Control of Metastases in the Nb Rat Prostatic Adenocarcinoma Model

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Chemotherapeutic agents, as well as acetyl-salicylic acid, heparin, and indocin, were evaluated in regard to their effect on the rate of metastasis in the androgen-insensitive Noble rat prostate andenocarcinoma system. Acetyl salicylic acid, heparin and indocin were similar to the chemotherapeutic agents cyclophosphamide and adriamycin in reducing the incidence of metastases, but had less effect on tumor volume. In the heparin and indocin groups, the rate of metastases was approximately one half that of the control group (25% vs. 59%). In all three experiments evaluating heparin and indocin, there was a decrease in the number of animals with metastasis compared with control groups.

Key words: metastasis, animal model.

J Androl 1984; 5:265–268.

About 75% of all cancer deaths are due to distant metastasis. Little progress has been made in increasing the survival of patients with distant metastases, even though such metastases are the principal cause of death from cancer. Chemotherapy and immunotherapy have not yet been proven to significantly improve the prognosis of those with distant metastases. It is for this reason that other alternatives are being sought (Henschke et al, 1977).

The process of hematogenous metastasis formation may be divided into four stages: 1) growth of the primary tumor, 2) invasion of the endothelial wall, 3) release of tumor cells into the circulation, and 4) entrapment or adhesion in the capillaries of distant organs, where penetration of the vessel wall and growth in perivascular tissues occurs. Anticoagulant therapy, an experimental method of decreasing distant metastasis, apparently acts on step 4 of the metastatic process (Fidler, 1978).

It has been observed in various animal systems that hypocoagulability and hyperfibrinolysis decrease metastasis; hypercoagulability and hypofibrinolysis increase the incidence of metastasis (Hoover and Ketcham, 1975; Lione and Bosmann, 1978; Dvorak et al, 1981). Two principal observations have led to the implication of the clotting system in the establishment of metastasis: 1) platelet aggregates and 2) the administration of anticoagulants and fibrinolytic agents to various tumor-animal host systems have decreased distant metastases (Warren, 1978; Pearlstein et al, 1980).

Ultrastructural studies have shown that platelets adhere to cell projections by means of pseudopodia extending from the platelet cell body (Warren, 1978). Circulating tumor cells have a close association with platelets (Lione and Bosmann, 1978; Warren, 1978; Kramer and Nicolson, 1979; Pearlstein et al, 1980; Dvorak et al, 1981). Tumor cell settling has been found to be delayed by the administration of anticoagulants such as heparin, and platelet aggregating inhibitors like acetyl-salicylic acid. It is hypothesized that preventing tumor cell settling and stripping the cells of their platelet armor may make the cell more susceptible to the host's immunologic defense, as well as to chemotherapeutic agents (Ambrus et al, 1978; Ambrus and Ambrus, 1980). It has also been observed that tumor cells that attach to endothelium are rapidly surrounded by fibrin and platelet aggregates, and are capable of penetrating the vessel wall in a matter of hours (Hoover and Ketcham,

Supported by grant no. CA 27380–04.

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Submitted for publication September 19, 1983; revised version received February 20, 1984; accepted for publication February 21, 1984.

1975). The important role played by the blood platelets in the development of distant metastases was demonstrated by Gasic and coworkers (Gasic et al, 1968), and impaired platelet function has been shown to interfere with the establishment of metastases (Gasic et al, 1978). The ability of a tumor cell line to induce platelets aggregation by secreting clot-forming enzymes, clot-stabilizing enzymes and a "cancer coagulative factor" resembling thromboplastin has been correlated with an increase in distant metastases (Hoover and Ketcham, 1975; Dvorak et al, 1981).

Materials and Methods

Three experiments were designed utilizing Nb rats. These rats have been an inbred colony for many years. All subjects were females weighing between 150 and 175 grams at the beginning of the experiment. The experimental design, which has been utilized previously by this laboratory, includes subcutaneous placement of tumor wedges (2 mm^3) into the flanks of control and experimental animals. The tumor used was the Nb-Pr-A.I.-III, an androgen-insensitive autonomous prostatic carcinoma maintained by this unit for the last five years. The experiment was initiated on day 11 ± 2 postimplantation, when the tumor volume had reached 90 ± 10 mm^3. Drugs were administered intraperitoneally. Metastases were evaluated by gross and microscopic determination, and were primarily pulmonary, with some mediastinal lymph node metastases as well; all metastases were recorded, independent of size.

In Experiment 1, there were 17 animals in the control group and 17 in the acetyl-salicylic acid group, which received doses of 54 mg/kg at weekly intervals. Heparin treatment consisted of 100 units/kg intraperitoneally at weekly intervals in 18 animals and indocin was used at a dosage of 1.43 mg/kg in 16 animals. All treatments were given at four weekly intervals.

In the second experiment, there were 11 control animals and ten animals in each experimental drug group. The dose of acetyl-salicylic acid was 18 mg/kg; heparin and indocin were used in dosages similar to those in Experiment 1. There were two additional treatment modalities; adriamycin (1.5 mg/kg) and cyclophosphamide (60 mg/kg) were combined, and cyclophosphamide was given alone at 60 mg/kg. These were all used at four weekly intervals.

In Experiments 1 and 2 it was determined that indocin and heparin had a significant effect on decreasing metastasis. Experiment 3 was designed to evaluate dose response to three doses of indocin and three doses of heparin. Sixty-three animals were used in this study. Indocin was administered at 1 mg/kg, 10 mg/kg and 100 mg/kg at weekly intervals for three treatment cycles; indocin was dissolved in alcohol plus DMSO and Na_2HPO_4 plus KH_2PO_4. Heparin was administered at 10 units/kg, 100 units/kg and 1000 units/kg every day for three weeks.

Tumor volume was measured twice weekly, and at the conclusion of the experiment all animals were necropsied for number of metastases per group (Tables 1

TABLE 1. Analysis of Effect of Treatments on Metastasis and Tumor Volume

Effects of Acetyl Salicylic Acid, Indocin, and Heparin on Number of Metastases and Tumor Volume: Experiment 1

Treatment	Dosage	No. with Metastases	% with Metastases	$\bar{X}$ Tumor Volume (mm^3)	*P* Value* No. with Metastases
Control		10/17	59	4580	
Acetyl salicylic acid	54 mg/kg	7/17	41	4081	N.S.
Heparin	100 units/kg	5/18	28	3704	<0.05
Indocin	1.43 mg/kg	4/16	25	3985	<0.05

Effects of Five Different Treatments on Incidence of Metastases and Tumor Volume: Experiment 2

Treatment	Dosage	No. with Metastases	% with Metastases	$\bar{X}$ Tumor Volume (mm^3)	*P* Value* Tumor Volume
Control		5/11	45	12418	
Acetyl salicylic acid	18 mg/kg	5/10	50	5823	<0.05
Heparin	100 units/kg	0/10	0	5048	<0.05
Indocin	1.43 mg/kg	0/10	0	5990	<0.05
Cyclophosphamide + adriamycin	60 mg/kg 1.5 mg/kg	3/10	30	1010	<0.025
Cyclophosphamide	60 mg/kg	2/10	20	1120	<0.025

* Student's T-test used for obtaining *P* values.

The evaluation of control group, acetyl-salicylic acid, heparin, and indocin reveals the number of metastases to be significantly less in both indocin and heparin treatment when compared with controls. The tumor volumes, however, were not statistically significant among these groups. This table reveals that heparin and indocin treatment resulted in the lowest number of animals with metastasis; however, the combination treatment with cyclophosphamide and adriamycin, as well as cyclophosphamide treatment alone, resulted in a significant tumor volume reduction when compared with these nonchemotherapeutic agents. In this experiment, however, the nonchemotherapeutic treatment groups did result in a significant lowering of final tumor volumes.

TABLE 2. Dose Response of Indocin and Heparin: Experiment 3

	Number Metastases	Animals Died	$\bar{X}$ Tumor Volume (mm^3)	Tumor Volume *P* Value
Controls	6/11	0	14,000 ± 1,350	
Indocin 1 mg/kg	2/8	0	7,183 ± 600	<0.05
10 mg/kg	2/8	2/10	16,000 ± 948	N.S.
100 mg/kg	1/5	5/10	12,460 ± 1,086	N.S.
Heparin 10 units/kg	1/8	0	5,608 ± 486	<0.04
100 units/kg	1/8	0	5,238 ± 240	<0.025
1000 units/kg	1/8	0	3,161 ± 310	<0.005

At all dosages, number of animals with metastasis was reduced; however, only with the low dose of indocin, at 1 mg/kg, was the tumor volume significantly affected. All heparin treatments resulted in a reduction of metastasis with no animals dying of toxicity, and also resulted in a significantly lower tumor volume when compared with controls.

and 2). All animals were sacrificed 42 days after the last treatment injection.

Results

Experiment 1

Ten of the 17 control animals had metastases, for a metastatic incidence of 59%. In the acetyl-salicylic acid treatment group, seven of 17 animals developed metastases, for a rate of 41%. The heparin group had a metastatic rate of 28% (5/18), and the indocin group had a rate of 25% (4/16). These metastases were predominantly found in the lungs and liver. The tumor volumes in Experiment 1 were significantly reduced by heparin and indocin, but not by acetyl-salicylic acid. The reason for this remains unclear. However, there were significant differences ($P < 0.05$) in the number of metastases in the indocin and heparin-treated groups (4/16 and 5/18, respectively) as compared with the untreated controls (Table 1).

Experiment 2

In this experiment, five of 11 control animals and five of the acetyl-salicylic acid treatment group had metastatic disease. None of the heparin treated group nor any of the indocin group demonstrated metastases at necropsy. In the chemotherapy group combining cyclophosphamide and adriamycin, three of the ten had metastases, and with cyclophosphamide only, two animals had metastases. Experiment 2 was terminated 42 days after the final chemotherapy treatment. All treatments significantly reduced tumor volume ($P < 0.05$). However, it is well known in this animal model system that cyclophosphamide and adriamycin have been effective in reducing tumor volume as well as reducing the number of metastases. In these preliminary experiments, antitumor effects of acetyl-salicylic acid, indocin and heparin have been observed, but the mechanism of this action is not clearly understood (Table 1).

Experiment 3

The control animals had an average tumor volume of 14,000 mm^3, and six of the 11 animals had metastatic disease. The indocin treatment of 100 mg/kg resulted in the deaths of 50% of the animals prior to the conclusion of therapy, possibly because of indocin toxicity. In this group, one of the five remaining animals had metastatic disease. The animals receiving 10 mg/kg did not have a significant reduction in tumor volume: however, only two of the eight developed metastases. Indocin treatment of 1 mg/kg did significantly reduce the tumor volume, no animals died during treatment, and only two of the eight animals had metastatic disease. Heparin treatment resulted in significant tumor volume reduction at all three dosages; no animal died during therapy or in the observation period following treatment. In each of these three heparin treatment groups, four of eight tumors underwent complete tumor regression. The exact mechanism of the antitumor effect of heparin still remains to be determined (Table 2).

Discussion

These experimental protocols have yielded some interesting information that suggest further study. They have shown that the indocin-heparin treatment led to a significant reduction in metastatic incidence and tumor volume in the Nb rat prostatic adenocarcinoma androgen-insensitive tumor III. Speculation exists as to the way heparin and indocin act, and includes the microthrombus prevention theory as well as inhibition of prostaglandins (Kramer and Nicolson, 1979; Dvorak et al,

1981; Honn et al, 1981). Other alternative theories might be entertained, and include the antiadherence theory. No explanation is rendered for the decrease in tumor volume in the acetyl-salicylic acid, heparin and indocin treatment groups in Experiment 2, or the dose response in Experiment 3. However, these are currently being reevaluated. In addition, this laboratory has not directly evaluated prostaglandins with respect to the tumor itself. Studies are currently planned to evaluate not only serum prostaglandins, but other platelet factors such as bleeding time, protime and PTT, as well as the factors of prostaglandin synthesis in the tumor itself.

Other investigators have studied acetyl-salicylic acid as an antimetastatic drug (Wood and Hilgard, 1972; Gasic et al, 1973). The mechanism of action for the antimetastatic effect of acetyl-salicylic acid apparently comes from its inhibition of platelet aggregation (Henschke et al, 1977). Heparin has also been shown to have an antimetastic property by several investigators (Ivarsson and Rudenstam, 1975; Dvorak et al, 1981). Several reports have shown that heparin may not work by its anticoagulating mechanism, and it has been suggested that heparin may act independently of blood coagulation (Retik et al, 1962; Dvorak et al, 1981). Rather, heparin may act to change the surface charges of particles (Wilkins, 1967; Suemaser and Ishikawa, 1970; Dvorak et al, 1981). Heparin, being a potent surfactant, can alter membrane function and adhesiveness and the ability of cells to aggregate, and may result in decreased tumor cell lodgement in the vascular endothelium (Ivarsson and Rudenstam, 1975). Although heparin's action still remains relatively unclear, the above theories may be able to provide some explanations for the results observed.

Indomethacin may exert its antimetastatic effect via its relationship with the prostaglandins. Much work has been done in this field and more is needed to answer the question of metastasis control (Kenhl, 1977; Editorial, 1979; Poste and Fidler, 1980; Dvorak et al, 1981; Honn et al, 1981). The anti-inflammatory agent, indomethacin, inhibits prostaglandin production and may therefore have an antimetastatic effect similar to that of aspirin. The study of this antimetastatic factor does seem to warrant further investigations, as outlined above. In our experiments, heparin and indocin were valuable agents in significantly reducing the number of metastases when compared with controls in the androgen-independent Nb rat prostatic adenocarcinoma model.

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