Interruption of Tumor-Associated Platelet Consumption With Platelet Enzyme Inhibitors

By Sherrill J. Slichter, Paul L. Weiden, Margaret R. O’Donnell, and Rainer Storb

Twenty dogs with naturally occurring metastatic tumors were treated with anticoagulants (Warfarin) or platelet enzyme inhibitor drugs (dipyridamole, dipyridamol plus aspirin, RA233, sulfipyrazone, or a combination of RA233 and sulfipyrazone) to determine if tumor-related reductions in platelet survival and concentration could be reversed. Anticoagulation was ineffective, while platelet enzyme inhibitors were able to produce improvements in platelet survival. Of the 18 dogs with metastatic tumor treated with platelet enzyme inhibitors, only 5 (28%) showed a reduction in platelet survival during the first week of observation on therapy compared to their baseline survivals. This is significantly different than the decreases in platelet survivals observed in 8 of 10 untreated dogs (80%) with metastatic tumor observed for the same inter-

Both hemorrhage and thrombosis are associated with malignant disorders. In most clinical situations, excessive bleeding is related to thrombocytopenia, which results from impaired platelet production secondary to either chemotherapy or radiation-induced marrow suppression or marrow invasion with tumor. However, hemorrhagic complications may also result from tumor-related increased utilization of hemostatic factors. The two major diseases associated with this type of bleeding are promyelocytic leukemia and metastatic prostatic adenocarcinoma. Prevention of hemostatic factor and platelet consumption (DIC) has been attempted by anticoagulation with heparin in patients with promyelocytic leukemia and by inhibition of fibrinolysis in patients with prostatic cancer. Other malignancies are known to be associated with consumptive processes, as evidenced by abnormalities in coagulation screening tests and platelet counts. These chronic DIC syndromes are generally “low-grade” and usually do not produce clinically significant bleeding. However, with the use of more aggressive chemotherapy and radiotherapy treatment programs, the incidence of thrombocytopenia and the requirement for platelet transfusion support has increased substantially. This makes it important to reverse any condition that accelerates platelet consumption. This would not only enhance the patient’s ability to maintain platelet counts but would prolong the survival of transfused platelets, thereby reducing the number of platelet transfusions required. Thus, platelet enzyme inhibitors with different mechanisms of action may have a synergistic effect in reversing the abnormal platelet hemostasis found in a variety of spontaneously occurring canine neoplasms.

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TUMOR-ASSOCIATED PLATELET CONSUMPTION

Platelet recovery was calculated by extending the survival curve to time zero (\(T_0\)). The radioactivity at \(T_0\) was multiplied by the blood volume of the animal estimated as 80 ml/kg, and this product was divided by the radioactivity of the platelet concentrate injected. Recovery in the 41 normal animals was 55% ± 1% of the platelets injected.

Platelet turnover (a measure of the number of platelets destroyed per day and, in a steady state with constant platelet counts, a reflection of platelet production) was determined by dividing the peripheral platelet count by the platelet survival and correcting for recovery.\(^{11}\) In the normal animals, platelet turnover was 105,000 platelets/μl/day ± 16,000.

Fibrinogen concentration. Twice weekly, a sample of blood was drawn into 0.1 ml of EDTA/EACA (epilone-amino-capric acid) and fibrinogen was determined by a method that measures total clottable protein.\(^{14}\) Normal fibrinogen concentration was 2.10 ± 0.10 mg/ml.

Fibrinogen kinetics. One hundred milliliters of blood were drawn from a single donor animal into ACD once a month to prepare purified fibrinogen for \(^{125}\)I labeling.\(^{17}\) An aliquot of the \(^{125}\)I-labeled purified dog fibrinogen was injected simultaneously with the radiolabeled autologous platelets. Blood samples were drawn from the animal at 1 hr postinjection, twice daily for the next 2 days, and then once a day for the ensuing 2 days to determine the disappearance rate of the radioactively labeled fibrinogen. Blood samples were analyzed for radioactivity in a Searle gamma counter. Survival was determined by least squares computerized fitting to determine the half-time disappearance, which was divided by the natural log of 2. Normal fibrinogen survival was 2.6 ± 0.1 days. Turnover was calculated by dividing fibrinogen concentration by survival. Fibrinogen turnover in normal dogs was 0.77 ± 0.05 mg/ml/day.

Drug Programs

Each dog served as his own control, i.e., a 1-wk baseline study was performed before starting any drug treatment. Drug therapy was initiated 3 days before the start of week 1 "on drug" survival study and continued for at least 2 wk, i.e., weeks 1, 2, etc. "on drug."

Statistics

Statistical evaluation for baseline data in each group of animals was done using a t statistic for two means comparing the data to the observations obtained in the 41 normal dogs (Table 1). For the serial measurements (Table 2) or the weeks of observation on a drug treatment (Table 3), a paired t test was used with control data being the dogs' baseline measurements. Statistics were not done for those treatment groups with less than two observations.

RESULTS

Untreated Dogs

The hemostatic data obtained in 15 dogs with localized tumors and in 23 dogs with metastatic tumors not given antithrombotic therapy are given in Table 1.\(^{11}\) Platelet survival in dogs with localized tumors was modestly reduced to 4.4 ± 0.2 days compared to the normal value of 5.4 ± 0.1 days (\(p < 0.05\)), but platelet concentration and turnover were not significantly different from normal.

In contrast, the 23 dogs with metastatic tumors had a significant decrease in both platelet concentration and survival, resulting in an increased platelet turnover.
of 1.6 times normal. When the platelet survival was reduced to less than 4.0 days, a proportionate decrease in platelet count was found (correlation coefficient of 0.76, Fig. 1).

Serial platelet and fibrinogen parameters were obtained in 6 animals with local tumors and 10 animals with metastatic disease (Table 2). The data in dogs with localized tumors, including platelet survival measurements (Fig. 2), remained essentially constant. In contrast, platelet survival decreased by more than half a day in 8 of the 10 dogs with metastatic disease in the second week of observation (p < 0.005) (i.e., week 1 after baseline) (Table 2 and Fig. 2). This resulted in a platelet turnover of 2.7 times normal; in 4 animals, platelet counts were reduced by at least 90,000/μl (Fig. 3). Other measurements were unchanged from baseline values.

Treatment Programs

Platelet Enzyme Inhibitors

Eighteen dogs were treated with one or more antiplatelet agents and had at least 1 wk of data obtained on treatment. In 10 dogs, data from 2 wk or more on treatment were available.

Four dogs were given RA233, a phosphodiesterase inhibitor (25 mg/kg b.i.d.). Only 1 of the 4 dogs showed an improvement in platelet survival from 1.5 days to 5.1 days (Fig. 2 and Table 3). This was associated with a minimal change in platelet count from 130,000/μl to 152,000/μl (Fig. 3). The 3 other dogs showed a constant platelet survival while on therapy (Fig. 2) and no improvement in platelet counts (Fig. 3). No significant changes in fibrinogen parameters occurred in any of the 4 dogs (Table 3). Two dogs died after 1 wk on treatment and one was euthanized. An additional week of treatment in the remaining dog did not result in any further improvements (Table 3, Figs. 2 and 3).

Five dogs received sulfinpyrazone, a cyclooxygenase inhibitor (500 mg/kg b.i.d.). During the period of treatment (ranging from 10 to 25 days), platelet survivals improved in each of the dogs (Fig. 2 and Table 3). However, of the 5 dogs studied, only 1 achieved a platelet survival within the normal range. Maximum improvement occurred during the second week on therapy, i.e., average platelet survival was 2.5 ± 0.4 days baseline, 3.0 ± 0.5 days during the first week of treatment (p > 0.05), and 3.6 ± 0.2 days during the second week on therapy (p < 0.05). In the 2 dogs studied for 3 treatment weeks, platelet survival

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<tr>
<th>Table 1. Baseline Platelet and Fibrinogen Kinetics</th>
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<tr>
<td>Normal</td>
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<tr>
<td>Local tumor</td>
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<tr>
<td>Metastatic tumor</td>
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For statistical evaluation, observations of tumor animals were compared to normal dogs. XN, Times normal value for turnover measurements.

* p < 0.05
† p < 0.005
‡ p < 0.0001

<table>
<thead>
<tr>
<th>Table 2. Serial Platelet and Fibrinogen Kinetics</th>
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Statistical evaluations for observations during weeks 1 and 2 are compared to the baseline data for the subset of animals under study. XN, times normal value for turnover measurements.

* p < 0.005.
Fig. 1.  Relationship between initial platelet count and platelet survival.  The relationship between platelet count and platelet survival in normal dogs (O) and dogs with localized tumors (A) is shown in the hatched area.  In those dogs with metastatic tumors that presented with platelet survivals of less than 4 days (B), there was a proportionate reduction in platelet count (correlation coefficient of 0.76) that can be described by the equation $y = 13.1 + 0.04x$ ($r^2 = 0.58$).  

Table 3.  Platelet and Fibrinogen Kinetics Before and After Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>Observations</th>
<th>Count/μl (x 10^11)</th>
<th>Platelets/μl (x 10^11)</th>
<th>Conc. (mg/ml)</th>
<th>Survival (Days)</th>
<th>Turnover (mg/ml/Day)</th>
<th>XN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>4</td>
<td>201 x 72*</td>
<td>62 x 7</td>
<td>131 x 24</td>
<td>4.49 ± 0.51</td>
<td>1.3 ± 0.3</td>
<td>2.2 ± 0.2</td>
<td>2.10 ± 0.17</td>
</tr>
<tr>
<td>RA233</td>
<td>1</td>
<td>176</td>
<td>58</td>
<td>104 ± 43</td>
<td>4.71 ± 0.22</td>
<td>1.0 ± 0.4</td>
<td>2.2 ± 0.2</td>
<td>2.37 ± 0.16</td>
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<tr>
<td>Sulfinpyrazone</td>
<td>5</td>
<td>273 x 83</td>
<td>35 x 6</td>
<td>278 ± 60</td>
<td>6.25 ± 1.43</td>
<td>2.8 ± 0.6</td>
<td>2.7 ± 0.2</td>
<td>2.14 ± 0.36</td>
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<tr>
<td>Warfarin</td>
<td></td>
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<td></td>
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<td>0.97 ± 0.35</td>
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<tr>
<td>RA233 + Sulfinpyrazone</td>
<td>8</td>
<td>259 x 46*</td>
<td>62 x 8</td>
<td>165 ± 36</td>
<td>2.87 ± 0.445</td>
<td>2.2 ± 0.2</td>
<td>1.33 ± 0.18</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>Baseline</td>
<td>8</td>
<td>323 x 46</td>
<td>61 x 9</td>
<td>173 ± 30</td>
<td>3.56 ± 0.82</td>
<td>2.3 ± 0.2</td>
<td>2.02 ± 0.68</td>
<td>2.6 ± 0.9</td>
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<tr>
<td>Warfarin</td>
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*Baseline observations were performed on no treatment, and statistical comparisons were made with data from normal dogs.  All dogs had metastatic tumors.  Studies done during weeks 1 and 2 were performed while the animal was receiving the indicated medications, and statistical evaluations were performed using baseline data for that subset of animals.  XN, times normal value for turnover measurements.

†Only 2 dogs received Warfarin treatment, and they are identified as A and B.

$p < 0.05$

$p = 0.005$

$p < 0.001$

Fig. 2.  Modification of platelet consumption with antithrombotic drugs.  Platelet survivals are plotted for individual dogs on a weekly basis.  The upper panels show results in untreated dogs (O) and dogs with localized tumors (A).  There was a proportionate reduction in platelet count (correlation coefficient of 0.76) that can be described by the equation $y = 13.1 + 0.04x$ ($r^2 = 0.58$).
did not improve further during the third week of therapy (Fig. 2). In spite of improved platelet survivals, however, platelet counts increased in only 1 dog, were unchanged in 1, and decreased in 3 (Fig. 3). Again, there were no significant changes in fibrinogen measurements with therapy (Table 3).

Eight dogs were treated with both sulfinpyrazone (500 mg/kg, b.i.d.) and RA233 (25 mg/kg, b.i.d.). Platelet survival was significantly increased from 2.7 ± 0.4 days baseline to 4.7 ± 0.6 days at the peak of treatment response \((p < 0.001)\). In 5 of the 8 dogs studied, platelet survivals became normal during treatment (Fig. 2). Two had normal platelet survivals after the first week on therapy, two had normal survivals after the second week, while the fifth dog required 5 wk of treatment before the platelet survival became normal. Of the three dogs whose platelet survivals did not normalize, two died after the first week, and the third was studied for 5 wk without a significant change in platelet survival. Seven of these dogs died during the course of these studies and the other was euthanized. As opposed to the other treatment programs, this therapeutic combination was associated with increased platelet counts, 259,000/µl ± 46,000 baseline versus 364,000/µl ± 48,000 at the peak of treatment response \((p < 0.05)\) (Fig. 3). Fibrinogen measurements were unchanged with treatment (Table 3).

A single dog was treated with dipyridamole, a phosphodiesterase inhibitor (25 mg/kg, b.i.d.) in combination with aspirin, a cyclooxygenase inhibitor (10 mg, b.i.d.). Platelet survival increased during the first week of therapy from 2.8 days to 5.0 days, and the platelet count rose from 329,000/µl to 382,000/µl. These changes resulted in a reduced platelet turnover from 1.7 to 1.2 times normal. There were no associated changes in fibrinogen survival or concentration.

**Anticoagulation**

Two dogs were given Warfarin in a dosage sufficient to prolong the prothrombin time to 2.5 times the control value. This resulted in an improved fibrinogen survival and concentration in one of the dogs. Other hemostatic parameters were either unchanged or more abnormal than baseline values (Table 2 and Fig. 2).

**DISCUSSION**

Observations of hemostatic parameters in dogs with localized tumors have shown only modest reductions in platelet survival and platelet counts that remain within the normal range. In contrast, in 23 dogs with multicentric or metastatic disease, 82% had a reduction in platelet survival of more than 2 standard deviations from normal, i.e., less than 4.2 days. In addition, the platelet destructive rate of 1.6 times normal resulted in a significant reduction in platelet concentration that varied with the intravascular platelet survival. None of the dogs with localized tumors and only 9 of the 23 dogs with metastatic disease had fibrinogen survivals of less than 2 standard deviations from normal, i.e., less than 2.1 days. The mean fibrinogen survival of even the dogs with metastatic tumor, however, was not significantly different than that found in normal animals. The only consistent abnormality in fibrinogen hemostasis was a nonspecific elevation in fibrinogen concentration that was not different between dogs with local or metastatic disease. Elevation of fibrinogen concentration in association with malignancy has been previously described in man.
In the present study, dogs with metastatic tumor were evaluated before and after administration of either platelet enzyme inhibitors or Warfarin therapy in order to determine whether the hemostatic abnormalities associated with extensive malignant disease could be reversed. These animals were chosen as they had severe abnormalities in platelet hemostasis that became progressively more abnormal with time. Thus, any observed improvements in platelet survival or concentration could be directly attributed to the treatment program.

Platelet enzyme inhibitors with differing mechanisms of action were selected for evaluation; dipyridamole and RA233 inhibit the platelet phosphodiesterase enzyme system while sulfipyrazone and aspirin inhibit cyclooxygenase. The total dosages administered were 2–10 times those given to human subjects but were chosen to ascertain the effects of the medications on hemostatic parameters rather than to determine a minimum effective level. Our results show that the use of drugs with different mechanisms of action on platelet enzyme systems may act synergistically in dogs with metastatic tumor to improve platelet survival. The beneficial effect of this combination is further supported by the observation that these drug regimens were the only ones that produced a significant increase in platelet count as a consequence of the improved platelet survivals. The studies concentrated on the combination of RA233 and sulfipyrazone, as these agents, in contrast to aspirin, produce no significant prolongation of the bleeding time in humans. Thus, there would be no contraindication to their use in thrombocytopenic patients.

Since there were few abnormalities in fibrinogen kinetics, administration of an anticoagulant (Warfarin) would be anticipated to have little effect. In the two treated animals, fibrinogen survival was improved in one but the major abnormalities in platelet parameters were unchanged.

There were too few dogs in each group to determine if tumor type had any effect on the hemostatic response to treatment. There was no indication that the antiplatelet drugs prolonged life or reduced tumor growth in these dogs with advanced tumors. However, there are studies in both patients and other animal tumor model systems that suggest that modification of the coagulation system may be effective in preventing metastasis formation. Since these dogs were referred late in the course of their disease, they did not lend themselves to studies of tumor spread.

Nevertheless, if platelets somehow promote tumor growth or metastasis by releasing growth factors or by serving as a nidus for tumor deposition, then treatment programs that normalize platelet hemostasis may be important in limiting tumor deposition.

This study has confirmed that the predominant hemostatic abnormality in dogs with malignant tumors is a disturbance of platelet homeostasis resulting in a reduction in platelet survival and an associated depression in platelet count. The mechanism(s) responsible for shortening of platelet survival in dogs with tumors are not known, but may include direct platelet–tumor cell interactions with platelet aggregation, adherence to incompletely endothelialized tumor vessels, or alterations of the platelet surface by tumor-related proteins resulting in aggregation and premature removal from the circulation. Tumor–platelet interactions in dogs with naturally occurring tumors are substantially different from those in mice bearing metastatic Lewis lung carcinoma. In mice, the thrombocytopenia was related to decreased production rather than accelerated destruction, as platelet survivals were normal. However, since not all the dogs in the present study showed a concomitant rise in platelet count with improved posttreatment platelet survivals, we cannot exclude the possibility that some dogs had an associated production defect similar to that found in the tumor-bearing mice. Alternatively, those animals whose platelet count did not improve following a change in platelet survival may simply have had a lag period between the normalization of platelet survival and improvement in platelet count. A 1-wk lag period was observed in two of the dogs who survived long enough to perform serial platelet studies on treatment.

Anticoagulants had no effect on platelet parameters, while platelet enzyme inhibitors were able to stabilize and, in some cases, improve platelet survival. Similar findings have been shown in human patients, indicating the validity of the dog model to investigate effective treatment programs. A combination of a phosphodiesterase and a cyclooxygenase inhibitor was consistently able to improve both platelet survivals and counts in dogs with metastatic disease. Whether similar drug regimens in man might be able to reduce platelet consumption in association with an underlying malignant process, thereby increase platelet counts, and thus prevent the need for, or alternatively, prolong the survival of transfused platelets remains to be determined.

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Interruption of tumor-associated platelet consumption with platelet enzyme inhibitors

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