The Effect of Human Fibrinolysin on Pulmonary Metastases of Walker 256 Carcinosarcoma*

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SUMMARY

1. A significant in vitro fibrinolytic activity could be obtained in plasma of rats given human fibrinolysin intravenously.
2. Pulmonary metastases were produced in a large percentage (81 per cent) of control rats given inoculations intravenously of 100,000 Walker 256 carcinosarcoma cells/ml. When cells were inoculated into animals with marked fibrinolytic activity produced by human fibrinolysin, the takes of tumor as measured by pulmonary metastases were markedly reduced to 25 per cent.

The demonstration of cancer cells in the blood in patients with resectable neoplasms has attracted a great deal of interest. Patients with primary colonic neoplasms have been studied most commonly, and in these a higher percentage of mesenteric vein blood samples showed tumor cells (5). This led to the use of chemotherapeutic agents at the time of surgery in an attempt to affect the showers of tumor cells in the systemic circulation (8). Unfortunately, little is known of the mechanism whereby tumor cells lodge in various organs. Some studies have been made with the Walker 256 carcinosarcoma of rats to ascertain the number of cells needed for successful inoculation in the systemic and portal circulations. It has been found that with this tumor the most successful route of inoculation is the intraportal, with 96 per cent positive takes in the liver. The intravenous inoculation yielded a lower percentage of takes (85 per cent). Nitrogen mustard has been shown to diminish the inoculation rate in these animals (3). This has been used as an additional reason for the use of this agent in humans during the surgery of malignant neoplasms.

It has been shown that the intravenous injection of as few as 250 cells will result in metastases (4). Other investigators have shown that there is an increase in pulmonary metastases after amputation of a tumor-bearing limb (7). The effect of trauma has been confirmed by the demonstration that cancer cells can be dormant in the liver and apparently begin to grow after celiotomy and liver trauma. Rats given injections intraportally of as few as 50 Walker 256 carcinosarcoma cells and examined 3 months later showed no evidence of hepatic tumor growth. If at this time the rats were subjected to repeated laparotomy and liver examinations at 7-day intervals, 100 per cent had a tumor within a few weeks (4).

Studies of the mechanisms of lodging of tumor cells in organs have been limited. The VX2 carcinoma of rabbits was shown to have a thromboplastic effect, severe enough to result in death of inoculated animals by pulmonary embolization. Heparin diminished the mortality caused by VX2 carcinoma (6), and later human fibrinolysin was shown to have the same effect (2). In this same series of experiments human fibrinolysin was also highly effective in preventing the production of pulmonary metastases by the inoculation of the VX2 carcinoma (2). The fibrinolysin-treated animals had 21 per cent gross pulmonary metastases, whereas the control animals had 91 per cent. It was thought that the lysis of small fibrin deposits by fibrinolysis or the prevention of fibrin deposits by anticoagulants at the time of intravascular inoculation of cancer cells might be of value in diminishing the number of visceral metastases. More recently it was shown that VX2 carcinoma...
cells aggregate in minute thrombi, and the fibrin meshwork then adheres to the capillary wall and a nidus of tumor is formed (10). Nonthromboplastic tumors were sought to determine whether this phenomenon was a general one with tumors. With the Brown-Pearce carcinoma the results were not quite so striking, but there was a definite decrease in liver metastases with fibrinolysin treatment (2). Since the Walker 256 carcinosarcoma produces metastases in both liver and lung and has been the subject of extensive studies on metastases, it seemed to be an excellent tumor with which to expand these studies.

MATERIALS AND METHODS

Adult, female, white Wistar rats weighing from 180 to 200 gm. were used. The animals were fed a stock diet of chow checkers and water.

The Walker 256 carcinosarcoma tumor is carried in the ascitic form in the laboratory of radio-biology and has been transplanted at weekly intervals for over 2 years in more than 200 generations. The ascitic fluid was aspirated under sterile precautions, and a thin cell suspension was made by dilution with saline. An aliquot was taken and a cell count carried out by a dilution and a direct smear counting method (9). The ascitic fluid was found to have 5–10 million cells/cu mm. Dilutions were made with saline so that the tumor suspension used had about 100,000 cells/ml. Frequent checks were made to assure a correct dilution of cell suspension. To insure maximal viability of tumor cells, no suspension was used longer than 1 hour after preparation. Several rats were given inoculations subcutaneously during each series of experiments to have proof of viability at the beginning and end of the transfers. If tumors did not grow in these animals then the entire experimental series was discarded.

**Human fibrinolysin.**—Twenty-five thousand (25,000) units were dissolved in 25 ml. of saline, and 1 ml. was given intravenously in 5 minutes to a 180–200-gm. animal. This was approximately 5,000–6,000 units of fibrinolysin/kg body weight.

The fibrinolytic activity of the plasma was determined by the euglobulin fibrinolytic method (1). The normal value in the rat with the bovine clot lysis time is over 24 hours. The antiplasmin fibrinolytic method (1) was used to evaluate the inhibitory level of the plasma.

At the start it was felt that tail vein injections would provide a good means of inoculation. However, we could never be certain that the inoculation was intravenous. The method of surgical ex-}

1 Human fibrinolysin, Thrombolysin, supplied by Merck, Sharp & Dohme, West Point, Pa.
lungs. The tumor nodules measured 0.5–1 cm. in diameter. Any lung containing even one tumor nodule was counted as positive. In the negative animals the lungs appeared grossly normal, and no gross metastases were detected (Fig. 1). In most of the positive animals, the lungs were loaded with large tumor nodules replacing the parenchyma, and yet grossly the liver, spleen, and kidneys were normal, without metastases. On occasion metastatic deposits were found in the myocardium and pericardium. These animals did not develop pleural effusion.

In the 113 animals given human fibrinolysin prior to the tumor suspension only 28 animals (25 per cent) were found to have gross evidence of metastatic deposits in the lungs. No deposits were found in the liver, spleen, or other organs inspected. The treated animals which had metastases were similar to the control animals with metastases. This 25 per cent incidence of positive takes in the lung is remarkable compared with that in the control group. The lungs of the treated animals which were grossly negative were sectioned and found to be resilient and normal in appearance. In a few animals tumor was found to grow in the subcutaneous tissues of the thigh near the site of femoral vein injection, probably owing to leakage of blood at this site. These animals were counted as negative if they did not have pulmonary metastases, because the criterion being used was that of blood-borne metastases in the lung.

**DISCUSSION**

There is no obvious explanation for the above findings. Since little is known of the mechanism of deposition of tumor cells in organs, a good deal of supposition and additional work is necessary. The data suggest that the existence of fibrinolytic activity at the time of intravascular inoculation of tumor cells prevents the lodging of the tumor cells in the lungs, and as a result there are few metastases. It is possible that clumping of cells is necessary for lodging of cells and that fibrin formation maintains them till growth can take place. Apparently this phenomenon may be of significance not only with the VX2 carcinoma of rabbit but also in other animal tumors, such as the Brown-Pearce carcinoma of the rabbit and the Walker carcinosarcoma of the rat.

The present findings pose more questions than they answer. If the tumor cells do not lodge in the lung, what becomes of them? Although there is transpulmonary passage of tumor cells, no liver metastases were found. It is not understood why the circulating cells do not lodge and produce metastases after the fibrinolytic enzyme activity ends in 2–3 hours. These data would suggest that in the rat Walker 256 carcinosarcoma the cells would disappear from the circulation within 1 hour and that fibrin deposition and microthrombi formation are significant mechanisms in the lodging of blood-borne tumor cells in the Walker 256 carcinosarcoma. However, no studies were done to...
elucidate the problem of the dormant cell which might begin to form gross metastases if mechanical or chemical stimulation were applied.

REFERENCES

Fig. 1.—Autopsy specimens. To the left, a lung of a rat that died of multiple pulmonary metastases after 100,000 cells of Walker 256 carcinosarcoma by intravenous inoculation. Note the multiple tumor nodules. To the right, a lung of a rat that was sacrificed 9 weeks after inoculation of 100,000 cells of Walker 256 carcinosarcoma intravenously preceded by plasmin, 5000 units/kg, intravenously. Note the absence of visible metastases. Both specimens were fixed in formalin for 2 weeks prior to photography, and this accounts for the apparent difference in size of the two lungs. The uninvolved lung tissue shrank when fixed.
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