Platelet and Fibrinogen Kinetics in Canine Tumors

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ABSTRACT

Fifty-three dogs with spontaneously occurring tumors were evaluated for abnormalities in the concentration and in vivo survival of platelets and fibrinogen. Thrombocytopenia occurred only in animals with extensive tumor involving spleen or marrow. Platelet survival was shortened in 6 of 15 (40%) dogs with localized tumor [mean 4.4 days ± 0.3 (S.E.); normal 5.4 days ± 0.1] and 30 of 35 (80%) dogs with metastatic tumor (mean 3.2 days ± 0.2). Platelet survival progressively shortened during studies performed in dogs with ongoing disease.

Fibrinogen concentration was increased (mean 420 ± 30 mg/dl) in 44 of 53 (83%) of tumor-bearing dogs (normal 210 ± 10 mg/dl). Neither histology nor extent of disease, including presence of hepatic metastases, appeared to influence fibrinogen concentration significantly. Fibrinogen survival was below the normal range in 3 of 15 (20%) dogs with localized tumor and in 9 of 34 (26%) dogs with metastatic tumors. Thus, platelet consumption appeared to be the most significant hematostatic abnormality in tumor-bearing dogs. This model may be useful in evaluating the efficacy of antithrombotic therapy in preventing tumor-related hematostatic abnormalities.

INTRODUCTION

Hemorrhagic and thrombotic events have been estimated to complicate the clinical course in 30% of patients with neoplastic disease, while laboratory evidence of altered hemostasis is even more common, occurring in 80 to 90% of patients (19). The mechanisms underlying these findings are still incompletely understood. In patients with leukemia or lymphoma, decreased marrow production of platelets (25), disseminated intravascular coagulation due to release of procoagulant material by malignant cells (10, 22), and immune mechanisms may produce severe thrombocytopenia leading to hemorrhage (23). Thrombotic events are more characteristic of patients with solid tumors. Factors implicated in the development of thromboses are tumor-induced platelet adhesion and aggregation (6), release of procoagulant material by tumors (9), and incomplete endothelialization of tumor vascular beds (28). The balance among these factors may vary with tumor type and growth rate.

In vivo studies of hemostasis in human subjects with tumors are often difficult to interpret, since it may be impossible to separate coagulation and platelet abnormalities intrinsic to the neoplastic process from those caused by chemotherapeutic agents or intercurrent infection. On the other hand, in vitro models may not accurately reflect the interaction of tumor cells with platelets and endothelium while rodent models generally involve transplantable rather than spontaneous tumors. To avoid these problems, we have studied dogs with naturally occurring tumors to evaluate the effect of tumor histology and the extent and progression of disease on platelet and fibrinogen homeostasis. Dogs with spontaneous tumors have already been shown to provide a large animal model suitable for studies clinically relevant to humans (4, 29, 31). Such dogs can be studied without the additional variables of concurrent surgery, chemo-, or radiotherapy and can also be treated with antithrombotic agents not yet suitable for trials in humans.

We thus sought to characterize a model system to use for future studies on the role of antithrombotic therapy in prevention of tumor-associated consumption of clotting factors, reduction of thromboembolic events, and prevention of metastasis.

MATERIALS AND METHODS

Dogs with Tumor. Fifty-two dogs with spontaneously occurring tumors were referred by veterinarians in Washington and Oregon to the Fred Hutchinson Cancer Research Center as described previously (4). One additional dog was studied after i.v. injection of transmissible venereal sarcoma (tumor cells supplied by Dr. R. Epstein, Abraham Lincoln School of Medicine, West Side V. A. Hospital, Chicago, III.) (3). This animal developed pulmonary and cutaneous metastases 14 weeks after inoculation. Histological diagnoses were obtained from either biopsy or autopsy material. Fourteen dogs had fibro- or osteosarcoma; 14 had lymphoma; and 9 had adenocarcinoma: 4 prostate, 4 breast, and 1 rectum. Other dogs had hemangioepithelioma (4), melanoma (3), mastocytoma (3), venereal sarcoma (3), chronic lymphocytic leukemia (2), and one had renal cell carcinoma.

All animals were evaluated for extent of disease by physical examination, complete blood counts, chest X-ray, and when indicated, determination of blood chemistry values. Autopsy confirmation of extent of disease was available in 40 dogs. Fifteen dogs with tumor limited to one site at autopsy were considered to have local disease; 38 dogs with either regional nodal involvement or widely disseminated metastases were considered to have metastatic disease. Dogs with clinical Stage III or IV lymphoma and chronic lymphocytic leukemia were included in the metastatic group.

None of the dogs had surgery or chemotherapy in the month preceding or during the period of study. There were no episodes of infection or septicemia as determined by clinical examination and weekly blood cultures.

Normal Dogs. Thirty-four dogs of mixed breeds were obtained from the King County, Washington, dog pound and 7 healthy, randomly bred dogs from Sunnyvale Kennel, Squimm, Washington.
Platelet Counts and Survivals. Platelet counts were determined twice weekly with a Coulter S counter (Coulter Electronics, Inc., Hialeah, Fla.) (2).

Autologous platelets, harvested from 50 to 100 ml of acid citrate dextran-anticoagulated blood, were radiolabeled with $^{51}$Cr (New England Nuclear, Boston, Mass.) and injected as described previously (30). Five-mi peripheral blood samples, anticoagulated with 0.2 ml of 1% EDTA, were drawn 2 hr postinjection and daily for the next 4 days to determine the disappearance rate of the $^{51}$Cr-labeled platelets. Platelet recovery and survival were calculated by the least-squares method, as outlined by Murphy et al. (17).

Fibrinogen Concentration and Survival. Fibrinogen concentrations were determined twice weekly as total clottable protein using the alkaline urea method described by Jacobsson (14).

Blood for fibrinogen labeling was drawn from a normal donor animal, and a fibrinogen fraction was prepared by ammonium sulfate precipitation. The purified fibrinogen was labeled with $^{125}$I (New England Nuclear) by the iodine monochloride technique (26). Weekly injections of labeled fibrinogen were performed simultaneously with the injection of the labeled autologous platelets. Separate 3-ml blood samples drawn into EDTA-$\epsilon$-aminocaproic acid at the same intervals as for the platelet samples were used to determine $^{125}$I-fibrinogen disappearance. Fibrinogen survival was determined as the half-life divided by the natural log of 2 (20).

Statistical Analysis. The standard error of the mean was used to express variance among groups while the standard deviation was used to express variance among animals within a group. Rank-order (Mann-Whitney-U) tests of statistical significance were used to assess differences between groups of normal and tumor-bearing dogs (27). The paired Wilcoxon signed-rank test was used in the serial studies (32).

RESULTS

Normal Dogs. In normal dogs, the mean platelet count was 334,000/$\mu$l (Table 1). The normal range, defined as the mean ± 2 S.D., was 104,000 to 564,000/$\mu$l. Mean platelet recovery after i.v. injection of $^{51}$Cr-labeled platelets was 55%, with a range of 45 to 65%. Mean platelet survival was 5.4 days with a range of 4.2 to 6.6 days. Mean fibrinogen concentration was 210 mg/dl, with a range of 190 to 230 mg/dl, while mean fibrinogen survival was 2.6 days with a range of 2.0 to 3.2 days.

Tumor-bearing Dogs. The platelet and fibrinogen data for all tumor-bearing dogs are shown in Table 1. Both mean platelet count and platelet survival were significantly decreased in dogs with tumor compared to normal dogs, while mean fibrinogen concentration was increased and fibrinogen survival was normal.

Effects of Histology. When dogs with tumors were subdivided into histological categories (Table 1), platelet counts in these categories were not significantly different from normal (partly because the number of dogs in each group was relatively small). However, platelet survival was significantly shorter than normal for dogs in each histological subgroup (Table 1). Mean platelet survival was somewhat less in dogs with adenocarcinoma or lymphoma than in dogs with sarcoma, although these differences were not statistically significant. However, when individual platelet survivals were analyzed, 44 and 42% of dogs with adenocarcinoma and with lymphoma, respectively, had values less than one-half of normal (<2.7 days) compared with only 7% of dogs with sarcoma.

Platelet recovery was normal when all dogs with tumor were considered together but was less than normal among dogs with lymphoma (Table 1). Seven of the 14 dogs with lymphoma had moderate to marked splenomegaly. Mean platelet recovery for these 7 dogs was 31% ± 2 and for the 7 dogs without splenomegaly was 46% ± 2, and associated platelet counts were 188,000/$\mu$l ± 34,000 and 245,000/$\mu$l ± 37,000, respectively. Thus, increased splenic pooling appeared to account for the decreased platelet recovery and contributed to the thrombocytopenia observed in some dogs with lymphoma (11).

Fibrinogen concentrations were abnormal in almost all tumor-bearing dogs. In 87%, the fibrinogen concentrations were above the normal range (>230 mg/dl), and 10% had fibrinogen concentrations below the normal range (>180 mg/dl). Fibrinogen concentration was significantly higher in dogs with sarcomas than with other tumors ($p < 0.01$).

In contrast, most of the affected dogs had fibrinogen survivals within the normal range. Only 12 dogs (23%) had low levels, and 10 of these 12 animals had concomitant shortening of platelet survival. However, this measurement in dogs with lymphoma and adenocarcinoma was statistically below normal (Table 1).

Effects of Extent of Disease on Platelet and Fibrinogen Homeostasis. Coagulation measurements in dogs with localized tumors were compared to those in dogs with metastatic disease (Table 2).
Mean platelet counts were not significantly different in dogs with local or metastatic tumor. However, in 6 dogs with metastatic disease, the platelet count was below the normal range (<104,000/μl) as compared with only one dog with localized disease. The latter dog had evidence of increased splenic pooling of platelets (20% recovery), and at autopsy, a recent large splenic infarct was found. Four of the 6 markedly thombocytopenic dogs in the metastatic disease group had evidence of marrow replacement (2 dogs with lymphoma, one with chronic lymphocytic leukemia had marrow tumor invasion, and one dog with prostate adenocarcinoma had marrow fibrosis). Marrow replacement could not be documented to account for the observed severe thrombocytopenia in the 2 other dogs, one with Stage IV lymphoma involving skin and liver and the other with breast carcinoma metastatic to liver and spleen. However, the dog with lymphoma had the shortest platelet survival observed in this study (i.e., 1.2 days). No reason for the thrombocytopenia of 92,000/μl could be found in the remaining dog with breast carcinoma. The platelet recovery was 39% and survival 3.7 days.

In addition to the 4 dogs discussed above, there were 2 other animals with evidence of marrow replacement. One dog with chronic lymphocytic leukemia had a normal platelet count. The second, a dog with lymphoma, developed a leukemic phase 2 weeks after initial study, and his platelet count decreased from 220,000 to 60,000/μl. Thus, 5 of 6 dogs with marrow involvement had or developed severe thrombocytopenia.

The mean platelet survival in the dogs with metastatic disease was 3.2 days, i.e., 1.2 days less than the platelet survival in the animals with local disease (p < 0.05) (Table 2). The tendency for platelet survival to be shorter among dogs with metastatic disease was also evident when the dogs were subdivided by histology, both among dogs with sarcoma and those with adenocarcinoma. Thirteen of 14 dogs with lymphoma had advanced disease; thus, the effects of the extent of disease could not be directly assessed in this category (Table 1).

Fibrinogen concentrations did not vary significantly between dogs with local or metastatic tumor, considering either all dogs or histological subgroups (Table 2). Among 10 dogs with hepatic metastases, the mean fibrinogen concentration was 360 ± 70 mg/dl, not significantly different from the dogs without hepatic involvement. One dog, whose liver was almost entirely replaced by melanoma, had marked hypofibrinogenemia (23 mg/dl) and a normal fibrinogen survival of 2.7 days. In contrast to fibrinogen concentrations, fibrinogen survivals were significantly shorter among dogs with metastatic disease than with local disease. This difference in fibrinogen survival between dogs with local and metastatic disease was more pronounced in the animals with adenocarcinoma than in those with sarcoma (p < 0.01).

When abnormalities in mean platelet survival were compared with those in mean fibrinogen survival (Chart 1), dogs with local tumor were found to have a modest shortening of mean platelet survival but no change in mean fibrinogen survival. Forty % of dogs with local tumors had platelet survivals <4.2 days and normal fibrinogen survivals, while the remaining 60% had normal values for both measurements. Dogs with metastatic tumor had shortening of both platelet and fibrinogen survival but relatively greater shortening of platelet than of fibrinogen survival. Sixty-three % of dogs with metastatic tumors had decreased platelet survival with normal fibrinogen survivals, while 22% had decreases in both platelet and fibrinogen survival.

Tumor Growth. In untreated dogs with tumors, weekly platelet and fibrinogen survivals were performed to assess the rate of platelet and fibrinogen consumption. Twenty-three dogs were studied twice, and 7 were studied 3 times (Chart 2). Platelet count and fibrinogen concentration remained essentially stable, while platelet survival and, to a lesser extent, fibrinogen survival decreased during the 3 weeks of observation.

Weekly platelet and fibrinogen measurements were made in one dog following i.v. injection of venereal sarcoma cells. At 14 weeks postinjection, the dog developed palpable cutaneous nodules and pulmonary metastases by X-ray. With the appearance of clinically evident disease, both platelet count and platelet survival decreased significantly (Chart 3), while fibrinogen concentration and survival remained about the same. The most dramatic changes in platelet count and survival occurred during the 2 weeks after development of palpable tumor. During those 2 weeks, both the number and size of the cutaneous and pulmonary nodules increased.

**DISCUSSION**

In the current studies, we investigated whether we could reduplicate in dogs with spontaneously occurring tumors results found previously in humans. In dogs, as in humans (24), we found that moderate to severe thrombocytopenia was un-

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**Table 2**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Extent of disease</th>
<th>No. of dogs</th>
<th>Platelet Count (x 10^3/μl)</th>
<th>Platelet Survival (days)</th>
<th>Fibrinogen Concentration (mg/dl)</th>
<th>Fibrinogen Survival (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All tumor dogs</td>
<td>Local</td>
<td>15</td>
<td>270 ± 30</td>
<td>4.4 ± 0.3</td>
<td>430 ± 50</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>Sarcoma</td>
<td></td>
<td>3</td>
<td>270 ± 20</td>
<td>4.6 ± 0.3</td>
<td>490 ± 60</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>Local</td>
<td>2</td>
<td>160 ± 30</td>
<td>4.6 ± 0.4</td>
<td>500 ± 80</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Metastatic</td>
<td>7</td>
<td>180 ± 30</td>
<td>2.7 ± 0.3</td>
<td>370 ± 50</td>
<td>1.7 ± 0.4</td>
</tr>
</tbody>
</table>

* p = 0.05.
* p = 0.001.
Chart 1. Effects of extent of disease on platelet and fibrinogen survivals. Only platelet survival was reduced in dogs with local tumor while both platelet and fibrinogen survivals were shorter in metastatic tumor. Proportionate decreases in survival of platelets and fibrinogen. Bars, S.E.

Chart 2. Sequential weekly platelet and fibrinogen data in tumor dogs. Twenty-three dogs had 2 studies, and 7 dogs had 3 studies. Hatched areas, normal mean ± S.E.; bars, S.E.

Chart 3. Weekly platelet counts and survivals before and after i.v. injection of venereal sarcoma cells. Fourteen weeks after inoculation, the dog developed pulmonary and cutaneous tumor nodules. In the next 2 weeks, rapid tumor proliferation (nodules increased from an average of 0.5 to 4.0 cm) was associated with decrease in platelet count and survival. Measurable tumor proliferation slowed after 2 weeks. +, animal sacrificed.

common in animals with solid tumors (7 of 52 dogs; 13%) and was predominately confined to dogs with either splenic or marrow disease (5 of 7 dogs; 71%). Thus, despite the frequent occurrence of diminished platelet survival (68%), megakaryocyte reserves were usually adequate to maintain normal platelet numbers. As in humans, short platelet survivals were found to be directly related both to extent of disease and to histology (24). Increased platelet consumption was more frequent in dogs with metastatic tumor (79%) than in dogs with localized tumor (40%), and progressive disease in untreated animals further reduced platelet survivals. Shortening of platelet survival was most pronounced in dogs with metastatic adenocarcinoma or advanced lymphoma; dogs with sarcoma had a lesser degree of platelet consumption. The shortened platelet survival in dogs with tumors may reflect direct platelet-tumor cell interactions with platelet aggregation (16), adherence to incompletely endothelialized tumor vessels (1), or alterations of the platelet surface by tumor-related proteins with in vivo aggregation and premature removal from circulation. For example, Gasic et al. (5) demonstrated that platelets incubated with supernatant from mouse mammary carcinoma cultures had high spontaneous aggregation and release of serotonin.

Hyperfibrinogenemia was the most common laboratory abnormality in these tumor-bearing dogs. However, since fibrinogen concentration is increased in most inflammatory processes (33), this measurement has limited usefulness in studies of tumor activity of antithrombotic therapy.

Fibrinogen survival was less likely to be abnormal than platelet survival in dogs with tumor, suggesting that these 2 events are not, in general, interdependent. The markedly shortened fibrinogen survival in dogs with metastatic adenocarcinoma, however, may reflect in vivo activation of the coagulation cascade by tumor products. Similar observations have been reported by Gordon et al. (8) in human patients with breast carcinoma with production of Factor X activator by the tumor. However, disseminated intravascular coagulation could not be implicated in the majority of animals with normal fibrinogen survivals and decreased platelet survivals.

In this study, we have demonstrated that platelet and fibrinogen kinetics in dogs with spontaneous tumors are similar to those in human subjects (15). The relationships observed may be important in designing clinical trials for prophylaxis of thrombosis or prevention of metastases in patients with tumors. For example, if platelet consumption predominates, platelet function inhibitors might provide adequate antithrombotic protection (12) or impair deposition of metastatic tumor cells (7). However, if there is parallel consumption of both platelets and fibrinogen, anticoagulants alone (12,13) or in combination with platelet function inhibitors might be needed. Further kinetic studies in the canine model and in humans may help to elucidate characteristic patterns of platelet and fibrinogen consumption in different histological subgroups of neoplasms and to their correction with antithrombotic drugs. Studies of other important platelet reactions, such as aggregation and granule secretion (21) and of the mechanism of fibrinogen catabolism (18) may also provide further insight into the relationship between hemostasis and malignancy.

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