Clinical Response and Adverse Event Profile of Bleomycin Chemotherapy for Canine Multicentric Lymphoma

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ABSTRACT

Although canine multicentric lymphoma is initially responsive to multidrug chemotherapy, resistance and relapse create a need for novel chemotherapeutics. Bleomycin is an antitumor antibiotic with a minimal adverse event profile; though commonly used for human non-Hodgkin’s lymphoma, its use is poorly characterized in dogs. The purpose of this retrospective case series was to describe the clinical response and adverse event profile of systemic bleomycin for canine multicentric lymphoma (n = 10). A partial response was noted in one dog that died 24 days later due to unrelated disease. Adverse events were infrequent and limited to grade 1 gastrointestinal and grade 1 constitutional toxicity. Although clinical response was minimal, systemic bleomycin was well tolerated when administered at 0.5 U/kg. Additional studies are warranted to determine the influence of administration schedule and dose on the efficacy of bleomycin for veterinary neoplasia. (J Am Anim Hosp Assoc 2017; 53:128–134. DOI 10.5326/JAAHA-MS-6598)

Introduction

Lymphoma is the most common hematopoietic malignancy in dogs with an estimated annual incidence rate of 107 per 100,000.1 The majority of dogs with multicentric lymphoma initially respond well to multidrug chemotherapy protocols and remission rates greater than 90% have been reported.2,3 However, relapse is common with less than 25% of dogs experiencing a remission greater than 2 yr.2 A variety of rescue protocols have been described with overall response rates of 0–87% and progression free intervals ranging from 1–4 mo.4–8 The prevalence of multidrug resistance, influenced by P-glycoprotein overexpression, results in significantly shorter progression free intervals for rescue protocols.9,10 Thus, there is an ever-present need for novel chemotherapeutic agents.

Bleomycin is an antitumor antibiotic isolated from the fungus Streptomyces verticillus.11 Bleomycin induces oxidative cleavage of DNA creating both single- and double-strand breaks with chromosomal gaps, deletions, and fragments occurring as a consequence.11 Although DNA is the main target, bleomycin is also capable of inhibiting RNA and protein synthesis.11,12 Bleomycin is an appealing antineoplastic drug as it is non-myelosuppressive and unaffected by P-glycoprotein-mediated multidrug resistance.11,13

Bleomycin has been included in human chemotherapy multidrug protocols for the treatment of Hodgkin’s and non-Hodgkin’s lymphoma, testicular cancer, malignant pleural effusions, and head and neck squamous cell carcinoma.11,14,15 Bleomycin is ALT (alanine aminotransferase); UWM-CHOP (University of Wisconsin-Madison cyclophosphamide, hydroxydaunorubicin, oncovin, prednisone)
often used within multidrug protocols, such as the ABVD protocol for Hodgkin’s lymphoma.\(^\text{16}\) Single-agent bleomycin has been utilized as a rescue for advanced Hodgkin’s lymphoma with a response rate of 37%.\(^\text{17}\) Despite its use in humans, veterinary data regarding bleomycin is mostly limited to pharmacokinetic studies. Bleomycin has been administered to healthy beagle dogs at doses of 0.5–9.0 U/kg IV q 4 days for 6–28 wk duration.\(^\text{18,19}\) In humans, bleomycin is administered at 0.5 U/kg via IV or intramuscular routes.\(^\text{11}\) A therapeutic dose has yet to be established in dogs. Reported side effects in dogs include epithelial lesions (e.g., foot pad ulceration, dermatitis, purulent conjunctivitis), gastrointestinal upset (e.g., anorexia, vomiting, diarrhea), pyrexia, reversible elevations in alanine aminotransferase (ALT), and nephrotoxicity.\(^\text{18–21}\) As these side effects occurred in pharmacokinetic studies, their true incidence is unknown. Idiosyncratic hypersensitivity reactions occur in 1% of human lymphoma patients, but have not been reported in dogs.\(^\text{22}\) The most severe side effect in both species is interstitial pneumonia that rarely progresses to fatal pulmonary fibrosis.\(^\text{21}\) In dogs, pulmonary toxicity appears both time-dependent and cumulative; lesions have been observed after a minimum of 38 days with total doses of 4 U/kg and above.\(^\text{19,21}\)

Few studies exist documenting the therapeutic use of bleomycin in dogs. Bleomycin has successfully been used intralesionally or with electrochemotherapy in dogs with anaplastic ameloblastoma and oral squamous cell carcinoma.\(^\text{23,24}\) One study evaluated bleomycin with electrochemotherapy as a treatment for localized lymphoma and a response was noted in all patients (n = 6) with durations of 1 week to 3 yr.\(^\text{25}\)

Anecdotally, bleomycin has been systemically administered as a rescue agent for canine multicentric lymphoma; however, no publication has determined the clinical response. The purpose of this retrospective case series was to describe the use of systemic bleomycin, evaluate clinical response, and identify any adverse events encountered subsequent to drug administration.

**Materials and Methods**

**Medical Records Review**

Data were retrieved retrospectively from the pharmacy records of dogs that received bleomycin at the University of Florida Veterinary Teaching Hospital between January 2009 and August 2014. Dogs were eligible for inclusion if they (1) had a diagnosis of multicentric lymphoma confirmed by means of cytologic or histologic examination, (2) received single agent systemic bleomycin at any point in their chemotherapeutic protocol, and (3) had adequate follow-up information available to assess clinical response and adverse events. Patients receiving concurrent corticosteroids were only eligible for inclusion if this was a pre-existing medication administered at the same or lower dose during bleomycin chemotherapy. Dogs were excluded if bleomycin was administered within 24 hr of other cytotoxic chemotherapy.

Data obtained from the medical records included breed, age, sex, body weight, stage, substage, and immunophenotype. Chemotherapy protocols administered prior to bleomycin were described by the number and names of chemotherapy agents received, addition of half body radiation therapy, and response to induction protocol. Information about bleomycin treatment included days from diagnosis to administration of bleomycin, reason for bleomycin administration, bleomycin dosage and route of administration, number of doses, and concurrent medications. The results of physical examinations (e.g., lymph node measurements) and diagnostics (e.g., complete blood counts, chemistry profiles, thoracic radiographs) performed before and after administration of bleomycin were obtained. Clinical response and reported side effects were recorded, and necropsy results were described when available. Follow-up data was obtained from the patient’s medical record at the University of Florida Veterinary Teaching Hospital or the primary care veterinarian.

**Assessment of Response and Toxicity**

Patients were evaluated with a physical examination and complete blood count before each treatment and 6 to 14 days post-treatment, depending on owner compliance. Chemistry profiles before each treatment and within 30 days thereafter were reviewed when available. Tumor response was determined at each recheck examination according to the Veterinary Cooperative Oncology Group Response Evaluation Criteria for Peripheral Nodal Lymphoma in Dogs v.1.0.\(^\text{26}\)

Potential toxic effects were recorded from the medical record history, physical examination findings, complete blood counts and chemistry profiles. Thoracic radiographs and necropsies, if
available, were evaluated for signs of pulmonary fibrosis. Toxicity grades were retrospectively assigned using the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events v.1.1.27

Statistical Analysis
Standard statistical software26 was used for analysis. The progression free interval was defined as the date of bleomycin administration to date of disease progression or date lost to follow-up. Death was assumed due to disease unless otherwise stated in the medical record. One dog was censored from analysis of clinical response as it was in complete remission prior to receiving bleomycin; this dog was included in the manuscript for the sole purpose of evaluating toxicity.

Results
Patients
The medical record search identified 10 dogs with multicentric lymphoma that received single agent systemic bleomycin. Eight dogs underwent staging at the time of presentation to the authors’ institution, which included a complete blood count, chemistry profile, urinalysis, thoracic radiographs, and abdominal ultrasound. A bone marrow aspirate was performed on one dog. Patient demographics, World Health Organization stage/substage, and immunophenotype results are described in Table 1.

Previous Chemotherapy
Nine dogs (90%) received previous chemotherapeutic drugs with a median of 3.5 drugs (range, 0–8 drugs) administered prior to bleomycin treatment. Six dogs were initially treated with the modified University of Wisconsin-Madison cyclophosphamide, hydroxydactinorubicin, oncovin, prednisone (UWM-CHOP) protocol using vincristine1, cyclophosphamide6, doxorubicin1, and prednisone8.28 Four of these dogs also received L-asparaginase1b as part of their induction protocol and one dog received half body low-dose rate irradiation.29,30 An additional dog was treated with a modified UWM-CHOP protocol substituting mitoxantrone1 for doxorubicin due to cardiac disease. The remaining two dogs were treated with single agent doxorubicin and L-asparaginase, respectively.

Of the nine pretreated dogs, 88.9% responded to their induction protocol with five achieving complete remission and three exhibiting a partial response. One dog did not respond to induction and developed progressive disease after receiving a single dose of doxorubicin. The median first remission duration was 50 days (range, 6–294 days).

Bleomycin Treatments
Bleomycin was administered to patients as part of induction (n = 2), during UWM-CHOP (n = 2), or as a rescue protocol (n = 6). Bleomycin was administered during induction to one treatment naive patient and one that previously responded to L-asparaginase. Both patients receiving bleomycin during UWM-CHOP had pre-existent myelosuppression. The remaining six dogs received bleomycin as a rescue agent. A median of three rescue protocols were utilized prior to bleomycin chemotherapy (range, 0–6 protocols). The median time from diagnosis to administration of bleomycin was 78 days (range, 1–777 days).

TABLE 1
Description of Dogs with Multicentric Lymphoma Undergoing Single Agent Bleomycin Chemotherapy

<table>
<thead>
<tr>
<th>Dog</th>
<th>Breed</th>
<th>Sex</th>
<th>Age (yr)</th>
<th>Weight (kg)</th>
<th>WHO stage/substage</th>
<th>Immunophenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Komondor</td>
<td>CM</td>
<td>7</td>
<td>43.5</td>
<td>IVb</td>
<td>N/A</td>
</tr>
<tr>
<td>2</td>
<td>Labrador retriever</td>
<td>SF</td>
<td>7</td>
<td>26.8</td>
<td>Va</td>
<td>B-cell</td>
</tr>
<tr>
<td>3</td>
<td>Mixed-breed</td>
<td>CM</td>
<td>8</td>
<td>44.0</td>
<td>IVb</td>
<td>T-cell</td>
</tr>
<tr>
<td>4</td>
<td>Labrador retriever</td>
<td>SF</td>
<td>10</td>
<td>33.7</td>
<td>Vb</td>
<td>B-cell</td>
</tr>
<tr>
<td>5</td>
<td>Cocker spaniel</td>
<td>CM</td>
<td>9</td>
<td>16.4</td>
<td>III+a*</td>
<td>N/A</td>
</tr>
<tr>
<td>6</td>
<td>English bulldog</td>
<td>CM</td>
<td>10</td>
<td>31.2</td>
<td>Vb</td>
<td>N/A</td>
</tr>
<tr>
<td>7</td>
<td>German shepherd dog</td>
<td>F</td>
<td>2.5</td>
<td>37.9</td>
<td>Ila</td>
<td>B-cell</td>
</tr>
<tr>
<td>8</td>
<td>Greyhound</td>
<td>SF</td>
<td>6</td>
<td>27.9</td>
<td>Ila</td>
<td>B-cell</td>
</tr>
<tr>
<td>9</td>
<td>Jack Russell terrier</td>
<td>SF</td>
<td>6</td>
<td>5.3</td>
<td>III+a*</td>
<td>B-cell</td>
</tr>
<tr>
<td>10</td>
<td>Golden retriever</td>
<td>SF</td>
<td>9</td>
<td>26.5</td>
<td>Ila</td>
<td>B- and T-cell†</td>
</tr>
</tbody>
</table>

CM, castrated male; F, female; N/A, not available; SF, spayed female; WHO, World Health Organization.
*Not fully staged, but known to be at least Stage III.
†Biphenotypic lymphoma on flow cytometry and PCR for antigen receptor rearrangement.
The most common reason for treatment with bleomycin was the presence of cytopenia presumed to be from prior chemotherapy or irradiation (n = 3) or risk thereof due to recent chemotherapy (n = 3). Five of these six dogs also exhibited progressive disease prior to bleomycin administration. The remaining dogs received bleomycin due to progressive disease in the absence of cytopenia (n = 1), unavailability of other preferred chemotherapy agents (n = 1), or for unknown reasons (n = 2).

A total of 13 doses of bleomycin were administered to 10 patients. The median number of treatments was 1 (range, 1–4 treatments). Bleomycin was administered at a median dose of 0.51 U/kg (range, 0.45–0.58 U/kg). It was diluted in sodium chloride for a total volume of 1.5 to 3.0 U/mL. The drug was administered through a subcutaneous injection (n = 11) or intramuscularly (n = 2) according to the clinician’s preference. Diphenhydramine1 (1 mg/kg intramuscularly) was administered prior to bleomycin administration in 2 of 13 cases (15%). Pretreatment antiemetics were administered in 3 of 13 cases (23%) and included standard doses of maropitant citrate2 alone (n = 2) or in combination with ondansetron3 (n = 1). Prednisone was continued in seven dogs already receiving this medication at a median dose of 1.3 mg/kg per os q 24 hr (range, 0.91–1.87 mg/kg/day).

**Response to Treatment**

Response to treatment was evaluated in 9 of 10 dogs (Table 2). Progressive disease occurred in eight dogs (89%) between 1 and 8 days after administration of bleomycin. One of these eight dogs was a naive lymphoma (dog number 7); no response to bleomycin was noted after 24 hr of in-hospital monitoring and UWM-CHOP chemotherapy was initiated thereafter.

A partial response was observed in one dog with peripheral lymphadenopathy following bleomycin administration (11%). Bleomycin was administered to this responder (dog number 10) on a weekly basis for four treatments at a cumulative dose of 2.3 U/kg. This patient’s response persisted for 24 days, at which point the dog was euthanized due to peritonitis secondary to a ruptured hepatic abscess. Necropsy and histopathology demonstrated chronic suppurative bacterial hepatitis with no evidence of hepatic lymphoma. Peripheral lymph node measurements at the time of death confirmed a persistent partial response to bleomycin.

Dog number 8 was censored from progression analysis as complete remission was present at the time of drug administration. Bleomycin was administered once to this patient during UWM-CHOP due to treatment-related neutropenia and thrombocytopenia. The dog remained in complete remission until conventional chemotherapy could be administered 8 days later.

**Toxicoses**

No immediate adverse effects were seen at the time of administration of bleomycin. Adverse events were reported by the owner for 2 of 10 dogs. Dog number 5 experienced grade 1 gastrointestinal toxicity characterized by two episodes of vomiting and less than 24 hr of soft stool occurring 3 days after treatment. Grade 1 lethargy was observed with dog number 10 for 2 days following therapy. No dogs were reported to experience skin lesions or pyrexia. In the single case of repeated bleomycin administration, dose reduction was not required.

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**Table 2**

<table>
<thead>
<tr>
<th>Dog</th>
<th>Time of Administration</th>
<th>Number of BLM</th>
<th>Adverse Event Description</th>
<th>Tumor Response</th>
<th>PFI (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rescue</td>
<td>1</td>
<td>None</td>
<td>Progressive</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>During UWM-CHOP</td>
<td>1</td>
<td>None</td>
<td>Progressive</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>Rescue</td>
<td>1</td>
<td>None</td>
<td>Progressive</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Rescue</td>
<td>1</td>
<td>None</td>
<td>Progressive</td>
<td>6</td>
</tr>
<tr>
<td>5</td>
<td>Rescue</td>
<td>1</td>
<td>Grade 1 Gastrointestinal</td>
<td>Progressive</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>Induction</td>
<td>1</td>
<td>None</td>
<td>Progressive</td>
<td>5</td>
</tr>
<tr>
<td>7</td>
<td>Induction</td>
<td>1</td>
<td>None</td>
<td>Progressive</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>During UWM-CHOP</td>
<td>1</td>
<td>None</td>
<td>N/A*</td>
<td>N/A*</td>
</tr>
<tr>
<td>9</td>
<td>Rescue</td>
<td>1</td>
<td>None</td>
<td>Progressive</td>
<td>8</td>
</tr>
<tr>
<td>10</td>
<td>Rescue</td>
<td>4</td>
<td>Grade 1 Constitutional</td>
<td>Partial Response</td>
<td>24†</td>
</tr>
</tbody>
</table>

BLM, Bleomycin; N/A, not available; PFI, Progression free interval; UWM-CHOP, University of Wisconsin-Madison CHOP.

*Patient was in complete remission prior to receiving bleomycin and was therefore censored from response and survival analysis.
†Patient censored due to death from unrelated disease (confirmed on necropsy).
Complete blood counts performed 6 to 14 days post-treatment were available for all 10 dogs. Hematologic side effects were not observed. Dogs with pre-existing cytopenia experienced improvement in cell lines following drug administration. Chemistry profiles performed within 30 days post-treatment were available for review for four dogs. A moderate elevation in ALT (399 U/L; reference range 18–64 U/L) was noted in dog number 10, which was later euthanized due to a ruptured hepatic abscess. Other hepatic or renal abnormalities were absent.

Thoracic radiographs were obtained for four patients after administration of bleomycin and were available for comparison to pretreatment radiographs. Time between bleomycin administration and imaging ranged from 16 to 372 days (median, 61 days); two dogs had radiographs performed greater than 3 mo after bleomycin. One asymptomatic patient demonstrated a mild diffuse bronchial pattern on thoracic radiographs, and imaging was normal for the remaining three dogs. Necropsy reports were available for two dogs, including the dog with the bronchial pattern noted on radiographs. In both cases, there was no evidence of interstitial pneumonia or pulmonary fibrosis per histopathology.

Discussion

Results of the present study suggest that the clinical response and adverse event profile of systemic bleomycin was minimal when administered at a dose of 0.5 U/kg as a subcutaneous or intramuscular injection. Adverse events were limited to grade 1 (gastrointestinal and constitutive) toxicities that occurred in two patients. No immediate reactions were noted at the time of administration. Dermatologic adverse events did not occur despite prior reports. Hematologic effects were also absent.

Biochemical abnormalities directly attributable to bleomycin administration were not observed. Although transient ALT elevations have been previously reported, the single patient exhibiting this abnormality was soon euthanized due to a hepatic abscess confirmed on necropsy. Although ALT increase may be attributed to the underlying disease process, histopathologic evidence of bleomycin hepatopathy has not been described and, thus, can not be definitively excluded as a contributor. No patient receiving bleomycin developed azotemia after treatment. However, prior reports of azotemia occurred late in the treatment period with high doses not achieved in the present study.

Historically, interstitial pneumonia has been reported as the treatment-limiting toxicity of bleomycin and occurred in 93% of dogs within a pharmacokinetics study. In the current study, there was no evidence of interstitial pneumonia or pulmonary fibrosis in any patient based on physical examination, thoracic radiographs, or necropsy. However, prior reports of pulmonary toxicity demonstrate a poor correlation with physical exam findings and thoracic radiographs, and only two dogs in the present study had necropsies performed. Further, bleomycin-induced pulmonary toxicity appears both cumulative and time-dependent and it is unlikely these criteria were met in the current study. Interstitial pneumonia has previously been reported in dogs at doses as low as 0.4 U/kg administered intravenously q 4 days for a total of 10 treatments. The development of lesions also appears related to the length of observation, as lung injury has been described after 38 days following bleomycin administration. Although four dogs had thoracic radiographs available, in only two cases were these performed greater than 38 days after drug administration. While pulmonary abnormalities were not present in the two patients necropsied, the remaining eight dogs were not examined postmortem. However, the patient receiving the largest cumulative dose of bleomycin (2.3 U/kg) was necropsied and did not exhibit any signs of pulmonary toxicity. Although pulmonary toxicity was absent in the present study, the incidence of this adverse event cannot be assessed as sufficient doses and length of follow-up were not achieved.

Results of the present study suggest that the clinical response to systemic bleomycin was minimal when administered at a dose of 0.5 U/kg as a subcutaneous or intramuscular injection. One dog exhibited a measurable decrease in lymph node size as a result of single agent bleomycin administration. This patient’s partial response persisted for 24 days, at which point the dog died of an unrelated disease. Necropsy confirmed this partial response to treatment and also identified the unrelated cause of death. The remaining eight dogs with measurable disease exhibited progression of lymphadenopathy following drug administration.

While the response rate is low (11%), it is plausible that results were negatively influenced by patient selection. Patients may have presented with undetected advanced stage since bone marrow aspirates were not routinely performed and diagnostic imaging was declined in 2 of 10 cases. Further, the median first remission duration of 50 days is notably shorter than previously described for patients receiving multidrug protocols. This short response duration may be spurious given the small sample size or attributed to inadvertent selection for advanced or aggressive disease. Bleomycin was often utilized after dogs became unresponsive to other chemotherapeutics, and patients receiving bleomycin as a rescue therapy (60%) had received a median of 3.5 prior rescue protocols. Thus, it is possible that advanced stage and/or rapidly resistant disease may have prejudiced treatment results.

Limitations of the study include its retrospective nature, small sample size, lack of complete staging at initiation of therapy, and empirical dosing of bleomycin. The dose and schedule of
bleomycin used in the present study were adapted from human medicine.\textsuperscript{11} Since side effects were minimal when bleomycin was systemically administered at 0.5 U/kg, a dose escalation trial would be valuable to determine if dose intensification is possible while minimizing side effects. While pharmacokinetic studies exist for intravenous and intramuscular dosing, future studies should evaluate the pharmacokinetics of subcutaneous administration. Lastly, many dogs in the current study were heavily pretreated, and future research efforts would ideally assess effectiveness of bleomycin for treatment naïve lymphoma patients.

Conclusion

In conclusion, the efficacy of systemic bleomycin appears limited at the administered dose in canine patients with multicentric lymphoma and remains poorly characterized. However, bleomycin was well tolerated at the doses utilized in this study and only minimal toxicities were described. Additional studies are warranted to examine the influence of bleomycin dosage and schedule of administration.

Footnotes

\textsuperscript{a} Bleomycin for Injection USP; Hospira, Lake Forest, Illinois
\textsuperscript{b} 0.9% Sodium Chloride Injection USP; Hospira, Lake Forest, Illinois
\textsuperscript{c} SigmaPlot V11.0; Systat Software Inc., San Jose, California
\textsuperscript{d} Vincristine Sulfate Injection USP; Hospira, Lake Forest, Illinois
\textsuperscript{e} L-Asparaginase; KRS Global Biotechnology Compounding Pharmacy, Boca Raton, Florida
\textsuperscript{f} DOXOrubicin Hydrochloride USP; Pfizer, New York, New York
\textsuperscript{g} Prednisone Tablets USP; WestWard Pharmaceuticals Corp., Eatontown, New Jersey
\textsuperscript{h} DOXOrubicin Hydrochloride USP; Hospira, Lake Forest, Illinois
\textsuperscript{i} Mitoxantrone Injection USP; Hospira, Lake Forest, Illinois
\textsuperscript{j} Cyclophosphamide Capsules; Roxane Laboratories Inc., Columbus, Ohio
\textsuperscript{k} L-Asparaginase; KRS Global Biotechnology Compounding Pharmacy, Boca Raton, Florida
\textsuperscript{l} Mitoxantrone Injection USP; Hospira, Lake Forest, Illinois
\textsuperscript{m} DOXOrubicin Hydrochloride USP; Pfizer, New York, New York
\textsuperscript{n} Prednisone Tablets USP; WestWard Pharmaceuticals Corp., Eatontown, New Jersey
\textsuperscript{o} DOXOrubicin Hydrochloride USP; Hospira, Lake Forest, Illinois
\textsuperscript{p} DOXOrubicin Hydrochloride USP; Pfizer, New York, New York
\textsuperscript{q} Prednisone Tablets USP; WestWard Pharmaceuticals Corp., Eatontown, New Jersey

References

27. Veterinary cooperative oncology group - common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or

