagmar and Boeryd, 1969a	Melanoma i.v.	↓ Lung	↑ Various organs
Hagmar and Boeryd, 1969b	Rhabdomyosarcoma i.v.	= Lung (N.S. ↓)	= Liver (N.S. ↑)
	Melanoma i.v.	↓ Lung	= Liver
Hagmar and Norrby, 1970	Rhabdomyosarcoma i.v.	= Lung	↑ Various organs
Irimura et al., 1986	Melanoma i.v.	↓ Lung	
Koike, 1964	Mammary carcinoma i.v.	↓ Lung	= Various organs
Lee et al., 1988	Mammary carcinoma i.v.	↓ Lung	
Lee et al., 1990a	Mammary carcinoma i.v.	↓ Lung	
Maat, 1978	Lewis lung carcinoma i.v.	↓ Lung	↑ Various organs
	Melanoma i.v.	↓ Lung	↑ Various organs
	Schwannoma i.v.	↓ Lung	↑ Various organs
	Astrocytoma i.v.	↓ Lung	=
Milas et al., 1985	Fibrosarcoma i.v.	↓ Lung	
	Mammary carcinoma i.v.	↓ Lung	
Nagawa et al., 1990	Colon carcinoma i.p.	= Liver	= Various organs (N.S. ↑)
Parish et al., 1987	Mammary carcinoma i.v.	↓ Lung	
Sciumbata et al., 1996	Melanoma i.v.	↓ Lung	
Smorenburg et al., 1999c	Colon carcinoma i.p.	= Liver	
Suemasu and Ishikawa, 1970	Anaplastic lung carcinoma i.v.	↓ Lung	= Various organs (N.S. ↓)
Vlodavsky et al., 1994	Melanoma i.v.	↓ Lung	
Wood et al., 1961	Lewis carcinoma i.v.	↓ Lung	=

i.v., intravenously; i.p., intraportally; N.S., not statistically significant.

some studies (Retik et al., 1962; Hagmar, 1968; Maat and Hilgard, 1981; Antachopoulos et al., 1996). Maat and Hilgard (1981) concluded that the effects of heparins and other anticoagulants on metastasis after intravenous administration of cancer cells cannot extrapolated to spontaneous metastasis. This conclusion was based on observations that fibrin was often present on circulating cancer cells after intravascular injection, whereas fibrin could not be found on cancer cells in the circulation that originated from primary solid tumors (Maat and Hilgard, 1981). In some studies, the incidence of spontaneous metastases was increased in heparin-treated animals (Retik et al., 1962; Hagmar, 1969, 1970). On the other hand, heparin treatment significantly reduced metastasis from subcutaneously implanted fibrosarcomas, lung, prostate, and mammary carcinomas (Wood et al., 1961; Drago et al., 1984; Milas et al., 1985; Lee et al., 1988, 1990a).

TABLE 2

Effects of UFH on subcutaneously implanted tumors and their spontaneous metastasis; only those studies are included that report effects of UFH alone as compared with placebo or no treatment

Reference	Tumor Type	Primary Tumor	Spontaneous Metastases
Antachopoulos et al., 1996	Human colon carcinoma (nude mice)	= (N.S. ↑)	=
Drago et al., 1984	Prostate carcinoma	=	↓ Various organs
Hagmar, 1968	Rhabdomyosarcoma	=	= Lungs
Hagmar, 1969	Rhabdomyosarcoma	=	↑ Lungs
			= Lymph nodes
Hagmar, 1970	Rhabdomyosarcoma	=	↑ Lungs
Lee et al., 1988	Mammary carcinoma	=	↓ Lungs
Lee et al., 1990a	Mammary carcinoma	=	↓ Lungs
Maat and Hilgard, 1981	Lewis lung carcinoma	=	= Lungs
	Melanoma	=	= Lungs
Milas et al., 1985	Fibrosarcoma	=	↓ Lungs
	Mammary carcinoma	=	↓ Lungs
Ohkoshi et al., 1993	Squamous cell carcinoma	↓	
Owen JR, 1982	Walker sarcoma	=	
Retik et al., 1962	Sarcoma (T-241)	=	↑ Lungs
	Sarcoma (DBA-49)	=	= Lungs
Wood et al., 1961	Lewis lung carcinoma	=	↓ Lungs

N.S., not statistically significant.

III. Effects of Heparins on the Various Steps in Cancer Progression

A series of coordinated steps are essential in cancer development and metastasis. These steps include 1) proliferation of cancer cells; 2) defense against attacks of the immune system; 3) formation of new blood vessels; 4) migration of cancer cells after detachment from their original site; 5) invasion of surrounding tissue requiring adhesion and subsequent degradation of extracellular matrix (ECM) components by controlled proteolysis; and 6) access of cancer cells to blood and lymph vessels, and subsequent adhesion to and invasion of the endothelium, allowing colonization at distant sites in the organism (Woodhouse et al., 1997; van Noorden et al., 1998b). Potential effects of heparins on these successive steps are discussed in the following paragraphs.

IV. Interference of Heparins with Proliferation of Cancer Cells

Heparins can inhibit proliferation of various cell types, including vascular smooth muscle cells, mesangial cells, fibroblasts, and epithelial cells (Tiozzo et al., 1989; Au et al., 1993; Bennett et al., 1994; Miralem et al., 1996). The antiproliferative effects of heparins are related to inhibition of expression of proto-oncogenes, such as c-*fos* and c-*myc*, via alterations in the protein kinase C-dependent signal transduction pathway (Castellot et al., 1989; Pukac et al., 1990, 1992; Imai et al., 1993; Miralem et al., 1996). Recent studies have shown that heparins selectively inhibit the phosphorylation of mitogen-activated protein kinase, an intermediate kinase in the protein kinase C signaling cascade (Ottlinger et al., 1993; Daum et al., 1997; Mishra-Gorur and Castellot, 1999). Only a few studies have evaluated the effects of heparins on proliferation of cancer cells. Results of these studies are inconclusive (Lee et al., 1988; Bertolesi et al., 1994; Lapierre et al., 1996; Sciumbata et al., 1996; Zvibel et al., 1998).

V. Interference of Heparins with the Immune System

Heparins can interfere with immune reactions by affecting adhesion of leukocytes to endothelium at sites of inflammation or tumor invasion. In addition, heparins may inhibit leukocyte activation and affect complement activation. The effects of heparins on the immune system have recently been reviewed by Tyrrell et al. (1999) and, therefore, will be discussed only briefly.

Leukocyte recruitment from the vasculature to sites of inflammation or tumors is a dynamic multistep process that starts with complex interactions between inflammatory cells and 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. Selectins are expressed on leukocytes (L-selectin), activated endothelium (E- and P-selectin), and platelets (P-selectin) (Carlos and Harlan, 1994; McEver, 1994) and serve to slow down leukocytes, a critical first step in their recruitment. Heparins and heparin oligosaccharides can interfere with the binding of selectins to their carbohydrate ligands (Handa et al., 1991; Ley et al., 1991; Nelson et al., 1993; Norgard-Sumnicht et al., 1993; Koenig et al., 1998) and have been found to inhibit adhesion of leukocytes to endothelium during acute inflammation (Nelson et al., 1993).

After 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, which include C5a, leukotriene-B4, and various chemokines such as interleukin-8 (IL-8), macrophage inflammatory protein-1β (MIP-1β), and the chemokine that is regulated on activation, normal T cell-expressed and secreted (RANTES) induce a second adhesion event in which leukocyte integrins firmly adhere to their counterligands on the endothelium. Chemokines can bind to heparan sulfate proteoglycans, and this binding is thought to enhance leukocyte responses to chemokines (Tanaka et al., 1993; Webb et al., 1993). Interference with binding of chemokines to heparan sulfates has been found to affect migration of immune cells through the endothelium and into the ECM. For instance, pretreatment of RANTES and MIP-1β with heparins or release of ECM-bound chemokines with heparinase have been shown to abrogate induction of T cell adhesion by chemokines (Gilat et al., 1994).

Heparins have also been found to affect the second more tightly integrin-dependent adhesion of leukocytes to endothelium. Mac-1 (CD11/CD1), a β2-integrin expressed on activated leukocytes, binds to several cell surface and soluble ligands, including intercellular adhesion molecule-1 (ICAM-1) that can be expressed by activated endothelium (Diamond et al., 1990). It has been shown that Mac-1, isolated from human granulocytes, also binds to heparins and that association of Mac-1 with heparins or cell surface heparan sulfate chains on endothelial cells complements other receptors such as ICAM-1 in the Mac-1-mediated neutrophil extravasation process (Diamond et al., 1995). In contrast to the previously mentioned studies of Nelson et al. (1993) and Norgard-Sumnicht et al. (1993), monoclonal antibodies to L-selectin did not inhibit neutrophil adhesion to heparins or heparan sulfate in the study of Diamond et al. (1995), and neutrophils of patients with leukocyte adhesion deficiency, which lack Mac-1 but express L-selectin, did not bind to heparins.

The results of these studies indicate that alterations in the binding of selectins, chemokines, or integrins to their respective heparan sulfate-binding sites on endothelium or in the ECM can reduce extravasation of leukocytes, for example by the effects of heparinases or

competitive glycosaminoglycans such as heparins. However, since various of these adhesion molecules or chemokines have other functions as well, it is unknown whether and how heparins ultimately affect tumor growth by these mechanisms. For example, IL-8 has not only an important role in leukocyte activation, but also acts as a promoter of tumor growth via its angiogenic properties (Arenberg et al., 1996), whereas production of RANTES by human melanoma cells has been found to be associated with increased tumor formation, irrespective of its possible role in recruitment of T cells and monocytes into tumors (Mrowietz et al., 1999).

Heparins can also modulate activation of leukocytes. Dependent on the concentration, heparins may increase or inhibit production of superoxide radicals in neutrophils (Leculier et al., 1993; Itoh et al., 1995). Moreover, heparins have been found to inhibit complement activation or complement-dependent experimental inflammation (Sharath et al., 1985; Ekre et al., 1986; Weiler et al., 1992). 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 (Rent et al., 1975; Weiler et al., 1978; Hennink et al., 1984; Linhardt et al., 1988; Pangburn et al., 1991). Furthermore, heparin and modified heparin with diminished anticoagulant activity have been shown to inhibit complement activation and hemolysis in vivo (Weiler et al., 1992).

In addition to direct effects of heparins on the immune system, Gorelik and colleagues (1984, 1987) have suggested that heparins inhibit metastasis by rendering cancer cells more vulnerable to cytotoxic effects of natural killer (NK) cells. Heparins did not inhibit NK cell activity in vitro in these experiments, but enhanced the inhibitory effects of stimulated NK cells on formation of experimentally induced B16 melanoma or Lewis lung carcinoma metastases in mice. In contrast, the antimetastatic effects of heparins were completely abrogated when NK cell reactivity in mice was suppressed by cyclophosphamide.

In conclusion, heparins can affect the immune system directly by their inhibitory effects on extravasation of leukocytes and the complement system or by enhancing the susceptibility of cancer cells to immunologic attacks. However, it is not yet known to what extent the various effects of heparins on the immune system contribute to their effect on cancer progression.

VI. Interference of Heparins with Angiogenesis

Angiogenesis, the formation of new blood vessels from existing vessels, is required for further development of tumors once they have reached a diameter of approximately 5 mm and for facilitating the escape of cancer cells from the primary tumor (Folkman, 1997). Angiogenesis is a complex multistep process involving endothelial cell activation, controlled proteolytic degradation of ECM, proliferation and migration of endothelial cells, and formation of capillary vessel lumina (Diaz-Flores et al., 1994). These processes can be initiated and controlled by a number of compounds that are secreted by cancer cells, including growth factors, inhibiting factors, proteolytic enzymes, and ECM proteins. 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.

A. Heparins and Angiogenic Growth Factors

Tumors release a number of angiogenic growth factors, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and scatter factor (Senger et al., 1986; Rosen and Goldberg, 1997; Kumar et al., 1998; Schmidt et al., 1999). 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 (Coughlin et al., 1988; Jaye et al., 1992). The angiogenic growth factors can bind to heparan sulfate proteoglycans that are present on the endothelial cell surface and in the ECM (Ruoslahti and Yamaguchi, 1991; Andres et al., 1992; Lyon and Gallagher, 1994; Colin et al., 1999). Binding to heparan sulfates results in stabilization and relative inactivation of the growth factors as well as prevention of their diffusion and proteolytic degradation (Saksela et al., 1988; Rusnati and Presta, 1996). Therefore, it has been proposed that heparan sulfates in the ECM have an important role in storing active growth factors that can be released when needed to exert their effects immediately upon release (Presta et al., 1989; Ishai-Michaeli et al., 1990; Vlodavsky et al., 1992). Soluble heparins compete with heparan sulfates for binding of growth factors and other proteins, and may cause release of these proteins from the ECM (Folkman et al., 1988; Vlodavsky et al., 1992). In man, therapeutic dosages of UFH can indeed cause an increase in plasma levels of growth factors, such as scatter factor and bFGF (D'Amore, 1990; Taniguchi et al., 1994; Yamazaki et al., 1997).

Binding of growth factors to heparins or heparan sulfates is also thought to have a crucial role in the modulation of activity of the high-affinity receptors (Mason, 1994; Tessler et al., 1994; Schlessinger et al., 1995; Colin et al., 1999). This phenomenon has been thoroughly studied for bFGF (Schlessinger et al., 1995). bFGF activates the high-affinity receptors by inducing dimerization, i.e., bridging of the specific signaling receptors on endothelial cells (Lemmon and Schlessinger, 1994). Formation of a multivalent complex of bFGF and heparins or heparan sulfates promotes bFGF receptor dimerization and activation (Schlessinger et al., 1995; Rusnati and Presta, 1996). Interestingly, it has been shown that LMWH, in contrast to UFH, can hinder binding of growth factors to their high-affinity receptors as a result of its smaller size. Indeed, in vitro heparin fragments of

less than 18 saccharide residues reduce activity of VEGF, and fragments of less than 10 saccharide residues inhibit activity of bFGF (Soker et al., 1994; Jayson and Gallagher, 1997). Small molecular heparin fractions have also been shown to inhibit VEGF- and bFGF-mediated angiogenesis in vivo, in contrast to UFH (Norrby, 1993; Lepri et al., 1994; Norrby and Ostergaard, 1996, 1997). Nevertheless, treatment with either UFH or LMWH had no effect on tumor-associated angiogenesis in an experimental model of colon cancer metastasis in rat liver (Smorenburg et al., 1999c).

Heparins can also interfere with the activity of growth factors other than VEGF and bFGF that are involved in angiogenesis and tumor development. Transforming growth factor-β (TGFβ) is a potent immunosuppressor (de Visser and Kast, 1999) and an important regulator of growth, differentiation, and adhesion of a wide variety of cells (Massague et al., 1992). In cooperation with VEGF and bFGF, TGFβ induces tumor-associated angiogenesis (Pepper, 1997; Nakanishi et al., 1997; Relf et al., 1997). Cancer cells have been found to produce TGFβ in vivo and in vitro (Constam et al., 1992; Nerlich et al., 1997) and production of TGFβ or levels of TGFβ in plasma often correlate with progression of the disease (Tsushima et al., 1996; Wikstrom et al., 1998; Wunderlich et al., 1998; Saito et al., 1999).

In vivo, TGFβ is complexed to alpha-2 macroglobulin and inactive (O'Connor-McCourt and Wakefield, 1987; Huang et al., 1988). Alpha-2 macroglobulin, which can be produced and secreted by cancer cells (Smorenburg et al., 1996), binds both various cytokines and growth factors and proteinases to inhibit them irreversibly. When heparins or heparan sulfates bind to inactive TGFβ/alpha-2 macroglobulin complexes, the binding site of TGFβ is exposed to cell surface receptors (McCaffrey et al., 1989; Lyon et al., 1997). As a result, biological activity of TGFβ is potentiated by heparins (Lyon et al., 1997).

B. Heparins and Other Processes Involved in Angiogenesis

Effects of heparins on angiogenesis have been explained mainly by their interference with activity of angiogenic growth factors, but heparins may modulate angiogenesis as well by either their anticoagulant function, interference with 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 (Bastida et al., 1984; Gordon, 1992; Gordon and Chelladurai, 1992; Gordon and Mielicki, 1997), which contribute to thrombin and fibrin formation (Costantini and Zacharski, 1993). TF appears to have an important regulatory role in tumor-associated angiogenesis (Zhang et al., 1994; Ruf and Mueller, 1996; Abe et al., 1999). It has been demonstrated that overexpression of TF in sarcoma and melanoma cells can enhance growth of well vascularized subcutaneous tumors and metastases, whereas low TF expression can result in reduced vascularization and poor tumor growth (Zhang et al., 1994; Bromberg et al., 1999). In the study of Zhang et al. (1994), VEGF was up-regulated by overexpression of TF, whereas expression of thrombospondin, an angiogenesis suppressor, was down-regulated. Moreover, TF and VEGF mRNA and protein have been found to colocalize in various cancers of the lung, and there seems to exist a strong relationship between synthesis of TF and VEGF levels in human breast cancer cell lines (Shoji et al., 1998). Heparins induce elevated levels of TF pathway inhibitor in plasma and have been shown to inhibit TF production in stimulated human monocytes (Novotny et al., 1991; Pepe et al., 1997).

In addition to TF, other coagulation proteins, including thrombin and fibrin, are necessary for the formation of new capillaries in tumors (Liu et al., 1990; Tsopanoglou et al., 1993; Van Hinsbergh et al., 1997). Deposition of fibrin in connective tissue, which occurs when fibrinogen is cleaved at thrombin-specific cleavage sites, provides a temporary scaffolding for activated endothelial cells. Furthermore, structural and mechanical properties of the fibrin matrix have been found to play a regulatory role in angiogenesis in vitro (Nehls and Herrmann, 1996; Shats et al., 1997). Heparins inhibit the function of thrombin by potentiation of antithrombin, resulting in suppression of fibrin formation. Moreover, recent in vitro experiments have indicated that heparins can also affect angiogenesis by altering the structure of fibrin matrices (Collen et al., 2000). When UFH and LMWH are present during polymerization of fibrin matrices, they respectively enhance or restrict formation of capillary-like structures after activation of microvascular endothelial cells.

Besides coagulation activation, activation of proteolytic enzymes is necessary for angiogenesis to enable endothelial cells to invade into the ECM. Three classes of proteases have been associated with angiogenesis: serine proteases, especially plasminogen activators (PAs), matrix metalloproteinases (MMPs), and cathepsinsin (Keppler et al., 1996; Mignatti and Rifkin, 1996; Rabbani, 1998). Stimulatory as well as inhibitory effects of heparins on the expression of PAs and MMPs have been reported, but not of cathepsins (Clowes et al., 1992; Kenagy et al., 1994; Putnins et al., 1996; Brunner et al., 1998; Gogly et al., 1998). 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 (McCarthy et al., 1990).

In addition, heparins may affect angiogenesis via inhibition of proliferation and migration of pericytes

(Hoover et al., 1980; Clowes and Clowes, 1985; Au et al., 1993). Pericytes are closely related to smooth muscle cells, and a gradual transdifferentiation of smooth muscle cells into pericytes occurs in walls of both terminal arterioles and venules (Diaz-Flores et al., 1991). Smooth muscle cells, which are present in the media of arteries and to a lesser extent of veins, give mechanical support and stability to the vessel wall and have a regulatory role in venular and capillary permeability. Pericytes are also thought to have an important regulatory role in the control of angiogenesis, particularly in the maturation of newly formed vessels (Diaz-Flores et al., 1991; D'Amore, 1992).

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 no or little effect (Folkman et al., 1983; Sakamoto and Tanaka, 1987; Pucci et al., 1988; Benrezzak et al., 1989; Madarnas et al., 1989; Lee et al., 1990b; Thorpe et al., 1993; Derbyshire et al., 1995). Although the mechanism by which this combination inhibits angiogenesis is unknown, it has been postulated that heparins concentrate the steroid on the surface of vascular endothelial cells by hydrophilic binding to sulfated polyanions. The steroid then suppresses endothelial cell proliferation (Folkman et al., 1989). The effects of a combined application of heparins and corticosteroids have also been studied in mouse models of cancer (Folkman et al., 1983). Folkman et al. (1983) showed that tumor growth was arrested or even regressed by combined administration of heparins and corticosteroids, whereas metastasis to the lungs was prevented. However, studies in other laboratories reported inconclusive results of this combined treatment (Milas et al., 1985; Penhaligon and Camplejohn, 1985; Ziche et al., 1985; Teale et al., 1987).

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 tumor-associated angiogenesis in man because of the complex and often opposite effects of heparins.

VII. Interference of Heparins with Migration of Cancer 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. Therefore, the structure of the ECM has functional consequences for migration or spread of cells (Ohtaka et al., 1996; Crowe and Shuler, 1999). Both cancer cells and endothelial cells adhere to and detach from components of surrounding ECM by regulated expression of specific cell surface molecules, including integrins (Albelda, 1993; Brooks, 1996; Mizejewski, 1999). Integrins bind to specific components of the ECM, such as collagen, laminin, fibrinogen, fibronectin, and vitronectin (Horwitz, 1997). These components possess specific binding domains that promote cell attachment and spreading. They also possess heparin-binding domains, which have affinities for heparins or heparin-like molecules (Skorstengaard et al., 1986; McCarthy et al., 1990; Liang et al., 1997). Interactions between heparin-like molecules on the cell surface and heparin-binding domains on fibronectin, vitronectin, or laminin can enhance cell migration (Newman et al., 1987; Khan et al., 1988; Yoneda et al., 1995; Kapila et al., 1997; Sung et al., 1997; Yoshida et al., 1999). It has been postulated that extracellular or soluble heparins act as inhibitors of such auxiliary interactions and may consequently lead to inhibition of cell migration. Indeed, heparins and other glycosaminoglycans such as chondroitin sulfate and dextran sulfate inhibit adhesion and migration of cancer cells on fibronectin and laminin substrates (Makabe et al., 1990; Saiki et al., 1990; Antachopoulos et al., 1995). In addition, heparins and heparin fractions may modulate biosynthesis of ECM proteins. Injections of UFH into the allantoic sac of chick embryo eggs induced overexpression of fibronectin (Ribatti et al., 1997). On the other hand, UFH reduced production and release of fibronectin by stimulated mesangial cells in vitro in a concentration-dependent manner, whereas LMWH treatment can decrease levels of laminin mRNA and protein (Asselot-Chapel et al., 1996; Wang et al., 1998).

In conclusion, heparins may restrain migration of cells by inhibiting adhesion of cells to ECM proteins. Moreover, heparins can either stimulate or inhibit synthesis of ECM proteins, which may indirectly modulate migration of cells. However, the net effects of heparins on in vivo migration of cells are not yet well established.

VIII. Interference of Heparins with Invasion of Cancer and Endothelial Cells

Cancer cells and endothelial cells use specific proteolytic enzymes during invasion of the ECM (Liotta, 1992; Mignatti and Rifkin, 1993; van Noorden et al., 1998b). Degradation of the matrix takes place in highly localized regions close to the cancer or endothelial cell surface, where active proteolytic enzymes outbalance natural protease inhibitors that are present in the extracellular environment (Basbaum and Werb, 1996). The proteinases are produced by either inflammatory cells, stromal cells, or the cancer cells themselves (Liotta, 1992; van Noorden et al., 1998a). An important enzyme in this process is plasmin, a serine proteinase, which catalyzes

degradation of a variety of proteins present in the ECM, including fibrin, fibronectin, and laminin (Schmitt et al., 1997). In addition, plasmin amplifies pericellular proteolysis by activating pro-enzymes of the MMP family, such as MMP-2 and MMP-9 or the pro-enzyme of urokinase-type PA (uPA), thereby catalyzing its own activation (Murphy et al., 1992; DeClerck and Laug, 1996; Baramova et al., 1997). uPA and tissue-type PA (tPA), a second activator of plasminogen, activate plasminogen to plasmin by proteolytic cleavage. Especially uPA is involved in cancer invasion and metastasis (Ellis et al., 1992). Elevated levels of uPA and its receptor uPA-R are associated with poor prognosis in man (Grondahl-Hansen et al., 1993; Pedersen et al., 1994; Schmitt et al., 1997).

Recently, it has been reported that sulfated glycosaminoglycans such as heparins and heparan sulfates enhance invasion of human melanoma cells into fibrin by stimulating activation of plasminogen (Brunner et al., 1998). Plasminogen activation was found to be enhanced in several ways. Glycosaminoglycans stimulated both pro-uPA activation by plasmin, and plasminogen activation by uPA. Furthermore, the glycosaminoglycans partially protected plasmin from inactivation by α2-antiplasmin. Stimulation of pro-uPA and plasminogen activation at the cell surface by heparins have been reported by others as well (Stephens et al., 1991; Bertolesi et al., 1997), and a specific binding site for heparins in the urokinase kringle domain has been described (Stephens et al., 1992). Thus, heparins may stimulate pericellular proteolysis and ECM degradation by activation of uPA and plasminogen.

On the other hand, heparins may reduce invasion of cancer cells by inhibition of heparanases, a family of endoglycosidases. Heparanases hydrolyze internal glycosidic linkages of heparan sulfates in basement membranes and ECM. Cancer cells secrete heparanases, which synergize with proteinases to achieve efficient degradation of host tissue and subsequent invasion (Nakajima et al., 1988; Nicolson et al., 1998; Eccles, 1999). Heparanase activity has been found to correlate with metastatic potential of various types of cancer cells (Nakajima et al., 1988). Although heparins are structurally related to heparan sulfates, heparins are poor substrates for heparanases and they interfere with heparan sulfate degradation (Nakajima et al., 1988). In several experimental studies, heparins inhibited heparanase activity of cancer cells in vitro and reduced metastasis to lungs in vivo after intravenous administration of cancer cells (Irimura et al., 1986; Coombe et al., 1987; Parish et al., 1987; Vlodavsky et al., 1994; Lapierre et al., 1996). Chemically modified heparins without anticoagulant properties were also found to inhibit metastasis, and a good correlation was found between the anti-heparanase and antimetastatic effects of the heparins. It has been suggested that the presence of sulfate groups at *N*- or *O*-positions as well as the number of saccharide units are important for the capacity of heparins to inhibit heparanase activity and metastasis (Vlodavsky et al., 1994; Parish et al., 1999).

In addition to effects on serine proteinases and heparanases, heparins have been found to inhibit various MMPs in vitro in a dose-dependent manner, including MMP-1, -2, -3, and -9 (Au et al., 1992; Kenagy et al., 1994; Gogly et al., 1998). MMP-2 and -9 are thought to play a major role in metastasis (Kugler, 1999; Westermarck and Kahari, 1999).

In conclusion, heparins may affect cellular invasion by modifying the activity of various proteolytic enzymes. They potentially stimulate uPA activity and plasminogen activation, but inhibit heparanases and MMPs. Since all these proteinases may be involved in invasion of cancer cells and endothelial cells, it is difficult to predict how heparins ultimately affect invasion in vivo.

IX. Interference of Heparins with Adhesion of Cancer Cells to Vascular Endothelium

The arrest of cancer cells in small vessels is an important step in metastasis. At present, it is still controversial whether cancer cells are arrested in small vessels simple by mechanical entrapment or by specific cancer cell-endothelial cell interactions (Weiss, 1994; Koop et al., 1996; Morris et al., 1997). Nevertheless, it is thought that cancer cells can adhere to vascular endothelium in a way that is similar to that in the regulated recruitment of leukocytes to tissue sites of damage and inflammation (Smith and Anderson, 1991). Cancer cells first attach loosely to the endothelium, using selectins as described above for leukocytes. Selectins bind to carbohydrate-ligands such as sialyl-Lewis^x and sialyl-Lewis^a. These ligands normally function as leukocyte enrollment receptors, but cancer cells have been found to express sialyl-Lewis^x and sialyl-Lewis^a as well (Fukushima et al., 1984; Walz et al., 1990; Renkonen et al., 1999). Expression of these ligands correlates with metastatic potential of the cancer cells (Kurahara et al., 1999). Moreover, serum levels of sialyl-Lewis^x have been found to correspond with survival time and number of metastases in patients with non-small cell lung cancer (Satoh et al., 1997). As discussed earlier, heparins can interfere with the binding of selectins to their carbohydrate ligands (Handa et al., 1991; Nelson et al., 1993; Norgard-Sumnicht et al., 1993). As a result, heparins not only can restrain enrollment of leukocytes but also initial adhesion of cancer cells to endothelium.

After initial adhesion, endothelial cells and cancer cells are activated by the release of chemokines by cancer and/or endothelial cells, as is the case for leukocytes (see above) (Rottman, 1999). Activation results in enhanced expression of integrins, which leads to a tighter adhesion of cancer cells to endothelium. Of special interest in this process is integrin αIIβ3, also known as glycoprotein IIb/IIIa. Various studies reported that cancer

cells express αIIβ3, an integrin that was thought to be exclusively expressed by platelets (Chang et al., 1992; Honn et al., 1992a; Chen et al., 1997). The physiological ligand for αIIβ3 is fibrinogen, which normally links the αIIβ3 on one platelet to the integrin receptor on another platelet, thereby mediating platelet aggregation. Interestingly, αIIβ3 also plays a major role in mediating adhesion of cancer cells to endothelial cells and to platelets (Honn et al., 1992b; Trikha et al., 1997, 1998). Pretreatment with antibodies against αIIβ3 inhibits both activated cancer cells from adhering to endothelial cells and fibronectin, and cancer cell-induced platelet aggregation (Chopra et al., 1988; Grossi et al., 1988; Honn et al., 1988).

Expression of αIIβ3 on cancer cells and platelets is stimulated by thrombin, which is either generated directly by cancer cells or as a result of vascular damage. Thrombin formation thus promotes adhesion of cancer cells to the endothelium (Wojtukiewicz et al., 1992; Nierodzik et al., 1995; Dardik et al., 1998). In addition, thrombin induces cancer cell-platelets interactions, platelet aggregation and thrombus formation, which enhance survival of the cancer cells that are arrested in the vessel by protection against mechanical stress and the attack by immunocompetent cells. Aggregated platelets also release various mediators, including adhesive glycoproteins, growth factors, cytokines, vasoactive amines, and arachidonic acid metabolites, which stimulate cancer cell proliferation, extravasation, and interactions between cancer cells and compounds of the ECM (Honn et al., 1992b). Production of thrombin and induction of platelet aggregation by cancer cells is positively correlated with cancer progression and metastatic potential (Nierodzik et al., 1991; Honn et al., 1992b; Walz and Fenton, 1994). In addition, pretreatment of cancer cells by thrombin before intravenous administration has been shown to enhance metastasis 10- to 160-fold (Nierodzik et al., 1995).

As a consequence, heparins and other anticoagulants may inhibit adhesion of cancer cells to the endothelium by inactivation of thrombin or inhibition of platelet aggregation and thrombus formation. Indeed, some studies have shown that heparins reduce arrest of cancer cells and subsequent metastases in lungs after intravenous administration without affecting development of extrapulmonary metastases (Coombe et al., 1987; Lee et al., 1988). In the study of Lee et al. (1988), heparin treatment also reduced spontaneous formation of lung metastases in mice from a subcutaneously implanted mammary carcinoma and improved survival of the animals.

In summary, the importance of platelets, thrombin and clot formation for intravascular arrest and survival of cancer cells has been demonstrated in vitro and in vivo (Honn et al., 1992b; Walz and Fenton, 1994; Nierodzik et al., 1995). Moreover, human cancer cells express procoagulants, and patients with cancer often show signs of intravascular activation of coagulation (Rickles et al., 1992; Gouin-Thibault and Samama, 1999). Therefore, it is conceivable that platelet aggregation and clot formation are also involved in extravasation and metastasis of cancer cells in men. As a consequence, antithrombotic drugs such as heparins may interfere with intravascular arrest and extravasation of metastasizing cancer cells. However, results of studies focused on the effects of heparins on these processes are still not conclusive.

X. Conclusions

Heparins affect progression of cancer in many ways. Due to their anticoagulant function, they can inhibit thrombin and fibrin formation induced by cancer cells. Therefore, heparins may potentially inhibit intravascular arrest of cancer cells and thus metastasis. Besides their anticoagulant function, heparins bind to growth factors and ECM proteins and consequently can affect proliferation and migration of cancer cells and angiogenesis in tumors. Furthermore, heparins have been found to inhibit expression of oncogenes and to affect the immune system. They also have both stimulatory and inhibitory effects on proteolytic enzymes, which are essential for invasion of cancer cells through the ECM.

As a result of the wide variety of activities of heparins, the ultimate effect of heparin treatment on cancer progression is unpredictable. This conclusion, based on experimental studies of the effects of heparins on cancer progression, is in agreement with the outcome of a recent systematic clinical review of the effects of UFH versus placebo or no treatment on survival of patients with malignancy (Smorenburg et al., 1999a). Significant effects of UFH could not be established. Various trials reported improved survival of UFH-treated patients with cancer, whereas others showed no or adverse effects of UFH.

Effects of LMWH on cancer are less thoroughly investigated than is the case for UFH. Some of the effects of LMWH may differ from those of UFH, especially on angiogenesis. Moreover, there is suggestive evidence from clinical trials that LMWH treatment, as compared with UFH, prolongs survival of cancer patients with venous thromboembolic complications. At present, both experimental and clinical studies are being performed to evaluate whether LMWH indeed affects cancer progression, both in patients with and without concurrent venous thromboembolism.

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