Clinical Studies on Dose Optimization and Adverse Event Management of Chemotherapeutic Agents in Dogs

（犬における化学療法剤の用量最適化および有害事象管理に関する研究）

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TABLE OF CONTENTS

	PAGES
GENERAL INTRODUCTION	3
CHAPTER 1 - Adverse events in small dogs treated with a single dose of vinblastine	8
CHAPTER 2 - Evaluation of adverse events in small-breed dogs treated with maropitant and a single dose of doxorubicin	25
CHAPTER 3 - Safety and pharmacokinetics of thalidomide in tumor-bearing dogs	43
GENERAL DISCUSSION	71
ACKNOWLEDGEMENTS	77
REFERENCES	78

GENERAL INTRODUCTION

Recently, malignant tumors have become one of the leading causes of death in dogs, and the importance of their treatment has been increasing in small animal clinical practice.[1] Opportunities to diagnose and treat malignant tumors in clinical settings have increased, and treatment options have become more diverse. As in human medicine, chemotherapy plays a central role in the treatment of various malignant tumors in dogs.[2] Chemotherapy is used either alone or as part of multimodal treatment in combination with surgery or radiation therapy, contributing to prolonged survival and improved quality of life (QOL) in animals.[2]

However, compared with human medicine, chemotherapy protocols and appropriate dose settings for individual drugs have not been sufficiently investigated in veterinary medicine.[2,3] Many chemotherapeutic agents currently used in small animal practice are based on studies reported in Europe and North America; however, these studies are often relatively small and frequently involve populations that differ from the small-breed dogs commonly kept in Japan.[4] Therefore, careful consideration is required when applying these findings directly to clinical practice in Japan.

Dose setting for chemotherapeutic agents is an extremely important factor for providing safe and effective treatment. In veterinary medicine, doses of chemotherapeutic agents are generally calculated based on body surface area.[2] This method was introduced from human medicine to account for differences in metabolic function and body fluid distribution.[5] However, dogs vary widely in body weight, from approximately 2 kg to over 50 kg, and this variation in body size is an important factor affecting pharmacokinetics and toxicity.[6] In particular, with body surface area–based dosing, small dogs may receive relatively excessive doses, raising concern that the risk

of adverse events may increase for chemotherapeutic agents with a narrow safety margin.[6,7] Previous studies have suggested that small dogs may be more susceptible to myelotoxicity and gastrointestinal toxicity after administration of cytotoxic anticancer drugs; however, these issues have not been sufficiently investigated.[6,7]

Vinblastine (VBL) is a cytotoxic anticancer drug used mainly for canine mast cell tumors, transitional cell carcinoma of the bladder, and lymphoma.[8-17] The dose of VBL was historically and empirically set at 2.0 mg/m² [8,9]; however, because adverse events were relatively infrequent, a phase I trial was subsequently conducted, and the maximum tolerated dose was established as 3.5 mg/m².[18] However, that phase I trial mainly included medium- to large-breed dogs, and dogs weighing less than 10 kg were not included.[18] Because the dose-limiting toxicity of VBL is neutropenia, safety evaluation of a dose specifically for small dogs is considered necessary.

Doxorubicin (DOX) is a representative cytotoxic anticancer drug frequently used in veterinary medicine.[19-26] The dose of DOX is adjusted according to body size; 30 mg/m² has been used in medium- to large-breed dogs, whereas 1 mg/kg has been recommended for small dogs to reduce adverse events. [6,22,27] The main adverse events associated with DOX include short-term gastrointestinal toxicity and myelosuppression.[4,19,26] Gastrointestinal toxicity includes inappetence, vomiting, diarrhea, and hematochezia.[26] Recently, maropitant, an NK1 receptor antagonist, has been reported to suppress vomiting after chemotherapy and is widely used as supportive care during DOX administration.[28] Maropitant has also been suggested to contribute not only to suppression of vomiting but also to reduction of diarrhea. [28] On the other hand, regarding myelosuppression, it has been empirically observed that the incidence after

administration of 1 mg/kg in small dogs is relatively low, and insufficient dosing may be a concern in some cases. However, because of concerns regarding worsening gastrointestinal toxicity, [6] dose escalation in small dogs has not been sufficiently investigated. Therefore, in this thesis, the incidence of adverse events was evaluated when DOX was administered at 25 mg/m² with maropitant in dogs weighing 5–10 kg.

In recent years, in addition to conventional cytotoxic anticancer drugs, there has been increasing interest in drugs with immunomodulatory and anti-angiogenic effects.[29-31] Thalidomide is one such drug; it has mechanisms of action including suppression of tumor necrosis factor-α production, modulation of immune responses, and inhibition of angiogenesis, and it is used mainly for multiple myeloma in human medicine.[31] Although clinical experience with thalidomide in dogs is limited, postoperative adjuvant thalidomide administration has been reported to potentially prolong survival in dogs with splenic hemangiosarcoma.[32] Lethargy and other adverse events have been reported [32]; however, because of limitations in case numbers and observation periods, safety has not been sufficiently evaluated. In addition, pharmacokinetic data are scarce, and such evaluations are essential for establishing appropriate dose settings.

As described above, although chemotherapeutic agents are widely used in dogs, investigation of basic dose settings remains insufficient.[6,18] In particular, evidence regarding optimal doses in small dogs is limited, and the balance between efficacy and toxicity has not been adequately evaluated. In addition, for new or less commonly used drugs such as thalidomide, safety and pharmacokinetics in dogs have not been sufficiently evaluated.[33] Addressing these issues is essential for improving treatment outcomes and advancing evidence-based veterinary oncology.

Based on this background, this thesis focused particularly on small dogs and investigated the safety of chemotherapeutic agents and optimization of dose settings in dogs. Specifically, adverse events after vinblastine administration in small dogs, adverse events associated with doxorubicin administered with maropitant, and the safety and pharmacokinetics of thalidomide in tumor-bearing dogs were evaluated. Through these studies, this thesis aimed to provide findings that contribute to optimization of chemotherapeutic dosing in dogs and to improvement of the safety and efficacy of cancer treatment in veterinary clinical practice.

Chapter 1

Adverse events in small dogs treated with a single dose of vinblastine

SUMMARY

Vinblastine (VBL) is a chemotherapeutic agent commonly used to treat malignant tumors in dogs; however, although a maximum tolerated dose of 3.5 mg/m² has been reported, its safety and tolerability in small dogs remain inadequately characterized. This study retrospectively evaluated adverse events (AEs) associated with a single intravenous administration of VBL at a dosage of 2.0 mg/m² in dogs weighing <10 kg, with the hypothesis that AE occurrence would be comparable to that reported in phase I studies involving larger breeds. Forty client-owned dogs weighing <10 kg with malignant tumors were included. Myelosuppressive and gastrointestinal AEs were graded according to VCOG-CTCAE v2.0 criteria. Neutropenia and thrombocytopenia were observed in 82.5% (33/40) and 5.0% (2/40) of dogs, respectively. Grade 3 or 4 neutropenia occurred in 32.5% (13/40), including 15.0% (6/40) with grade 3 and 17.5% (7/40) with grade 4 events. Inappetence, vomiting, and diarrhea were reported in 7.5% (3/40), 7.5% (3/40), and 25.0% (10/40) of dogs, respectively, and were generally mild. Febrile neutropenia was noted in 5.0% (2/40) of dogs.

Based on these findings, VBL administered at a dosage of 2.0 mg/m² in small dogs with malignant tumors resulted in gastrointestinal toxicity within clinically acceptable limits; however, close monitoring for neutropenia is warranted, and dose escalation of VBL, as reported in larger dogs, may not be feasible in smaller dogs.

INTRODUCTION

Vinblastine (VBL) is a vinca alkaloid that binds to tubulin in microtubules, thereby disrupting spindle formation and inducing cell cycle arrest.[34-36] It is commonly used in dogs for the treatment of various malignant tumors.[8-14] Historically, it has been employed in the management of mast cell tumors,[8-12,15] but more recently, its efficacy has also been reported in urothelial carcinoma[13,16] and large cell lymphoma.[14,17,18] Several studies have empirically used VBL at a dosage of 2.0 mg/m^2, typically administered every 1-2 weeks.[8,9] Because of the relatively low incidence of adverse events (AEs) observed at this dosage, a phase I dose-escalation study was conducted in 2008, increasing the dosage to 3.0, 3.5, and 4.0 mg/m^2 q2 weeks.[18] The maximum tolerated dose (MTD) was determined to be 3.5 mg/m^2 q2 weeks.[18] However, the median body weight of dogs enrolled in that study was 35.5 kg, and none weighed <10 kg. Conversely, a study evaluating VBL in dogs with transitional cell carcinoma (TCC) found a higher incidence of neutropenia in dogs weighing <15 kg treated at 3.0 mg/m^2 (75%), compared with those ≥15 kg (23.1%).[13] These findings suggest that small breed dogs may have increased susceptibility to VBL-related toxicity and that alternative dosing strategies may be warranted.

In veterinary oncology, chemotherapeutic doses typically are calculated based on body surface area (BSA) rather than body weight to account for inter-breed size variation. However, several studies have shown that small dogs may experience a higher incidence of AEs despite BSA-based dosing.[6,7,37-40] Agents such as doxorubicin, mitoxantrone, melphalan, and carboplatin are known to require lower recommended doses in small breed dogs than in larger breeds.[6,7,39,40] Although the pharmacologic

profile of VBL has been well described in large dogs,[41] its safety in smaller dogs has not been adequately investigated.

Our objective was to retrospectively evaluate AEs after a single administration of VBL at 2.0 mg/m^2 in tumor-bearing dogs weighing <10 kg. I hypothesized that the frequency of AEs in these small dogs would be comparable to that reported in phase I studies involving larger dogs.

MATERIALS AND METHODS

Study animals

A retrospective review was conducted of dogs weighing < 10.0kg presented to the Japan Small Animal Cancer Center, affiliated with the Japan Small Animal Medical Center Foundation, between May 2012 and December 2022. Dogs were eligible for inclusion if they had a cytologic or histopathologic diagnosis of malignancy and received VBL at a dosage of 2.0mg/m^2.

Dogs were excluded if they had a prior history of VBL administration, were receiving concurrent cytotoxic chemotherapy or molecularly targeted therapy, or exhibited severe clinical signs attributable to gastrointestinal disease or the primary tumor, such as mast cell tumors with Darier's signs or systemic illness. Dogs also were excluded if they were suspected of having clinically relevant hepatic failure, had a pre-treatment total bilirubin concentration exceeding 1.5 times the upper limit of the reference interval,[41] or belonged to breeds with a high likelihood of the MDR1 gene mutation (ie, Collies and Australian Shepherds). In addition, dogs that had received toceranib or imatinib within 4 days before VBL treatment were excluded. However, dogs that had received prior

cytotoxic chemotherapy were included if the interval between their most recent treatment and VBL administration exceeded the standard dosing interval.

Study design

A dose of VBL equivalent 2.0 mg/m^2 was added to 5-10 mL of normal saline and administered IV over approximately 5 min. Data collected included signalment, tumor type, concomitant medications, presence or absence of febrile neutropenia (FN), and AEs related to myelosuppression and gastrointestinal toxicity.

Myelosuppression was assessed by evaluating the severity and frequency of neutropenia and thrombocytopenia. Febrile neutropenia was defined as a body temperature ≥39.2 °C with a neutrophil count ≤2500 cells/μL.[38] Gastrointestinal toxicity was evaluated by scoring appetite on a 10-point scale, with owners documenting the daily frequency of vomiting and diarrhea.

I evaluated the frequency of hematologic and gastrointestinal toxicities according to the presence or absence of macroscopic lesions.

The observation period was defined as the interval between VBL administration and the next chemotherapy treatment. All toxicities were graded according to the Veterinary Cooperative Oncology Group–Common Terminology Criteria for Adverse Events (VCOG-CTCAE v2.0).[42]

Statistical analysis

The frequency of grade ≥3 neutropenia (severe neutropenia) was compared between dogs weighing below and above the median, as well as between those younger and older than the median age,[43] using a χ^2 test. Statistical significance was defined as $P < 0.05$.

Analyses were performed using Stata Statistical Software, version 14.2 (StataCorp, College Station, TX, United States).

RESULTS

Study demographics

Forty dogs were included in the study. All dogs that received VBL were included in the evaluation of AEs. The median age was 9.5 years (range, 5-15 years) and median body weight was 4.8 kg (range, 1.7-9.4 kg). The study population consisted of 8 intact males, 9 neutered males, 4 intact females, and 19 spayed females. Breeds represented included Chihuahua (n = 6), Jack Russell Terrier (n = 5), Toy Poodle (n = 5), Miniature Dachshund (n = 5), mixed breed (n = 3), Maltese (n = 3), Miniature Schnauzer (n = 2), Pug (n = 2), Shiba Inu (n = 2), and one each of English Bulldog, Boston Terrier, French Bulldog, Italian Greyhound, Shih Tzu, Pomeranian, and Yorkshire Terrier. Tumor types included cutaneous or SC mast cell tumors (n = 34), TCC of the bladder (n = 4), nasal mast cell tumor (n = 1), and gastrointestinal mast cell tumor involving the small intestine, ileum, and liver (n = 1). At the time of VBL administration, 18 dogs had macroscopic disease, whereas 22 had microscopic disease only.

Concomitant medications included prednisolone (n = 25), enrofloxacin (n = 25), maropitant (n = 17), famotidine (n = 14), diphenhydramine (n = 14), omeprazole (n = 3), cephalexin (n = 2), and ursodeoxycholic acid (n = 2). The following medications each were administered to 1 dog: ondansetron, firocoxib, lansoprazole, metronidazole, piroxicam, telmisartan, torasemide, and benazepril (some dogs received multiple agents; see Table S1-1). All antimicrobial agents were prescribed

prophylactically at the discretion of the attending veterinarian, and maropitant likewise was administered prophylactically in all cases. With respect to the timing of administration, one dog received IV maropitant only on the day of VBL administration; 6 dogs received IV maropitant on the day of VBL treatment followed by PO administration (duration, 2 days; n = 1; 6 days, n = 5); and 10 dogs received PO maropitant only after VBL administration (duration, 4 days; n = 2; 5 days, n = 3; 6 days, n = 4; 14 days, n = 1). Given the potential for proton pump inhibitors (PPIs) to influence VBL metabolism,[41,44] only 3 dogs received concurrent PPI treatment. In 1 dog with a cutaneous mast cell tumor presenting as a macroscopic lesion, a PPI was administered as supportive care, but no objective response to VBL was observed, and treatment was discontinued after a single dose. Another dog had a mast cell tumor without macroscopic lesions, and omeprazole was prescribed prophylactically to prevent prednisolone-associated gastrointestinal toxicosis. This dog received 8 VBL treatments without evidence of recurrence during the treatment course. In the remaining dog, a PPI had already been prescribed before VBL administration for management of firocoxib-associated gastrointestinal toxicosis. Although gastrointestinal signs had nearly resolved by the time of VBL administration, no therapeutic response to VBL was observed, resulting in discontinuation after a single dose.

Chemotherapeutic agents administered within 1 month before VBL included toceranib (n = 3), imatinib (n = 2), and bleomycin and intralesional cisplatin for electrochemotherapy (n = 1).

Adverse events

Neutropenia and thrombocytopenia were observed in 82.5% (33/40) and 5.0% (2/40) of dogs, respectively. Grade ≥ 3 neutropenia occurred in 32.5% (13/40) of dogs. The distribution of neutropenia grades is presented in Table 1-1. The mean and median time to neutrophil nadir were 6.9 and 7 days, respectively (range, 3-13 days). Of the 2 dogs in which neutropenia was identified on day 3, one was presented with gastrointestinal signs, whereas the other was presented for evaluation of fever and found to be neutropenic on complete blood count (CBC) performed at that visit. The day on which the neutrophil nadir was observed was day 7 in 80% (32/40) of dogs, earlier than day 7 in 10% (4/40), and later than day 7 in 10% (4/40). The distribution of nadir confirmation days is shown in Figure 1-1. With a minimum neutrophil count of 2000/μL required for VBL administration,[45,46] 21 of 36 dogs (58.3%) had neutrophil counts ≤ 2000/μL on day 7, precluding subsequent VBL administration. Thrombocytopenia was observed in 2 dogs, both on day 7 after VBL administration. Febrile neutropenia was documented in 2 dogs (5.0%), both of which developed Grade 4 neutropenia. One dog had a mast cell tumor, and the other had TCC. Their body weights were 2.3 and 4.1 kg, respectively. The frequency of Grade ≥ 3 neutropenia was 8 of 20 dogs (40%) in dogs with body weights below the median, and 5 of 20 dogs (25%) in those with body weights at or above the median. No significant difference was observed in the frequency between the two groups (P = 0.3; 95% confidence interval [CI], 0.43-9.82).

Regarding age, the frequency of Grade ≥ 3 neutropenia was 7 of 20 dogs (35%) in dogs younger than the median age, and 6 of 20 dogs (30%) in those at or above the median age. No significant difference was observed between the groups (P = 0.7; 95% CI, 0.27-5.85).

Inappetence was observed in 3 dogs. Two dogs were classified as Grade 3 and occurred concurrently with FN. The 1 dog with Grade 1 diarrhea had pre-existing soft feces before VBL administration. One dog developed Grade 3 diarrhea during FN. The distribution of gastrointestinal AEs by grade is presented in Table 1-2. One dog with a mast cell tumor originating in the small intestine developed Grade 2 neutropenia, but no gastrointestinal toxicities were observed.

The frequency of hematologic and gastrointestinal toxicities according to the presence or absence of macroscopic lesions is shown in Table 1-3.

DISCUSSION

My objective was to evaluate AEs associated with a single administration of VBL at 2.0 mg/m^2 in dogs weighing <10 kg. The findings indicate that neutropenia was the primary AE, whereas gastrointestinal toxicity generally was mild. In this cohort, the frequency of Grade ≥ 3 neutropenia was 32.5%, which is comparable to the 25% frequency reported in medium- to large-breed dogs treated with VBL at 3.5 mg/m^2 q2 weeks.[18] Additionally, approximately half of the dogs failed to demonstrate adequate neutrophil recovery (> 2000/μL) by day 7 after VBL administration. These results suggest that weekly administration of VBL at 2.0 mg/m^2 may not be feasible in some small breed dogs.

When chemotherapy agents are dosed based on BSA, small breed dogs may be more susceptible to AEs.[6,7,37-40] In particular, some chemotherapy agents, such as doxorubicin, mitoxantrone, melphalan, and carboplatin, are dosed based on body weight to mitigate toxicity in small dogs.[6,7,39,40] A previous study of VBL in dogs with TCC

reported that those weighing <15 kg required dose reductions from 3.0 mg/m^2 q2 weeks because of myelotoxicity.[13] In our study, 32.5% of dogs developed Grade ≥ 3 neutropenia at a dosage of 2.0 mg/m^2, further suggesting that increasing the dose above this threshold may not be tolerable for dogs weighing <10 kg.

The appropriate dosing interval for VBL also may differ based on body size and drug tolerance. In protocols for treating mast cell tumors in dogs, VBL at a dosage of 2.0 mg/m^2 often is administered weekly in combination with prednisolone.[8,9] However, the phase I study that established the MTD of 3.5 mg/m^2 q2 weeks recommended a biweekly dosing interval.[18] In our study, CBCs obtained 7 days after treatment indicated that 45% (18/40) of dogs had neutrophil counts < 1500 cells/μL, precluding administration of the next dose. These findings suggest that the severity of myelosuppression at 2.0 mg/m^2 in small dogs may approximate that seen in larger dogs treated at the MTD. At present, I consider a dosage of 2.0 mg/m^2 every 2 weeks appropriate for small breed dogs.

Neutropenia is the primary dose-limiting toxicity of VBL,[18,47,48] and the development of FN poses a serious clinical concern. In previous studies, FN occurred in 1 of 16 dogs (6.3%) and 2 of 26 dogs (7.7%) treated with 3.5 mg/m^2 VBL q2 weeks.[18,49] In our study, FN occurred in 2 of 40 dogs (5.0 %), both of which had Grade 4 neutropenia. Although the frequency is similar to prior reports, most dogs in our study received prophylactic antibiotics, which may have decreased the risk of infection. Therefore, the incidence of FN in the absence of prophylactic treatment may be underestimated.

I examined whether hematologic toxicity associated with VBL is more prevalent in dogs with lower body weights by comparing the frequency of severe neutropenia between dogs with body weights below and above the median. Although no significant

difference was observed between the two groups, these findings should be interpreted with caution. The limited sample size and low number of severe neutropenia events raise the possibility of Type II error (false-negative results). Notably, both cases of FN occurred in dogs with body weights below the median. A VBL dosage lower than 2.0 mg/m^2 may be appropriate in individual dogs, and ongoing monitoring for hematologic toxicity is warranted in small dogs receiving more than a single dose of VBL.

Vinblastine generally is regarded as well tolerated, with low rates of gastrointestinal toxicity.[18,48,49] In a phase I study of VBL at 3.0-4.0 mg/m^2 q2 weeks (n = 23), vomiting occurred in 2 dogs (Grades 1 and 2) and diarrhea in 1 dog (Grade 1).[18] Another study using 2.0-3.0 mg/m^2 (n = 24) q1 week reported vomiting in 3 dogs (Grades 1-3) and diarrhea in 3 dogs (Grades 1-2).[50] In our study, most gastrointestinal AEs were < Grade 2 and clinically manageable. However, because prophylactic antiemetics and antidiarrheals were administered concurrently in many cases, the true incidence of gastrointestinal toxicity may have been underestimated. Future studies in dogs not receiving prophylactic medications are warranted to better assess VBL-associated gastrointestinal effects.

Our study had some limitations. First, its retrospective design and the evaluation of AEs limited to the initial administration of VBL may have introduced bias. The inclusion of cases that had received prior cytotoxic or molecularly targeted treatments could have influenced the assessment of toxicity. In addition, heterogeneity in tumor types, lack of standardized tumor staging and mast cell tumor grading, and the absence of controls on concomitant medications further limit the generalizability of our findings. The latter, in particular, may have introduced confounding variables affecting the

frequency of gastrointestinal toxicity or FN. Gastrointestinal toxicity was assessed based on owner-reported observations, raising the possibility of underreporting. Moreover, because CBCs were not monitored on a daily basis after VBL administration, the exact nadirs of neutrophil and platelet counts could not be determined, which may have led to an underestimation of the actual incidence of neutropenia and thrombocytopenia.

In conclusion, AEs observed after administration of VBL at 2.0 mg/m^2 in dogs weighing <10 kg appear comparable to those reported in larger dogs treated at the MTD of 3.5 mg/m^2 q2 weeks. Our findings suggest that small dogs may require distinct dosing strategies. Prospective evaluation using a Phase I dose escalation study is warranted to establish an appropriate and safe dosing regimen for this population.

Table 1-1. Incidence of cytopenias in small dogs (<10 kg) treated with a single dose of vinblastine (VBL) 2.0 mg/m^2.

	VBL 2.0 mg/m^2	
	n = 40	%
Neutropenia	33	82.5
Grade 1	13	32.5
Grade 2	7	17.5
Grade 3	6	15
Grade 4	7	17.5
Thrombocytopenia	2	5.0
Grade 1	1	2.5
Grade 2	0	0
Grade 3	1	2.5
Grade 4	0	0
Febrile Neutropenia	2	5.0

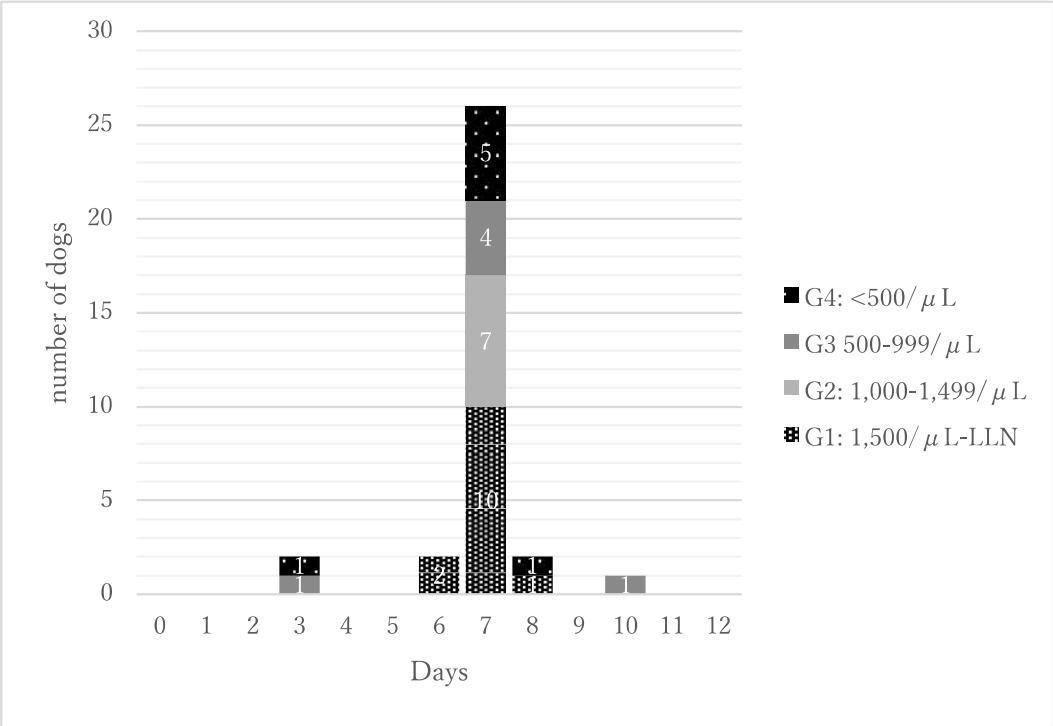
Table 1-2. Incidence of clinical signs associated with gastrointestinal toxicosis in small dogs (<10 kg) treated with a single dose of vinblastine (VBL) 2.0 mg/m^2.

	VBL 2.0 mg/m^2	
	n = 40	%
Anorexia	3	7.5
Grade 1	1	2.5
Grade 2	0	0
Grade 3	2	5
Grade 4	0	0
Vomiting	3	7.5
Grade 1	3	7.5
Grade 2	0	0
Grade 3	0	0
Grade 4	0	0
Diarrhea	10	25
Grade 1	6	15
Grade 2	3	7.5
Grade 3	1	2.5
Grade 4	0	0

Table 1-3. Incidence of adverse events according to the presence or absence of gross lesions.

Gross lesions	Neutropenia	Thrombocytopenia	Anorexia	Vomiting	Diarrhea
Yes n=18 (%)	13 (72.2)	2 (11.1)	1 (5.5)	1 (5.5)	4 (22.2)
No n=22 (%)	20 (90.9)	0	2 (9.0)	2 (9.0)	6 (27.2)

Figure 1-1. The days on which neutrophil nadirs were confirmed after vinblastine administration.



Supplementary Table 1-1. Concomitant medications given to dogs in this study.

No.	Concomitant medications
1	Cefalexin, Firocoxib
2	Prednisolone, Famotidine
3	Prednisolone, Famotidine, Diphenhydramine, Ursodeoxycholic acid
4	Prednisolone, Enrofloxacin, Telmisartan
5	Prednisolone, Famotidine, Diphenhydramine, Enrofloxacin
6	Cefalexin,
7	Prednisolone, Famotidine, Diphenhydramine, Enrofloxacin
8	Prednisolone, Famotidine, Diphenhydramine, Enrofloxacin
9	Prednisolone, Famotidine, Diphenhydramine
10	Prednisolone, Enrofloxacin
11	Enrofloxacin
12	Ursodeoxycholic acid, Enrofloxacin
13	Maropitant, Omeprazole, Metronidazole
14	Prednisolone, Famotidine, Diphenhydramine, Enrofloxacin
15	Prednisolone, Famotidine, Diphenhydramine, Enrofloxacin
16	Prednisolone, Famotidine, Diphenhydramine, Enrofloxacin
17	Prednisolone, Enrofloxacin
18	Prednisolone, Enrofloxacin, Diphenhydramine, Lansoprazole
19	Maropitant, Omeprazole, Prednisolone, Enrofloxacin
20	Prednisolone, Diphenhydramine, Famotidine, Maropitant
21	Enrofloxacin, Benazepril, Torasemid
22	Prednisolone, Enrofloxacin
23	Prednisolone, Enrofloxacin, Maropitant
24	Prednisolone, Enrofloxacin, Famotidine
25	Prednisolone, Omeprazole, Diphenhydramine, Enrofloxacin, Maropitant
26	Enrofloxacin, Maropitant, Piroxicam
27	Prednisolone, Famotidine, Diphenhydramine, Enrofloxacin, Ondansetron
28	Famotidine, Enrofloxacin
29	Maropitant
30	Maropitant, Prednisolone
31	Maropitant, Prednisolone
32	Maropitant, Enrofloxacin
33	Maropitant, Enrofloxacin
34	Prednisolone, Famotidine, Diphenhydramine, Maropitant
35	Prednisolone, Enrofloxacin, Diphenhydramine
36	Maropitant
37	Maropitant, Enrofloxacin
38	Maropitant
39	Maropitant
40	Maropitant, Prednisolone

Chapter 2

Evaluation of adverse events in small-breed dogs treated with maropitant and a single dose of doxorubicin

SUMMARY

The recommended doxorubicin (DOX) dose for small dogs is 1 mg/kg. Recent data suggest that DOX-induced gastrointestinal (GI) toxicosis can be reduced with maropitant treatment. This study investigated the incidence of adverse events (AEs) in small-breed dogs administered a single 25 mg/m^2 DOX followed by administration of maropitant (DOX25). The primary aim was to assess myelo- and GI toxicoses for 2 weeks after DOX administration. The secondary aim was to compare the incidence and grades of AEs found in the DOX25 group with a historical control group (DOX 1 mg/kg without administration of antiemetic or antidiarrheal medications).

Nineteen small-breed tumor-bearing dogs were included. This was a prospective, observational study of tumor-bearing dogs, weighing 5 to 10 kg, that received a single intravenous dose of DOX (25 mg/m^2), followed by administration of maropitant for 5 days.

Inappetence, vomiting, and diarrhea occured in 7/19, 2/19, and 6/19 dogs, respectively. Neutropenia and thrombocytopenia occured in 12/19 and 3/19 dogs, respectively. Most AEs were grades 1 and 2, except for grades 3 and 4 inappetence and neutropenia in 3 and 4 dogs, respectively. Furthermore, febrile neutropenia occurred in 3/19 dogs in the DOX25 group. There were no significant differences in the incidence or severity of AEs between the DOX25 and historical control groups.

Based on these findings, vomiting and diarrhea were deemed acceptable with 25 mg/m^2 DOX followed by maropitant treatment in 5 to 10 kg dogs; however, additional supportive care might be needed for dogs with inappetence and neutropenia.

INTRODUCTION

Doxorubicin (DOX) is a chemotherapeutic agent used to treat a variety of malignancies in dogs and cats.[19-26] Adverse events (AEs) associated with DOX include short-term toxicoses, such as gastrointestinal (GI) and myelotoxicosis.[19,26] The incidence of DOX-induced GI toxicosis is 24% to 64%. [19,26,28] The most common signs related to GI toxicosis include inappetence, vomiting, diarrhea, and colitis that usually occur 3 to 5 days after DOX administration.[26] Neutropenia is the primary consequence of myelotoxicosis with an incidence of 11% to 13.2%, usually occurring 5 to 10 days after DOX administration.[4,19,26]

Maropitant is an antiemetic used in dogs. It is an NK1 (neurokinin 1) receptor antagonist effective against chemotherapy-induced vomiting.[28,51,52] Administration of maropitant before cisplatin treatment is effective in cancer bearing dogs.[51] Maropitant reduced incidence of vomiting in 59 tumor-bearing dogs from 35% to 8%.

Doxorubicin is generally administered at 30 mg/m^2 in dogs weighing ≥10 to 15 kg and at 1 mg/kg in dogs weighing ≤10 to 15 kg.[22,27] This body weight-based dosing regimen is based on the observation that GI toxicosis and neutropenia (<1000 cells/μL) was more often seen in dogs weighing ≤10 kg than in those weighing ≥10 kg after the administration of 30 mg/m^2 DOX.[6] Depending on body weight, the dose calculated with mg/kg could actually be lower than that calculated with mg/m^2 and therefore might not be a sufficient therapeutic dose. Without an appropriate phase 1 clinical trial evaluating DOX dosing in small dogs, few attempts have been made to increase dosing in small dogs over concerns about AEs, especially GI toxicosis.

This study aimed to prospectively investigate the incidence of GI and bone marrow AEs in dogs weighing 5 to 10 kg, treated with the initial single DOX dose at 25 mg/m^2,

followed by maropitant treatment (DOX25). The secondary aim was to compare the incidence and grades of AEs found in the DOX25 group with a historical control group (DOX 1 mg/kg without antiemetics and antidiarrheals). I hypothesized that the combined use of maropitant with DOX would allow the DOX dose to be increased in small dogs.

MATERIALS AND METHODS

Study Animals

This prospective observational study included dogs weighing 5.0 to 10.0 kg that were presented to the Japan Small Animal Cancer Center at the Japan Small Animal Medical Center between May 2012 and September 2017. Dogs were included in the study if they were to receive an initial DOX dose of either a single agent or as part of a multiagent protocol for the treatment of various malignancies diagnosed by cytology, histopathology, or diagnostic imaging (ie, cardiac hemangiosarcoma). Dogs were excluded from the study if they had an earlier history of DOX treatment, received any antiemetic or antidiarrheal agents after DOX treatment, or had preexisting GI toxicosis before DOX administration, such as inappetence, vomiting, and diarrhea, or had a life expectancy of less than 1 month. However, I did not restrict drugs that were related to an underlying disease and had been prescribed before DOX administration or drugs prescribed for febrile neutropenia (FN) prophylaxis. Complete blood count, serum chemistry panels, thoracic radiographs, and abdominal ultrasound imaging were performed in all dogs at the initial visit for staging. Before the study, written informed consent was obtained from all owners.

Study design

All dogs were administered a 25 mg/m^2 DOX dose (Doxorubicin Hydrochloride; ADRIACIN Injection 10, Aspen Japan, Tokyo, Japan) by IV infusion over 30 to 60 minutes without any premedication. Doxorubicin was diluted in 25 to 100 mL physiologic saline. Maropitant (Maropitant citrate monohydrate, Cerenia, Zoetis Japan, Inc, Tokyo, Japan) was administered PO once a day at a dose of 2 mg/kg for 5 days from the day after DOX administration. To assess the general condition of the dogs, owners were asked to record their dog's activity level, appetite, and water intake throughout the day on a 10-point scale. We asked the owners to measure body temperatures by rectal temperature, heart rates by palpable heartbeat in the left thorax or femoral artery, and respiratory rates by abdominal movement with breathing. All measurements were taken at rest and at home twice a day. The measured items were written on a sheet and submitted weekly. For GI toxicosis evaluations, we asked about the severity of vomiting (frequency and duration per day) and diarrhea (a 4-level severity scale about the frequency and duration per day) for 2 weeks after DOX administration. To assess myelotoxicosis, CBC was performed 7 and 14 days after DOX administration to determine the incidence of neutropenia and thrombocytopenia. Febrile neutropenia was defined as a body temperature of ≥39.2 °C and neutrophil count of ≤2500 cells/μL,[38] and its incidence during the study period was recorded. Gastrointestinal toxicosis and myelotoxicosis were graded according to the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE v1.1).[53] Treatments for AEs were performed at the discretion of attending veterinarians and was not standardized. In addition, the incidences of GI toxicosis and myelotoxicosis were compared with those in the historical control group.

Historical control group inclusion criteria

The historical control group consisted of dogs weighing 5 to 10 kg that were presented to the Japan Small Animal Cancer Center at the Japan Small Animal Medical Center, or Kobayashi Animal Hospital, Saitama, between January 2003 and December 2009. These dogs received DOX at 1 mg/kg to treat various malignancies and received no antiemetics or antidiarrheals after DOX administration. However, I did not restrict drugs related to an underlying disease and had been prescribed before DOX administration, or drugs prescribed for FN prophylaxis. I obtained signalment, tumor types, concomitant medications, presence of vomiting or diarrhea, and CBC on days 7 and 14 after the DOX administration.

Statistical analysis

For statistical comparison between the DOX25 and historical control groups, the Wilcoxon rank-sum test was used for age and body weight. The Chi-square test or Fisher's exact test was used for sex ratios and AE incidences. Differences were considered significant at a $P < .05$. All statistical analyses were performed using Stata Statistical Software Ver. 14.2 (StataCorp, College Station, Texas).

RESULTS

Study demographics

A total of 19 dogs were included in the DOX25 group. The median age was 10 years (range, 5-14 years). There were 3 intact and 6 castrated males and 2 intact and 8 spayed females. There were 9 Miniature Dachshunds, 2 Miniature Schnauzers, and 1 of a different breed. The mean body weight was 6.5 kg (range, 5.03-10.0 kg). The most

common tumor type was high-grade lymphoma (multicentric = 4, hepato-splenic = 2), followed by hemangiosarcoma (right atrium = 1, subcutaneous = 2, spleen = 1), transitional cell carcinoma of the bladder (n = 3), subcutaneous soft tissue sarcoma (n = 2), sarcoma (spleen = 1, liver = 1), mandibular osteosarcoma (n = 1), and mammary gland adenocarcinoma (n = 1). As for the disease status at the time of DOX administration, 4 dogs with multicentric lymphosarcoma (LSA) and 1 dog with hepato-splenic LSA had complete remission, and 1 dog with hepato-splenic lymphoma did not have abdominal ultrasonography or a cytologic examination immediately before the DOX administration because of a complete resolution of clinical signs. For tumors other than lymphoma, 4 dogs had gross lesions, and 9 had microscopic lesions at the time of DOX administration. Six dogs received DOX as part of a multidrug protocol, and 13 dogs received DOX as a single agent. The mean and median doses in the DOX25 group were 8.9 and 8.8 mg, respectively. If these dogs had been administered 1 mg/kg dose, the mean and median dose would have been 6.7 and 6.5 mg, respectively. Compared with DOX at 1 mg/kg, the mean dose intensity of DOX at 25 mg/m^2 was 1.32 times higher and the median was 1.35 times higher. Complete blood count on day 7 were performed on day 7 after the DOX administration in 14 dogs and on day 7 ± 1 in the remaining 5 dogs for owner convenience. Complete blood count on day 14 were performed on day 14 in 11 dogs and on day 14 ± 2 in the remaining 8 dogs for the same reason.

Adverse events

The incidence of signs of GI toxicosis in the DOX25 group was 7/19 for inappetence, 2/19 for vomiting, and 6/19 for diarrhea. Most GI toxicosis assessments were grades 1

and 2, except for grade 3 inappetence in 3 dogs (Table 2-1). The incidence of neutropenia and thrombocytopenia in the DOX25 group was 12/19 and 3/19, respectively. The majority of neutropenic events were grades 1 and 2, except for grades 3 and 4 neutropenia observed in 1 and 3 dogs, respectively (Table 2-2). The incidence of FN in the DOX25 group was 3/19, and FN was observed in all 3 dogs with grade 4 neutropenia. All dogs with FN recovered without any problems.

Concomitant treatments

Aside from maropitant treatment, the following concomitant medications were used after DOX administration: drugs given as diseaserelated were prednisolone (n = 5), firocoxib (n = 4), meloxicam (n = 1), enalapril (n = 1), and levothyroxine (n = 1). Famotidine was prescribed to 6 dogs receiving long-term treatments with prednisolone or NSAIDs. The drugs given for FN prophylaxis were enrofloxacin (n = 16), cephalexin (n = 1), fosfomycin (n = 1), and ofloxacin (n = 1). One of the 2 dogs that had vomiting was receiving enrofloxacin as a concomitant medication, but the other dog had not received any concomitant medication at the time vomiting was observed. Five of the 6 dogs that had diarrhea received either enrofloxacin, ofloxacin, or fosfomycin, and 3 of the 6 dogs received either famotidine, firocoxib, or prednisolone concomitantly (partially overlapping). One dog did not receive any concomitant medication at the time diarrhea was observed. Six of the 7 dogs that had inappetence were receiving either enrofloxacin or ofloxacin, and 3 of the 7 were receiving famotidine, firocoxib, or meloxicam (partially overlapping). One dog did not receive any concomitant medication at the time inappetence was observed. On the other hand, 10 dogs that did not experience vomiting, diarrhea, or inappetence received either enrofloxacin or cephalexin in all cases, and

famotidine, prednisolone, firocoxib, enalapril, or levothyroxine in 7 dogs (partially overlapping). Enrofloxacin was administered to 2 of the 3 dogs with FN and ofloxacin in 1 dog. Enrofloxacin was administered to 2 of the 3 dogs with FN and ofloxacin in 1 dog (Table S2-1).

Demographics of the historical control group

A total of 18 dogs were included in the historical control group. The median age was 9.5 years (range, 3-14 years). There were 7 intact and 4 castrated males and 4 intact and 3 spayed females. There were 6 Shih Tzu, 4 Miniature Schnauzers, 2 Miniature Dachshunds, 2 mixed dogs, and 1 of a different breed. The mean body weight was 6.4 kg (range, 5.2-9.5 kg). There were no significant differences in age, sex, or body weight between the DOX25 and historical control groups (Table 2-3). The tumor types included high-grade lymphoma (multicentric= 5, GI = 1, cutaneous = 2, hepatic = 1, oral lymphoma with bone metastasis = 1), carcinoma (oral undifferentiated adenocarcinoma or squamous cell carcinoma = 1, cutaneous apocrine adenocarcinoma = 1), mammary gland adenocarcinoma (n = 2), nasal adenocarcinoma (n = 1), hepatic liposarcoma (n = 1), fibrous histiocytic nodule (n = 1), and subcutaneous undifferentiated sarcoma (n = 1). As for the disease status at the time of DOX administration, 9 dogs with lymphoma had complete remission, and 1 dog with cutaneous lymphoma had gross disease. For tumors other than lymphoma, 6 dogs had gross lesions and 2 had microscopic lesions at the time of DOX administration. In the historical control group, 8 dogs received DOX as a single agent, and 10 dogs received DOX as a part of a multidrug protocol. Doxorubicin was administered as the initial dose in all cases. The following concomitant medications were used after DOX

administration: drugs given as disease-related were prednisolone (n = 6), piroxicam (n = 3), meloxicam (n = 1), thalidomide (n = 2), ursodeoxycholic acid (n = 1), and enalapril (n = 3). Famotidine was prescribed in 6 dogs receiving long-term treatment with prednisolone or NSAIDs. The drugs given for FN prophylaxis were cephalexin (n = 3), enrofloxacin (n = 1), minocycline (n = 1), and ampicillin (n = 1). In the historical control group, all dogs had CBC on day 7 ± 1 after DOX administration. Thirteen dogs had CBC on day 14 ± 1, 2 on day 11, and 1 on day 12 after DOX administration. Two dogs did not have CBC on day 14. One dog had a gastrostomy tube and was excluded from the inappetence analysis.

Comparison of AEs with the historical control group

The incidence of GI toxicosis in the historical control group was as follows: 7/17 for inappetence, 5/18 for vomiting, and 9/18 for diarrhea. There was no significant difference compared with the DOX25 group for any GI toxicosis (Table 2-1). The incidence of neutropenia and thrombocytopenia was 6/18 and 1/18, respectively, and were grades 1 and 2 in all cases. There was no significant difference compared with the DOX25 group for myelotoxicosis (Table 2-2). The incidence of FN in the historical control group was 1/18, and the dog had a grade 2 neutropenia. There was no significant difference in the incidence of FN between the 2 groups ($P = 0.60$; Table 2-2).

DISCUSSION

Our results show that all vomiting and diarrhea was grades 1 and 2 range and was clinically acceptable when DOX 25 mg/m^2 was administered in combination with

maropitant to dogs weighing 5 to 10 kg. On the other hand, inappetence was seen in 36.8% of the dogs, 3 of which were grade 3. In the historical control group, inappetence was also seen in 41.2% of the dogs, 3 of which were grade 3. These results suggest that inappetence after DOX administration should be a concern at any dose. Regarding neutropenia in the DOX25 group, 3 dogs had grade 4 neutropenia, which developed into FN in all of these dogs. This suggests that close monitoring, including temperature pulse respiration measurements at home and supportive care, such as prescription of prophylactic antibiotics, are needed when DOX 25 is administered to dogs weighing 5 to 10 kg.

I showed that vomiting in small dogs weighing 5 to 10 kg, treated with DOX at 25 mg/m^2 with concomitant maropitant, was acceptable. The incidence of vomiting is proportional to dose intensity,[26] meaning that higher dose intensities are associated with greater incidence of vomiting. Therefore, because the incidence of vomiting, which was expected to be higher in the DOX25 group, was actually comparable with that in the DOX 1 mg/kg (historical control) group, it might suggest that maropitant administration with DOX could reduce vomiting at this higher DOX dose in dogs weighing 5 to 10 kg. In the present study, the incidence of vomiting was 10.5% (2/19), and all grades of vomiting were mild. In addition, I did not find a significant difference in the incidence of vomiting between the DOX25 and historical control groups DOX 1 mg/kg without antiemetics or antidiarrheals. However, the results should be interpreted with caution, as the incidence in the DOX25 group might not have been completely comparable with that of the historical control group as a type II error because of small sample sizes. Also, the lack of a significant difference does not mean that they are equivalent.

Our results suggest that maropitant could also reduce diarrhea caused by DOX. The

concomitant use of maropitant with DOX could also reduce diarrhea and vomiting.[28] Neurokinin 1 receptors, where maropitant binds, exist not only in the central chemoreceptor trigger zone but also in the intestinal tract.[54,55] Other studies have shown that substance P plays a significant role in the development of diarrhea in ulcerative colitis and Crohn's disease by binding to NK1 receptors. [56,57] These facts suggest that NK1 receptor antagonists, such as maropitant, can also act on peripheral receptors to inhibit the development of diarrhea. The incidence of diarrhea in the present study was comparable to that reported previously (33%)[28] but showed no significant difference between the DOX25 and historical control groups. In any case, all diarrheal events observed in the 6 dogs of the present study were grades 1 and 2 and considered clinically manageable.

Our data showed that maropitant effectively reduced the incidence of DOX-induced vomiting but did not improve DOX-induced inappetence. The incidence of inappetence after treatment with DOX with concomitant maropitant was 36.8%. In addition, grade 3 inappetence was observed in both the DOX25 and historical control groups, suggesting that it might be necessary to treat inappetence at any dose after DOX administration. The effects of ondansetron, maropitant, and metoclopramide on cisplatin-induced vomiting and nausea in dogs were reported in a single-blind crossover study.[58] Ondansetron, maropitant, and metoclopramide were administered to 8 dogs each immediately after cisplatin and compared the incidence of vomiting and nausea between treatment groups. The results showed that both ondansetron and maropitant effectively reduced vomiting, whereas only ondansetron was effective in improving nausea. Capromorelin is a ghrelin receptor agonist approved by the Food and Drug Administration as an appetite stimulant in dogs. The efficacy of capromorelin for dogs

with anorexia was evaluated in a prospective, randomized, masked, placebo-controlled clinical study and confirmed its appetitestimulating effects.[59] In addition, mirtazapine has been used as a treatment for inappetence in dogs.[60] It is thus important to consider the concomitant use of maropitant with other antiemetics, such as ondansetron, and appetite-stimulating agents, such as capromorelin or mirtazapine for the management of DOX-induced inappetence and nausea.

In the DOX25 group, 4 dogs had grade 3 or higher neutropenia, and all 3 dogs with grade 4 neutropenia developed FN, suggesting that more aggressive strategies for neutropenia may be needed when administering DOX at 25 mg/m^2 to dogs weighing 5 to 10 kg. Different DOX doses in dogs and reported that treating dogs weighing ≤10 kg with DOX at a dose of 30 mg/m^2 resulted in severe neutropenia.[6] Based on this observation, it has been recommended that dogs weighing ≤10 kg be treated with DOX at a dose of 1 mg/kg. These results suggest that when administering DOX at 25 mg/m^2 to dogs weighing 5 to 10 kg, appropriate supports should be taken for the possible occurrence of FN, such as prescribing prophylactic antibiotics and carefully monitoring animal conditions at home. In our study, although there was no significant difference in neutropenia compared with the historical control group, the results should be interpreted with caution as a type II error because of small sample sizes could have occurred.

Limitations to this study include the nonrandomized design and the fact that the study only evaluated AEs after the initial DOX administration but not after additional doses of DOX at 25 mg/m^2. The tumor types and chemotherapy protocol (ie, DOX as a monotherapy or as a part of a multiagent protocol) were not standardized in our study, and the results might have been different if the protocols had been standardized. Moreover, I did not restrict drugs that were related to the underlying disease and had

been prescribed before DOX administration or drugs prescribed for FN prophylaxis. These drugs might have been confounding factors for GI toxicosis. Because of the nature of the historical control group, the staging before DOX, monitoring after DOX, description of vomiting, diarrhea, and inappetence were not as complete as those in the DOX25 group. It is generally accepted in clinical studies to compare study groups with a group of historical control animals; however, the drug doses should be the same between the 2 groups. Before the start of the present study, a 1 mg/kg dose was used in dogs weighing less than 10 kg at our center and the Kobayashi Animal Hospital. Therefore, it was not possible to include dogs weighing >10 kg that were treated with a DOX 25 mg/m^2 dose in the historical control group.

In conclusion, although a DOX dose of 25 mg/m^2 combined with maropitant treatment was considered acceptable in terms of the incidence of vomiting and diarrhea, the fact that FN was observed in 15.8% of dogs suggests that additional management for neutropenia are needed when administering DOX at this dose. Although maropitant was effective in reducing vomiting, it did not show sufficient efficacy against inappetence. Therefore, it is important to consider additional management for inappetence, such as the concomitant use of ondansetron, mirtazapine, and capromorelin.

Table 2-1. A comparison of incidence of signs of gastrointestinal toxicosis between the study and historical control group dogs.

	DOX 25mg/m^2 n = 19	%	DOX 1mg/kg n =18 (17*)	%	Odds ratio	95% CI	P-value
Inappetence	7	36.8	7	41.2	0.8	0.2-3.9	1.0
Grade 1	2	10.5	3	17.6			
Grade 2	2	10.5	1	5.9			
Grade 3	3	15.8	3	17.6			
Grade 4	0	0	0	0			
Vomiting	2	10.5	5	27.8	0.3	0.03-	0.23
Grade 1	2	10.5	4	22.2		2.3	
Grade 2	0	0	1	5.6			
Grade 3	0	0	0	0			
Grade 4	0	0	0	0			
Diarrhea	6	31.6	9	50.0	0.46	0.1-2.1	0.32
Grade 1	5	26.3	7	38.9			
Grade 2	1	5.3	2	11.1			
Grade 3	0	0	0	0			
Grade 4	0	0	0	0			

* One dog in the historical control group had a gastrostomy tube and was excluded from the analysis of inappetence.

Table 2-2. A comparison of incidence of signs of cytopenia between the study and historical control group dogs.

	DOX 25 mg/m^2 n =19	%	DOX 1 mg/kg n =18	%	Odds ratio	95% CI	P-value
Neutropenia	12	63.2	6	33.3	3.4	0.7-	0.1
Grade 1	7	36.8	4	22.2		16.5	
Grade 2	1	5.3	2	11.1			
Grade 3	1	5.3	0	0			
Grade 4	3	15.8	0	0			
Thrombocytopenia	3	15.8	1	5.6	3.2	0.2-	0.6
Grade 1	1	5.3	0	0		177.0	
Grade 2	2	10.5	1	5.6			
Grade 3	0	0	0	0			
Grade 4	0	0	0	0			
Febrile Neutropenia	3	15.8	1	5.6	3.2	0.2- 177.0	0.6

Table 2-3. A comparison of signalments between the study and historical control group dogs.

	DOX 25 mg/m^2 n =19	DOX 1 mg/kg n =18	P-value
Age, yrs	10.0	9.5	0.40*
IQR (range)	(8-13)	(6.5-12.2)	
Weight, kg	6.5	6.4	0.58*
IQR (range)	(5.4-8.0)	(6.0 -7.7)	
Gender			
Male	9	11	0.51**
Female	10	7	

*Wilcoxon rank-sum test.
** chi-square test.

Supplementary Table 2-1. Adverse event descriptions.

No.	Concomitant treatments	Inappetence	Vomiting	Diarrhea	FN
1	enrofloxacin	1 (grade3)	1	1	1
2	enrofloxacin	1 (grade2) *	1 *	1 *	
3	ofloxacin, famotidine, firocoxib	1 (grade3)		1	1
4	enrofloxacin	1		1	
5	enrofloxacin	1			1
6	meloxicam, enrofloxacin	1 (grade2)			
7	enrofloxacin	1 (grade3)			
8	enrofloxacin, prednisolone			1 (grade2)	
9	firocoxib, famotidine, fosfomycin			1	
10	enrofloxacin				
11	prednisolone, famotidine, enrofloxacin, levothyroxine				
12	enrofloxacin				
13	prednisolone, famotidine, enrofloxacin				
14	enrofloxacin				
15	firocoxib, cephalexin				
16	prednisolone, enrofloxacin, famotidine				
17	enrofloxacin, enalapril				
18	prednisolone, enrofloxacin				
19	firocoxib, famotidine, enrofloxacin				

* Before administration of enrofloxacin

Chapter 3

Safety and pharmacokinetics of thalidomide in tumor-bearing dogs

SUMMARY

Thalidomide, an angiogenesis inhibitor, has recently been used to treat malignant canine tumors. This study retrospectively investigated the adverse events (AEs) of thalidomide administered to tumor-bearing dogs. I investigated the pharmacokinetics of thalidomide after administration and the rate of body weight change before and after administration. The initial thalidomide dose was 5 mg/kg per os once daily, which was increased to 10 mg/kg once daily in dogs with no significant AEs. Pharmacokinetics were measured in four dogs after the 5 mg/kg or 10 mg/kg dose. I evaluated AEs related to clinical signs in 51 patients; 9/51 had lethargy, 6/51 had tremor, 4/51 had dizziness, 31/51 had decreased appetite, 8/51 had vomiting, and 16/49 had soft stool/diarrhea. I evaluated hematologic toxicity in 44 patients with grade 3 or higher adverse events; 1/44 had thrombocytopenia, 1/44 had increased blood urea nitrogen concentrations, and 5/44 had increased alanine aminotransferase activities. The mean thalidomide blood levels were Cmax=1.4 ± 0.7 μg/mL (Area under the curve $[AUC]_{0–24}$=8.5 ± 4.7 μg•hr /mL) and Cmax=3.2 ± 2.1 μg/mL ($AUC_{0–24}$=22.0 ± 14.7 μg•hr/mL) in the 5 mg/kg and 10 mg/kg groups, respectively. The Cmax and AUC in the 10 mg/kg group were comparable to the effective blood concentrations seen in humans administered thalidomide. The weight fluctuation rates were assessed in 24 dogs approximately 1 month after the start of thalidomide administration; more than half showed weight maintenance or gain. Most AEs were clinically acceptable; however, peripheral nerve signs were seen in some dogs.

INTRODUCTION

Thalidomide (alpha-phthalidoglutarimide) was marketed in more than 40 countries as a sedative in the late 1950s and prescribed to pregnant women with morning sickness symptoms from 1957 to 1961.[61] However, newborns born to mothers who had taken thalidomide often developed deformities of the limbs, ears, heart, and gastrointestinal tract.[62,63] These teratogenic properties led to the withdrawal of thalidomide from the global markets in 1961. However, thalidomide was remarketed in 1965 due to its remarkable effectiveness against lepromatous erythema nodosum, an acute inflammatory reaction of leprosy.[64-66]

Thirty years later, scientists observed that thalidomide inhibited cachexia-inducing tumor necrosis factor (TNF)-α in stimulated human monocytes,[67] and angiogenesis in rabbit corneas and chorioallantoic membranes of chickens, indicating the possible therapeutic use of this drug for malignant tumors.[29,30] Then, in 2006, the Food and Drug Administration (FDA) approved the combination of thalidomide and dexamethasone for the treatment of multiple myeloma.[31]

In dogs, thalidomide has been used as part of a multimodal treatment for hemangiosarcoma, mammary gland tumors, and lung tumors.[32,68,69] Thalidomide administered as adjuvant chemotherapy after splenectomy for splenic hemangiosarcoma may provide a survival benefit compared with using doxorubicin as an adjuvant,[32] which is supported by the observation that the vascular endothelial growth factor produced by hemangiosarcoma tumor cells is reduced after thalidomide administration.[70]

Adverse events associated with thalidomide, other than teratogenicity and lethargy, have been reported in humans, including neurologic effects, such as dizziness, tremor, and lip and hand numbness, and hematologic effects, such as neutropenia, anemia, and

thrombocytopenia.[66,67,71,72] In dogs, thalidomide has been reported as a well-tolerated drug, and when 43, 200, and 1,000 mg/kg were administered orally to healthy beagle dogs for 53 weeks, no deaths occurred.[73] The same study reported a "No Observed Adverse Effect Level" for thalidomide in dogs receiving 200 mg/kg/day. A retrospective study of 15 dogs with hemangiosarcoma also found no adverse events associated with thalidomide other than lethargy.[32] In contrast, in a study investigating the pharmacokinetics of thalidomide in experimental dogs, tremor and dizziness were observed after thalidomide administration,[33] indicating that dogs can develop peripheral nerve signs similar to those observed in humans.

The main objective of this study was to retrospectively investigate adverse events after thalidomide administration in dogs with malignant tumors. In addition, pharmacokinetics after thalidomide administration and the rate of change in body weight before and after thalidomide administration were also investigated.

MATERIALS AND METHODS

Adverse events associated with thalidomide.

Study animals

Dogs that were presented to the Japanese Small Animal Cancer Center between December 2004 and August 2010 and prescribed thalidomide for the treatment of malignant tumors were retrospectively studied. Malignancy was diagnosed by cytology or histopathology. Dogs that received at least one dose of thalidomide and had at least one visit after the dose were included in the study. Dogs with concomitant radiation therapy or cytotoxic anticancer agents and those with incomplete medical records were excluded.

Thalidomide treatment and follow-up

When thalidomide was prescribed, we explained that thalidomide is a teratogenic drug and obtained the owner's written consent. When administering thalidomide, owners were instructed to wear gloves to prevent direct contact with the drug. The initial dose of thalidomide (Talizer®, Laboratories Serral, Ciudad de Mexico, Mexico) was 5 mg/kg per os once daily before bedtime. After several days of treatment, the dose was increased to 10 mg/kg in dogs with no apparent adverse events. Physical examination and blood tests were performed 1–2 weeks after the start of treatment, followed by regular monthly checkups.

Assessment of adverse events

Thalidomide adverse events were examined for the presence and severity of lethargy, tremor, dizziness, decreased appetite (anorexia), vomiting, diarrhea, and hematologic toxicity. Packed cell volumes (PCVs) and complete blood counts (CBCs) were measured to assess for hematologic toxicity. Serum chemistry panels were also measured to assess for liver and kidney toxicities. Adverse events were evaluated using the Veterinary Cooperative Oncology Group Common Terminology Criteria for Adverse Events (VCOG-CTCAE v1.1).[53] The timing of adverse events was subdivided into short-term (within 7 days after the start of treatment), medium-term (8–28 days), and long-term (29 days or later).

Rates of weight change after thalidomide administration

To compare the weight before and approximately one month after thalidomide administration, the percentage change in weight (weight after thalidomide

administration/weight at the start of administration ×100) was calculated and analyzed. A Wilcoxon matched pairs signed-rank test was used to compare body weights before and after thalidomide administration using STATA version 14.2 software (StataCorp, College Station, TX, USA). In addition, dogs were examined for changes in body weight, decreased appetite, gross lesions, tumor types, and concomitant medications.

Thalidomide pharmacokinetics study

Patient selection: Dogs that presented to the Japanese Small Animal Cancer Center between August 2011 and April 2013 and were prescribed thalidomide for the treatment of malignant tumors were included in the study. The pharmacokinetic study was approved by the hospital board, and written consent was obtained from the owners. The pharmacokinetics of a single oral dose of thalidomide (5 or 10 mg/kg) were analyzed. The first four patients were given 10 mg/kg thalidomide, and the next four were administered 5 mg/ kg. Thalidomide dispensers wore gloves, masks, caps, and gowns, and the splitting was carried out in a biologic safety cabinet. Dogs were fasted for 2 hr before thalidomide administration and provided food 12 hr after administration. Dogs with a body condition score of 5/5, weighing less than 3 kg, or with gastrointestinal signs, such as diarrhea or vomiting, were excluded from the pharmacokinetic study. In addition, dogs with a history of be administered thalidomide and those with blood chemistry tests showing total protein, albumin, alanine aminotransferase (ALT), T-bilirubin, creatinine, calcium, and glucose levels deviating from the reference values were excluded from the pharmacokinetic study. There were no restrictions on concomitant medications at the time of the survey.

Pharmacokinetic analysis

One milliliter of blood was drawn immediately before and 0.5, 1, 2, 4, 6, 8, 10, 12, and 24 hr after thalidomide administration. However, since measurements taken from the third subject receiving the 10 mg/kg dose revealed that serum concentrations were below detectable limits at the 24 hr time point, an 18-hr blood draw was included in the fourth and following (5 mg/kg) subjects. Following blood collection, the blood samples were immediately centrifuged at 6,200 rpm for 15 min. Serum was mixed with an equal volume of 25 mM citrate buffer (pH 1.5), and the mixed samples were stored at −20°C for up to 1 month until analysis.[74] From the time of thalidomide administration until 24 hr post-administration, the animals were supervised by a registered nurse and veterinarian at the hospital, and adverse effects were noted. The mixed sample (200 μL) was added to 50 μL of an internal standard solution (100 μg/mL phenacetin in methanol), and 1 mL of dichloromethane and n-hexane (1:1 vol/vol), and the mixture was shaken for 10 min. After the solvent layers were separated by centrifugation at 3,000 rpm for 10 min, the organic layer was transferred to another tube and evaporated under reduced pressure. using a centrifuge evaporator. The residue was dissolved in 100 μL of the mobile phase, and 30 μL of the solution was injected into the high-performance liquid chromatography system. Separation was carried out using a Symmetry C18 Column (150 × 4.6 mm ID, Waters, MA, USA), and the column temperature was maintained at 40 °C. The mobile phase consisted of a mixture of acetonitrile and water (32:68, vol/vol) at a flow rate of 1.0 mL/min. Absorbance was measured at 220 nm using an ultraviolet absorption detector.[74] A signal-to-noise between 2:1 and 3:1 is generally considered acceptable for estimating the limit of detection.[75] Therefore, 0.01 mg/mL, which had a signal-to-noise ratio of 3 or higher, was deemed to be the detection limit. As pharmacokinetic parameters,

time of peak concentration (Tmax), peak concentration (Cmax), terminal half-life (t1/2,) mean residence time (MRT), oral total clearance (CL/F), volume of distribution (Vd/F), and area under the curve (AUC) were calculated by non-compartmental analysis.[76] Since the third subject in the 10 mg/kg group could not be analyzed using the serum concentration at 24 hr after administration, the pharmacokinetic parameters were analyzed for n=3, except for the third subject. Terminal half-life was calculated using serum concentrations at 12 to 24 hr after administration.

RESULTS

Study demographics

Fifty-one dogs with various malignancies were included in this study. Age, weight, sex, breed, and tumor type are listed in Table 3-1. The median initial thalidomide dose was 5.3 mg/kg (range 3.4–8.1). The median drug dose after increasing the thalidomide dose was 9.4 mg/kg (range 6.7–13.7). Thalidomide was not increased in 16 cases: seven had tumor progression, one was started on radiotherapy, one had tremor, one had dizziness, one had lethargy, one had tremor and dizziness, and one had anorexia. The reason for not increasing the dose was not given in three of the dogs. The median overall duration of thalidomide treatment was 27 days (mean 79 days, range 5–449 days). Prednisolone and Non-steroidal anti-inflammatory drugs (NSAIDs) were given concomitantly while receiving thalidomide in 7 and 33 dogs, respectively.

Body weight change rate

Rates of body weight change were calculated in 24 dogs during thalidomide administration. Tumor type, presence of gross disease, appetite, type of concomitant

medications, and body weight before and after thalidomide administration were described in Table S3-1. The median time from thalidomide administration to the date of the weight change was 30 days (mean 32 days, range 20–49 days). The median and mean percent changes in weight were 100.6 and 101.3, respectively, with no significant differences in weight before and after thalidomide administration ($P = 0.3$). The rate of change in body weight is shown in Figure S3-1.

Clinical signs

Adverse events related to clinical signs were available in 51 dogs. The incidence of clinical sings and timing of the adverse events are summarized in Table 3-2. Two dogs in which lactulose was used in combination as a stool softener were excluded from the evaluation of soft stools and diarrhea. The clinical signs observed prior to the initiation of thalidomide and concomitant medications (prednisolone, NSAIDs and antimicrobials) were described in Table 3-2 and Table S3-2. In one dog with lethargy and one dog with dizziness, thalidomide was reduced back to the initial dose before the dose increase.

Hematologic toxicity

Hematologic toxicity was only investigated in 44/51 dogs because thalidomide was withdrawn after less than 2 weeks of administration in seven of the dogs, and no blood tests were performed during that time. In one case, blood tests could not be performed within 14 days and were performed on day 16. Blood tests were performed every month after the thalidomide prescription, but one dog had not been tested for 56 days, one for 57 days, and two for 77 days. The incidence of hematologic toxicity and timing of the adverse events are summarized in Table 3-3. The adverse events related to CBCs included

20.4% (9/44) that had deviations from baseline measurements before thalidomide initiation (no duplicates) (Table 3-3): six dogs had decreased PCVs (grade 1=4, grade 2=2), one dog had neutropenia (grade 1=1), and two dogs had thrombocytopenia (grade 2=1, grade 3=1). One dog with grade 3 thrombocytopenia had persistent thrombocytopenia that occurred after lomustine treatment; the thrombocytopenia progressed to grade 4 after thalidomide treatment was started. Among the adverse events related to serum chemistry tests, 20.4% (9/44) of the dogs had measurements that deviated from reference values before thalidomide initiation (overlapping); hypoalbuminemia (grade 1=1) was observed in one dog, elevated ALT (grade 1=3, grade 2=1, grade 3=2) in six dogs, elevated total bilirubin (grade 1=1) in one dog, elevated blood urea nitrogen (BUN) (grade 1=2) in two dogs, elevated plasma creatinine (grade 1=1) in one dog, and hypoglycemia (grade 1=1) in one dog. Three of the 19 dogs with confirmed elevated ALT activities were diagnosed with hepatocellular carcinoma. The concomitant medications in dogs with adverse events in ALT, BUN and creatinine are shown in Table 3-3. The variation of each adverse event is also shown graphically (Figure S3-2).

Pharmacokinetic serum analysis

Pharmacokinetic analyses after thalidomide administration were measured in four patients, each in the 5 mg/kg and 10 mg/kg groups. The 5 mg/kg group included one Bernese Mountain Dog, one Welsh Corgi Pembroke, one Miniature Dachshund, and one Shetland Sheepdog. The median weight was 11.3 kg (range 5.5–25.5 kg), and the sexes consisted of one each of a male, castrated male, female, and spayed female. The median age was 11 years (range 10–13 years). The tumor types included prostatic carcinoma (n=1), oral adenocarcinoma (n=1), oral malignant melanoma (n=1), and transitional cell

carcinoma of the urinary bladder (n=1). The median drug dose was 5.4 mg/kg (4.5–6.0 mg/kg). The 10 mg/kg group included one Golden Retriever, two Welsh Corgi Pembroke, and one Cavalier King Charles Spaniel. The median weight was 13.8 kg (range 8.42–39.8 kg), and the sexes consisted of one male, one castrated male, and two spayed females. The median age was 11.5 years (range 10–13 years). The tumor types were hemangiosarcoma (n=1), mammary gland carcinoma (n=1), soft tissue sarcoma (n=1), and sarcoma (suspected osteosarcoma) (n=1). The median drug dose was 9.9 mg/kg (range 9.7–10.2 mg/kg). One Cavalier King Charles Spaniel was treated with pimobendan, enalapril, spironolactone, gabapentin, and firocoxib on thalidomide days for mitral regurgitation and cancer pain relief. None of the other dogs received any concomitant medication. The mean change in thalidomide serum concentration for both the 5 mg/kg and 10 mg/kg groups is shown graphically (Table 3-4, Figure 3-1). From the measured thalidomide serum concentrations, the mean values of each item were calculated. In the 5 mg/kg group, Tmax=1.5 ± 0.6 hr, Cmax=1.4 ± 0.7 μg/mL, t1/2=10.6 ± 6.1 hr, and $AUC_{0–24}$=8.5 ± 4.7 μg·hr/mL; in the 10 mg/kg group, Tmax=2.3 ± 1.5 hr, Cmax=3.2 ± 2.1 μg/mL, t1/2=4.8 ± 1.2 hr, and $AUC_{0–24}$=22.0 ± 14.7 μg·hr/mL. Lethargy was observed 2 hr after administration in one patient treated with 10 mg/kg thalidomide.

DISCUSSION

The results of the present study suggest that adverse events are considered clinically acceptable when thalidomide is administered in tumor-bearing dogs at 5–10 mg/kg. In addition, I considered an initial thalidomide dose of 10 mg/kg once daily to be appropriate, based on the blood levels of thalidomide obtained in this study and the frequency and

severity of adverse events. However, since a strong tendency toward lethargy and peripheral nerve signs was observed in some dogs, the dose should be adjusted depending on the severity of the adverse events. In more than half of the dogs, weight was maintained or increased one month after thalidomide administration, suggesting that thalidomide might contribute to weight maintenance in tumor-bearing dogs.

Adverse events associated with thalidomide have been reported in people and include peripheral neurologic symptoms, such as numbness and tremor.[67,71,77] Although there are reports of no peripheral nerve signs in dogs,[32,73] tremor, stiff walking, dizziness, and whining have been observed experimentally after a single dose of thalidomide.[33] However, these signs were transient after administration, and their exact relationship with thalidomide remains unclear. In our study, tremor and dizziness were observed in six and four dogs, respectively. In some dogs, these signs improved with thalidomide reduction or withdrawal, suggesting that thalidomide may also cause peripheral neurologic symptoms in dogs. Peripheral nerve signs after thalidomide administration have been reported in people to develop cumulatively.[78,79] Three of the six dogs with tremor identified in this study showed signs after receiving thalidomide for more than a month rather than immediately after starting thalidomide, raising the possibility that some adverse events are cumulative in dogs.

Thalidomide has been reported to have low gastrointestinal toxicity.[32,73] Most anorexia in the present study was also grades 1–2 and given the use of thalidomide in tumor-bearing dogs, I considered these adverse events clinically acceptable. In contrast, grade 3 appetite loss was also observed in four dogs in this study; a dog with advanced lung metastases from mammary gland carcinoma, a dog with advanced inflammatory mammary carcinoma, a dog with pharynx pain from squamous cell carcinoma of the

tonsils, and a dog with prostate cancer and pain from vertebral metastases. Since tumor-bearing dogs often experience pain,[80-82] I cannot rule out that part of the loss of appetite after thalidomide administration may be related to cancer pain. Vomiting and diarrhea were observed in 15.7% and 32.7% of the patients, respectively; however, similar to anorexia, most adverse events were mild (grade 1 to 2). Six of the eight patients with vomiting were treated with concomitant NSAIDs or steroids. Of the 16 dogs with diarrhea, 11 received concomitant NSAIDs, steroids, or antibacterial agents, and the possibility that some of these adverse events were adverse events caused by concomitant medications could not be ruled out. The current study included dogs with relatively advanced cancer that had been on thalidomide for a long duration. Therefore, it was difficult differentiating between tumor-associated effects, the effects of concomitant medications, or those caused by thalidomide.

Hematologic toxicity has been reported in dogs treated with thalidomide.[68,83] In the present study, most hematologic toxicities were grade 2 or lower, and hematologic toxicity due to thalidomide was considered acceptable, although thrombocytopenia was observed as grade 4 in one dog. Grade 3 or higher adverse events were also observed in dogs with increased ALT activity and BUN concentration. One patient with thrombocytopenia was thrombocytopenic before thalidomide administration; lomustine-induced thrombocytopenia was considered the primary cause. The possibility of nephrotoxicity due to piroxicam could not be ruled out in one dog, in which a grade 3 BUN increase was observed. A grade 3 or higher ALT activity was seen in five patients (11.3%): three dogs with increased ALT activity prior to treatment, three dogs receiving concomitant prednisolone, and one dog with a liver mass (overlapping). In people, hepatotoxicity due to thalidomide is rare; however, it has been reported to cause severe

liver damage.[84,85] Based on the present study, the possibility of thalidomide induced ALT elevations could not be completely excluded, and I suggest that liver enzymes be monitored regularly during thalidomide administration.

Thalidomide has both immunomodulatory and anti-inflammatory effects and may be beneficial in the treatment of cachexia in patients with cancer.[86,87] In the present study, the rate of change in the body weight of dogs taking thalidomide for approximately one month was investigated to determine the weight-maintaining effects of thalidomide. The results showed that more than half of the dogs maintained or increased their body weight. Interestingly, in dogs with decreased appetites and a large number of tumors, weight was maintained for approximately one month. However, our results do not clarify the anti-cachexia effect of thalidomide or its underlying mechanism. The sample size of our study was also relatively small, and the possibility of a Type II error due to sample size or that weight was maintained by concomitant medications cannot be ruled out. In future studies, we will evaluate the anti-cachexia effect of thalidomide in a larger population against a control group not receiving the drug.

Blood levels of thalidomide have not been reported in dogs in clinical medicine. We found that the Cmax (3.2 ± 2.1 μg/mL) and $AUC_{0–24}$ (22.0 ± 14.7 μg-hr/mL) of thalidomide administered at 10 mg/kg were comparable to those at a therapeutic dose in human patients (Cmax=1.68–2.0 μg/mL, AUC=15.8–20.1 μg•hr /mL).[72,77,88] It is unclear whether the effective blood levels for malignant tumors in dogs are comparable to those in humans. However, we used an equivalent dose of thalidomide (8.4 mg/kg) in a study where thalidomide was administered postoperatively for splenic hemangiosarcoma in dogs, showing potentially prolonged survival.[32] Thalidomide has a wide safety margin, and the maximum tolerated dose in dogs has not been determined. Based on blood levels

that were similar to the therapeutic dose in humans and the frequency and extent of adverse events in tumor-bearing dogs, we considered it reasonable to set the initial dose of thalidomide in dogs at 10 mg/kg.

In our study, the half-life was shorter in the 10 mg/kg group than in the 5 mg/kg group. One possible reason for this is that, in general, the lower the blood concentration of a drug, the longer the half-life. In other words, when the pharmacokinetics of the drug can be approximated by a two-compartment model, the half-life is longer in the beta phase than in the alpha phase, and the non-compartment analysis calculates the half-life by calculating the slope of the serum concentration from the latter three points. Therefore, the half-life is shorter in the 10 mg/kg group, but the slopes from 18 to 24 hr are similar, which means that the half-lives are considered to be similar. The small number of cases may be another reason why the results are difficult to interpret.

In healthy dogs, thalidomide was reported to increase blood levels of thalidomide when administered with food. In this study, thalidomide dosed at 400 mg/head=11.74 ± 0.56 mg/kg with food had an AUC of 42.46 ± 6.64 mg·hr/L, whereas the AUC after overnight fasting was only 12.38 ± 1.13 mg·hr/L, indicating that thalidomide dosed with food was significantly higher than when it was dosed in a fasted state.[33] In our study, the $AUC_{0–24}$ at 10 mg/kg of thalidomide was 22.0 ± 14.7 μg·hr /mL at an intermediate state between a non-fasting and fasting state, as performed in the study by Pierini et al.[33] Thalidomide also causes drowsiness relatively early after administration, making it difficult for dogs and families to share meaningful time together if administered during the day. Therefore, we directed the timing of thalidomide dosing to be at the owner's bedtime, and many dogs were dosed several hours after eating. However, if thalidomide is administered to dogs

with their meals, the timing of meals and thalidomide dosing should be carefully considered, as the incidence and severity of adverse events may change as thalidomide blood concentrations increase.

The limitations of this study include the retrospective nature of the investigation into adverse events and the possibility that the assessment of adverse events as related to clinical signs may have been underestimated (recall bias). It was also difficult to differentiate the side effects of thalidomide from those caused by tumors or concomitant medications. Because thalidomide pharmacokinetic measurements are lengthy, few owners were willing to agree to hospitalization, and it was not possible to assess pharmacokinetics in a sufficient number of dogs. In addition, because the tumor type, underlying disease, and concomitant medications were not standardized, the effects of these parameters on pharmacokinetics could not be examined.

Thalidomide is a drug that has caused worldwide drug-related harm due to teratogenicity, and veterinarians are obligated to use thalidomide appropriately when it is used in tumor-bearing dogs. The drug dose for tumor-bearing dogs in this study was considered appropriate at 10 mg/kg once daily, based on the blood levels obtained and the frequency and extent of adverse events. On the other hand, excretion in dogs is still unknown and great care should be taken when handling excreta after thalidomide administration. As a next step, I will investigate the anti-tumor effects of thalidomide as a phase 2 study and evaluate the potential contribution of thalidomide to weight maintenance in tumor-bearing dogs. I will include a larger population of dogs and a control group.

Table 3-1. Patient's characteristics.

Age, Weight	n = 51
Median age (years) (range)	11 (3-15)
Median weight (kg) (range)	9.4 (3.5-54)
Gender	
Spayed female	21
Castrated male	9
Intact female	15
Intact male	6
Breed	
Miniature Dachshund	11
Beagle	6
Shih Tzu	6
Mixed breed dog	5
Golden Retriever	4
Papillon	3
Others	16

Table 3-1. Patient's characteristics (continued).

Tumor	n = 51
Mammary gland carcinoma (Inflammatory mammary carcinoma)	9 (3)
Hemangiosarcoma	4
Hepatocellular carcinoma	4
Thyroid carcinoma	3
Mast cell tumor	2
Osteosarcoma	2
Renal cell carcinoma	2
Sarcoma of unknown origin	2
Squamous cell carcinoma	2
Transitional cell carcinoma or prostatic carcinoma	2
Liposarcoma	2
Adenocarcinoma in the oral cavity	1
Adrenal adenocarcinoma	1
Anal sac adenocarcinoma	1
Apocrine gland adenocarcinoma	1
Chondrosarcoma	1
Cutaneous hemangiosarcoma and hepatocellular carcinoma	1
Fibrosarcoma	1
Histiocytic sarcoma	1
Cutaneous malignant melanoma	1
Malignant melanoma or olfactory neuroblastoma in the nasal cavity	1
Malignant thymoma	1
Oral malignant melanoma	1
Sarcoma (suspected extraosteal osteosarcoma)	1
Soft tissue sarcoma	1
Transitional cell carcinoma	1
Undifferentiated carcinoma	1
Undifferentiated germ cell tumor	1

Table 3-2. The grades and timings of adverse events after thalidomide administration (n=51).

	n	%	Day 0～7	Day 8～28	Day 29～	Concomitant medications n (%)	Number of dogs that had events prior to the start of thalidomide n (%)
Anorexia	31	60.8					
Grade 1	15	29.4	6	4	5	24 (77.4)	15 (48.4)
Grade 2	12	23.5	6	2	4	Pred (n=3), NSAIDs (n=21),	
Grade 3	4	7.8	2	2	0	AB (n=5)	
Grade 4	0	0	0	0	0		
Diarrhea	16*	32.7					
Grade 1	15	30.6	4	7	4	11 (68.8)	2 (12.5)
Grade 2	1	2	1	0	0	Pred (n=1), NSAIDs (n=10),	
Grade 3	0	0	0	0	0	AB (n=2)	
Grade 4	0	0	0	0	0		
Vomiting	8	15.7					
Grade 1	5	9.8	2	1	2	6 (75)	0
Grade 2	3	5.9	0	1	2	Pred (n=1), NSAIDs (n=5),	
Grade 3	0	0	0	0	0	AB (n=1)	
Grade 4	0	0	0	0	0		
Lethargy	9	17.6					
Grade 1	7	13.7	3	3	1	N/A	0
Grade 2	2	3.9	2	0	0		
Grade 3	0	0	0	0	0		
Grade 4	0	0	0	0	0		
Tremor	6	11.8					
Grade 1	6	11.8	2	1	3	N/A	0
Grade 2	0	0	0	0	0		
Grade 3	0	0	0	0	0		
Grade 4	0	0	0	0	0		
Dizziness	4	7.8					
Grade 1	4	7.8	4	0	0	N/A	0
Grade 2	0	0	0	0	0		
Grade 3	0	0	0	0	0		
Grade 4	0	0	0	0	0		

*Two dogs in which lactulose was used in combination as a stool softener were excluded from the evaluation of soft stools and diarrhea.

Pred: Prednisolone, NSAIDs: Non-steroidal anti-inflammatory drugs, AB: Antibiotic. N/A: Not applicable

Table 3-3. The grades and timing of hematologic adverse events. (n=44)

	n	%	Day 0～7	Day 8～28	Day 29～	Concomitant medications n (%)	Number of dogs that had events prior to the start of thalidomide n (%)
Packed cell volume	14	31.8					
Grade1	11	25	3	5	3	N/A	6 (42.8)
Grade2	3	6.81	1	1	1		
Grade3	0	0	0	0	0		
Grade4	0	0	0	0	0		
Neutropenia	5	11.4					
Grade1	5	11.4	1	1	3	N/A	1 (20)
Grade2	0	0	0	0	0		
Grade3	0	0	0	0	0		
Grade4	0	0	0	0	0		
Thrombocytopenia	4	9.1					
Grade1	2	4.5	2	0	0	N/A	2 (50)
Grade2	1	2.3	0	0	1		
Grade3	0	0	0	0	0		
Grade4	1	2.3	1	0	0		
Albumin, low	7	15.9					
Grade1	6	13.6	1	2	3	N/A	1 (14.3)
Grade2	1	2.3	0	0	1		
Grade3	0	0	0	0	0		
Grade4	0	0	0	0	0		
ALT	19	43.2					
Grade1	5	11.4	1	3	1	5 (26.3)	6 (31.6)
Grade2	9	20.5	0	4	5	Pred (n=5)	
Grade3	3	6.8	1	0	2		
Grade4	2	4.5	0	1	1		

ALT: Alanine aminotransferase, Pred: Prednisolone, N/A: Not applicable.

Table 3-3. The grades and timing of hematologic adverse events (n=44) (continued).

	n	%	Day			Concomitant medications n (%)	Number of dogs that had events prior to the start of thalidomide n (%)
			0～7	8～28	29～		
Bilirubin	7	15.9					
Grade1	6	13.6	3	2	1	N/A	1 (14.3)
Grade2	1	2.3	0	1	0		
Grade3	0	0	0	0	0		
Grade4	0	0	0	0	0		
BUN	15	34.1					
Grade1	12	27.2	3	2	7	8 (53.3)	2 (13.3)
Grade2	2	4.5	0	1	1	NSAIDs (n=7), Aspirin (n=1)	
Grade3	1	2.3	0	0	1		
Grade4	0	0	0	0	0		
Creatinine	3	6.8					
Grade1	3	6.8	1	2	0	3 (100)	1 (33.3)
Grade2	0	0	0	0	0	NSAIDs (n=3)	
Grade3	0	0	0	0	0		
Grade4	0	0	0	0	0		
Glucose, low	13	29.5					
Grade1	12	27.2	4	4	4	N/A	1 (7.7)
Grade2	1	2.3	0	1	0		
Grade3	0	0	0	0	0		
Grade4	0	0	0	0	0		
Calcium, low	5	11.4					
Grade1	4	9.1	2	1	1	N/A	0
Grade2	1	2.3	0	1	0		
Grade3	0	0	0	0	0		
Grade4	0	0	0	0	0		

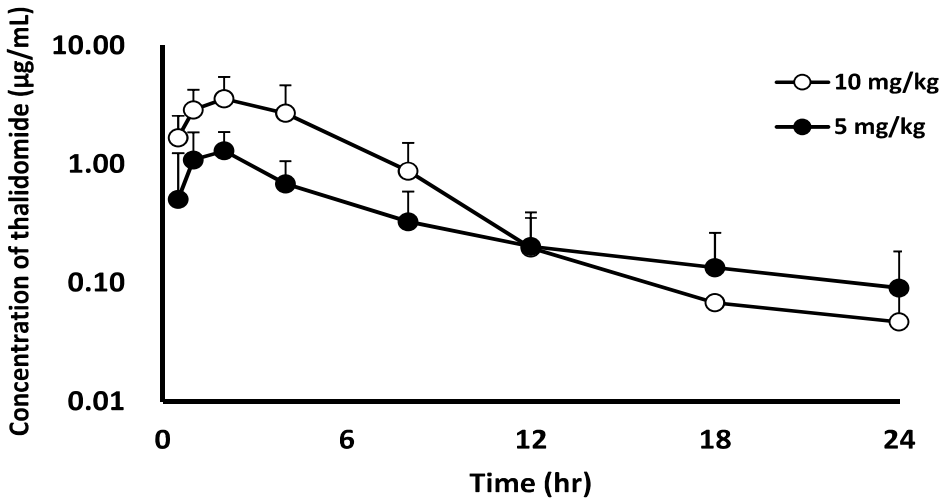
BUN: Blood urea nitrogen, NSAIDs: Non-steroidal anti-inflammatory drugs, N/A: Not applicable

Table 3-4. Pharmacokinetic parameters of thalidomide administered at doses of 5 and 10 mg/kg.

Pharmacokinetic parameters		5 mg/kg	10 mg/kg
T_{max}	(hr)	1.5 ± 0.6	2.3 ± 1.5
C_{max}	(μg/mL)	1.4 ± 0.7	3.2 ± 2.1
$t_{1/2}$	(hr)	10.6 ± 6.1	4.8 ± 1.2
MRT	(hr)	11.8 ± 7.2	5.6 ± 0.5
CL/F	(L/hr/kg)	0.7 ± 0.2	0.7 ± 0.5
Vd/F	(L/kg)	8 ± 5.3	3.9 ± 3.5
AUC_{0-24}	(μg▪hr/mL)	8.5 ± 4.7	22 ± 14.7
$AUC_{0-\infty}$	(μg▪hr/mL)	10.1 ± 5.8	22.4 ± 15

Tmax, time of peak concentration; Cmax, peak concentration; t1/2, terminal half-life; MRT, mean residence time; CL/F, oral total clearance; Vd/F, volume of distribution; F, bioavailability; AUC_{0-24}, area under the curve from 0 hr to 24 hr; AUC0-∞, area under the curve from 0 hr to infinity. Data are presented as the means ± standard deviations of n=3 for the 10 mg/kg group and n=4 for the 5 mg/kg group.

Figure 3-1. Serum concentration-time profiles of thalidomide after oral administration in dogs with various malignancies. Each point represents the mean and standard deviation of n=4, except for the 10 mg/kg dose group with n=1 for 18 hours and n=3 for 24 hours. The limit of detection was 0.01 μg/mL.



Supplementary Table 3-1. Tumor type, presence of gross disease, appetite, type of concomitant medications, and body weight before and after thalidomide administration in dogs studied for rate of weight change.

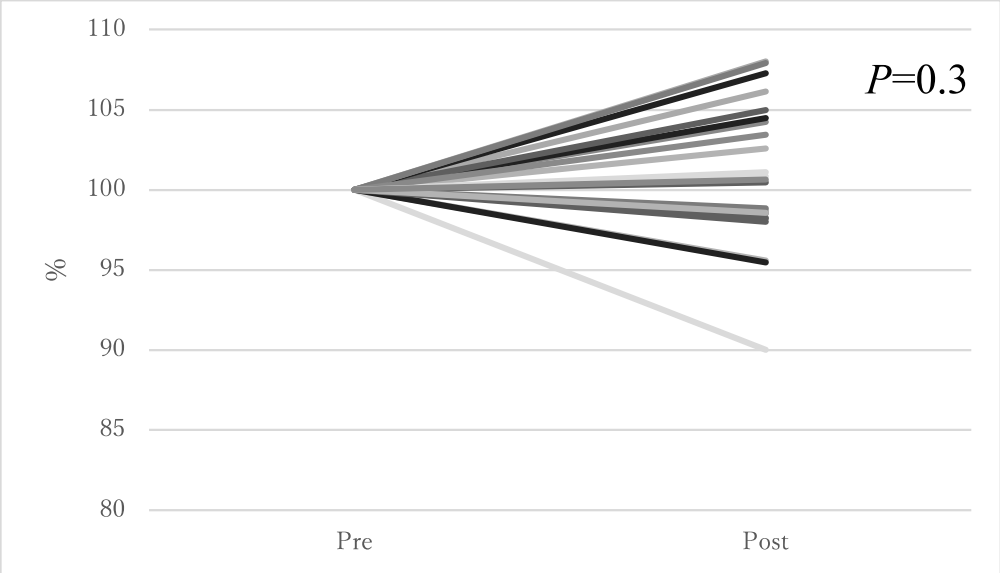
Tumor	Gross lesions	Pre BW (kg)	Post BW (kg)	Concomitant medications	Anorexia
Adenocarcinoma in the oral cavity	+	4.25	4.67	Piroxicam, Famotidine, Enrofloxacin	+
Adrenal adenocarcinoma	+	11.1	11.15	Prednisolone, Enalapril, Spironolactone	
Fibrosarcoma	+	4.67	4.79	Meloxicam	
Hemangiosarcoma	+	10.65	11.1	Carprofen	+
Hemangiosarcoma	+	24.6	25.7		+
Hepatocellular carcinoma		3.66	3.7		
Hepatocellular carcinoma		5.7	6.05		
Hepatocellular carcinoma	+	5.19	5.1		
Histiocytic sarcoma	+	24.1	25.3	Meloxicam	+
Malignant thymoma		7.4	7.3		
Mammary gland carcinoma		6.04	5.95	Piroxicam, Famotidine	
Mammary gland carcinoma	+	5.37	5.76	Piroxicam, Famotidine	+
Mammary gland carcinoma	+	5	4.5	Piroxicam, Famotidine	+
Mammary gland carcinoma (Inflammatory mammary carcinoma)	+	14.35	15.5	Piroxicam, Famotidine	+
Osteosarcoma	+	13.9	15	Carprofen	
Sarcoma of unknown origin	+	30.4	29.85		+
Sarcoma of unknown origin	+	14.7	14.05	Prednisolone, Famotidine, Diphenhydramine	+
Soft tissue sarcoma	+	27.7	28.65		
Squamous cell carcinoma	+	8.8	8.4	Piroxicam, Famotidine	+
Thyroid carcinoma		11.85	11.95	Piroxicam, Famotidine, Levothyroxine	
Thyroid carcinoma		7.65	7.55	Levothyroxine	
Thyroid carcinoma	+	13	12.85	Piroxicam, Famotidine	
Transitional cell carcinoma	+	6.52	6.39	Piroxicam, Famotidine	
Undifferentiated carcinoma	+	13.9	13.7	Piroxicam, Famotidine, Cephalexin	

Supplementary Table 3-2. The clinical signs observed prior to the initiation of thalidomide, and the concomitant medications givenat the time of gastrointestinal (GI) toxicity.

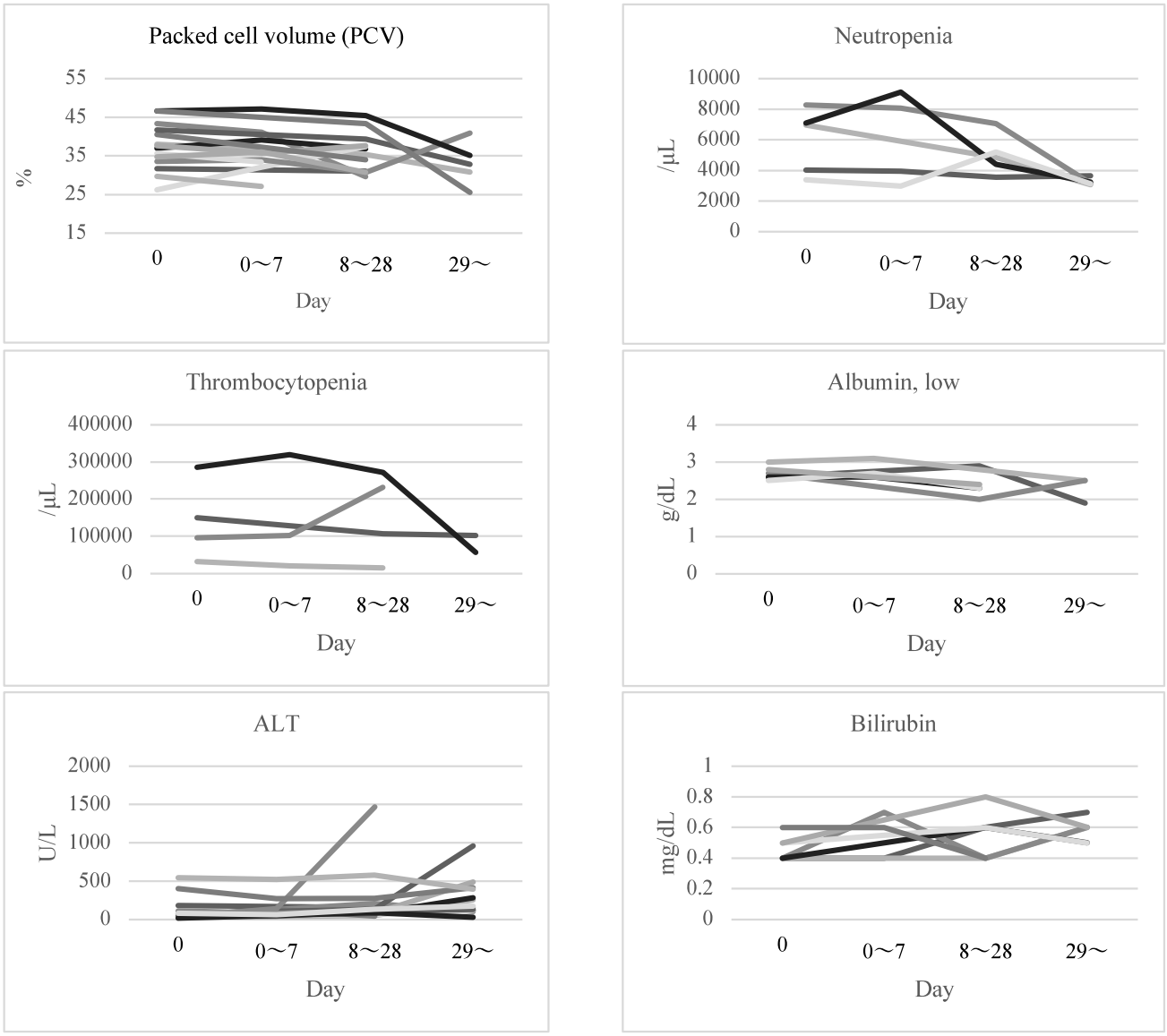
No.	GI toxicoses	Concomitant treatments	GI toxicoses	Concomitant treatments	GI toxicoses	Concomitant treatments
1	Anorexia	Prednisolone	Soft stools	Prednisolone	Vomiting	Prednisolone
2	Anorexia	Carprofen	Soft stools		Vomiting	Carprofen
3	Anorexia	Meloxicam	Diarrhoea	Firocoxib	Vomiting	Firocoxib
4	Anorexia	Piroxicam	Soft stools	Piroxicam, Enrofloxacin	Vomiting	Piroxicam, Enrofloxacin
5	Anorexia	Carprofen	Soft stools			
6	Anorexia*	Piroxicam	Diarrhoea*	Piroxicam		
7	Anorexia*	Piroxicam	Soft stools*	Piroxicam		
8	Anorexia	Prednisolone	Soft stools			
9	Anorexia*	Piroxicam	Soft stools	Piroxicam		
10	Anorexia*		Soft stools			
11	Anorexia		Soft stools			
12	Anorexia*	Piroxicam, Enrofloxacin	Soft stools	Piroxicam, Enrofloxacin		
13	Anorexia*	Piroxicam	Soft stools	Piroxicam		
14	Anorexia*	Piroxicam	Diarrhea	Piroxicam		
15	Anorexia*	Piroxicam			Vomiting	Piroxicam
16	Anorexia	Piroxicam			Vomiting	Piroxicam
17	Anorexia				Vomiting	
18	Anorexia	Carprofen				
19	Anorexia*	Meloxicam, Cefalexin				
20	Anorexia	Meloxicam, Cefalexin				
21	Anorexia*	Piroxicam				
22	Anorexia	Piroxicam				
23	Anorexia	Piroxicam				
24	Anorexia	Piroxicam, Enrofloxacin				
25	Anorexia*	Piroxicam, Enrofloxacin				
26	Anorexia	Prednisolone				
27	Anorexia					
28	Anorexia*					
29	Anorexia*					
30	Anorexia*					
31	Anorexia*	Meloxicam				
32					Vomiting	
33			Soft stools	Meloxicam		
34			Soft stools	Piroxicam		

* The clinical signs observed prior to the initiation of thalidomide. NSAIDs: Non-steroidal anti-inflammatory drugs.

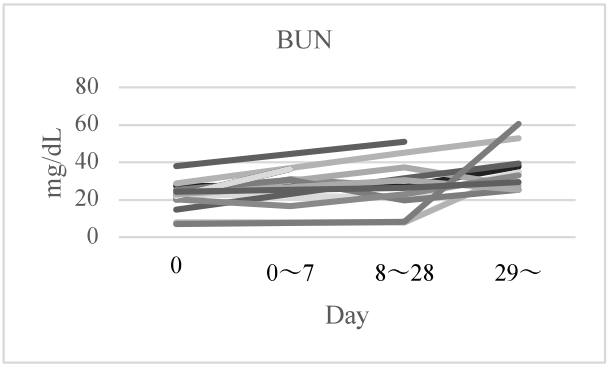
Supplementary Figure 3-1. The rate of change in body weight before and after thalidomide administration.

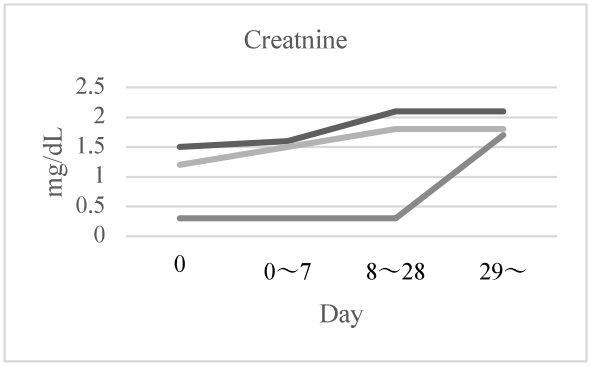


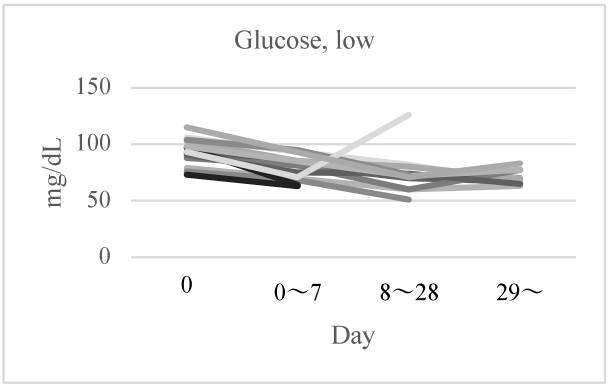
Supplementary Figure 3-2. The variability of the adverse events in each blood test during the administration of thalidomide.

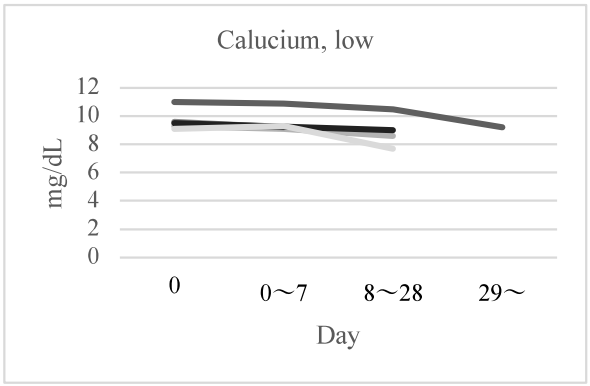


Supplementary Figure 3-2. The variability of the adverse events in each blood test during the administration of thalidomide (continued).









GENERAL DISCUSSION

This thesis aimed to investigate the optimization of chemotherapeutic dosages in dogs by evaluating adverse events and pharmacokinetics. The agents examined were vinblastine (VBL), doxorubicin (DOX), and thalidomide. For VBL and DOX, adverse events were evaluated with a particular focus on small dogs, whereas for thalidomide, adverse events and pharmacokinetics were investigated in tumor-bearing dogs.

A key finding from the three studies comprising this thesis was that dosage selection for chemotherapeutic agents in dogs should be based on comprehensive consideration of drug-specific adverse event profiles, body size, supportive care, and pharmacokinetics. In particular, when conventional body surface area–based dosing is applied directly to small dogs, relative overdosing may occur, potentially increasing the risk of adverse events such as myelotoxicity and gastrointestinal toxicity.[6,7] The VBL and DOX studies demonstrated these challenges in dosage selection for small dogs, whereas the thalidomide study showed that safety assessment and pharmacokinetic analysis are essential for determining appropriate dosages.

For VBL, the maximum tolerated dose in dogs has been reported to be 3.5 mg/m² in a phase I study.[18] However, dogs weighing <10 kg were not included in the study that provided the basis for this dosage, and the safety of VBL in small dogs had not been adequately evaluated. In the present study, adverse events were retrospectively investigated after a single intravenous administration of VBL at 2.0 mg/m² in dogs weighing <10 kg. The results showed that neutropenia occurred frequently in small dogs, even at the relatively low dosage of 2.0 mg/m², which has been used conventionally. This finding indicates that it is difficult to simply extrapolate the results

of phase I studies conducted mainly in medium- to large-breed dogs to small dogs. Therefore, when VBL is administered to small dogs, hematologic monitoring after treatment should be emphasized even when using an initial dosage of 2.0 mg/m², and the dosage and dosing interval should be adjusted according to the response of each individual dog.

In the DOX study, adverse events were prospectively evaluated in small dogs weighing 5–10 kg after a single intravenous administration of DOX at 25 mg/m² with concurrent maropitant. Although a dosage of 1 mg/kg has traditionally been recommended for small dogs,[6,22,27] insufficient dose intensity has been a clinical concern in some cases. Conversely, increasing the dose may worsen gastrointestinal toxicity and myelotoxicity.[6] In the present study, vomiting and diarrhea were relatively manageable with maropitant; however, grade 3 or 4 adverse events were observed for inappetence and neutropenia, and febrile neutropenia was also identified. Therefore, when DOX is administered at 25 mg/m², additional supportive care for gastrointestinal signs, hematologic monitoring for neutropenia, and observation at home are considered important.

Taken together, the results of the VBL and DOX studies suggest that, in dosage selection for cytotoxic chemotherapeutic agents in small dogs, not only gastrointestinal toxicity but also myelotoxicity, particularly neutropenia, represents an important limiting factor.[6] Although gastrointestinal toxicity may be partially mitigated by supportive care,[28] myelotoxicity requires post-treatment hematologic monitoring and individualized dose adjustment.

One possible reason for the increased risk of adverse events in small dogs is the limitation of body surface area–based dosing.[6,7] Dogs are a species with marked variation in body weight, and body surface area–based dosage calculation may not be uniformly applicable across all body sizes. In particular, when the same mg/m² dosage is administered, small dogs may have relatively higher drug exposure, which may increase the risk of adverse events.[6,7,37-40] In addition to body weight, multiple factors such as breed differences, metabolic capacity, hepatic and renal function, genetic predisposition, and concomitant medications may influence pharmacokinetics and toxicity. However, these factors are diverse and interrelated, and it is not easy to evaluate their individual effects in detail in clinical studies. Therefore, when investigating chemotherapeutic dosages, it is important to first stratify cases according to clinically assessable factors such as body weight and body size and to evaluate safety accordingly.[5-7]

In the thalidomide study, adverse events and pharmacokinetics were investigated in tumor-bearing dogs. Basic data on thalidomide in dogs were limited, and pharmacokinetic information was particularly insufficient.[33] In addition, unlike cytotoxic chemotherapeutic agents, thalidomide is not a drug for which dosage selection can be based solely on gastrointestinal toxicity or myelotoxicity; therefore, evaluation based on pharmacokinetic data was considered important.

In the present study, the maximum plasma concentration (Cmax) and area under the plasma concentration–time curve from 0 to 24 hours ($AUC_{0–24}$) after administration of thalidomide at 10 mg/kg were comparable to those observed in human patients receiving therapeutic doses.[72,77,88] Considering these pharmacokinetic findings together

with the observed adverse events, an initial dosage of 10 mg/kg was considered appropriate in dogs. However, some dogs developed adverse events such as increased liver enzyme activities and tremors after more than 1 month of administration. Therefore, when long-term administration is anticipated, continuous monitoring is considered important.

The three studies have several limitations. First, the number of cases in each study was limited, and plasma concentration measurements for thalidomide were performed in only four dogs in each dosage group. In future pharmacokinetic studies, more cases should be accumulated, and, if possible, differences in plasma concentrations among body weight groups and breeds should be evaluated. Second, the VBL and thalidomide studies were retrospective, and adverse events may have been underestimated or affected by recording bias. In particular, in the VBL study, some dogs received concomitant antimicrobial agents or medications for gastrointestinal signs, which may have led to underestimation of the incidence or severity of adverse events. Third, in the DOX study, retrospectively collected data were used as a control group, and differences in case background, supportive care, and observation methods may have affected the results. Fourth, pharmacokinetic analyses were not performed in the VBL and DOX studies; therefore, it remains unclear whether the adverse events observed in small dogs were caused by increased drug exposure or by host-related factors such as bone marrow sensitivity, metabolic capacity, or breed differences.

Future studies evaluating chemotherapeutic dosages in dogs should include phase I trials or prospective safety studies in which dogs are stratified at least by body weight. This is particularly important in Japanese small animal practice, where small-breed dogs

are commonly seen; therefore, dosage evaluations should reflect the clinical population in Japan rather than directly applying data obtained mainly from medium- to large-breed dogs in Western countries. In addition, evaluation systems should incorporate not only body weight but also factors that may influence pharmacokinetics and toxicity, such as breed, age, sex, concomitant medications, hepatic and renal function, nutritional status, and general condition.

In conclusion, chemotherapy in dogs, particularly in small dogs, requires individualized treatment strategies that consider body size, drug characteristics, adverse events, supportive care, and pharmacokinetics, rather than mechanical application of conventional standard dosages. The findings of this thesis provide evidence that may contribute to the optimization of chemotherapeutic dosages in dogs and to the implementation of safe and effective chemotherapy in veterinary oncology.

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