การใช้ยาเคมีบำบัดวินคริสไทน์ในการรักษาสุนัข 60 ตัวที่ป่วยด้วยโรค Transmissible Venereal Tumor

Vincristine Chemotherapeutic Treatment for Transmissible Venereal Tumor in 60 Dogs

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ABSTRACT

Sixty dogs with naturally occurring canine transmissible venereal tumor were examined at Veterinary Teaching Hospital, Kasetsart University and at Animal Shelter, Ratchaburi province, Thailand. They were treated with single-chemotherapeutic drug, vincristine at 0.7 mg/m^2 body surface area (BSA) given intravenously at weekly intervals until a response was noticeable. Data to be collected in this study included breed, sex, neuter status, age, weight, heartworm disease status, blood parasites, hematocrit, blood chemistry (i.e. creatinine, BUN, SGPT), primary tumor location, and other anatomical sites of tumor involvement. The results showed that all dogs (59 female intact dogs and 1 male intact dog) were mixed breed and tumors located at the genitalia. Dogs' age ranged from 1 to 5 years. The mean weight was 15.6 kg. During the treatment period, there were no therapy-related deaths and intensive care was not required. Vincristine-monotherapy ranged from one to eleven weekly cycles that resulted in complete tumor regression, with a median of four weekly cycles. Fifty-eight dogs (96.67%) were found more than 50% remission after the first week of intravenously

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vincristine treatment. In conclusion, the response to chemotherapy with vincristine 0.7 mg/m^2 intravenously at weekly interval was very excellent without relapse after 6 months.

Key words: canine, transmissible venereal tumor, vincristine

บทคัดย่อ

สุนัขป่วยด้วยโรค transmissible venereal tumor จำนวน 60 ตัว ที่เข้ารับการรักษาที่โรงพยาบาลสัตว์ มหาวิทยาลัยเกษตรศาสตร์ และที่ป่วยจากสถานสงเคราะห์สัตว์ จังหวัดราชบุรี ได้รับการรักษาด้วยยาเคมีบำบัด vincristine ขนาด 0.7 มิลลิกรัมต่อพื้นที่ผิวของร่างกายหน่วยตารางมิลลิเมตรด้วยการฉีดเข้าทางเส้นเลือดดำ ติดต่อกันทุกสัปดาห์ โดยให้สัปดาห์ละ 1 ครั้ง จนกระทั่งไม่พบมะเร็งที่บริเวณอวัยวะสืบพันธุ์จากการดูด้วยตาเปล่า ทำการเก็บรวบรวมข้อมูลของสุนัขป่วยที่เข้ารับการรักษา ได้แก่ พันธุ์ เพศ การทำหมัน อายุ น้ำหนัก รวมถึงการตรวจสอบสภาวะการเป็นโรคพยาธิหนอนหัวใจ โรคพยาธิเม็ดเลือด ตรวจค่าทางโลหิตวิทยา ชีวเคมีของโลหิต (เช่น creatinine, BUN, SGPT) ตำแหน่งของมะเร็ง และบริเวณอื่นๆ กรณีที่มะเร็งมีการแพร่กระจายไป ผลการศึกษาพบว่าสุนัขที่ได้รับการรักษาทั้งหมด (เป็นเพศเมียที่ยังไม่ได้รับการทำหมัน 59 ตัว เพศผู้ที่ยังไม่ได้รับการตอน 1 ตัว) เป็นพันธุ์ผสม มะเร็งที่พบเกิดที่บริเวณอวัยวะสืบพันธุ์ทั้งหมด ช่วงอายุที่พบอยู่ระหว่าง 1 ถึง 5 ปี ค่าเฉลี่ยของน้ำหนักสุนัขป่วยอยู่ที่ 15.6 กิโลกรัม ระหว่างให้การรักษาสุนัขป่วย ไม่พบมีสุนัขเสียชีวิตจากการใช้ยาเคมีบำบัด และไม่พบอาการแทรกซ้อนอื่นๆ ที่ต้องให้การเฝ้าดูแลเป็นพิเศษขณะที่มีการให้หรือหลังการให้ยาเคมีบำบัด จำนวนครั้งของการใช้ยาเคมีบำบัดที่ตอบสนองต่อการรักษาได้ดีพบได้ตั้งแต่ 1 ถึง 11 สัปดาห์ โดยมีค่ากึ่งกลางอยู่ที่ 4 สัปดาห์ นอกจากนี้ยังพบสุนัขป่วยจำนวน 58 ตัว (96.67 เปอร์เซ็นต์) ให้ผลตอบสนองต่อยาเคมีบำบัดได้ดี โดยที่ทำให้ขนาดของมะเร็งเล็กลงได้มากกว่า 50 เปอร์เซ็นต์ ตั้งแต่สัปดาห์แรกของการให้ยาเคมีบำบัด vincristine เข้าทางเส้นเลือดดำ โดยสรุปการศึกษานี้ พบว่าการใช้ยาเคมีบำบัด vincristine ขนาด 0.7 มิลลิกรัมต่อพื้นที่ผิวของร่างกายหน่วยตารางมิลลิเมตรให้ผลตอบสนองต่อการรักษาที่ดี และไม่พบการกลับมาเกิดซ้ำใหม่หลังให้การรักษาผ่านไป 6 เดือน

คำสำคัญ: สุนัข มะเร็ง เคมีบำบัด

INTRODUCTION

Canine transmissible venereal tumor (TVT) is a naturally occurring contagious round cell neoplasm and is transplantable on the external genital mucosa of male and female dogs (Brown *et al*., 1980; Cohen, 1985; Goldschmidt and Hendrick, 2002; Richardson, 1981). Transmission is by sexual contact, sniffing, licking, or free roaming sexually intact mature dogs with poorly controlled (Mukaratirwa and Gruys, 2003). TVT affects a slightly younger, sexually active or breeding female and is the predominant tumor type in areas of the world in which it is enzootic (Feldman and Nelson, 1987; Das and Das, 2000). TVT does not commonly metastasis, less than 5% of reported cases (Kroger *et al*., 1991). Metastasis of TVT to the skin, face or nose by trauma and licking, regional

lymph nodes, tonsils, eye, brain, liver, spleen, peritoneum, mammary region, kidney, lung, and bone marrow has been reported (Oduye *et al*., 1973; Feldman and Nelson, 1987; Yang, 1987; Bright *et al*., 1983; Rogers *et al*.,1998; Mukaratirwa and Gruys, 2003; Nak *et al*., 2004; Mello *et al*., 2005). The diagnosis of a TVT is easy and is slightly suggested by a genital location. Signalment, history, clinical signs and physical examination may provide a presumptive diagnosis of TVT in dogs with the classic presentation. Definitive diagnosis is obtained with cytopathologic or histopathologic analysis of the tumor cells and tissues. The ultimate goal of treatment of the tumor is complete cure, which can be achieved by surgical excision, radiotherapy, immunotherapy and chemotherapy (Misirlioglu *et al.,* 1999; Das and Das, 2000). Surgery has been reported for solitary or metastatic lesions and can be effective in selected cases. Recurrence following surgical management has been recorded in 12-68% of cases (Das and Das, 2000). The most effective treatments are radiotherapy and chemotherapy, particularly with vincristine sulfate (Thrall, 1982; Calvert *et al*., 1982; Amber and Henderson, 1982). This single chemotherapeutic agent is presently most effective, safe, inexpenxive and convenient agent for therapy (Amber *et al*., 1990; Singh *et al*., 1996; Das and Das, 2000). Although, TVT is generally quite responsive to chemotherapeutic treatment, but there have been few reports concerning the response of chemotherapy-resistant cases to vincristine treatment modality.

This present investigation describes the signalment, clinical status, incidence of metastasis, and evaluated the effect of vincristine in 60 dogs naturally affected with TVT.

MATERIALS AND METHODS

During an 18-month period (January 2008 - June 2009), sixty dogs with naturally occurring canine TVT were examined either at Veterinary Teaching Hospital, Kasetsart University or at Animal Shelter, Ratchaburi province, Thailand. Fine needle aspiration cytology was taken using 23-25 G needle and 2-5 ml syringe. Biopsy was performed to confirm by preserving tissue in 10% formalin and processed routinely for haematoxylin and eosin staining. Histopathologically, the untreated TVT was characterized by sheets or bundles of mostly rounded cells having a large, highly basophilic nucleus with a prominent, highly basophilic nucleolus (Figures 1 and Figures (2A-B)). After biopsy, all dogs were treated with single-chemotherapeutic drug, vincristine sulphate at 0.7 mg/m^2 body surface area (BSA) given intravenously at weekly intervals until a response was noted. Data to collect in this study including breed, sex, and neuter status, age, weight, heartworm disease status, blood parasites, hematocrit, blood chemistry (i.e. creatinine, BUN, SGPT), primary tumor location, other anatomical sites of tumor involvement.Chemotherapy-induced hematological toxicity (Hammer *et al.,*1994) was evaluated at each visit (Table 1) In each case, complete remission (CR) was defined as total tumor regression with no gross evidence

of disease. Partial remission (PR) was defined as greater than 50%, but less than 100%, regression of lesion size was seen, while no response (NR) was defined as less than 50% regression of lesion size or continued tumor growth. Descriptive statistics (mean and median) were calculated in this study.

Table 1 Hematological toxicity grading (Hammer *et al.*,1994)

Hematological Toxicity Grading			
Grade			
G_0	No toxicity		
G_1	2,000 < WBC < 3,000/μL;	1,000 <neutrophils <1,500/μL;	100,000 <platelets <150,000/ μL
G_2	1,500 < WBC < 2,000/μL;	800 <neutrophils <1,000/μL;	50,000 <platelets <100,000/ μL
G_3	1,000 < WBC < 1,500/μL;	500 <neutrophils <800/μL;	25,000 <platelets < 50,000/ μL
G_4	WBC < 1,000/μL;	neutrophils < 500/μL;	platelets < 25,000/ μL
	(WBC = white blood cell)		

RESULTS

Sixty dogs with naturally occurring canine TVT were included in the study, and all were mixed breed. Dogs' age ranged from one to five years, with a median of 3 years. The mean body weight was 15.6 kg (range, 7.0-30.2 kg). There were 59 female intact dogs and 1 male intact dog (Table 2). All dogs had found tumors located at the genitalia of both sexes (Figures $3A_1,B_1,C_1,D_1$).

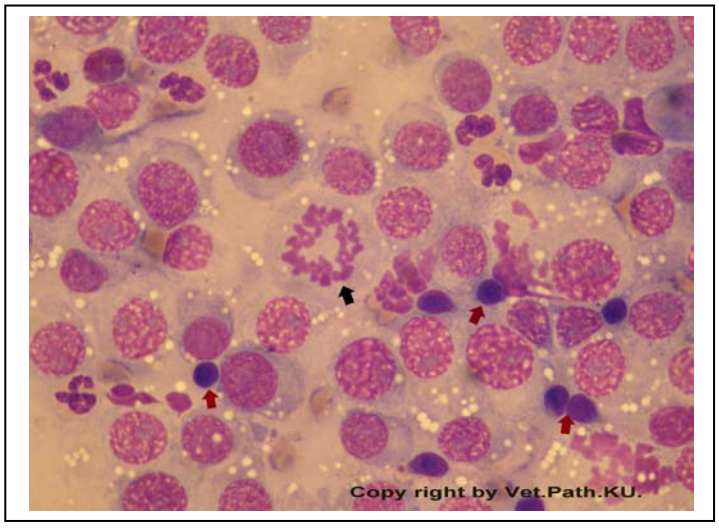
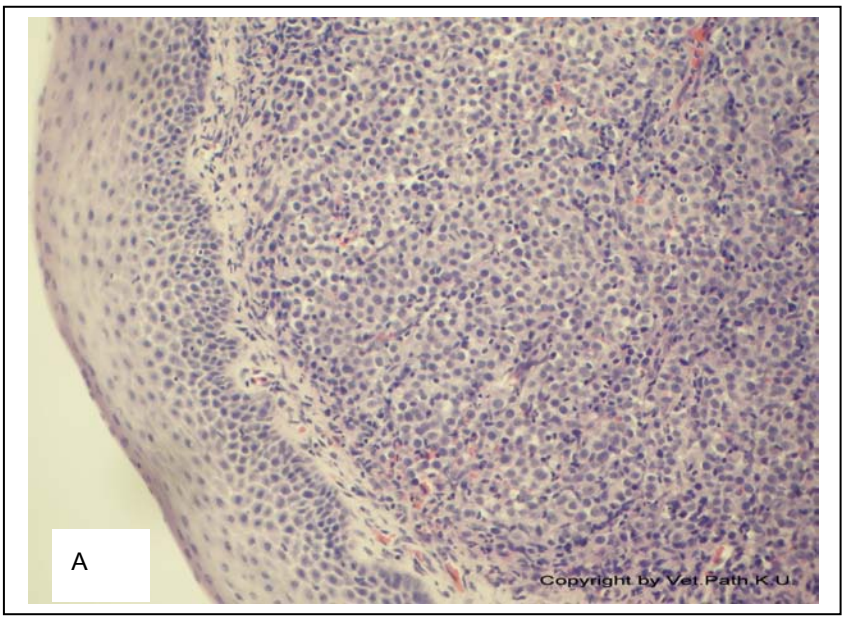
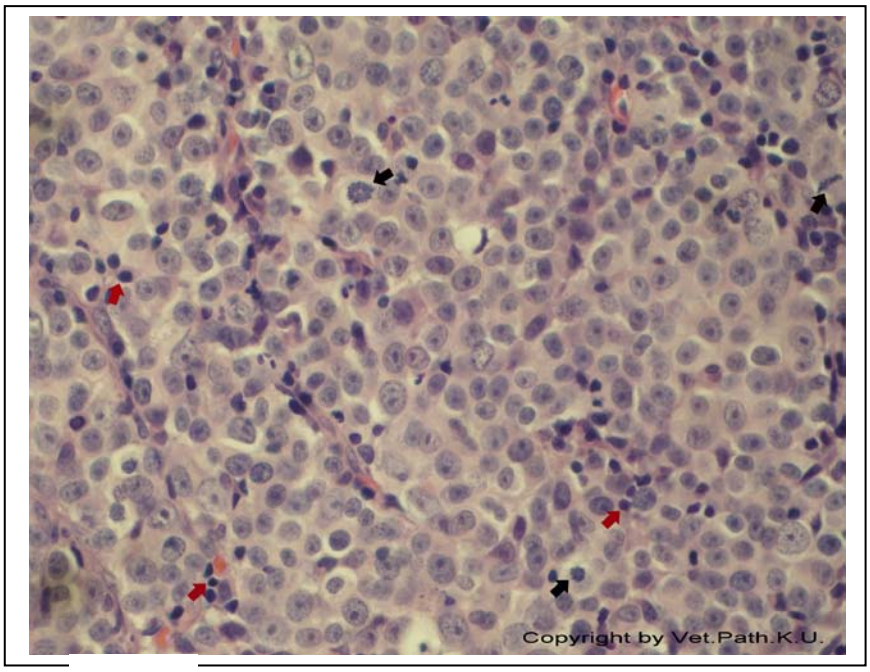


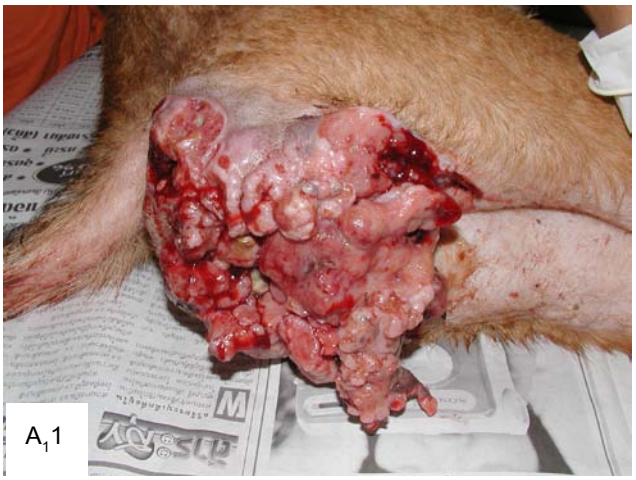
Figure 1 Dog# 16/51, Transmissible venereal tumors (TVT), vaginal mass, imprint. Neoplastic cells are large and round, prominent one to two nucleoli, reticular nuclear chromatin, abundant light basophilic cytoplasm which usually have cytoplasmic vacuoles. Noted an atypical mitotic figure (black arrow) and infiltration of lymphocytes (red arrows). (100x)

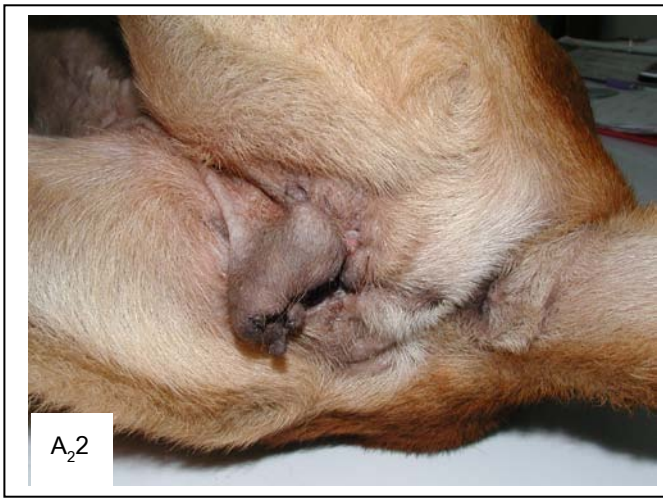




B

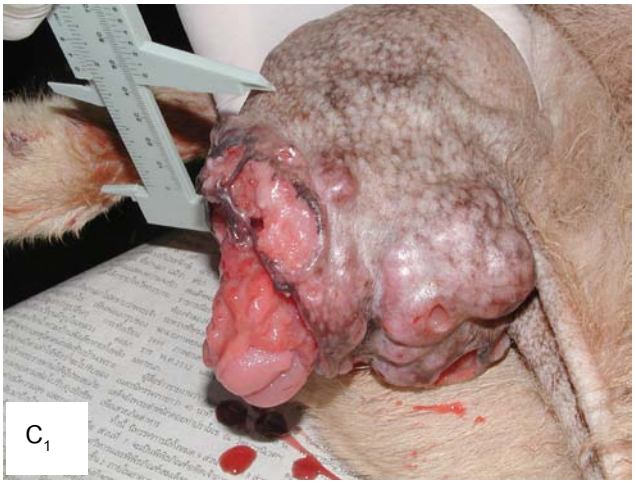
Figure 2(A-B) Dog# 16/51, **A**: An encapsulated and well-demarcated dermal mass which arranges in thick sheet pattern is separated in many small groups by fibrous connective tissues, (4x) **B**: The tumor cells have large, round, hyperchromatic vesicular nuclei and large, prominent central nucleoli. They have moderate amount of eosinophillic cytoplasm, cytoplasmic vacuoles and abundant cytoplasmic border. Many mitotic figures are marked (black arrows). Infiltrative of lymphocytes (red arrows), (40x)


A1 1


A2 2


B1


B2


C1


C2

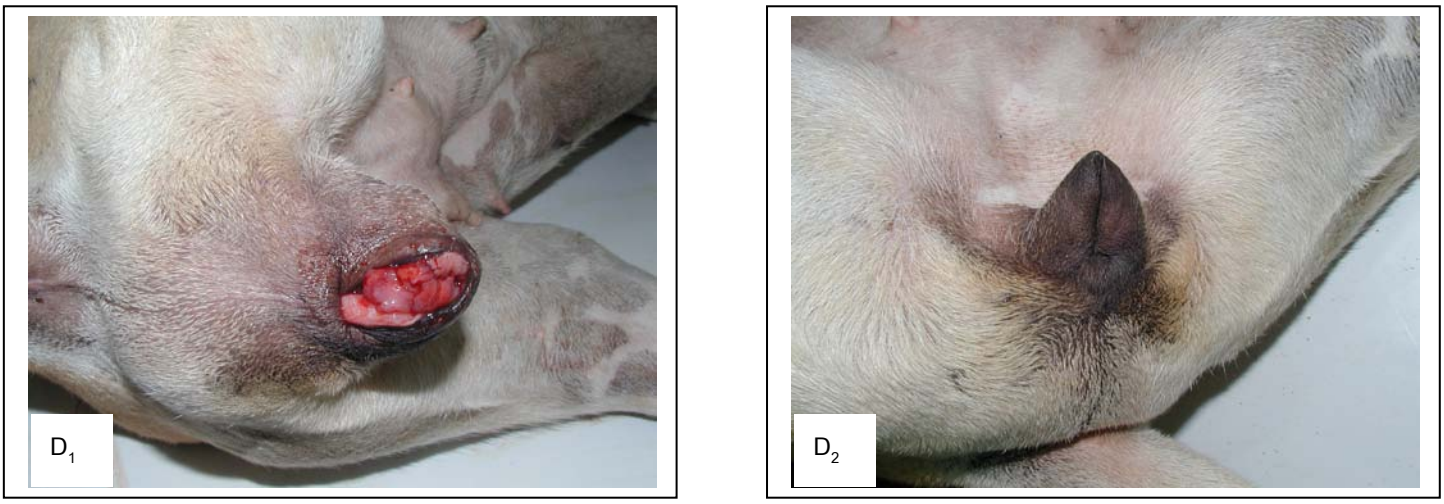


Figure 3(A_{1-2}-D_{1-2}) Dogs with TVT presented to Veterinary Teaching Hospital and complete response (CR) after various weekly cycles of vincristine monotherapy **A_1**: Dog # 16/51 with TVT at vulva, **A_2**: after seven weekly cycles of vincristine; **B_1**: Dog # 15/51 with TVT at vulva, **B_2**: after eight weekly cycles of vincristine; **C_1**: Dog # 17/51 with TVT at penis & prepuce, **C_2**: after seven weekly cycles of vincristine; **D_1**: Dog # 21/51 with TVT at vulva, **D_2**: after six weekly cycles of vincristine

Table 2 Patient Characteristics

Factor	Number of Dogs (n)	%
Breed		
Mixed	60	100
Age (years)		
1 yr- 3yr	50	83.3
> 3 yr – 5yr	10	16.7
Gender		
Male	1	1.7
Female	59	98.3
Weight (kg)		
≤ 10	1	1.7
> 10 – 20	52	86.7
> 20	7	11.6

The response to chemotherapy with vincristine 0.7 mg/m^2 intravenously at weekly interval was very excellent (Figures 3A_2,B_2,C_2,D_2). There was no therapy-related deaths, and intensive care was not required. Chemotherapy-induced hematological toxicity was evaluated at each case. All dogs were in G_0 grade during the vincrisitne monotherapy period. No dogs developed hematological toxicity (such as leucopenia (less than 3,500 /µl), thrombocytopenia (less than 100,000/µl)) or neurological toxicity, and no dogs died from treatment related causes. Adverse responses to medication, anorexia, vomiting, diarrhea, were not seen. The response rate of all 60 dogs was 100% achieved complete remission (Figure 4) without relapse after 6 months. After CR, ovariohysterectomy or neutering was performed using standard KU protocols for the procedure in all dogs, including postoperative pain management.

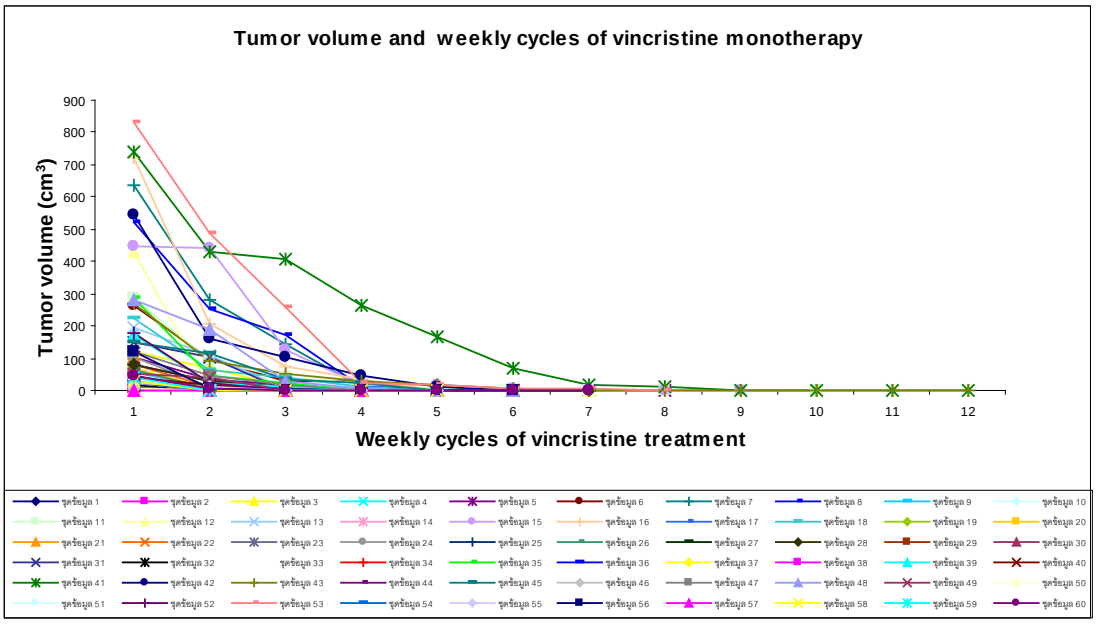


Figure 4 Tumor volume (cm^3) and weekly cycles of vincristine monotherapy in 60 dogs

Dogs treated weekly with vincristine monotherapy at the dose of 0.7 mg/m^2 body surface intravenously from one to eleven weekly cycles that resulted in complete tumor regression, with a median of four weekly cycles. Around forty-two cases treated effectively with two to five weekly cycles of vincristine that resulted in complete resolution of TVT masses in approximately ½ - 1 month (Table 3). There were tumor size-related weekly cycles of vincristine monotherapy. Dogs with small tumors (size $\leq$ 100 cm^3) treated effectively with one to six weekly cycles of vincristine. Dogs with large tumors (size $>$ 100 cm^3) treated effectively with three to eleven weekly cycles of vincristine. Fifty-eight dogs (96.67%) were found more than 50% remission after the first week of intravenously vincristine treatment (Table 4).

Table 3 Number of dogs (n) and weekly cycles of vincristine monotherapy that resulted in complete tumor regression

Weekly cycles of vincristine	Number of Dogs (n)
1	2
2	9
3	10
4	13
5	10
6	6
7	4
8	2
9	2
10	-
11	2

Table 4 Number of dogs and percentage remission after the first week of intravenously vincristine treatment

number of dogs	% remission (after the 1st week intravenously vincristine treatment)
58	> 50% remission
2	$\leq$ 50% remission

DISCUSSIONS

A study showed that the use of vincristine at the dosage of 0.5 mg/m^2 (BSA) was effective in the complete remission of TVT in dogs, but tumor recurrence and no remission were still observed (Amber *et al.*, 1990). Our study used higher dose (0.7 mg/m^2, BSA) than that of Amber *et al.* (1990) and complete response without drug-induced hematological toxicity had been found in all TVT-dogs. In addition, tumor recurrence and TVT resistance were not seen within 12 months.

Previous studies demonstrated the use several other chemotherapeutic agents for TVT treatment including cyclophosphamide (5 mg/kg, PO, for 10 days or given in association with prednisolone 3 mg/kg, for 5 days); vinblastine (0.1 mg/kg IV for 4-6 weeks); methotrexate (0.1 mg/kg, PO, every other day); and a combination of the these drugs, or combination of cyclophosphamide (0.1 mg/kg) and vincristine (0.025 mg/kg) daily for 10-12 days (Brown *et al.*, 1981; Richardson, 1981; Idowu *et al.*, 1984; Vermooten, 1987). These studies appeared to be more complicated than using vincristine monotherapy. In addition, there is no apparent advantage in the combination of chemotherapy over using vincristine alone.

This study has shown that one to eleven weekly, with a median of four weekly cycles of intravenously administration of vincristine monotherapy at the dosage of 0.7 mg/m^2 (BSA) was very effective in complete remission of TVT in dogs. After the first week of treatment 96.67% of dogs had more than 50% remission of TVT masses. There were no drug-induced toxic side effects found in all dogs during the TVT therapeutic period. This study indicated that vincristine is relatively safe, inexpensive, and provide a complete response of treated dogs within approximately one week to 1 ½ months.

Canine TVT is most commonly seen in sexually active dogs in tropical and subtropical climates. As we known, this disease is spread when dogs mate, and it can even be transmitted to other canine species, such as foxes and coyotes (Mukaratirwa and Gruys, 2003). Transmission occurs by inoculation of intact neoplastic cells in the damaged mucosa or skin. In this study all dogs had tumors located at the genitalia of both sexes. In order to protect transmission and recurrence, each treated dogs was also performed spayed in females and castrated in males after complete remission.

In conclusion, chemotherapy is very effective against canine TVT. The prognosis for complete remission with chemotherapy is excellent. The most common chemotherapy agent used for canine TVT is vincristine 0.7 mg/m^2 (BSA) intravenously administration provided a complete and durable response in over 90% to 95% of treated dogs typically following one to six weekly treatments with no drug-induced hematological toxicity.

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REFERENCES

Amber, E.I. and R.A. Henderson. 1982. Canine transmissible venereal tumor: evaluation of surgical excision of primary and metastatic lesions in Zaria-Nigeria. J. Am. Anim. Hosp. Assoc. 18: 350-352.

Amber, E.I., Henderson, R.A., Adeyanju, J.B., and E.O. Gyang.1990. Single-drug chemotherapy of canine transmissible venereal tumour with cyclophosphamide, methotrexate or vincristine. J. Vet. Intern. Med. 4:144-147.

Bright, R.M. N.T. Gormanm, C.W. Probst, *et al*. 1983. Transmissible venereal tumor of the soft palate in a dog. J. Am. Vet. Med. Assoc.183(8): 893-895.

Brown, N.O.C., E.G. Calvert, MacEwenm. 1980. Chemotherapeutic management of transmissible venereal tumors in 30 dogs. J. Am. Vet. Med. Assoc. 176: 983-986.

Brown, N.O., E.G.MacEwen, C.A. Calvert. 1981 Transmissible venvereal tumor in the dog. California Vet 3: 6-10.

Calvert, C.A., C.E., Leifer, E.G. MacEwen, 1982. Vincristine for treatment of transmissible venereal tumor in the dog. J Am Vet Med Assoc 181: 163-164.

Cohen D. 1985. The canine transmissible venereal tumor: a unique result of tumor progression. Adv Cancer Res 43:75-112.

Das, U. and A.K. Das .2000. Review of canine transmissible venereal sarcoma. Vet. Res. Commun. 24:545-546.

Feldman, E.C., R.W. Nelson. 1987. Canine and feline endocrinology and reproduction. WB Saunders Company, Philadelphia, PA, pp 475-477.

Goldschmidt, M.H. and M.J. Hendrick. 2002. Tumor of the skin and soft tissues. In: Tumors in domestic animals, ed. Meuten DJ, 4th ed., pp 45-118. Iowa State Press, Ames, IA.

Hammer, A.S., C.G., Couto R.D., Ayl., K.A. Shank. 1994. Treatment of tumor bearing dogs with actinomycin D. J Vet Int Med 8: 236-239.

Idowu, A.L., A., Adetunji, G.O., Babalola, 1984. Cyclophosphamide and vincristine chemotherapy for canine transmissible venvereal tumor. Trop Vet 2: 159-163.

Kroger, D., R.M., Grey, J.W., Boyd. 1991. An unusual presentation of canine transmissible venereal tumor. Canine Practice, 57(3): 19-21.

Mello Martins, M.I., Ferreira de Souza, F., Gobello, C.2005. The canine transmissible venereal tumor: etiology, pathology, diagnosis and treatment. In: Concannon PW, England G, Verstgegen J, Linde-Forsberg C, Eds. Recent Advances in Small Animal Reproduction. International Veterinary Information Service, Ithaca NY

(www.ivis.org).25-Apr 2005;A1233.0405.

Misirlioglu, D., K., Seyrek-Intas, M.M., Kahraman, S., Ozbilgin, M.O., Ozyigit, I.T., Cangul, A., Sevimli, M. Buyukcoban. 1999. A study about the effects of paramunity activator Baypammun N on the prophylaxis and treatment of canine transmissible venereal tumor (CTVT): macroscopical findings. Kafkas Univ Vet Fak Derg 5: 1-8.

Mukaratirwa, S., E., Gruys. 2003. Canine transmissible venereal tumour: cytogenetic origin, immunophenotype, and immunobiology. A review. Vet Q 25: 101-111.

Nak, D., D., Misirkioglu, Y., Nak, K., Seyrek-Intas, H.B. Tek. 2004. Transmissible venereal tumor with mammary gland metastase in a bitch. Vet Bilimleri Derg 20: 99-102.

Oduye, O.O., B.O.,Ikede, G.O., Esuruoso, J.U. Akpokodje.1973.Metastatic transmissible venereal tumour in dogs. J. Small Anim. Pract. 14: 625-637.

Richardson, R.C. 1981. Canine transmissible venereal tumor. Compend Contin Educ Pract. Vet. 3: 951-959.

Rogers, K.S., M.A., Walker, H.B. Dillon. 1998. Transmissible venereal tumor: a retrospective study of 29 cases. J Am Anim. Hosp. Assoc. 4: 463-470.

Singh, J., J.S., Rana, N., Sood, G.R., Pangawkar, P.P. Gupta. 1996. Clinico-pathological studies on the effect of different antineoplastic chemotherapy regimens on transmissible venereal tumours in dogs. Vet. Res. Commun. 23: 217-219.

Vermooten, M.I. 1987. Canine transmissible venvereal tumor (TVT): a review. J. S. Afr. Vet. Assoc. 58(3):147-150.

Thrall, D.E. 1982. Orthovoltage radiotherapy of canine transmissible venereal tumors. Vet Rad 23:217-219.

Yang, T.J. 1987. Metastatic transmissible venereal sarcoma in a dog. J. Am. Vet. Med. Assoc. 190(5): 555-556.

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