การใช้เคมีบำบัดรักษาด้วย ไซโคลฟอสฟาไมด์ วินครีสไทน์ และเพรดนิโซโลน ร่วมกับโพรโตคอลรักษาดำเนินต่อไปในสุนัขที่ป่วยด้วยมะเร็งลิมโฟม่า

Chemotherapy with cyclophosphamide, vincristine, and prednisolone (COP) and maintenance protocols in dogs with lymphoma

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ABSTRACT

Dogs with lymphoma confirmed by cytopathologically and histopathologically were treated with cyclophosphamide, vincristine and prednisolone (COP) for induction, followed by methotrexate, cyclophosphamide and prednisolone for maintenance were evaluated for remission rate and hematological, neurological, and gastrointestinal toxicities. Remission rate for the studied dogs was 100%. Complete remission (CR) and partial remission (PR) were 75% and 25%, respectively.The overall toxicity was very low, and the treatment protocol was well tolerated by 75% of the dogs treated. There were no found hematological and neurological toxicities for dogs treated in this study.The chemotherapy protocol based on COP with maintenance phase has achieved some of the highest rates of complete response in the treatment of lymphoma in dogs, while treatment-related toxicity is minimal. The median time of survival was 62 weeks (range 32 - 248 weeks).

Keywords: dogs, canine, lymphoma, chemotherapy, COP

บทคัดย่อ

สุนัขป่วยด้วยมะเร็งลิมโฟม่าเข้ารับการรักษาด้วยสารเคมีบำบัดร่วมกันของ cyclophosphamide, vincristine, prednisolone และ methotrexate โดยศึกษาถึงการตอบสนองของสุนัขต่อการรักษา ความเป็นพิษของยาต่อระบบเลือด ระบบประสาท และระบบทางเดินอาหาร จากการศึกษาพบว่า สุนัขให้ผลตอบสนองต่อการรักษาได้ถึง100% โดยให้ผลการรักษาเป็นแบบสมบูรณ์ (complete remission) 75% และเป็นแบบบางส่วน (partial remission) 25% ส่วนความเป็นพิษของยาพบว่า 75% ของสัตว์ป่วยไม่แสดงอาการใดๆ หลังการรักษา

ด้วยสารเคมีบำบัด โดยไม่พบอาการจากความเป็นพิษของยาทางระบบเลือด และระบบประสาท จากการศึกษานี้สรุปได้ว่า การรักษาสุนัขที่ป่วยด้วยมะเร็งลิมโฟม่าด้วยสารเคมีบำบัดกลุ่มนี้ให้ผลตอบสนองต่อการรักษาได้ดีและมีความเป็นพิษต่ำ ค่าเฉลี่ยกลางของการมีชีวิตอยู่ 62 สัปดาห์ (ช่วงระหว่าง 32 ถึง 248 สัปดาห์)

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Introduction

Lymphoma is one of the most common hematopoietic malignant tumors that occurs in the dogs (Couto,1985; Madewell,1985; Leifert and Matus,1986; Weller,1986; MacEwen *et al.*,1977; Carter and Valli,1988; Postorino *et al.*,1989). It represented approximately 83% of all hematological malignancies (Couto,1985) and accounted for approximately 7% to 24% of all canine neoplasia (Kaiser,1981; Moulton and Harvey,1990). Reported an estimated annual incidence rates range from 6 to 30 per 100,000 dogs at risk (Backgren,1965; Dorn *et al.*,1968; Mac Vean *et al.*,1978; MacEwen *et al.*,1981; Weller,1986; Rosenthal and MacEwen,1990), which is approximately twice the reported incidences in humans (MacEwen,1989).

The malignant lymphomas in dogs can be classified according to their anatomic location as multicentric, alimentary, mediastinal (thymic), cutaneous, and extranodal such as renal, central nervous system (Carter,1987*a*; Madewell and Theilen,1987; Gorman,1991). The ideal goal of cancer chemotherapy is to reduce the tumor cell population to zero. Canine lymphoma is a highly chemosensitive neoplasm with many chemotherapy protocols have been used. Many different drugs have been utilized either singly or in combination for the treatment of lymphomas in dogs. The advantage of combination chemotherapy over single-agent chemotherapy is that combining drugs with different mechanisms of action and differing dose-limiting toxicities may kill more cells and decrease or delay the emergence of chemoresistant clones (DeVita,1994). Using drugs with differing dose-limiting toxicities allows higher dose intensity of the individual chemotherapeutic agents.

The objective of this study was to evaluate a standard-dose chemotherapy protocol based on cyclophosphamide, vincristine and prednisolone (COP) for the treatment of canine lymphoma.

Materials and Methods

Eight dogs with multicentric lymphoma were evaluated. Diagnosis was based on cytopathological and histopathological examination of affected lymph nodes in all cases. Diagnostic evaluation included a complete blood count (CBC), serum biochemistry, thoracic and abdominal radiographs. All dogs were treated with cyclophosphamide, vincristine and prednisolone for induction, followed by methotrexate, cyclophosphamide and prednisolone for maintenance (Table 1) by the Oncology Service at the Veterinary Teaching Hospital of Kasetsart University (VTH-KU).After maintenance phase, the treatments with COP were continuted as alternate week treatment for 4 months, then 1 week in 3 for 6 months, and reduce to 1 week in 4 after 1 year.

Table 1 Combinations of Chemotherapy Protocols

Drug	Dose	Route	Frequency
Induction Phase:			
Cyclophosphamide	50 mg/m^2	PO	every 24 hours for the first 4 days of each week
Vincristine	0.7 mg/m^2	IV	every 7 days
Prednisolone	20 mg/m^2	PO	every day
Maintenance Phase:			
Methotrexate	5.0 mg/m^2	PO	on days 1 & 5
Cyclophosphamide	100 mg/m^2	PO	on day 3 of each week
Prednisolone	20 mg/m^2	PO	every other day

IV = ntravenously; PO = per os

All dogs were observed on weekly intervals during the induction and maintenance periods. A CBC was performed before each chemotherapy treatment. A white blood cell count below 3,000 cells/μl or a neutrophil count below 1,500 cells/μl resulted in treatment delay. Physical examination findings and results of diagnostic evaluation were used to establish clinical stages for these animals according to the World Health Organization (WHO) guidelines (Owen,1980) for canine lymphoma (Table 2) Staging was based on the results of physical examination, clinical laboratory testing, imaging studies, cytologic evaluation of fine needle aspirates of affected lymphoid tissues, histologic evaluation of affected lymphoid tissues and ophthalmologic evaluation. If central nervous system signs were presented, cerebrospinal fluid examination was recommended

Table 2 Clinical Stage Classification of Canine Lymphoma: World Health Organization (WHO)

Clinical Stage	Criteria
I	Involvement limited to single node or lymphoid tissue in single organ (excluding bone marrow)
II	Regional involvement of many lymph nodes, with or without involvement of the tonsils
III	Generalized lymph nodes involvement
IV	Involvement of the liver, spleen, or both, with or without generalized lymph node involvement
V	Involvement of blood, bone marrow, other organs, or a combination

Dogs were categorized on the basis of response to chemotherapy. Response rate was defined as the percentage of dogs with complete remission, partial remission, and no response; complete remission was diagnosed when complete regression (100%) of all palpable nodes or tumor mass for extranodal lymphoma occurred; partial remission occurred

when greater than 50%, but less than 100%, regression of palpable nodes or tumor mass for extranodal lymphoma was seen; and no response was defined as less than 50% regression of lymph nodes or spleen or tumor mass for extranodal lymphoma or progression of the disease was seen. Chemotherapy-induced toxicity was evaluated at each visit using the National Cancer Institute (NCI) Criteria (Table 3) Descriptive statistics (mean and median) were calculated in this study

Table 3 Toxicity Grading System: National Cancer Institute Criteria

Toxicity Grading System
Hematological Toxicity
Grade 0 No toxicity
Grade 1 WBC < 3,000/μL; neutrophils < 1,500/μL; platelets < 100,000/ μL
Grade 2 WBC < 2,000/μL; neutrophils < 1,000/μL; platelets < 70,000/ μL
Grade 3 WBC < 1,500/μL; neutrophils < 800/μL; platelets < 40,000/ μL
Grade 4 WBC < 1,000/μL; neutrophils < 500/μL; platelets < 20,000/ μL
Gastrointestinal Toxicity
Grade 0 No toxicity
Grade 1 Nausea, anorexia
Grade 2 Vomiting 1 to 5 times/day; 5 to 7 loose stools/day; < 5% weight loss
Grade 3 Vomiting 6 to 10 times/day; > 7 loose stools/day; 5-10% weight loss
Grade 4 Intractable vomiting or diarrhea; > 10% weight loss
Neurological Toxicity
Grade 0 No toxicity
Grade 1 Mild depression
Grade 2 Marked depression
Grade 3 Confusion, disorientation, agitation
Grade 4 Coma, seizures
*WBC = white blood cell count

Results

Eight dogs (3 male and 5 female dogs) with multicentric lymphoma were treated with the COP protocol for induction, followed by methotrexate, cyclophosphamide and prednisolone for maintenance. Breed, age, gender, weight and clinical stage are shown in Table 4. Breeds represented include mixed breed (n=3); Doberman pincher (n=1); golden retriever (n=1); poodle (n=1); Pit Bull (n=1); and Terrier (n=1) in the patient population. The ages of affected dogs ranged from 4 to 17 years (mean, 8 years; median, 7.5 years); 2 (25%) dogs were less than six years of age, 5 (62.5%) dogs were between six to ten years of age, and 1 (12.5%) dog was greater than ten years of age. The median body weight was 17.5 kg (mean 19.2 kg; range, 6-39 kg); 5 (62.5%) dogs weighed less than 20 kg, 3 (37.5%) dogs weighed between 20 and 40 kg, and no dog was greater than 40 kg in body weight.

Table 4 Patient Characteristics

Factor	Number of Dogs
Breed	
Mixed	3
Doberman pinscher	1
Golden retriever	1
Poodle	1
Pit Bull	1
Terrier	1
Age (years)	
<6	2
6-10	5
>10	1
Gender	
Male	3
Female	5
Weight (kg)	
<20	5
20-40	3
>40	-
Stage	
I	1
II	4
III	2
IV	1
V	-

One (12.5 %) dog was classified as stage I, 4 (50%) dogs were stage II, 2 (25%) dogs were stage III, and 1 (12.5 %) dog was stage IV. During the first induction treatment, two (25%) dogs developed anorexia (gastrointestinal toxicity) that included nausea and vomiting. These two dogs had toxicities classified as grade 2 by the NCI criteria and required supportive therapy. One of these dogs was found blood parasite (*Escherichia canis*). In additional, gastrointestinal toxicity was seen only one time at the first induction-treatment. No dogs developed hematological toxicity (such as leucopenia (less than 3,000 total WBC), thrombocytopenia (less than 100,000/µl) or neurological toxicity, and no dogs died of treatment related causes. There was no significant effect of the chemotherapy protocol on PCV, neutrophil, and platelet counts. There were no dogs requiring hospitalization among treatment. Treatment delays occurred after week 1 of induction phase in two dogs.

Response was achieved in 8 (100%) dogs in this study. A complete remission was obtained in 6 (75%) dogs, partial remission in 2

(25%) dogs. No dog was in no response. The median time of survival was 62 weeks (range 32 - 248 weeks).

Discussion

The most common method of treatment for lymphoma is combination chemotherapy. Since, single agents protocols, except for doxorubicin, have a lower response rate that is not as durable as combination chemotherapy (Vail and Young, 2007). Without treatment, most dogs with lymphoma die of the disease in four to six weeks (MacEwen *et al.*, 1977). Many chemotherapy protocols for dogs with lymphoma have been developed over the past 15 to 20 years (Madewell,1975; MacEwen *et al.*,1981; MacEwen et *al.*1987; Postorino *et al*.,1989; Greenlee *et al.*,1990; Keller *et al.*,1993; Boyce and Kitchell,2000).The protocol in this study was designed to induce remission with a chemotherapeutic regime, proceed to maintenance treatment once remission is complete, and to maintain quality of life for the patient. Doxorubicin is generally accepted to be the most effective single agent for the treatment of canine lymphoma (Carter *et al*.,1987*b*; Postorino *et al*.,1989). However, this protocol does not use doxorubicin because it is cytotoxic by mechanisms involving free-radical formation and topoisomerase-II-dependent damage (Doroshow,1996). Adverse effects of doxorubicin include myelosuppression, gastrointestinal signs, cardiac toxicity, and severe local injury when extravasation occurs (Couto,1990). The COP low dose protocol was selected for induction because they were inexpensive and of low toxicity. Thus, the authors hoped that this protocol would minimize toxicity from doxorubicin. An increased prevalence of severity is reported for a number of breeds such as golden retrievers, boxers, Labrador retrievers, Saint Bernards, poodles, beagles, basset hounds, terriers, and other hounds (MacEwen,1989, Rosenthal,1982) while a decreased risk is reported for others such as Dachshunds, and Pomeranians (Dorn *et al.*,1967; Priester and McKay,1980; Couto,1985; Rosenthal,1984; Keller,1993; Teske,1994; Edwards,2003), however lymphoma can occur in any breed. Almost 40% of the dogs in our study was mixed breed. Males and females were in similar proportions in this study. Most reports show that gender is not an important risk factor (Jarrett,1966; Parodi,1968). The median age of dogs with lymphoma in the present study was 7.5 years, which was slightly higher than the reported by Boyce and Kitchell (2000) ; Garrett *et al.(*2002), and a little lower than that reported by Baskin *et al.* (2000). and MacEwen (1989) showed that multicentric lymphoma predominantly affected dogs of four to nine years old, with an average age of six to seven years at diagnosis. Our report found that the ages of affected dogs of four to seventeen years, and the average age was eight years. However, the reason for age distribution is still unknown.

A complete remission was obtained in 6 (75%) dogs, and partial remission in 2 (25%) dogs with lymphoma. The overall response (complete remission plus partial remission) rate of 100% seen in this study is comparable to previously reported overall response rates of 96% (Teske *et al.*,1990; MacEwen *et al.*,1992), 94.7% (Postorino,1989), 92% (Boyce and Kitchell,2000), 91% (Day,1986), and 81% (Goldie *et al.*,1982) using similar protocols.Therefore, the response rate

achieved by COP protocol in the present study was higher than those achieved by the previous studies. The overall toxicity of the studied dogs was very low, and the treatment protocol was well tolerated by 75% of the dogs treated. Only two dogs required supportive therapy for gastrointestinal toxicity.There were no hematological and neurological toxicities of dogs treated in this study. None of the dogs died of chemotherapy-related complications. Thus, the hematological and neurological toxicities are not a contraindication for using COP protocol in dog with malignant lymphoma. Additionally, treatment cost, time, therapeutic response, and the owners' cooperation are important factors influencing accomplishment. It is therefore difficult to accurately assess the contribution of survival time achieved by the COP protocol without further research.

In conclusion, the chemotherapy protocol based on COP with maintenance phase has achieved some of the highest rates of complete response in the treatment of lymphoma in dogs, while treatment-related toxicity is minimal.

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