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Effect of Defibrination on Tumor Growth and Response to Chemotherapy[1]

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SUMMARY

Other investigators have demonstrated fibrin deposition in tumors. Experiments were therefore designed to test whether systemic defibrination would alter tumor growth or tumor response to chemotherapy with cyclophosphamide. Defibrination with Ancrod, a venom extract of *Agkistrodon rhodostoma*, did not significantly affect tumor sensitivity to chemotherapy. Similarly, defibrination plus fibrinolytic therapy with streptokinase did not affect responsiveness to cyclophosphamide. Long-term defibrination did not affect tumor growth. These results suggest three possible interpretations: (*a*) the coagulation system may not be important in tumor growth and response to chemotherapy; (*b*) adequate clearing of fibrin from the tumor was not accomplished in our experiments; or (*c*) other factors such as platelet deposition may be involved and platelet function was not inhibited by the therapies used in our experiments.

INTRODUCTION

Although an increased tendency to thrombosis in cancer patients has been recognized since Trousseau's classic observation (28), the coagulation mechanism has only recently been found to have a significant role in the growth of primary tumors and their metastases (5, 6, 16, 24, 25). O'Meara and Jackson (25) first noted the deposition of fibrin in a variety of tumors, especially at the invading periphery. He subsequently found various coagulants and fibrin in tumors at higher concentrations than in the surrounding tissues and suggested that a fibrin latticework facilitates vascularity and growth of the tumor tissue (24). This concept is supported by other demonstrations of the presence of factors X (15), XIII (31), and other "thromboplastins." Wood (30) observed the association between local fibrin formation and the successful experimental tumor embolization in animals. He suggested that the development of microthrombi enveloping tumor cells is an important mechanism by which tumor cells adhere to the vascular lining and migrate through vessel walls at metastatic sites. Accordingly, various attempts to reduce the growth and spread of tumors by anticoagulants and fibrinolysins have been made in man and in experimental animals (11, 12, 18). The results of these attempts are difficult to evaluate, since none of the therapeutic manipulations were sufficiently effective to abolish completely the fibrin deposition within the tumor.

Recently, the polypeptide Ancrod, extracted from the venom of the Malayan pit viper (*Agkistrodon rhodostoma*) and known to convert fibrinogen to fibrin, has been used i.v. to produce a complete defibrination in man and animals (3, 4, 19, 21). This preliminary study utilizes this defibrinating agent in association with cyclophosphamide in an attempt to prevent formation of fibrin deposits in tumors, thereby enabling the assessment of the role of fibrin deposition upon tumor sensitivity to chemotherapy. Additionally, a thrombolytic agent, streptokinase, is also used further to decrease the amounts of tumor fibrin by fibrinolysis.

MATERIALS AND METHODS

Drugs. Ancrod (Venacil) was kindly supplied by Abbott Laboratories, North Chicago, Ill. Defibrination was initiated with a dose of 100 units/kg s.c. and followed by 200 units/kg after a 12-hr interval. A maintenance dose of 400 units/kg was given s.c. every 12 hr subsequently. Rapid death, presumably due to the production of disseminated intravascular coagulation, occurred when the defibrination was initiated with the full 400 units/kg s.c. dose.

Streptokinase (Varidase, Lederle Labs., Pearl River, N. Y.) was given in doses of 800 IU s.c. every 8 hr.

Cyclophosphamide (NSC 26271) was kindly supplied by Mead Johnson Laboratories, Evansville, Ind. The dose was given s.c. as a single injection in a volume of 0.01 mg/g body weight.

Animals. Eight- to 12-week-old male C57BL × DBA/2 F_1 mice, 20 to 26 g, were housed in groups of 5 in plastic cages (Chart 1). On Day 0, they were given injections of 10 Lewis lung carcinoma cells (6) in the right hind leg muscle by a previously described method (8). After the carcinoma cells were given on Day 0 and Ancrod was given from Days 6 through 10, mice of Groups I, II, and III received cyclophosphamide s.c. on Day 10 in a dose of 80, 160, and 240 mg/kg, respectively.

In another series of experiments, mice of Group IV received 160 mg cyclophosphamide per kg on Day 17 with no defibrination therapy. Mice of Group V received Ancrod from Days 13 through 17 and also 160 mg cyclophosphamide per kg on Day 17. Mice of Group VI received Ancrod on Days 13 to 17, Ancrod plus streptokinase on Days 16 and 17, and 160 mg cyclophosphamide per kg on Day 17. Mice of Group VII received Ancrod every 12 hr from the time of tumor implant until death.

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Blood Studies. Blood was collected by cardiac puncture. The whole-blood clotting time, by the capillary method of Creskoff (7), was determined at 24-hr intervals on animals treated with Ancrod and on their controls. Fibrinogen levels were determined from platelet-poor plasma by Astrup's modification (2) of the method of Ratnoff and Menzies, using blood collected in 3.8% trisodium citrate; the lower limit of sensitivity of this fibrinogen assay was found to be 25 mg/100 ml.

Tumor Measurements. Tumor growth was determined by measuring tumor diameter 3 times weekly; the tumor diameter was converted to tumor weight as previously described (8).

RESULTS

With progressive defibrination, a significant prolongation of the whole-blood clotting time was produced so that, by 8 hr, the blood was incoagulable. Twelve hr after the initial doses of 100 or 200 units/kg, however, the clotting time had returned to pretreatment values. Effective and persistent defibrination was achieved, however, after doses of 400 units/kg every 12 hr. A significantly reduced plasma fibrinogen level was evident by 48 hr and, thereafter, fibrinogen was not detectable (Table 1).

The effect of Ancrod treatment from Days 6 to 10 on cyclophosphamide treatment on Day 10 is shown in Chart 2A. A dose of Ancrod was given just before cyclophosphamide and another dose was given 12 hr later so that defibrination would persist over the interval during which cyclophosphamide was present in the blood. Ancrod in this experiment had no effect on tumor growth *per se*. With increasing doses of cyclophosphamide, a greater antitumor effect was seen as manifested by a greater degree of tumor shrinkage. Pretreatment with Ancrod had no consistent effect on sensitivity to cyclophosphamide, and there was a suggestion of greater shrinkage after the use of cyclophosphamide in the higher dose (240 mg/kg; Chart 2*C*). The opposite effect seen after the use of cyclophosphamide, at a dose of 160 mg/kg (Chart 2*B*), may be explained by a 40% smaller tumor burden measurable initially, a ratio that persisted for the duration of the observations.

These results led us to speculate that a period of 4 days of defibrination might not have been sufficient to alter the fibrin content of the tumor. Thus, treatment regimens combining defibrinating and fibrinolytic agents were carried out. In experiments using this combination therapy, Lewis lung carcinoma of a more advanced stage was tested. More advanced stages of this tumor are known to be less sensitive to cyclophosphamide (8), and fibrin deposition might be a factor in this decreased sensitivity with increasing

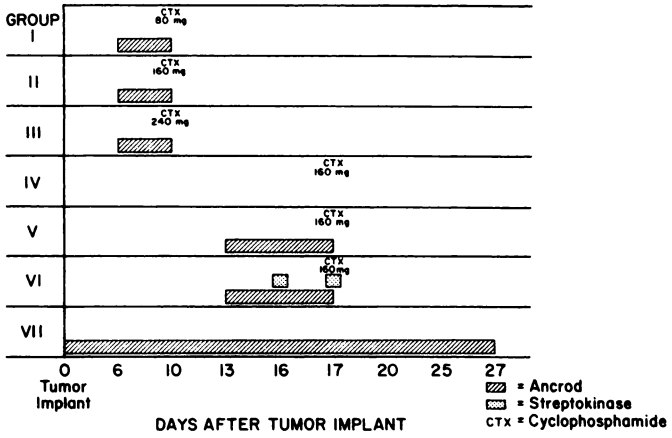

Chart 1. Schematic diagram of different therapeutic regimens used in the different groups of animals.

Table 1

Effect of Ancrod treatment on whole-blood clotting times and fibrinogen levels in tumor-bearing mice[a]

Sampling time (hr after initial dose)	Clotting time (min)	Fibrinogen[b] (mg/100 ml)
0	3.5	217
24	8.0	100
48	8.0	<25
72	12.0	<25
96	5.5	<25

[a] Mice received 400 units/kg s.c. every 12 hr.
[b] The lower limit of sensitivity of this assay in 25 mg/100 ml.

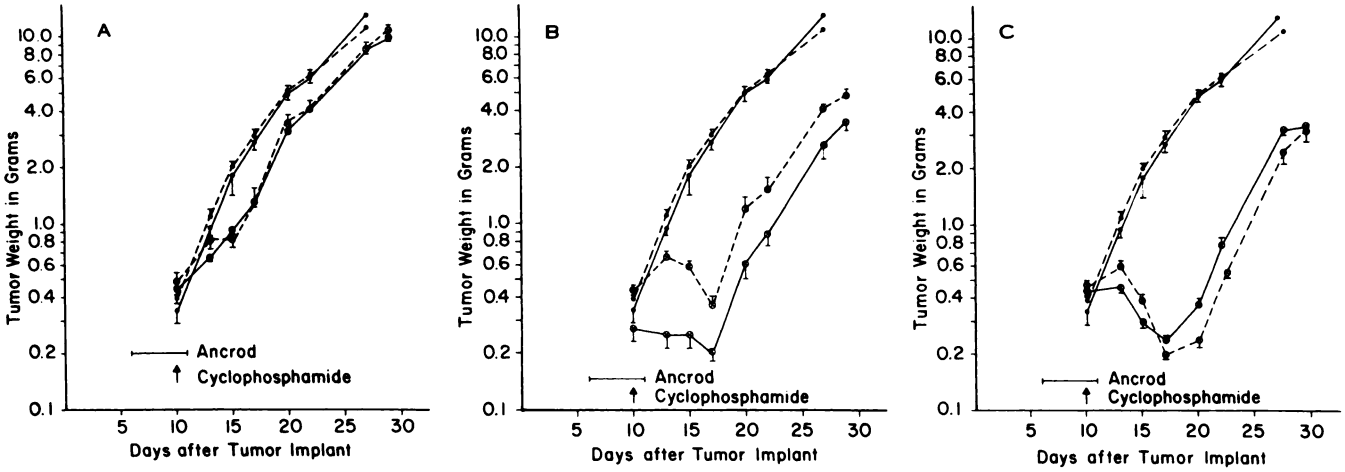

Chart 2. Effect of Ancrod pretreatment (Days 6 to 10) on tumor sensitivity to cyclophosphamide given on Day 10. Cyclophosphamide dose was 80 mg/kg. *A*, 160 mg/kg; *B*, 240 mg/kg; *C*, 240 mg/kg. ●——●, untreated control; ●- - -●, Ancrod only; ⊗——⊗, cyclophosphamide only; ⊗- - -⊗, Ancrod plus cyclophosphamide. *Vertical bars* show when S.E. is larger than symbol drawn. Sensitivity was not significantly affected by pretreatment with Ancrod.

tumor size. In the group receiving cyclophosphamide alone (Group IV), the tumor regressed slightly (less response than seen in Chart 2*B*) and then regrew (Chart 3). In the 2 groups (V and VI) receiving Ancrod before cyclophosphamide, tumor regression was of a similar degree as in the cyclophosphamide group (IV), with reference to tumor size on the day of cyclophosphamide treatment (Chart 3). The addition of streptokinase did not affect tumor response to cyclophosphamide (Group VI).

In the experiment shown in Chart 3, Ancrod *per se* (prior to cyclophosphamide) appeared to have a slowing effect on tumor growth, and this suggested that a longer duration of Ancrod treatment might significantly slow tumor growth. To evaluate this possibility, Group VII was treated with Ancrod, 400 mg/kg every 12 hr, beginning from the time of tumor implantation and continuing until death. As shown in Chart 4, prolonged Ancrod treatment had no effect on tumor growth.

It was observed that on Days 13 to 15, in the groups of animals receiving Ancrod (V and VI), a loss of body weight occurred, averaging 2 to 3 g. This was interpreted as due to a nonspecific loss of caloric intake as a result of the multiple injections of Ancrod. Such reduced caloric intake has been shown to slow tumor growth (9).

DISCUSSION

A pathophysiological approach to tumor microanatomy focuses our attention upon the components of the hemostatic system that are focally related within a tumor and may be postulated to play a role in tumor growth or spread. Because fibrin deposition along the walls of blood vessels has been observed to be an important mechanism for trapping metastatic tumor cells (30), efforts have been made to vary the fibrin available to the growing tumor and to the micrometastasis (1, 12, 16, 18). In addition to this postulated matrix role, intravascular or extravascular fibrin deposition in the tumor can conceivably provide a barrier to chemotherapeutic agents reaching the tumor from the hematogenous route. The systemic hemostatic parameters, unfortunately, may not reflect the hemostatic activity focally surrounding a tumor, because in addition to thromboplastic substances (5, 6, 24, 26), clotting factors (15, 31), and fibrin (25), malignant tissues contain inhibitors of fibrinolysis (22) and factors that may enhance platelet aggregation (14). Thus, in addition to fibrin, platelet depositions such as those observed around carcinoma cells (13, 14, 17, 29) may perform the same roles as are postulated for fibrin alone. The attempt in this study to investigate the sensitivity of Lewis lung carcinoma to cyclophosphamide was made under conditions of impaired fibrin deposition following defibrination by Ancrod and of increased fibrin removal following fibrinolysis by streptokinase. Our results suggested that this attempt did not succeed in changing the rate of growth or the response of the tumor to chemotherapy.

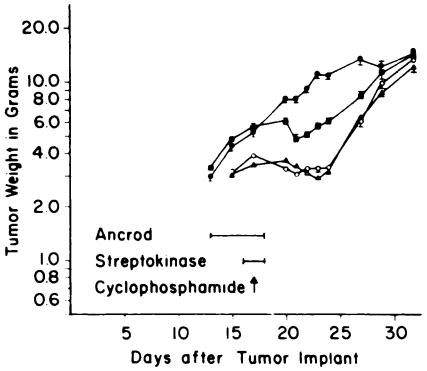

Chart 3. Effect of pretreatment with Ancrod and streptokinase on sensitivity to cyclophosphamide. ●, untreated control; ■, cyclophosphamide only; ○, Ancrod plus cyclophosphamide; ▲, Ancrod, streptokinase, and cyclophosphamide.

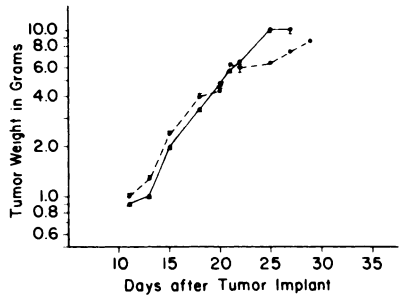

Chart 4. Effect of Ancrod treatment from the time of tumor implant until death in mice bearing Lewis lung carcinoma cells. ●——●, untreated control; ●- - -●, Ancrod treated.

Our findings may be interpreted in several ways. Ancrod produces systemic defibrination by converting fibrinogen into fibrin, after which the fibrin is removed by fibrinolysis and by the reticuloendothelial system (10, 11, 20). After the initial defibrination in man or animals, the continued long-term administration of Ancrod keeps the plasma fibrinogen concentration low for the extended duration of treatment. Whether extravascular fibrinogen can be depleted is not certain, but previous studies, using an immunofluorescent technique, have indicated that fibrin deposition in various viscera may continue even after Ancrod administration if the fibrinolysis is impaired by inhibitors of fibrinolysis such as ε-aminocaproic acid (27). Since it has been shown that tumor tissues of various types contain high levels of inhibitors of fibrinolysis, the local fibrin deposition in the tumor can be anticipated to occur in spite of systemic defibrination. Similarly, such local concentrations of fibrinolytic inhibitors would blunt the lytic effect of streptokinase upon the fibrin within the tumor. Thus, the failure of both Ancrod and streptokinase to influence the tumor sensitivity to cyclophosphamide does not necessarily preclude the role of fibrin in tumor growth.

It is known that platelets and tumor cells may adhere closely *in vitro* and *in vivo* (13, 14, 17, 29) and masses of aggregated platelets have been demonstrated around tumor cells. Platelets provide a source of fibrin that persists in spite of systemic Ancrod therapy. Thus, these platelets may not only provide fibrin and otherwise form a barrier to the transport of chemotherapeutic agents to the tumor, but they also provide a rich source of fibrinolytic inhibitors (23) and would thus prevent the lysis of fibrin by streptokinase in this study. A recent experiment in which the entrapment of tumor cells in the vascular endothelium of rats following i.v. inoculation of Walker 256 carcinoma cells was successfully

inhibited by the use of the cycloheptane derivative bencyclane hydrogen fumarate, which is a strong inhibitor of platelet function (14), supports the interest in this platelet role. A trial of platelet-inhibiting agents in this model of tumor growth and of response to cyclophosphamide seems warranted.

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