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Efficacy and Toxicity of a Dose-Intensified Doxorubicin Protocol in Canine Hemangiosarcoma

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The purpose of this study was to evaluate the efficacy and toxicity of a single-agent, dose-intensified doxorubicin protocol in canine hemangiosarcoma (HSA). Canine HSA is a highly malignant tumor, and most affected dogs die within 6 months of diagnosis. Doxorubicin is the most, and possibly the only, effective chemotherapeutic drug for this malignancy, but it provides only moderate improvement in survival. On the basis of previous studies reporting similar survival in dogs treated with doxorubicin as a single agent and doxorubicin-based combination chemotherapy and the concept of summation dose intensity, a dose-intensified single-agent doxorubicin protocol was initiated. Twenty dogs with HSA were recruited to participate in this study. Workup and staging were performed according to standard practice. Chemotherapy was initiated within 3 weeks of surgery. Doxorubicin was scheduled to be administered at 30 mg/m^2 IV every 2 weeks for a total of 5 treatments. The dogs were monitored for toxicity and signs of recurrence during and at regular intervals after chemotherapy. The protocol was tolerated well. No dogs were hospitalized because of adverse effects or developed clinical signs consistent with doxorubicin-induced cardiomyopathy. There was a significant difference in survival in dogs with stage I and II HSA compared with dogs with stage III HSA, with median survival times of 257, 210, and 107 days, respectively. These results are slightly better than the historical control with respect to toxicity and efficacy but are not statistically different from what is achieved with standard treatments. There was no association between dose intensity and outcome.

Key words: Chemotherapy; Dogs; Dose intensity; Malignant endothelioma.

Hemangiosarcoma (HSA) is more common in the dog than in any other species and accounts for 7% of all canine malignancies.[1,2] HSA is a highly malignant soft tissue sarcoma characterized by local invasion and early distant metastasis, and most dogs with this disease die within 6 months of the initial diagnosis.[1–7] HSA typically affects middle-aged to older, larger breed dogs, males more than females, and certain breeds such as German Shepherds and Golden Retrievers are predisposed to this disease. Common primary sites include spleen, liver, right atrium, and subcutaneous tissues, but HSA can occur at any site with a vascular supply.[8–10] Dogs with HSA at primary visceral sites typically present with signs associated with acute tumor rupture and internal hemorrhage such as collapse, weakness, anemia, and tachycardia, and most of them present with advanced stage disease (stage II or higher). Treatment options include surgery alone, surgery combined with chemotherapy, or palliative chemotherapy. Surgery alone has been associated with a short survival of a few weeks to a few months.[4,6] Adjuvant chemotherapy can prolong survival, but despite treatment, most dogs with this malignancy develop progressive, drug-resistant metastatic disease within 6 months of the initial diagnosis. Several chemotherapy protocols have been evaluated in dogs with HSA. Protocols containing doxorubicin have been found to have moderate efficacy, whereas protocols without doxorubicin have been found to have limited or no efficacy.[8–11] The doxorubicin-containing protocols used in canine HSA include the VAC protocol (vincristine, cyclophosphamide, and doxorubicin), the AC protocol (doxorubicin and cyclophosphamide), and doxorubicin alone, and there does not appear to be an important difference in survival among dogs treated with VAC, AC, or doxorubicin alone.[9–11]

The efficacy of chemotherapeutic protocols is influenced by the dose, the dose intensity, and the combination of effective chemotherapeutic drugs.[12–13] Response to chemotherapy is characterized by a steep dose-response curve, and optimal tumor response typically is observed when the maximum tolerated dose is administered. Combination protocols are likely to be more effective than single-agent chemotherapy, but in order to provide a broader coverage against a heterogeneous tumor cell population, the combination protocols should consist of several drugs with single-agent efficacy.[14–16] A previous study showed that vincristine and cyclophosphamide without doxorubicin did not provide significant improvement in survival in dogs with HSA compared to dogs treated with surgery alone, suggesting that cyclophosphamide and vincristine might not contribute to the improvement in survival reported with the VAC and AC protocols.[8]

The dose intensity of a chemotherapeutic protocol is not only determined by the actual dosage of the drugs, but also of the frequency of administration.[17] In order to improve the dose intensity, the intervals between treatments can be shortened to allow only for the minimal time required for recovery of sensitive normal tissues. Doxorubicin is given every 3 weeks in the standard VAC and AC protocols, but overlapping toxicities might prohibit shortening of the treatment intervals and improve the dose intensity with these protocols. In contrast, doxorubicin as a single agent can be given on a schedule of every 2 weeks instead of the standard every 3 weeks.[18] A previous study documented a dramatic difference in the efficacy of doxorubicin in canine appendicular osteosarcoma by shortening the treatment in-

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terval from 3 to 2 weeks. Dogs treated with doxorubicin every 3 weeks had a median survival of 104 days, whereas dogs treated every 2 weeks had a median survival of 366 days.[18, 19] The dose intensity of doxorubicin given at a dosage of 30 mg/m^2 every 3 weeks for 5 cycles is 1.6 mg/m^2/d compared to a dose intensity of 2.4 mg/m^2/d if the dose interval is reduced to 2 weeks.

The purpose of this pilot study was to evaluate the efficacy and toxicity of an every-2-weeks doxorubicin protocol in canine HSA.

Materials and Methods

Twenty dogs with a HSA of any primary site (except superficial dermal HSA) and any stage were recruited to participate in this study. Eligible dogs were required to have a histologic diagnosis of HSA and an owner-signed informed consent form detailing the risks, benefits, and responsibilities associated with participation in this trial. All dogs had surgical resection of their primary tumor if deemed appropriate according to the stage of disease and clinical status of the dog. Work-up and staging were performed according to standard practices for dogs with HSA, and included CBC, serum biochemistry, urinalysis, 3-view thoracic radiography, abdominal ultrasonography, and cardiac echocardiography. The dogs were staged according to a modified World Health Organization (WHO) staging system, in which stage I is a tumor confined to the primary site, stage II is a tumor that has ruptured or spread to the regional lymph node, and stage III is a primary tumor with measurable metastasis or multicentric disease. The cardiac echocardiogram was performed for staging purposes in addition to establishing baseline shortening fraction measurements before doxorubicin therapy.[20]

Enrollment had to be completed and chemotherapy initiated within 3 weeks of surgery for the primary tumor. All dogs were scheduled to receive doxorubicin at a dosage of 30 mg/m^2 every other week for a total of 5 treatments. The dogs were monitored closely for toxicity and recurrence during and after completion of the protocol by performing a CBC 7 days after, and immediately before, doxorubicin treatment. The hematologic toxicity, specifically the granulocyte grade, was assigned according to a standard hematologic grading system (grade 0, neutrophil count >2,500 cells/μL; grade 1, 1,500–2,499 cells/μL; grade 2, 1,000–1,499 cells/μL; grade 3, 500–999 cells/μL; grade 4, <500 cells/μL). Dose reductions were performed in dogs with a neutrophil count lower than 1,800 cells/μL at the nadir. The dose was reduced from 10 to 25% according to the severity of neutropenia and in dogs with signs of gastrointestinal toxicity such as prolonged anorexia, excessive vomiting, and diarrhea. Treatment was delayed in dogs with persistent neutropenia and neutrophil counts <2,000 cells/μL on the day they were due to receive doxorubicin.

In addition, the dogs were scheduled to have repeated echocardiograms, thoracic radiography, and abdominal ultrasonography after the completion of the 5 doxorubicin cycles and every 2 months thereafter for 1 year after initial diagnosis. Continued chemotherapy or rescue chemotherapy was offered for dogs that had residual disease after the initial 5 cycles of doxorubicin or that developed progressive disease after completion of the protocol. A complete postmortem examination was requested for all dogs that died during or after completion of this protocol. In addition to evaluating the extent and distribution of metastasis, sections of cardiac tissue were evaluated and graded for presence and severity of doxorubicin-induced cardiomyopathy according to a modified grading system.[21] Endpoints included survival and development of clinical signs consistent with doxorubicin-induced dilated cardiomyopathy. This protocol was reviewed and approved by the Institutional Animal Use and Care Committee (IACUC) and the Committee on the Use of Client Owned Animals (COAP).

Statistical Analysis

Survival was calculated from the date of surgery to the date of death. Dogs that were still alive were censored at the last date they were reported to be alive. The Kaplan-Meier product limit method was used to estimate the portion of dogs that were alive or had died from HSA in each stage of disease group. Statistical differences in survival between stages were assessed by the log rank test. The Kaplan-Meier product limit method and the log rank test were used to assess whether there was a difference in outcome between dogs that received the maximum dose intensity and dogs that received a less intensive dose protocol. Statistical significance was defined as $P < .05$. All analyses were performed with SAS statistical software.[a]

Results

Twenty dogs were recruited to participate in this clinical trial. The breeds represented were typical for a population of dogs with HSA and consisted of 6 mixed breeds, 5 Golden Retrievers, and other mostly larger dog breeds. The majority of the dogs were middle-aged to older with a mean age of 9.8 years, (range 3–13 years); 13 of the dogs were female, all spayed, and 7 dogs were male, 5 of which were castrated. Fourteen of the dogs in this study had primary splenic HSA, 4 had primary subcutaneous HSA, and the right atrium and kidney were represented with 1 dog each. Most of the dogs had advanced disease when they were diagnosed with HAS: 6 dogs had stage III disease, 9 dogs had stage II disease, and 5 dogs had stage I disease. Four of the 5 dogs with stage I disease had primary subcutaneous HSA.

Toxicity

The protocol was tolerated well. Seventeen of the 20 dogs completed the treatment protocol. The treatment protocol was discontinued in 2 dogs after 2 and 4 cycles, respectively, because of progressive disease, and in 1 dog because of toxicity after receiving only 2 treatments. The latter dog had gastroenteritis as a result of a concurrent *Campylobacter* infection, but the owners were concerned that chemotherapy had caused some of the gastrointestinal signs. One dog received an additional 2 cycles of doxorubicin after the initial 5 treatments because of residual tumor presence. None of the dogs received other rescue chemotherapy. One dog had grade 3 neutropenia, 3 dogs had grade 2 neutropenia, and 5 dogs had grade 1 neutropenia. Five dogs had dose reductions because of neutropenia or gastroenteritis, but only 1 of these dogs required treatment delay because of prolonged neutropenia. Four of these 5 dogs had their doses reduced after the 1st cycle of doxorubicin, and the 5th dog had the dose reduced after the 4th cycle. None of the neutropenic dogs developed fever or other signs consistent with sepsis or required hospitalization for treatment of associated toxicities. These dogs did not develop clinical signs or echocardiographic changes consistent with doxorubicin-induced cardiotoxicity, and 3 of the 4 long-term survivors (>1 year) completed the protocol and survived for 1 year or longer after the initial diagnosis without developing signs of dilated cardiomyopathy.

Results from histopathologic grading of the cardiac changes secondary to doxorubicin toxicity were available in 11 dogs. Grading could not be performed in 1 of the

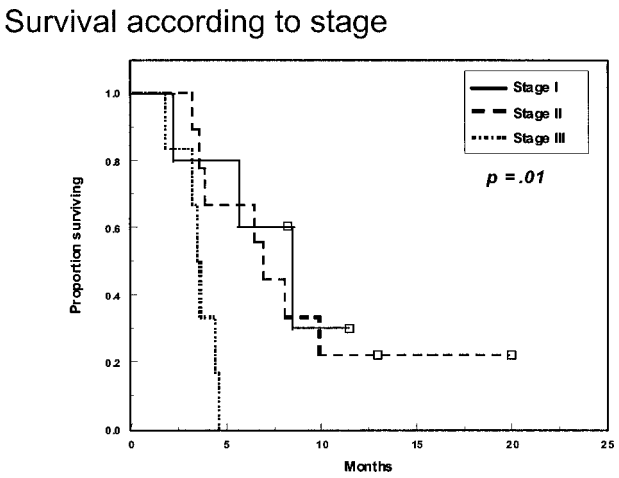

Fig 1. Survival according to stage.

dogs because of severe autolysis and bacterial overgrowth. Five of the 11 dogs had moderate to severe fibrosis and mild to moderate vacuolar changes consistent with grade 2 toxicity according to the modified grading scale. One of these dogs received 7 doxorubicin treatments; the other 4 received 5 treatments each. Four of the remaining 6 dogs had no evidence of cardiac toxicity, and 2 had mild changes consistent with grade 1 toxicity. Two of the dogs with no histologic changes completed the protocol and had 5 treatments of doxorubicin, whereas the 2 remaining dogs received only 2 and 4 doxorubicin treatments, respectively.

Both of the dogs with grade 1 cardiac toxicity completed the protocol.

Dose Intensity

The study treatment protocol was designed to consist of doxorubicin at 30 mg/m^2 every 2 weeks. However, this dosage was modified to 25 mg/m^2 in 3 dogs that weighed less than 15 kg according to the individual attending clinician's discretion. Dose intensity was not calculated for the 2 dogs that received only 2 cycles of doxorubicin. Eleven of the remaining 18 dogs received the maximum dose intensity protocol of 2.4 mg/m^2/d, and 7 dogs received a less dose intensive protocol: the original dosage was modified because of low body weight or toxicity. The mean and median dose intensity was 2.2 and 2.4 mg/m^2/d, respectively (range 2.4–1.7). None of the dogs had their dose intensity reduced to 1.6 mg/m^2/d, which is the calculated dose intensity of the standard every-3-week protocol. There was no association between dose intensity and outcome ($P = .6$).

Survival

Sixteen of the 20 dogs in this trial died or were euthanized because of their tumor, and 4 dogs were alive without signs of recurrence at 243, 351, 384, and 605 days. Two of the dogs that were alive had stage I HSA (1 primary subcutaneous and 1 renal), and 2 of the dogs had stage II splenic HSA. Dogs with stage I and II HSA had a significantly longer survival than dogs with stage III HSA and had a median survival of 257 days (stage I) and 210 days (stage II) versus 107 days (stage III), $P < .01$ (Fig 1).

Complete postmortem examinations were performed on 12 of the 16 dogs that died, and all of them had metastatic HSA. Furthermore, all of the dogs with primary splenic HSA that died or were euthanized had recurrent intra-abdominal hemorrhage secondary to disseminated hepatic, omental, and mesenteric metastases.

Discussion

The dose-intensified doxorubicin protocol was tolerated well but did not result in significant improvements in survival in dogs with HSA. Despite dose intensification, most dogs in this study died or were euthanized because of recurrent HSA, and only 4 dogs were long-term survivors, of which 2 had stage II splenic HSA; the other 2 dogs had stage I HSA, 1 subcutaneous and 1 primary renal (Fig 1). All of the dogs with stage III disease died from progressive HSA. This suggests that dose intensification might be a more effective strategy in patients with microscopic disease than in patients with established metastasis. The probability of cure is inversely proportional to the tumor cell burden. A larger tumor mass consists of de novo resistant subclones, whereas microscopic disease is more likely to be eradicated by chemotherapy.[14,15]

We compared these results with historical controls. The AC protocol resulted in median survival of 250, 186, and 87 days in dogs with stage I, II, and III HSA, respectively, and the VAC protocol resulted in a median survival of 145 days in dogs with splenic HSA.[9,10] The study that reported the single-agent doxorubicin protocol at the standard interval of every 3 weeks did not provide survival information according to stage of disease or site of primary tumor, but according to completeness of surgical excision; the median survival was 172 days in dogs that had complete removal of all tumors before chemotherapy.[11] In this study, there was no significant difference in survival between dogs with stage I HSA, of which 4 of the 5 dogs had primary subcutaneous HSA, and dogs with stage II splenic HSA, with a median survival of 257 and 210 days, respectively. Three of the 4 dogs with subcutaneous HSA died from metastatic disease despite chemotherapy, suggesting that primary subcutaneous HSA has an aggressive biological behavior. These results are in contrast to a previous retrospective study of cutaneous HSA treated with surgery alone that reported a median survival of 307 days in 5 dogs with primary dermal HSA with invasion into the underlying musculature.[22] It is not clear whether primary cutaneous HSA with secondary invasion into the underlying subcutaneous musculature and primary subcutaneous HSA have a similar biological behavior or whether these results are skewed because both of these reports contain small numbers of dogs.

A comparison of the results from the new dose-intensified doxorubicin study with historical controls that use AC, VAC, or doxorubicin alone reveals that the results from the current study are slightly favorable, but not statistically significant. In order to fully compare the efficacy of this dose-intensified doxorubicin protocol to standard protocols, a prospective randomized trial would be necessary.

These results are not as impressive as the improvement in survival from 104 to 366 days reported in dogs with

appendicular osteosarcoma treated with a similar dose-intensified doxorubicin protocol, but the previous study reporting a survival of only 104 days might have underestimated the efficacy of the standard every-3-week doxorubicin protocol in canine appendicular osteosarcoma. A more recent study reported a longer survival in dogs with the standard single-agent doxorubicin protocol.[23]

This protocol was tolerated well and none of the dogs required hospitalization and supportive care to treat acute adverse effects. However, the incidence of grade 2 cardiac toxicity is concerning. The clinical relevance of the cardiac changes is unclear because none of these dogs developed signs of dilated cardiomyopathy and heart failure, which is a serious and often fatal complication of doxorubicin-induced cardiotoxicity. This result might be attributable to the death of dogs as a result of their tumor before they developed clinical signs of dilated cardiomyopathy or might indicate that the modified grading system did not identify dogs that have sufficient structural damage to cause functional changes that lead to cardiomyopathy and heart failure. The onset of doxorubicin cardiomyopathy can be delayed, and it has been reported to occur years after completion of chemotherapy in human patients. There is a clinical impression that this occurs much sooner in treated dogs. Furthermore, 4 dogs, of which 3 completed the protocol, were long-term survivors and had no signs of dilated cardiomyopathy. A previous study evaluating the same protocol in canine appendicular osteosarcoma reported a 10% incidence of cardiomyopathy.[18] The median survival of dogs in that study was significantly longer than the survival of the dogs in this study and might therefore have provided the necessary time for functional changes to develop.

The efficacy of chemotherapeutic protocols is influenced by the dose intensity, availability of effective drugs, and drug resistance. Resistance to chemotherapeutic drugs can be classified as apparent and absolute resistance.[24] Apparent resistance is relative resistance caused by inadequate absorption, inadequate dosing, suboptimal scheduling, drug interactions, and kinetic resistance. Absolute or intrinsic resistance, de novo or acquired, is conferred by cellular protective mechanisms involving decreased drug uptake, increased drug excretion, poor drug activation, development of alternative pathways, and increased drug catabolism.[24,25] Apparent drug resistance might be more common in veterinary than human oncology because of differences in treatment philosophy and lower chemotherapy dosages because of concerns about quality of life. Several of the dogs had dosage modifications because of low body weight or toxicity, but despite these dosage reductions, the mean and median dose intensity was close to the calculated maximum dose intensity for this protocol. Such dosage reductions are not ideal and likely decrease the efficacy of the protocol, but they are sometimes necessary to prevent premature discontinuation of chemotherapy. Dose intensification might improve the efficacy of chemotherapeutic protocols and prolong remission and survival by overcoming apparent resistance and possibly delaying the development of acquired intrinsic resistance. However, in order to eradicate all subclones with de novo intrinsic resistance and effectively cure cancer patients, a dose-intensive combination of several effective chemotherapeutic drugs is necessary.[13–15] Most tumors consist of heterogeneous cell populations at the time of clinical detection, and it might be unrealistic to expect that a dose-intensified, single-agent doxorubicin protocol would result in dramatic improvements in survival. The lack of significant improvement in survival of the dose-intensified, single-agent doxorubicin protocol might indicate that the major cause of treatment failure and drug resistance in canine HSA is absolute de novo resistance, and novel effective drugs or treatment strategies are needed to improve the outcome in this malignancy.

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Footnote

[a] SAS software, SAS Institute, Cary, NC

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