Reduced Incidence of Spontaneous Metastases with Long-Term Coumadin * Therapy

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THE IMPORTANCE of the coagulation mechanism in the intravascular metastatic spread of tumors has been established by the extensive work of Wood,[9] and Cliffton and Agostino.[2] Anticoagulation by various agents reduced the incidence of pulmonary embolic tumor growths after intravenous tumor cell injection, while agents inducing a hypercoagulable state increased this incidence. A similar effect of long term anticoagulation with Coumadin on the incidence of spontaneous pulmonary metastases in tumor bearing mice was noted by Orme.[6] Though the relationship has been well established, the conditions and parameters of treatment under which it will persist have not been. Anticoagulation has preceded tumor implantation, the level of anticoagulation has infrequently been determined, and anticoagulation has usually been of short duration. To assess the breadth of this effect of anticoagulation, several host-tumor systems were studied with long-term Coumadin therapy instituted, following establishment of the primary tumor and the level of anticoagulation held constant in the usual therapeutic range of two to three times the mean prothrombin time of normal subjects.

Materials and Methods

The host-tumor systems studied were the anaplastic sarcoma T241 of Lewis in C57BL/6N mice and the mammary adenocarcinoma in C3H/HeN mice. The former was induced in this strain by methylcholanthrene in 1938 and carried by transplantation. The latter, a spontaneous tumor, was in the 117th transplant generation. Both have a uniform, rapid growth rate and metastatic pattern well established by previous study.[3] All mice were females, 6 to 8 weeks old, housed individually, and fed on purina chow.

For each host-tumor system 100 mice were inoculated with tumor and then divided into an untreated control group and a Coumadin treated group of 50 mice each. The tumor inoculum was 0.05 ml. of a cell suspension prepared by passage of 1 Gm. of tumor and 10 cc. of 0.85% sodium chloride solution through a Snell cytocieve. Inoculation was subcutaneously into the left thigh. Anticoagulation was attained by use of Coumadin added to the drinking water which the mice took *ad lib.* This was begun the second day after inoculation, and water and Coumadin were changed alternately each third and fourth day thereafter. Dosages used were 9.325 mg. of Coumadin/l. of drinking water for the C57 mice and 9.215 mg./l. for the C3H strain. Prothrombin time determinations were done by Miale's micro-method using periorbital blood.[4] The effect of the above drug dosages on the prothrombin time had been previously determined by daily prothrombin times on mice identical to those under study. For mice in the study the prothrombin time was determined immediately prior to their being killed. For the Lewis T241-C57 system ten mice from each group were killed on days 7, 9, 11, 13, and

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TABLE 1. *Percentage of C57BL/6N Mice with Pulmonary Metastases when Sacrificed*

Groups	Days after Tumor Innoculation					Cumulative Total
	7	9	11	13	15	
1. Control	60%	50%	75%	90%	90%	73%
2. Coumadin treated:	10%	10%	15%	25%	15%	15%

Note: 20 mice in each group each interval totaling 100 for each group.
* Difference signif. $p < 0.001 - \chi^2$ test of difference between series of proportions.

TABLE 2. *Percentage of C3H/HeN Mice with Pulmonary Metastases when Sacrificed*

Groups	Days after Tumor Innoculation					Cumulative Total
	9	12	15	18	21	
Control	50%	60%	65%	65%	70%	62%
Coumadin treated	5%	15%	15%	20%	20%	15%

Note: 20 mice in each group each interval totaling 100 for each group.
* Difference signif. $p < 0.01 - \chi^2$ test of difference between a series of proportions.

15 following tumor inoculation. For the mammary adenocarcinoma the intervals were 9, 12, 15, 18, and 21 days. These times were chosen to divide the interval between the onset of detectable metastatic lesions and their maximum incidence into equal and progressive increments. Previous study had documented these times.[7] After the mice were killed their lungs were inflated with India ink, fixed in Fekete's solution and pulmonary metastatic lesions detected macroscopically by differential staining of tumor and normal lung tissue.[8] Each study was then repeated to determine reproducibility and degree of correlation.

Results

All 400 mice developed tumor enlargement of the inoculated thigh. Enlargement was first evident on the third or fourth day and reached a diameter approximating three times normal by completion of the study.

The daily mean prothrombin time of both Coumadin treated and untreated tumor bearing mice identical to the two systems here studied are shown in Figures 1 and 2. Among the mice under study the mean prothrombin time of the C57 control mice was 12.2 seconds, individual values ranging from 11.4 to 13.8 seconds. The prothrombin time of the C57 mice receiving Coumadin ranged from 2.1 to 3.0 times this control mean, 26.0 to 36.7 seconds. The mean among C3H untreated mice was 11.4 seconds (range from 10.3 to 11.9 seconds) with the prothrombin times of the C3H Coumadin treated mice ranging from 2.2 to 3.3 times this mean value, from 24.6 to 37.5 seconds. Prolongation of the prothrombin time beyond one-minute, at which level spontaneous fatal hemorrhage would occur, was entirely avoided.

Comparison between the percentage of mice with pulmonary metastases in the initial and repeated studies for both treated and untreated groups shows a high degree of correlation for both host-tumor systems (Figs. 3 and 4). In both host-tumor systems, the group and interval being the same, any result from the repeated study falls within the 95% confidence intervals of the comparable value from the initial study. Thus the results of the initial and repeated studies were pooled for subsequent analysis.

The incidence of metastases was signifi-

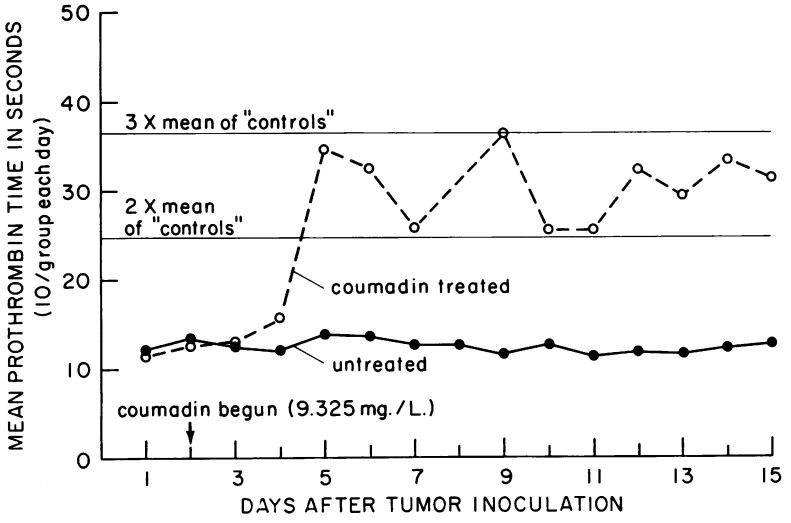

FIG. 1. Mean prothrombin times of Coumadin treated and untreated C57BL/6N mice with T241 tumor.

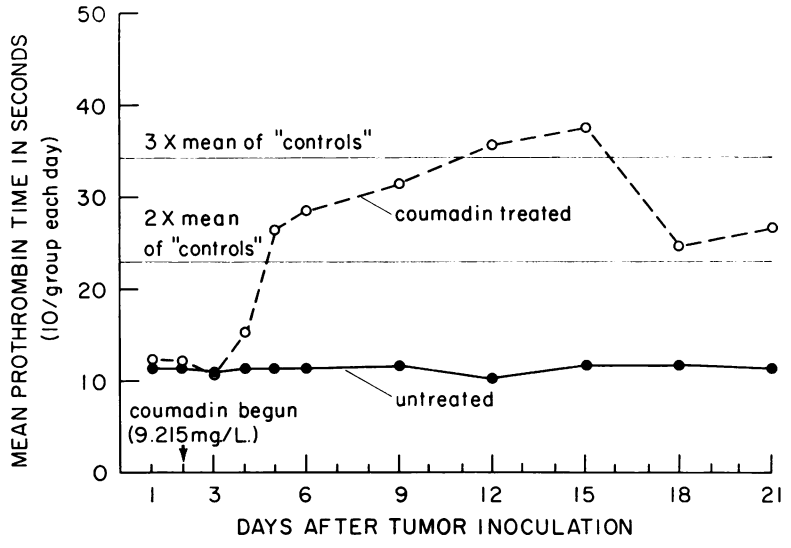

FIG. 2. Mean prothrombin times of Coumadin treated and untreated C3H/HeN mice with mammary adenocarcinoma.

cantly less among the Coumadin treated mice in both systems studied. At every interval in both host-tumor systems the frequency of metastases among untreated mice was much higher than among the treated group, from 3.2 to 10.0 times increased (Tables 1 and 2). While in each host-tumor system 15% of the Coumadin treated mice evidenced metastatic spread, 73% of the untreated C57 mice and 62% of the untreated C3H mice were so affected. These differences are all significant statistically at a level where $p < 0.01$. For each metastatic lesion found in the Coumadin treated C57 mice there were 12 in the untreated controls, and in the C3H mice 6 lesions among the controls for each one among the treated mice (Tables 3 and 4). Among the C57 mice developing metastatic lesions, those receiving Coumadin averaged fewer lesions, 2.0 as compared to 5.0 in the untreated mice.

Discussion

The effectiveness of anticoagulation in lowering the incidence of pulmonary metastatic spread in numerous host-tumor sys-

TABLE 3. *Pulmonary Metastatic Spread from Lewis T241 in Coumadin Treated and Untreated C57BL/6N Mice*

Groups	No. of Mice	No. with Metastases	Total No. of Metastases	Metastases/ Mouse with Metastases*
Control	100	73	365	5.0
Coumadin treated	100	15	29	2.0

* Difference significant, $p < 0.02$, t test.

TABLE 4. *Pulmonary Metastatic Spread from Mammary Adenocarcinoma in Coumadin Treated and Untreated C3H/HeN Mice*

Groups	No. of Mice	No. with Metastases*	Total No. of Metastases	Metastases/ Mouse with Metastases
Control	100	62	236	3.8
Coumadin treated	100	15	41	2.7

* Difference signif. $p < 0.01$ — 99% confidence interval computation.

tems was well summarized by Wood, Holyoke, and Yardley.[9] Using intravenous tumor cell injection pulmonary embolic tumor growth was reduced by acute anticoagulation with various agents. Agents used were heparin, dicoumerol and fibrinolysin; hosts have been rats, mice, and rabbits; and tumors have included Walker 256, Lewis 150 and 241, V2 carcinoma, Brown-Pierce carcinoma and Guerin TA carcinoma. When the tumor was inoculated subcutaneously, thus giving rise to spontaneous metastatic spread, heparin and plasmin effected a reduction among mice with T241 tumor, heparin was effective in rats with Walker 256 and Lewis 150 tumor, and Coumadin was effective in rats with Guerin TA carcinoma.

Though the association of anticoagulation and retarded metastatic spread has been documented in many host-tumor systems, the details of the level of anticoagulation and its constancy have been sparse. Agostino using intravenous injections of Walker 256 in rats lowered the incidence of pulmonary embolic tumor growth and improved survival by Coumadin therapy

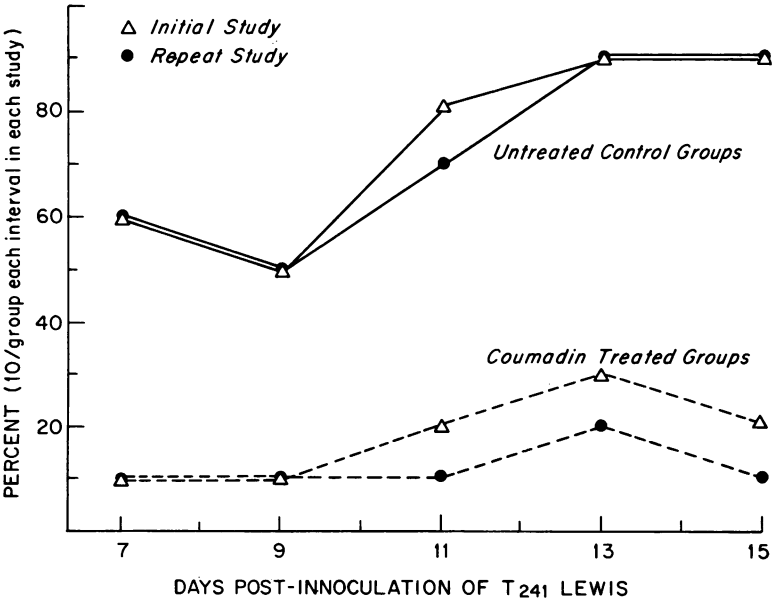

FIG. 3. Percentage of C57BL/6N mice in the initial and repeat studies with pulmonary metastases when sacrificed.

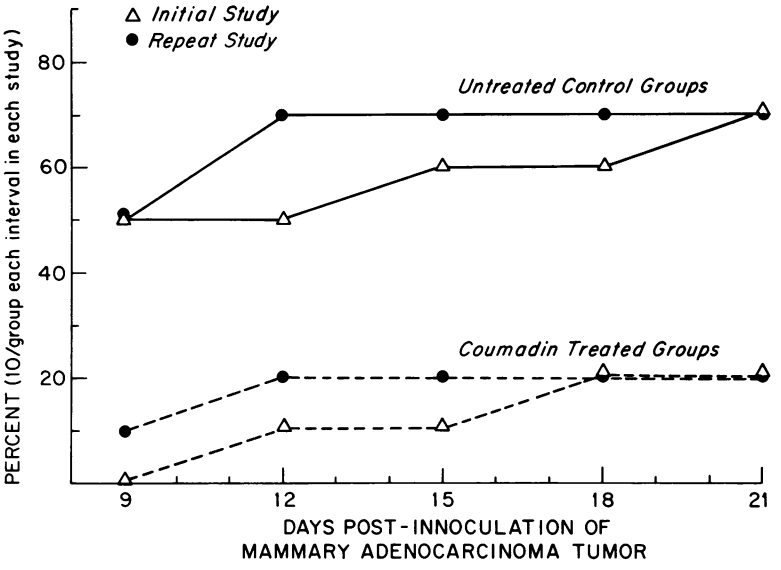

FIG. 4. Percentage of C3H/HeN mice in the initial and repeat studies with pulmonary metastases when sacrificed.

which prolonged the prothrombin time more than twice the average value in normal rats.[1] In the same host-tumor system Cliffton obtained similar results with an average prothrombin time of 93 seconds.[2] It is encouraging to see the persistence of this retarding effect of Coumadin on pulmonary metastatic spread when the level of anticoagulation is constantly maintained in a range which is in common therapeutic use. Excessive levels of anticoagulation with resultant unacceptable hemorrhagic complications would not appear necessary to achieve this desired effect.

This effectiveness of Coumadin and other anticoagulants in reducing the incidence of metastatic spread in a wide variety of animal host-tumor systems is suggestive that it may influence some aspect of the metastatic process common to many malignant tumors. The illuminating work of Wood on the pathogenesis of metastasis formation assigns a decisive role to the formation of an intra-capillary microthrombus about the embolic tumor cells shortly following initial endothelial adherence.[10] In instances where an enveloping microthrombus with evident fibrin-like material did not form, the tumor cells did not penetrate the endothelium but were dislodged and carried away. As each patient with a malignant growth may well be a biologically different host-tumor system, an effect against a common mechanism of metastasis formation could be very advantageous. The retrospective study of Michaels lends encouragement that anticoagulants may favorably influence metastatic spread in human cancer as well.[5] Further studies are currently in progress to evaluate the use of Coumadin as an adjunct to standard cancer therapy.

Summary

Long-term oral Coumadin therapy instituted after establishment of the primary tumor and producing a constant and safe level of anticoagulation significantly reduced the incidence of spontaneous pulmonary metastases and significantly increased the number of mice free of pulmonary metastatic disease.

Summary

The effect of a constant, safe level of Coumadin anticoagulation on the incidence of spontaneous pulmonary metastatic spread was studied in two mouse tumor systems. Prothrombin time prolongation

was held constant in the range of two to three times the normal average value. The period of observation extended from shortly after the onset of metastatic spread into the range of its maximum incidence. The Coumadin treated mice had a significantly reduced incidence of metastases and a significantly increased number were free of pulmonary metastatic disease at each interval of comparison in both tumor systems. Thus a significant reduction of spontaneous pulmonary metastatic spread was achieved by a level of Coumadin anticoagulation in common therapeutic usage.

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