Trauma as a Cause of Localization of Blood-Borne Metastases: *

Preventive Effect of Heparin and Fibrinolysin

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THE EFFECT of trauma on the development, spread or localization of malignant tumors has long been a subject of dispute among clinicians. Occasional cases of metastasis occurring at the site of injury have been striking and suggest that trauma at least predisposes to localization of metastatic lesions.

It is obvious that cancer cells must be circulating in the blood stream at some time in order to produce distant metastases. Cancer cells have been demonstrated in the blood of patients with resectable and unresectable cancers.[9] Their significance has not been determined. Cancer cells in the blood certainly do not always lead to metastasis.[8, 9] It is believed that the vast majority are destroyed while others lodge in the capillaries of the lung and other tissues. Once lodged in the capillaries they may grow rapidly to produce metastases,[14, 17] they may be destroyed [15, 17, 21, 24] or they may remain dormant for years before becoming reactivated by means as yet unknown.[10, 13] Hormonal changes may be responsible,[24, 28] or stress either of operation, trauma or disease may precipitate metastases or cause the tumor to grow rapidly.[4, 19, 23] The effect of the primary tumor may be significant. It has been suggested that the removal of a primary tumor itself results in a more rapid growth of metastases.[22]

We have been particularly interested in the effect of alterations in the clotting mechanism on the development of metastases. Heparin and fibrinolysin have been shown to be effective in decreasing the transplantability of circulating cancer cells [1, 12] and in decreasing pulmonary metastases.[2, 3, 5, 11, 12, 16, 29, 31] Trauma and stress of various kinds predispose to a hypercoagulable state with increased sludging, increase in platelet counts and fibrinogen levels all of which predispose to clotting. The present experiment was set up to investigate the effect of local trauma on the development of metastases by blood borne tumor cells (Walker carcinosarcoma 256) and to evaluate the effectiveness of fibrinolysin and heparin in preventing such metastases.

Materials

Adult Wistar Rats: Female, weighing approximately 200 Gm.

Walker 256 Carcinosarcoma: The tumor has been carried in the ascitic form in our laboratory for many years. For these experiments the fluid was diluted with saline

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TABLE 1. *Incidence of Liver and Leg Metastases after Inoculation of 25,000 Walker 256 Carcinosarcoma Cells in the Control Group (A) and Turpentine Group (B). (Turpentine was Given into the Right Leg 48 Hours Prior to the Cance Cell Suspension)*

	Control—Group A				Turpentine Only—Group B			
Survival (weeks)	Liver Metast.	Unilat. Metast.	Bilat. Metast.	No Tumor	Liver Metast.	Unilat. Metast.	Bilat. Metast.	No Tumor
1	—	—	—	—	—	—	—	—
2	—	1	—	—	2	2	—	—
3	5	—	4	—	12	21	8	—
4	21	2	9	—	12	12	5	—
5	2	—	1	—	2	1	—	—
6	3	2	—	—	—	1	—	—
7	—	—	—	—	—	—	—	—
8	—	—	—	—	—	—	—	—
9*	—	—	—	2	—	—	—	2
(%)	32(60%)	5(10%)	14(28%)	2(4%)	28(51%)	35(64%)	13(23%)	2(3%)
	Unilateral + bilateral metastases 38%					87.1%		
	Total no. rats 50				55			

* Survived nine weeks.

to a count of 25,000 cells/ml. Viability of each batch was proven by subcutaneous inoculation.

Fibrinolysin:* 50,000 units diluted in 10 ml. of saline (5,000 units/ml.).

Heparin Aqueous:** 1,000 units/ml. diluted with saline to 50 units/ml.

Turpentine:*** 0.5 ml.

Methods

To determine its local effects, 0.5 ml. of turpentine was injected into the right thigh of each of ten rats which were observed for 9 weeks. Severe irritation of the skin was noted 24 hours following the injection of the turpentine. The inflammation disappeared after approximately 10 days, leaving a slight epilation. There was no obvious general toxicity.

The experimental animals were anesthetized with Nembutal. A laparotomy was performed, the aorta exposed and 1 ml. of the cancer cell suspension (25,000 cells) was injected directly into the aorta below the take-off of the renal arteries. The laparotomy incision was then closed in the conventional manner.

The 307 rats were divided as follows:

Group A (50 rats)—Controls: These animals were injected only with 1 ml. (25,000 cells) of the cancer cell suspension.

Group B (55 rats): Turpentine (0.5 ml.) was injected into the muscles of the right thigh. Forty-eight hours later 1 ml. of the cancer cell suspension (25,000 cells) was injected into the aorta. These animals had no other treatment.

Group C (96 rats): This group of rats was treated with turpentine as in Group B. Forty-eight hours later 5,000 units of fibrinolysin was injected into the vena cava and 15 minutes later 1 ml. of the cancer cell suspension (25,000 cells) was inoculated into the aorta as in Group A and B.

Group D (96 rats): Turpentine was injected as in Group B and C. Forty-eight hours later 50 units of heparin were injected into the vena cava and ten minutes later 1 ml. of the cancer cells suspension (25,000 cells) was injected into the aorta.

Postmortem examination was made as

* Thrombolysin, Merck Sharp & Dohme, West Point, Pa.

** Heparin, Organon Inc., W. Orange, N. J.

*** Turpentine, Hyden Newport Chemical Corp., New York, N. Y.

TABLE 2. *Incidence of Liver and Leg Metastases after Inoculation of 25,000 Walker 256 Carcinosarcoma Cells in the Fibrinolysin Group (C) and Heparin-Treated Group (D). (Both of these Groups were Inoculated with 0.5 ml. of Turpentine into the Right Leg 48 Hours Prior to the Inoculation of the Cancer Cell Suspension).*

	Turpentine and Fibrinolysin—Group C				Turpentine and Heparin—Group D			
Survival (weeks)	Liver Metast.	Unilat. Metast.	Bilat. Metast.	No Tumor	Liver Metast.	Unilat. Metast.	Bilat. Metast.	No Tumor
1	—	—	—	—	—	—	—	—
2	2	5	—	—	1	2	—	—
3	1	8	—	—	6	14	2	—
4	6	18	4	—	6	19	1	—
5	3	8	2	—	3	7	1	—
6	1	4	—	—	—	—	—	—
7	—	2	—	—	—	—	—	—
9*	1	—	—	13	2	—	—	15
(%)	14(15%)	45(47%)	6(6%)	13(14%)	18(19%)	42(44%)	4(4%)	15(16%)
	Unilateral + bilateral metastases 53%					48%		
	Total no. rats 96					96		

* Survived nine weeks.

soon as possible after death (less than 12 hours) in all animals which did not survive for 9 weeks. All animals were sacrificed at 9 weeks and postmortem examinations made immediately. The survival time and incidence of metastases of each group are reported in Tables 1 and 2.

Results

The survival time and the presence of gross hepatic and leg metastases were determined in the animals of each group.

Group A: controls. Of 50 animals, 32 (60%) had obvious liver metastases. Five (10%) had a unilateral leg tumor and 14 (28%) had bilateral leg tumors. Two (3%) survived nine weeks. At postmortem these two rats showed no evidence of metastatic tumor (Table 1).

Group B: turpentine only. Of 55 animals, 28 (51%) had gross hepatic metastases. Thirty-five (64%) had a unilateral leg tumor on the side of turpentine injection and thirteen (23%) had bilateral leg tumors. Two (3%) survived nine weeks with no evidence of tumor (Table 1).

Group C: turpentine and fibrinolysin. Of 96 animals, only 14 (15%) had gross liver metastases, 45 (47%) had unilateral leg tumor and six (6%) had bilateral leg tumors. Thirteen (14%) survived nine weeks without evidence of metastatic tumor (Table 2).

Group D: turpentine and heparin. Of 96 animals, only 18 (19%) had gross liver metastases, 42 (44%) had unilateral leg tumor and four (4%) had bilateral leg tumors. Fifteen (16%) survived nine weeks without evidence of tumor at postmortem (Table 2).

About 10 per cent of the animals in all groups developed solid tumor at the site of injection; others developed peritoneal effusion due to spill of tumor cells at this site, and 5 to 8 per cent in all groups developed pulmonary metastases. There was no gross evidence of metastases in the spleen or kidney in any animals.

Discussion

In experimental animals there is evidence that trauma increases the incidence of metastases or the rate of growth. Fisher and Fisher [10] demonstrated an increase in metastases in the liver after trauma and suggested that this was due to activation of

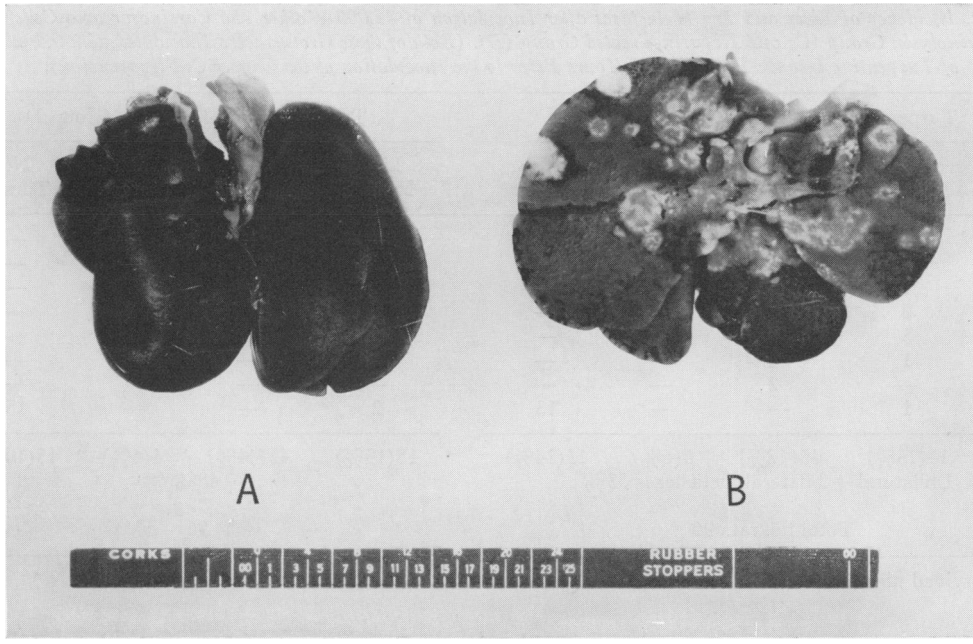

FIG. 1. Autopsy specimens: A) Liver of a rat that was sacrificed at nine weeks after receiving 5,000 units of fibrinolysin. Note the complete absence of metastatic nodules; B) liver of a rat that died at 4 weeks following the intravenous inoculation of 25,000 cancer cells. Note the presence of multiple metastatic nodules.

dormant tumor cells. An increase in the incidence of tumor takes produced by injected tumor cells after celiotomy was reported by Buinauskas *et al.*[4] Lewis and Cole[18] reported an increase in metastases after the surgical stress of amputation of a limb containing a primary tumor. They attributed this to the lowered resistance of the animals. Schatten[22] also noted an increase in metastases when the tumor bearing limb was removed. He suggested that the primary tumor had an inhibiting effect on the growth of the metastases. Whether the increase of metastasis with operations is due to trauma itself, anesthesia or blood loss has not been determined.[27]

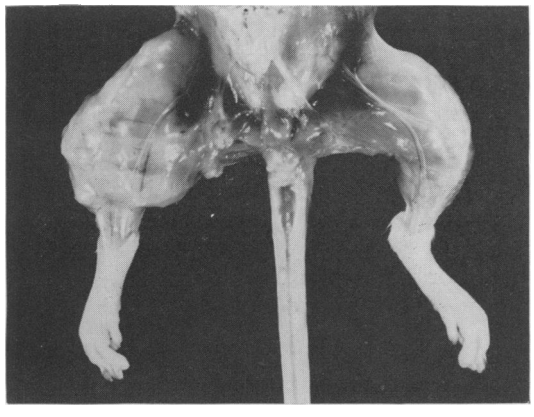

FIG. 2. Autopsy specimen of a rat that died with massive leg tumor. This rat had received turpentine into the leg 48 hours prior to the cancer cell suspension. Note the huge tumor on the left leg where turpentine was given, as compared with no metastatic involvement of the right leg.

These studies of an animal tumor with a well known potential for metastasis would support the impression that trauma does lead to localization of the metastases. This study does not indicate that trauma increases general metastases since the overall rate of metastases and the survival rate in the controls and the turpentine injected animals were almost identical. The inflammatory reaction induced by turpentine in an extremity causes an increase in the incidence of local metastases under the con-

ditions of this experiment. The incidence of malignant tumor growth in the leg increased from 38 per cent in the control group to 87.1 per cent in the animals injected with turpentine. However bilateral leg metastases were similar in the two groups (28% in the controls and 23% in the turpentine group), and liver and lung metastases were also unchanged. This indicates that the turpentine trauma did not increase the tendency to general metastasis. It was only in the unilateral leg metastases that a significant increase occurred (from 10 to 64%). The rise in all leg metastasis from 38 per cent in the controls to 87.1 per cent in the turpentine-treated animals is due entirely to this increase in metastasis in the traumatized leg.

The effectiveness of fibrinolysin and heparin in preventing metastases is confirmed. However, it should be noted that the greatest effect was on the liver metastases and the bilateral leg metastases. The effect on the turpentine-induced unilateral metastasis was significant but less than that on the liver and bilateral leg metastases, indicating that the inflammatory effect is so strong that it cannot be overcome by this small standard dose of heparin and fibrinolysin. The improvement in rate of survival from 4 per cent in the controls and 3.1 per cent in the turpentine-treated animals to 14 per cent with turpentine plus fibrinolysin and 16 per cent with turpentine plus heparin is significant.

The mechanism of the turpentine is not explained by these experiments. Perhaps the inflammatory reaction, by increasing the rate of blood flow to the part, acts in a mechanical way to bring more cancer cells to the area. It must also be considered that the inflammatory reaction results in more fibrin formation and stickiness of the intima of the vessels, trapping the cells. It has been shown that sterile abscess formation in an extremity, as by turpentine injection, results in an increase in systemic blood fibrinogen and fibrinolysin inhibitor levels.[19] Both these phenomena would tend to enhance metastasis and inhibit the effectiveness of fibrinolysin and heparin.

Summary

The number of metastases in the area of local trauma due to injection of turpentine was greatly increased following intra-aortic injection of Walker 256 carcinosarcoma cells in the rat.

When the animals injected with turpentine before tumor cell infusion were treated with heparin or fibrinolysin: 1) the incidence of bilateral leg metastases was greatly reduced; 2) metastases in the traumatized leg were decreased, though less than with the bilateral leg and hepatic metastases; 3) hepatic metastases were reduced significantly; and 4) survival for nine weeks without obvious tumor was increased five to six times.

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