Inhibition of Metastases by Anticoagulants

Michael Hejna, Markus Raderer, Christoph C. Zielinski

Metastasis involves several distinct steps, including one in which the tumor cell, after entry into the bloodstream, comes to rest in a capillary located at the distant site where a metastatic tumor will ultimately form. Components of the blood-clotting pathway may contribute to metastasis by trapping cells in capillaries or by facilitating adherence of cells to capillary walls. Conceivably, anticoagulants could interfere with this step in the metastatic process. In this review, we have summarized current knowledge on the interaction of malignant cells, clotting factors, and anticoagulants. We used computerized (MEDLINE®) and manual searches to identify studies done in humans, in animals, and in in vitro systems that were published in English between 1952 and 1998. We found many reports that the formation of metastatic tumors could be inhibited by heparin, a vitamin K antagonist (warfarin), and inhibitors of platelet aggregation (prostacyclin and dipyridamole). Despite these encouraging preliminary results and a compelling biochemical rationale, only limited information exists on the clinical use of anticoagulants for the prevention or treatment of metastatic cancer because there have been so few controlled and prospectively randomized studies on this topic. In view of the preliminary results, anticoagulants may hold promise for the prevention and treatment of metastases. We believe that larger controlled investigations are strongly warranted to evaluate the clinical potential of anticoagulants for the prevention and treatment of metastases in humans. [J Natl Cancer Inst 1999;91:22–36]

The controversy as to whether anticoagulants inhibit metastases is reflected in an extensive body of literature. It has been known for more than a century that thromboembolism may complicate the course of cancer (1) and that fibrin is associated with some types of intravascular tumors in humans (2). In addition, the changes that are indicative of disseminated intravascular coagulation occur in many cancer patients (3), but the biologic significance of these changes is not clear.

The metastatic process involves a series of distinct steps that are required before a metastatic tumor is firmly established in a new environment. After intravasation, a metastatic cell must survive its passage through the bloodstream, come to rest in a blood vessel in the potential target organ, and finally pass through the epithelium of that vessel into the new organ where it will form a tumor. The metastatic tumor must then form a blood supply that branches from the host’s circulatory system and is sufficient for continued growth. Although it seems logical that the bloodstream carries tumor cells to sites in distant organs, the mechanisms that the malignant cells use to adhere to and finally migrate into these organs involve cellular and molecular interactions that are not completely elucidated. Apart from the intrinsic properties of the tumor cell, it has been hypothesized (4–7) that vascular factors and components of the clotting pathways play crucial roles throughout the natural history of metastatic tumors. On the basis of recent experimental and clinical data, including therapeutic interventions with anticoagulant drugs, some investigators have argued that components of the clotting pathway(s) might contribute to the progression of malignancy (4–11).

In this review, we critically evaluate the current knowledge of the role played by the components of the clotting pathways in the formation of metastatic tumors and the therapeutic potential of inhibiting metastases with anticoagulants. To this end, we reviewed the English-language literature on this subject that was published between 1952 and 1998 through the use of computerized (MEDLINE®) and manual searches.

IN VITRO EXPERIMENTS AND ANIMAL STUDIES

Anticoagulants and Fibrinolytic Agents

Large numbers of tumor cells are released into the blood during the metastatic process. Liotta et al. (12) found that the number of cells released from an implanted MTW9 mammary carcinoma increased as the vascularized tumor grew, with $1.4 \times 10^3$ cells released or shed from the tumor per 24 hours on day 5, $3.0 \times 10^4$ cells shed on day 10, and $1.5 \times 10^5$ cells shed on day 15. Butler and Gullino (13) quantitated cell shedding into the efferent blood of a rat mammary adenocarcinoma and found that $3–4 \times 10^6$ cells were shed in 24 hours per gram of tumor or $1.6–2.0 \times 10^4$ cells were shed per milliliter of blood. Glaves (14) showed that Lewis lung carcinoma cells and B16 melanoma cells were detectable in the systemic circulation 1–3 days after initiation of primary tumor growth. The median number of cells shed throughout the whole tumor development was $1 \times 10^5$ cells for Lewis lung carcinoma cells and $2.4 \times 10^6$ cells for B16 melanoma cells. In human patients with cancer, circulating tumor cells have been identified in the venous blood (15–19). Although transport of tumor cells in the bloodstream is an essential component of the metastatic process, it is important to note that the mere presence of tumor cells in the circulation does not inevitably lead to the formation of metastases.

The physiologic conditions of the blood provide a hostile

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lymphocytes

Heterotypic interactions of tumor cells with platelets (42–45), with appropriate reluctance. The identification of fibrin by rou-

tion of metastases have been shown to decrease the probability of the forma-

and/or motility could enhance the metastatic process (49–51).

Various treatments that interfere with tumor–platelet interactions have been shown to decrease the probability of the forma-

tion of metastases (52–58).

Circulating tumor cells are thromboplastic; i.e., tumor cells can cause or accelerate clot formation in the bloodstream. Vari-

ous tumor cell-associated procoagulant activities have been de-

scribed, such as an activity similar to tissue factor (59,60), an

activity that can activate factor X (61–64), and an activity that is

associated with plasma membrane vesicles and that stimulates

the generation of prothrombinase (65,66). O’Meara (60) dem-

onstrated that fibrin is always found in and around all cancerous

lesions, particularly at the growing edge. According to these
data, cancer cells actually use the fibrin lattice structure as a

support, and fibrin appears to “attract” new blood vessels in the

tumor. O’Meara (67) also demonstrated that cancer cells have

increased coagulative activity as a result of “cancer coagulative

factor,” a compound thought to have thromboplastic activity.

Subsequently, fibrolysin was found to counteract cancer coagu-
lative factor (68,69), and plasmin was found to be the most

active inhibitor of cancer coagulative factor. The plasmin-

induced inhibition of cancer coagulative factor can be reversed

by plasmin inhibitors. Thones and Martin (70) also reported that

plasmin has a cytopathic effect on cancer cells, an effect that was

not found with streptokinase or plasminogen, the two compo-

nents of plasmin.

However, more convincing evidence that fibrinogen-related proteins are localized in solid tumors came from the work

(71,72) showing that, when fibrinogen and anti-fibrinogen anti-

body were administered systemically to animals with tumors or
to humans with tumors, both proteins were later detected in the
tumors. Laki and Yancey (73) demonstrated that, when placed in a
fibrinogen solution, cultured malignant cells could directly

convert fibrinogen to fibrin. After the inoculation of tumor cells into mice, fibrin was observed, by electron microscopy, to be

adjacent to the tumor cells. Other investigators (74,75) using

immunofluorescence techniques have identified fibrinogen-

related proteins in several experimental and human neoplasms.
The conclusions from these early reports, which were interpreted

as being indicative of fibrin deposition within tumors, were met

with appropriate reluctance. The identification of fibrin by rou-
tine histologic studies is problematic, and heterogeneous antisera
developed against fibrinogen used for the immunolocalization
studies were not able to distinguish fibrin from fibrinogen,

which is also a component of the tumoral stroma. Furthermore,

the demonstration that fibrin is located in the tumoral stroma is

not sufficient evidence to support a direct role for fibrin in the

progression of cancer, because the deposition of fibrinogen and/or

fibrin is a general phenomenon in diseases other than cancer
(e.g., inflammation, atherosclerosis, and renal disease) (68),

the deposition could also be associated with focal zones of tumor

necrosis, or the deposition could represent an artifact resulting

from clot formation during the procurement of the specimen.

More recent data have resolved certain of these objections.
The use of antiserum, and in particular monoclonal antibodies,
defined specificity (69,76–78) with immunohistochemistry

and the use of electron microscopic analysis (79,80) to charac-
terize fibrin patterns have established that fibrin is a constituent

of the stroma of some experimental tumors and some human

tumors (78–88). In addition, fibrin deposition related to tumor

necrosis may be distinguished from artifacts caused by tumor

manipulation by the examination of the microanatomic distribu-
tion and by the fact that coagulation activation in situ is strictly

associated with specific anatomic features in tumor masses (79–

89). The occurrence of fibrin in some tumor types and its ab-
sence in other tumor types, even when both tumor types were

obtained in the same manner, argue against it being an artifact of

the procurement procedure. In addition, the existence of cause-

and-effect relationships between activated coagulation and can-
cer progression is supported by a growing body of literature that

provides evidence that therapeutic manipulation of the coagula-
tion pathways alters either the dissemination of experimental
cancer or the progression of certain types of cancer in the patient

(6,8,90–94).

The deposition of fibrin, which occurs when fibrinogen is
cleaved at thrombin-specific cleavage sites, in the connective
tissue surrounding viable tumor may influence the progression
of the tumor in several ways. Fibrin deposits around tumor cells
may serve as a barrier that keeps host inflammatory cells from

invading and destroying the tumor (9,81,95). This hypothesis is

based on histologic evidence that inflammatory cells, parti-
cularly lymphocytes, are confined to the tumor–host interface and
do not substantially penetrate the tumor (81,95). Recent studies

of macrophage migration in fibrin gels offer some explanation of
these findings. Depending on the concentrations of fibrin and

thrombin, fibrin matrices can either enhance or inhibit macro-

phage migration (96). In addition, studies by Gorelik and col-
leagues (97–99) suggested that fibrin can protect tumor cells
from the cytotoxic activity of natural killer or lymphokine-

activated killer cells. A protective effect was obtained only when
coagulation occurred before formation of target–effector conju-
gates, probably because contact between target and effector cells

was prevented rather than because of some change in the lytic
phase of lymphokine-activated killer cytotoxicity (99).

Fibrin might also enhance angiogenesis in the metastatic tis-
mor. Olander et al. (100) reported that endothelial cells became

organized into vascular channels when cells were cultured in a

fibrin gel. It has also been shown that tumor cells can be fixed
in the capillaries by fibrin (101–103). In addition, Wood (104)
demonstrated that infused tumor cells became fixed in a clump

with fibrin and penetrated the vascular wall to reach the extra-

vascular space. Locally injected fibrinolysin readily reversed the
clumping of tumor cells and the penetration of the vessel wall by
tumor cells (105).

Alternatively, the thrombin, which is available to the surface of
tumor cells, might have a fibrin-independent effect on the
progression of cancer. Studies in various model systems have
shown that thrombin or thrombin–protein complexes bind to the
surface of certain normal and transformed cell types (106–109).
Thrombin-initiated signal transduction across the cell membrane
results in protein kinase C-mediated enhancement of phosphoino-
sitol turnover and calcium mobilization (110–112) and is asso-
ciated with a reduction in the level of intracellular cyclic aden-
osine monophosphate (113). Thrombin can also stimulate cell
contraction, cellular secretion, and the expression of tissue factor
(114) and the c-myc proto-oncogene (115). These effects of
thrombin could contribute to metastasis by promoting cell mi-
gration through tissues, stimulation of autocrine growth factor
secretion, or preservation of local fibrin through the enhanced
production of plasminogen activator type I. In addition, because
thrombin can initiate synthesis of deoxyribonucleic acid and
mitosis (115,116), it has been referred to as a growth factor.

Cliffton and Grossi (117) demonstrated that heparin markedly
prolonged survival of rabbits that were given an injection of VX2
carcinoma cells (118). Both heparin and fibrinolysin, when
given before the injection of tumor cells, diminished and pre-
vented, respectively, the appearance of pulmonary and liver me-
tastases (118,119). In addition, studies were performed (120–
131) to evaluate the effect of heparin and fibrinolysin on the
intravascular inoculation of cell suspensions of Walker 256 car-
cinosarcoma in the rat. All of these studies indicate that lodging
of cancer cells introduced into the circulation depends on a fibrin
mesh that allows implantation (120–131). Fisher and Fisher
(123) have also reported and confirmed the use of anticoagulants
in the prevention of metastases in rats. Furthermore, an accu-
mulating body of evidence demonstrates that fibrinolytic agents
are effective agents for the reduction of metastases (117,122–
126).

The combination of fibrinolysin with other types of treat-
ment, such as radiation, seems to provide enhanced therapeutic
activity. Similar incidences of pulmonary metastases were ob-
served in animals given an injection of irradiated tumor cells
(1000 rad) and in animals given an injection of nonirradiated
tumor cells. However, the formation of metastases was markedly
reduced (125) when fibrinolysin was added to cell suspensions
before intravenous injection.

In an attempt to simulate a clinical situation (e.g., the release
of cells from a tumor during colonic resection), an artificial cecal
tumor was generated with the Walker 256 carcinosarcoma (120)
in animal models. The tumor was explored at a resectable stage,
massaged, and finally resected. Liver metastases were found in
34% of control animals compared with 19% of fibrinolysin-
treated animals. In addition, inhibitors of fibrinolysis tend to
increase the number of metastases (126,127) in some models.

Inhibitors of Platelet Aggregation

The results were analyzed statistically using the unpaired Stu-
dent’s t test; all P values were two-sided. Schwelke et al. (132)
evaluated the effect of prostacyclin on hepatic metastases of a
human pancreatic cancer in a nude mouse model system. The
surface of the implanted tumor was measured after a defined
time as an objective indicator of the influence of prostacyclin on
tumor growth. The mean surface area of the liver covered with
tumor was statistically significantly reduced in all treatment
groups. Specifically, in the untreated control group, the mean
surface area of the liver covered with tumor was 485 mm².
Animals treated with 200 μg of prostacyclin 0.5 hour before the
injection of tumor cells had tumors covering a surface area of
only 21 mm² (P = .004), and animals treated with 400 μg of
prostacyclin had tumors covering a surface area of 20 mm² (P = 
.004). Thus, there appeared to be no substantial difference in
response to the lower dose versus the higher dose. If 200 μg of
prostacyclin was administered 4.0 hours after injection of the
tumor cells, the surface area of tumor was 85 mm² (P = .017).
The maximal reduction of tumor surface area (to only 11 mm²)
was observed when 200 μg of prostacyclin was given 0.5 hour
before and 4.0 hours after the injection of tumor cells (P = 
.003). These results indicate that prostacyclin has statistically
significant antitumoral activity on hepatic metastases from hu-
man pancreatic adenocarcinoma in the nude mouse.

Prostacyclin, which is among the most potent inhibitors of
platelet aggregation, was also used by Honn et al. (56), who
reported a reduction in pulmonary metastases and a complete
elimination of hepatic metastases in the B16 amelanotic melano-
ma model. Additional studies by the same group (57,58)
found that cathepsin B levels were associated with both platelet
aggregation and metastatic potential. Local proteolysis of the
extracellular matrix by proteinases released from tumor cells has
been implicated in tumor invasiveness and metastasis. A cathep-
sin B-like cysteine proteinase has been shown to be released
from tumors, and cathepsin B levels are elevated in the serum of
patients with several types of cancer. There is a positive asso-
ciation between cathepsin B activity and the metastatic potential
of tumor cells, as well as a positive association between the
ability of tumor cells to promote platelet aggregation and their
metastatic potential. The mechanism by which tumor cell ca-
thepsin B induces platelet aggregation is as yet unidentified
(133,134). Cathepsin B-induced platelet aggregation can be ef-
ectively inhibited with prostacyclin. Of particular interest to the
study of human pancreatic carcinoma was the observation that
cathepsin B levels were elevated in the pancreatic secretions of
a patient with pancreatic cancer (135).

Additional detailed studies revealed that prostacyclin reduced
phorbol 12-myristate 13-acetate- and 12-(S)-hydroxycosa-
tetraenoic acid-stimulated adhesion of tumor cells to subendo-
thelial cell matrix and fibronectin and that prostacyclin reduced
endothelial cell retraction (136). Prostacyclin analogues, such as
iloprost and cicaprost, had the same effects in vitro (137), and
iloprost reduced the number of colonies in the lungs of animals
given an intravenous injection of B16 melanoma and Lewis lung
carcinoma cells. After a single intravenous administration, il-
oprost reduced metastases and increased the survival time of an
animal with a spontaneously metastasizing Lewis lung carci-
noma tumor (137). Another analogue, TEI 8153, inhibited the
formation of colonies in the lung of intravenously injected fi-
brosarcoma cells.

The first data from a continuous-treatment schedule were
obtained with eptaloprost, a prodrug derived from cicaprost. In
rats, treatment with eptaloprost led to a substantial reduction in
the number of metastases from subcutaneously implanted R3327
MAT Lu prostate carcinoma tumors without inhibiting the
growth of primary tumors (137). When the antimetastatic effects
of eptaloprost and cicaprost were compared, effects of both were
equivalent (138). It is interesting that cicaprost did not affect the
growth of primary tumors, exerted a weak effect on Lewis lung carcinoma (50%), and reduced the median number of metastases from most other tumor types investigated (137–141).

Schirner and Schneider (140) evaluated the effect of cicaprost on the growth of mammary carcinomas (SMT2a and the 13762 NF MTLn3 line) that preferentially metastasize to the lung and lymph nodes of experimental animals. Cicaprost substantially reduced the median number of metastases from SMT2a tumors. All rats in the control group had between 10 and 45 lung metastases, whereas five of 10 animals in the group treated with cicaprost were free of metastases and the remaining five had only a small number of lymph node lesions. The most intriguing result, however, was an approximately 40% reduction in the weight of axillary lymph nodes. (Weight is a measure of the number of metastatic cells in an organ, such as the lymph nodes.) The MTLn3 line is a tumor that predominantly metastasizes to both regional and distant lymph nodes. Cicaprost, when tested in this model at a daily oral dose of 0.01, 0.03, or 0.1 mg/kg, caused a dose-dependent decrease in the median number of metastases. Although all control animals developed metastases, 43% of the rats treated with cicaprost at 0.1 mg/kg were free of metastases. The weight of the lung was significantly decreased (two-sided test) by the two higher doses of cicaprost used. Cicaprost also reduced the weight of the ipsilateral axillary lymph nodes to about 50% at all three doses used and reduced the weight of the contralateral lymph nodes to the level of lymph nodes from a non-tumor-bearing animal (141). Cicaprost was administered from day 11 after orthotopic MTLn3 tumor cell implantation until the end of the experiment (i.e., day 21). In fact, therapy was started when palpable primary tumors and infiltrated lymph nodes were already present. Cicaprost given at a daily oral dose of 0.1 mg/kg substantially suppressed the median number of metastases to about 20, compared with more than 500 metastases in control animals. Surgical removal of the primary tumor at day 12, on the other hand, did not have a statistically significant effect on this parameter (142). A similar experiment was performed in the SMT2a mammary tumor model. Animals were treated from day 10 until day 32. Cicaprost (0.1 mg/kg orally) reduced the median number of metastases to about 20% of the control value (143). These data show that prostacyclin analogues have antimetastatic activity in advanced disease. Prostacyclin and its stable analogues act by binding to a specific receptor (144). In vivo, cicaprost exerts a prominent antimetastatic effect on MTLn3 mammary carcinomas (141), which have functional prostacyclin-binding sites, but cicaprost has no effect on RUCA endometrial carcinomas, which lack functional receptors. In vitro, transendothelial migration of MTLn3 cells is inhibited by cicaprost (145).

In an extension of the experiments performed with cicaprost, Schirner and Schneider (146) evaluated the combined use of cicaprost and various chemotherapeutic agents in the MXT-OVEX mouse mammary carcinoma model system. Cicaprost at daily doses of 0.5 mg/kg and 1.0 mg/kg given orally had no effect on the growth of subcutaneously implanted tumors. Cisplatin at a dose of 1.5 mg/kg strongly inhibited tumor growth, but cisplatin at a dose of 0.75 mg/kg had only weak effects. The efficacy of both concentrations of cisplatin was not altered by addition of cicaprost (0.5 mg/kg orally) administered in two different schedules. Cyclophosphamide (400 mg/kg subcutaneously) completely inhibited the growth of an MXT-OVEX tumor, but doxorubicin (2.5 mg/kg subcutaneously) and 5-fluorouracil (10 mg/kg subcutaneously) had only minimal efficacy. The combination of cicaprost with cyclophosphamide, doxorubicin, or 5-fluorouracil did not alter the antineoplastic activity of any of the drugs. Although an additive or synergistic effect seems unlikely, Schirner and Schneider (146) concluded that the antimetastatic agent cicaprost can be used in combination with cytostatic regimens without decreasing their therapeutic efficacy.

Apart from prostacyclins, agents widely used for pain management or inhibition of platelet aggregation have been implicated as inhibitors of metastases on the basis of experimental data suggesting that metabolites of arachidonic acid may influence the metastatic process (147,148). Increased levels of thromboxane and decreased levels of prostacyclin have been observed in the plasma or in the tumors of patients with cancer of the lung, ovary, breast, or bone (149–152). Increased thromboxane and decreased prostacyclin are associated with an enhanced potential for platelet aggregation. The leukotrienes, which are other arachidonic acid metabolites, have profound effects on vascular integrity (153–155). Nardone et al. (156) demonstrated that ketoconazole inhibited pulmonary metastases in the B16 melanoma model. Ketoconazole, apart from its antimycotic potential, acts as a selective inhibitor of the thromboxane synthase and 5-lipoxygenase pathways. Furthermore, Nardone et al. (156) postulated that a combination of the inhibition of platelet aggregation and the protection of vascular epithelial integrity was responsible for these observations.

Conde et al. (157) found that prostaglandin D2 inhibited malignant glioma cells by biomodulation of cell morphology and the cytoskeleton. This effect was dose-dependent and could be seen only above a prostaglandin D2 threshold of 2.5 μg/mL. Prostaglandin D2 at greater than 10 μg/mL was clearly cytotoxic and produced the highest cell killing within 12–24 hours of administration. These findings are in line with the results obtained in a mouse glioma model (158) and a human glioma model (159), although these latter groups reported a cytototoxic effect but did not report cytotoxicity. More recently, prostaglandin J2, a serum-mediated catabolite, was postulated to be the metabolite responsible for the antitumoral action of prostaglandin D2 (160,161).

A statistically significant potentiation of the antineoplastic effect of cytotoxic drugs was found when flurbiprofen, an inhibitor of prostaglandin synthase, was added, as shown by an increased survival of mice bearing syngeneic metastasizing tumors (162). In similar experiments, Maca (163) found that non-toxic doses of indomethacin augmented the sensitivity of cultured tumor cells to etoposide (VP-16). Although an increased intracellular accumulation of VP-16 was observed, the mechanism through which the combination of VP-16 and indomethacin act has not been elucidated (163).

Furthermore, there are possible antimetastatic and antiangiogenic effects of many agents that inhibit membrane-active platelet aggregation and have prostacyclin-releasing activity, including flubiprofen (164), dipryridamole (165–167), mepipadomole (166), pentoxifylline (167), ticlopidine (165,167), diltiazem (165), and trapidil (165). Some of these agents have also been shown to potentiate the antineoplastic effect of interferons (168–170).

**Clinical Trials**

Results from clinical trials have been mixed. Michaels (128) reported a marked decrease in metastases in patients who had
received long-term treatment with anticoagulants for cardiovascular disease. A study performed in Copenhagen reported that treatment with porcine plasmin resulted in clinical improvement in five of 11 cancer patients over a period of several weeks (129). In contrast, Dahl (130) reported no improvement when porcine plasmin was used. Clifton (118) reported that patients with cancer who were treated for thromboses showed improvement of the thrombosis and also showed improvement in their general condition. Specifically, when a group of 31 patients with various advanced cancers was treated with fibrinolytic agents (118), there was an important clinical improvement in 43% of patients, no change in 50% of patients, and a definitive shrinkage of the tumor in 16% of patients (five patients), including one complete remission in a patient with inguinal nodules from an epidermoid carcinoma of the cervix.

The hypothesis that anticoagulants have antitumor activity was further supported by the results of a study by Salsali and Clifton (131), who showed that, for an obstruction of the superior vena cava that was caused by a cancer of the lung, a combined treatment that included anticoagulants increased survival. These authors (131) reported that, in 136 patients treated with x-ray therapy alone or with x-ray therapy plus nitrogen mustard, there was only one survivor after 5 years. Of 15 patients who were treated with fibrinolytic agents and radiotherapy, one patient survived 5 years and another patient survived 4 years. The latter patient had a primary brain tumor at autopsy but had no evidence of residual or metastatic lung cancer. In six of these patients, there was documented recanalization of the superior vena cava after treatment with fibrinolysin and radiotherapy. However, a direct influence of antithrombotic treatment on survival resulting in a diminished rate of (lethal) cardiovascular complications cannot be completely ruled out.

Another study (171) evaluated the use of anticoagulants and conventional anticancer therapy in 50 patients with a malignant obstruction of the superior vena cava. Thirty-one of the patients had carcinoma of the lung, 11 patients had lymphoma, six patients had carcinoma of the stomach, one patient had soft tissue sarcoma of the chest wall, and one patient had melanoma. Twenty-five patients were treated with irradiation and/or chemotherapy only, and 25 patients received anticoagulants in addition to irradiation and/or chemotherapy. Heparin was initially given as a continuous intravenous drip that was later changed to intermittent intravenous administration. After 10–14 days, the patients were placed on coumarin for the rest of their radiotherapy. The overall survival of the entire group was poor. Thirteen patients died after 1 month, and only 11 patients survived more than 1 year. The patients who received heparin, coumarin, and conventional therapy had a better clinical course, as shown by a shorter hospital stay, but the prognosis of the disease was not changed significantly. In the 31 patients with lung cancer, none of the patients treated conventionally survived more than 17 months. In the group treated with anticoagulants, three patients survived longer than 17 months. Thus, survival was prolonged, although for only a few months, by the anticoagulants.

Thornes (172) in studies on the use of fibrinolytic therapy to treat leukemia reported encouraging results that suggest that plasmin and the anticoagulant warfarin (sodium salt) have a direct effect on cancer cells. In a controlled trial using warfarin for maintenance therapy in patients with advanced cancer, 26 patients who received the anticoagulant were alive after 24 months compared with only 11 patients in the untreated control group (173).

In fact, only a few studies have been performed in a controlled randomized way. Apart from the differences in the designs of the studies reported in the literature (case reports; phase I, II, and III studies; and largely retrospective epidemiologic reviews), most studies were done in combination with cytotoxic drugs. Tables 1–3 give an overview of reports of phase I, II, and III studies, as well as largely retrospective epidemiologic reviews.

**Colorectal Cancer**

Nonsteroidal anti-inflammatory drugs appear to warrant further study for prevention of colonic polyposis and subsequent colorectal cancer (174–179). Dipyridamole, a coronary vasodilator and inhibitor of thrombocyte aggregation, interacts with the pyrimidine salvage pathway of 5-fluorouracil. The multimodal biochemical modulation of 5-fluorouracil by folinic acid, interferon, and dipyridamole did not change response rates; in fact, it seems to increase the response but not statistically significantly (180–182). The injection of protease inhibitors into a localized colorectal carcinoma lead to the infiltration of inflammatory cells and to the regression of the tumor, as observed by subsequent tumor resection (183).

Protease inhibitors (183) and also the combination of dipyridamole, interferon, and 5-fluorouracil (181,182) merit additional consideration for definitive investigation in colon cancer.

**Lung Cancer**

Data from randomized trials (see Table 2) suggest that small-cell lung cancer (SCLC) consistently responds (184–187) to heparin and vitamin K antagonists but that non-small-cell lung cancer (NSCLC) does not respond (184,188) to these anticoagulants. Other randomized clinical trials suggest that NSCLC consistently responds to mepipdamole (RA-233), an analogue of dipyridamole (189,190). On the basis of existing knowledge of mechanisms (191–193) and favorable pilot data (194), fibrinolytic agents in SCLC and protease inhibitors in NSCLC (195) merit additional consideration for definitive investigation.

**Conclusions**

Interpretation of the literature dealing with the effects of anticoagulant or fibrinolytic agents on tumor progression is complicated by the fact that such agents may alter immunologic mechanisms or directly inhibit tumor cell motility and growth. Nonetheless, pharmacologic and histologic data suggest a role of fibrin depositions in the metastatic process. On the other hand, only small amounts or transient formations of fibrin are found around certain tumors, and in many cases, fibrin formation is not required for the arrest of cells in circulation or the metastatic spread of tumor cells (196).

The experimental evidence strongly suggests that anticoagulants or agents interfering with platelet aggregation can prevent metastases. Despite uncertainties about the exact nature of the interaction between anticoagulants and cancer cells, anticoagulants may inhibit the adherence (or trapping) of cancer cells to capillary walls, so that cancer cells remain in systemic circulation and thus are exposed for a prolonged period to a hostile environment with the potential to eliminate cancer cells. The rationale for the use of anticoagulants seems to be at least partly based on the hypothesis that circulating cancer cells need a fibrin
<table>
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<th>Investigational regimen</th>
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<th>Protocol design</th>
<th>Comment</th>
<th>Reference No.</th>
</tr>
</thead>
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<td>Low-dose heparin for 7 days</td>
<td>Dukes’ A, B, and C colorectal cancer</td>
<td>Approximately 75% of patients reported were part of a double-blind trial for prevention of thromboembolism.</td>
<td>No treatment-related differences in overall survival observed</td>
<td>(205)</td>
</tr>
<tr>
<td>Aspirin (600 mg given twice daily for 2 y)</td>
<td>Dukes’ B2 and C colorectal cancer</td>
<td>Double blind</td>
<td>No treatment-related differences observed in disease-free or overall survival</td>
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<td>Advanced colorectal cancer</td>
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<td>Aspirin (400 mg/day) + dipyridamole (75 mg thrice daily)</td>
<td>Resected Dukes’ B2 and C colorectal cancer</td>
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<td>Prevention of colorectal cancer mortality</td>
<td>Risk assessment based on epidemiologic evaluation of drug surveillance data</td>
<td>Statistically significant reduction in risk of subsequent death from colon cancer in NSAID users</td>
<td>(174)</td>
</tr>
<tr>
<td>Aspirin (NSAID)</td>
<td>Reduction in risk of colorectal cancer mortality</td>
<td>Risk assessment based on epidemiologic evaluation of drug surveillance data</td>
<td>No reduction in risk of colon cancer development in aspirin users</td>
<td>(174)</td>
</tr>
<tr>
<td>Aspirin (NSAID)</td>
<td>Risk of developing colon cancer</td>
<td>Risk assessment based on epidemiologic evaluation of drug surveillance data</td>
<td>No reduction in risk of colon cancer with aspirin use</td>
<td>(174)</td>
</tr>
<tr>
<td>Mopidamole (RA-233) (150 mg/day)</td>
<td>Disseminated colorectal carcinoma</td>
<td>Double blind</td>
<td>No effect in disseminated colorectal cancer</td>
<td>(189)</td>
</tr>
<tr>
<td>Sulindac (NSAID)</td>
<td>Familial and nonfamilial colonic adenomatous polyposis</td>
<td>Report of 11 cases</td>
<td>Regression of polyposis with drug therapy</td>
<td>(175)</td>
</tr>
<tr>
<td>No further treatment vs. portal vein infusion of 5-fluorouracil + heparin vs. portal vein infusion of urokinase</td>
<td>Resected localized colorectal carcinoma</td>
<td>Prospective randomized trial</td>
<td>Statistically significant reduction in incidence of subsequent hepatic metastases but no change in survival in 5-fluorouracil + heparin group; no group received 5-fluorouracil alone</td>
<td>(208)</td>
</tr>
<tr>
<td>5-Fluorouracil and folinic acid with or without dipyridamole</td>
<td>Advanced colorectal cancer</td>
<td>Prospective randomized trial</td>
<td>No statistically significant effect of dipyridamole on response or survival</td>
<td>(209)</td>
</tr>
<tr>
<td>5-Fluorouracil, folinic acid, allopurinol, and dipyridamole</td>
<td>Advanced colorectal cancer</td>
<td>Pilot study in 28 patients</td>
<td>No effect of treatment</td>
<td>(210)</td>
</tr>
<tr>
<td>Warfarin, urokinase, both, or neither</td>
<td>Resected Dukes’ B2 and C colorectal cancer</td>
<td>Prospective randomized trial</td>
<td>No effect on overall or disease-free survival</td>
<td>(211)</td>
</tr>
<tr>
<td>Aspirin (NSAID)</td>
<td>Prevention of colorectal cancer mortality</td>
<td>Risk assessment based on epidemiologic evaluation of drug surveillance data</td>
<td>Statistically significant reduction in risk of subsequent death from colorectal cancer in users of aspirin but not in users of acetaminophen</td>
<td>(176)</td>
</tr>
<tr>
<td>NSAIDs</td>
<td>Prevention of colorectal cancer</td>
<td>Risk assessment based on epidemiologic evaluation of drug surveillance data</td>
<td>Statistically significant reduction in risk of subsequent death from colorectal cancer in NSAID users</td>
<td>(177)</td>
</tr>
<tr>
<td>Sulindac (NSAID)</td>
<td>Familial and nonfamilial colonic adenomatous polyposis</td>
<td>Report of seven cases</td>
<td>Marked reduction in size and number of polyps that recurred after cessation of treatment</td>
<td>(178)</td>
</tr>
<tr>
<td>Sulindac (NSAID)</td>
<td>Familial and nonfamilial colonic adenomatous polyposis</td>
<td>Report of 10 cases</td>
<td>Regression of polyposis with drug therapy</td>
<td>(179)</td>
</tr>
<tr>
<td>Dipyridamole + interferon and 5-fluorouracil</td>
<td>Advanced colorectal cancer</td>
<td>Pilot study in 15 patients</td>
<td>Response rate of 40%</td>
<td>(181)</td>
</tr>
<tr>
<td>Dipyridamole + combination chemotherapy</td>
<td>Advanced colorectal cancer</td>
<td>Pilot study in 15 patients</td>
<td>Overall response rate of 67% with projected median survival &gt;11 mo</td>
<td>(212)</td>
</tr>
</tbody>
</table>
haps this drug would be an ideal anticoagulant for the prevention of metastases, such as being convenient to give to patients and having a favorable safety record. From the treatment and prevention of thromboembolism in a woman who was receiving chemotherapy for advanced breast cancer. They comment, however, that warfarin had no effect on the natural history of breast cancer, in contrast to observations with SCLC. We thus add breast cancer to the list of tumor types (colon carcinoma, prostate carcinoma, and NSCLC) that can be considered unresponsive to warfarin. Correspondingly, breast cancer cells also apparently do not produce thrombin in situ. Instead, these cells produce urokinase (202) and its receptor (203), which have been implicated in growth and promotion of breast cancer (193,202). These molecules are potential therapeutic targets for the treatment of breast cancer. Similarly, because thrombin can enhance tumor growth by several mechanisms, perhaps the partial beneficial effects found for warfarin and heparin therapy of SCLC may be improved by use of more potent and specific inhibitors of blood coagulation (such as hirudin, antistatin, and low-molecular-weight heparin) and extended to the treatment of renal cell carcinoma (193), malignant melanoma (193), and ovarian carcinoma (204), cancers that also produce thrombin.

From the data obtained so far, we draw the following conclu-
Conclusions: 1) For SCLC, therapy with warfarin may lead to a longer period of disease-free survival and overall survival. 2) Therapy with RA-233, a dipyridamole analogue, leads to an increased overall survival in patients with localized NSCLC. 3) The efficacy of low-dose acetylsalicylic acid in colorectal cancer is still the subject of controversy. Treatment with sulindac, another nonsteroidal anti-inflammatory drug, leads to a statistically significant reduction in amount and size of colon polyps in adenomatosis coli.

Further studies are needed to confirm the existing (mostly preclinical) data and to determine whether vitamin K antagonists, heparin, low-molecular-weight heparin, or platelet aggre-

**Table 2. Clinical trials of antithrombotic drugs in small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC)**

<table>
<thead>
<tr>
<th>Investigational regimen</th>
<th>Disease studied</th>
<th>Protocol design</th>
<th>Comment</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Warfarin (conventional doses)</td>
<td>Advanced SCLC and NSCLC</td>
<td>Unblinded</td>
<td>Statistically significant increase in survival in SCLC; no effect in NSCLC</td>
<td>(184)</td>
</tr>
<tr>
<td>Warfarin (conventional doses)</td>
<td>Disseminated SCLC</td>
<td>Unblinded</td>
<td>Statistically significant increase in disease-free and overall survival and incidence of tumor regression</td>
<td>(216)</td>
</tr>
<tr>
<td>Aspirin (1 g/day)</td>
<td>Limited or disseminated SCLC</td>
<td>Unblinded</td>
<td>No effect on survival for either limited or disseminated disease</td>
<td>(217)</td>
</tr>
<tr>
<td>Mopidamole (RA-233) (150 mg/day)</td>
<td>Resected NSCLC</td>
<td>Double blind</td>
<td>Statistically significant prolongation of survival</td>
<td>(190)</td>
</tr>
<tr>
<td>Mopidamole (RA-233) (150 mg/day)</td>
<td>NSCLC limited to one hemithorax</td>
<td>Double blind</td>
<td>Statistically significant prolongation of survival; no effect in disseminated SCLC and NSCLC</td>
<td>(189)</td>
</tr>
<tr>
<td>Warfarin, levamisole, and tranexamic acid + combination chemotherapy</td>
<td>Stage III inoperable NSCLC</td>
<td>Concomitant but nonrandomized controls treated with chemotherapy alone</td>
<td>Apparent statistically significant prolongation of survival in experimental therapy group</td>
<td>(195)</td>
</tr>
<tr>
<td>Combination chemotherapy with or without mopidamole (RA-233)</td>
<td>SCLC</td>
<td>Prospective, randomized, double-blind trial</td>
<td>No effect of experimental treatment on tumor response rates or survival</td>
<td>(218)</td>
</tr>
<tr>
<td>Combination chemotherapy with or without warfarin</td>
<td>Extensive-stage SCLC</td>
<td>Prospective, randomized trial</td>
<td>Statistically significant increase in objective response rate in the warfarin group</td>
<td>(185)</td>
</tr>
<tr>
<td>Combination chemotherapy with or without unfractionated heparin given subcutaneously in adjusted dose two or three times daily for 5 wk</td>
<td>SCLC</td>
<td>Prospective randomized trial</td>
<td>Statistically significant improvement in the incidence of tumor regression and survival in heparin-treated group</td>
<td>(186)</td>
</tr>
<tr>
<td>Intermittent urokinase + combination chemotherapy</td>
<td>SCLC</td>
<td>Pilot study in 51 patients</td>
<td>Increase in incidence of tumor regression and in survival compared with historical controls</td>
<td>(187)</td>
</tr>
<tr>
<td>Combined modality chemotherapy and radiation therapy with warfarin</td>
<td>Limited-stage SCLC</td>
<td>Pilot study</td>
<td>Showed the feasibility of such a treatment</td>
<td>(174)</td>
</tr>
<tr>
<td>Combination chemotherapy and subcutaneously unfractionated heparin treatment</td>
<td>Extensive-stage SCLC</td>
<td>Unblinded</td>
<td>Improved complete response rate of 37% compared with 23% for those who received heparin</td>
<td>(219)</td>
</tr>
<tr>
<td>Dipyridamole and cisplatin</td>
<td>Advanced NSCLC</td>
<td>Prospective trial</td>
<td>No advantage in tumor regression and in survival compared with historical controls</td>
<td>(188)</td>
</tr>
<tr>
<td>Combination chemotherapy and radiation therapy with or without warfarin</td>
<td>Limited-stage SCLC</td>
<td>Prospective, randomized trial</td>
<td>No statistically significant differences in response rates, survival, failure-free survival, disease-free survival, or patterns of relapse between the warfarin-treated and control groups</td>
<td>(220)</td>
</tr>
<tr>
<td>Investigational regimen</td>
<td>Disease studied</td>
<td>Protocol design</td>
<td>Comment</td>
<td>Reference No.</td>
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<tr>
<td>---------------------------------------------------------------</td>
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</tr>
<tr>
<td><strong>Renal cell carcinoma</strong></td>
<td></td>
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<tr>
<td>Dipyridamole + vinblastine</td>
<td>Advanced renal cell carcinoma</td>
<td>Pilot study in 15 patients</td>
<td>No improvement in tumor response</td>
<td>(221)</td>
</tr>
<tr>
<td>Interferon alfa with or without aspirin (600 mg given four times per day)</td>
<td>Advanced renal cell carcinoma</td>
<td>Prospective randomized trial</td>
<td>No effect on response rates or survival</td>
<td>(222)</td>
</tr>
<tr>
<td>5-Fluorouracil, leucovorin, zidovudine, and dipyridamole</td>
<td>Advanced renal cell carcinoma</td>
<td>Phase I/II trial</td>
<td>No objective response</td>
<td>(215)</td>
</tr>
<tr>
<td><strong>Melanoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coumarin</td>
<td>Resected stage Ib and II melanoma</td>
<td>Double blind</td>
<td>Four recurrences in the coumarin-treated group of 13 patients and 10 recurrences in the placebo group of 14 patients (two-sided P&lt;.01)</td>
<td>(223)</td>
</tr>
<tr>
<td>5-Fluorouracil, leucovorin, zidovudine, and dipyridamole</td>
<td>Advanced melanoma</td>
<td>Phase I/II trial</td>
<td>No objective response</td>
<td>(215)</td>
</tr>
<tr>
<td><strong>Head and neck cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Warfarin</td>
<td>Advanced head and neck cancer</td>
<td>Unblinded</td>
<td>No effect in overall survival</td>
<td>(184)</td>
</tr>
<tr>
<td><strong>Stomach cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipyridamole + combination chemotherapy</td>
<td>Advanced stomach cancer</td>
<td>Pilot study</td>
<td>Administration of this combination is feasible.</td>
<td>(224)</td>
</tr>
<tr>
<td>Heparin, methotrexate, and 5-fluorouracil</td>
<td>Advanced stomach cancer</td>
<td>Pilot study in 10 cases</td>
<td>“Remarkable improvement” described in three patients and symptomatic improvement in others</td>
<td>(225)</td>
</tr>
<tr>
<td><strong>Ovarian carcinoma</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Tranexamic acid (an inhibitor of plasminogen activator)</td>
<td>Advanced ovarian carcinoma</td>
<td>Pilot study</td>
<td>Reduction of bulk disease and of ascites and effusion</td>
<td>(226)</td>
</tr>
<tr>
<td>Intraperitoneal tranexamic acid</td>
<td>Advanced ovarian carcinoma</td>
<td>Pilot study in 11 patients</td>
<td>Reduction of ascites</td>
<td>(227)</td>
</tr>
<tr>
<td>Combination chemotherapy and radiation therapy with or without mopidamole (RA-233)</td>
<td>Resected ovarian carcinoma</td>
<td>Prospective, randomized, double-blind trial</td>
<td>No effect of experimental treatment on time to recurrence or survival</td>
<td>(228)</td>
</tr>
<tr>
<td><strong>Breast cancer</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benoral (aspirin–acetaminophen conjugate) (4 g twice daily)</td>
<td>Stage II or anaplastic primary breast cancer</td>
<td>Double blind</td>
<td>No treatment-related differences observed in probability of bone metastasis or overall survival</td>
<td>(229)</td>
</tr>
<tr>
<td>Stanozolol (an androgen derivative that enhances fibrinolysis)</td>
<td>Advanced breast cancer</td>
<td>Pilot study in 18 cases</td>
<td>Partial disease regression was observed in three patients, and disease stabilization occurred in five patients.</td>
<td>(230)</td>
</tr>
<tr>
<td><strong>Cervix carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxyphenbutazone (100 mg thrice daily)</td>
<td>Squamous cell carcinoma of uterine cervix</td>
<td>Unblinded radiation therapy adjuvant study</td>
<td>Statistically significant prolongation of 5- and 10-y survival</td>
<td>(231)</td>
</tr>
<tr>
<td><strong>Prostate cancer</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Warfarin (conventional doses)</td>
<td>Advanced prostate cancer</td>
<td>Unblinded</td>
<td>No effect in overall survival</td>
<td>(184)</td>
</tr>
<tr>
<td><strong>Desmoid tumor</strong></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Testolactone, sulindac (NSAID), warfarin, and vitamin K1</td>
<td>Unresectable desmoid tumors</td>
<td>Pilot study in 10 patients</td>
<td>Disease regression or stabilization was observed in several of the patients.</td>
<td>(232)</td>
</tr>
<tr>
<td><strong>Various advanced cancers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dipyridamole + acivicin</td>
<td>Various advanced cancers</td>
<td>Pilot study</td>
<td>Administration of this combination is feasible.</td>
<td>(233)</td>
</tr>
<tr>
<td>Dipyridamole + methotrexate</td>
<td>Various advanced cancers</td>
<td>Pilot study</td>
<td>Statistically “significant improvement” in several patients</td>
<td>(234)</td>
</tr>
</tbody>
</table>
gation inhibitors effectively prevent the formation of metastatic tumors.

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(29)


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