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Pansawut Pansawut

Patrakrit Theewasutrakul

Sirichai Techarungchaikul

Suppawiwat Ponglowhapan

Kaywalee Chatdarong

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Short Communication

Treatment of Canine Transmissible Venereal Tumor Using Vincristine Sulfate Combined with *L*-Asparaginase in Clinical Vincristine-resistant Cases: A Case Report

Pansawut Sudjaidee[1] Patrakrit Theewasutrakul[2] Sirichai Techarungchaikul[2]
Suppawiwat Ponglowhapan[1] Kaywalee Chatdarong[1*]

Abstract

Three female mongrel dogs were cytologically diagnosed as transmissible venereal tumor (TVT) and had clinically developed resistance to vincristine treatment. One dog was treated with four treatments of 10,000 IU/m^2 body surface area of *L*-asparaginase combined with 0.025 mg/kg body weight of vincristine sulfate every two weeks while this combination was administered to the others once a week for four weeks and only vincristine sulfate once a week for four treatments. Both treatments resulted in a complete remission. Side effects such as gastrointestinal upset, diarrhea, depression, and decrease in appetite were observed in the dogs administered with the later protocol. Hematologic disturbance was observed in one out of two dogs showing leukopenia three weeks after the treatments. Although the complete regression of tumor was observed in both treatment courses, two-week treatment interval is recommended to avoid undesirable effects.

Keywords: canine, *L*-Asparaginase, vincristine resistant TVT, vincristine

[1] *Department of Obstetrics, Gynecology and Reproduction*
[2]*Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University, Pathumwan, Bangkok, Thailand*
***Corresponding author: E-mail:** kaywalee.c@chula.ac.th*

บทคัดย่อ

การรักษาเนื้องอกระบบสืบพันธุ์ติดต่อในสุนัขที่มีประวัติการดื้อต่อยาวินคริสตีนซัลเฟต ด้วยวินคริสตีนซัลเฟตร่วมกับแอล-แอสปาราจิเนส : รายงานสัตว์ป่วย

*พรรษวุฒิ สุดใจดี[1] ภัทรกฤช ธีวสุตระกูล[2] ศิริชัย เตชะรุ่งชัยกุล[2] ศุภวิวัธน์ พงษ์เลาหพันธุ์[1] เกวลี ฉัตรดรงค์[1]**

สุนัขพันธุ์ผสมเพศเมียสามตัวตรวจพบเนื้องอกระบบสืบพันธุ์ติดต่อในสุนัข และวินิจฉัยยืนยันด้วยวิธีทางเซลล์วิทยา ได้รับการรักษาด้วยยาวินครีสทีนซัลเฟตและไม่ตอบสนองต่อการรักษา สุนัขตัวแรกได้รับยาแอลแอสพาราจีเนส ขนาด 10,000 หน่วยสากล/ตารางเมตร (พื้นที่ผิวร่างกาย) ร่วมกับวินครีสทีนซัลเฟตขนาด 0.025 มิลลิกรัม/กิโลกรัม (น้ำหนักตัว) ทุกสองสัปดาห์ จำนวนสี่ครั้ง ขณะที่สุนัขอีกสองตัวได้รับยาสองชนิดร่วมกันทุกสัปดาห์ จำนวนสี่ครั้ง ตามด้วยยาวินครีสทีนซัลเฟตชนิดเดียว ทุกสัปดาห์ จำนวนสี่ครั้ง ภายหลังการรักษาทั้งสองวิธี พบว่า เนื้องอกยุบจนหมดและตรวจไม่พบเซลล์เนื้องอกด้วยวิธีทางเซลล์วิทยา ผลข้างเคียงจากการใช้ยาเคมีบำบัดร่วมกัน เช่น ซึม ถ่ายเหลว กินอาหารลดลง พบในสุนัขที่ได้รับเคมีบำบัดที่ความถี่ทุกสัปดาห์ สุนัขหนึ่งในสองตัวพบจำนวนเม็ดเลือดขาวต่ำสามสัปดาห์ภายหลังการรักษา แม้ว่าจะให้ผลการรักษาที่เหมือนกัน แต่การให้เคมีบำบัดที่ความถี่ทุกสองสัปดาห์สามารถหลีกเลี่ยงผลข้างเคียงจากยาเคมีบำบัดได้

คำสำคัญ: สุนัข แอลแอสพาราจีเนส เนื้องอกติดต่อระบบสืบพันธุ์ที่ดื้อยาวินครีสทีนซัลเฟต วินครีสทีนซัลเฟต

[1]*ภาควิชาสูติศาสตร์ เธนุเวชวิทยา และวิทยาการสืบพันธุ์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330*

[2]*โรงพยาบาลสัตว์เล็ก คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330*

***ผู้รับผิดชอบบทความ E-mail:** kaywalee.c@chula.ac.th*

Introduction

Naturally occurring canine transmissible venereal tumor (TVT) is an important contagious neoplasm that commonly attacks the reproductive tract. This tumor widely spreads in free-roaming dogs (Batamuzi et al., 1992; Rogers et al., 1998). It is classified into two groups, genital TVT and extragenital TVT, according to the locations of the tumor mass present (Das and Das, 2000). Genital TVT is transmitted via natural mating while extragenital TVT is occurred by social contact, like sniffing or licking (Otomo et al., 1981). Prevalence varied upon the areas, for example, 11% in Kenya, 32% in Sri Lanka, 10% in Marryland (USA) and 23.5 to 28.6% in India (Das and Das, 2000). The clinical presentations for TVT are visible cauliflower-like mass in genital area or on skin surface with the presence of bloody discharge, ocular or nasal deformation from tumor invasion (Rogers, 1997; Mello Martins et al., 2005). Cytological method is commonly used to diagnose the tumor because it is easy, less painful and less time consuming than biopsy (Santos do Amaral et al., 2007). Treatments used to cure TVT are surgery, radiation or chemotherapy. Surgical tumor removal does not only provide unsatisfactory response but also causes tumor recurrent. Although radiotherapy yields complete regression, it requires trained workers, special equipments and expenses (Boscos and Ververidis, 2004). However, chemotherapy yields similar good response and tumor regression to radiotherapy. Vincristine sulfate has been widely accepted as an efficient single chemotherapeutic agent for treatment of TVT (Mello Martins et al., 2005).

Vincristine sulfate acts by binding to tubulin dimer which is necessary for mitosis of spindle fibers, contributing to cellular division arrested in metaphase stage (Coppoc, 2009). The typical course of vincristine treatment is four to eight week of intravenous administration at 0.5 to 0.7 mg/m^2 body surface area (BSA) (Boscos and Ververidis, 2004) or 0.025 mg/kg body weight (BW) (Das and Das, 2000; Kunakornsawat et al., 2009). Non-responsive vincristine cases have been occasionally reported (Rogers et al., 1998, Das and Das, 2000) which suggested alternative treatments such as radiotherapy (Rogers et al., 1998; Boscos and Ververidis, 2004), surgery (Kunakornsawat et al., 2010), and other chemotherapeutics such as doxorubicin, vinblastine, methotrexate, prednisolone or cyclophosphamide as a single or in combination between 2 to 3 drugs. However, side effects usually occur when the combined chemotherapeutics are used and recurrence is seen in cases treated by surgical removal (Das and Das, 2000; Boscos and Ververidis, 2004; Kunakornsawat et al., 2010).

L-asparaginase is one of the chemotherapeutic agents used for pediatric acute lymphoblastic leukemia (ALL) and lymphoma in human (Narta et al., 2007). It has been applied also to treat canine leukemia, lymphoma (Barton, 2001) and cutaneous lymphoma (Theewasutrakul et al., 2007).

Asparagine is a non-essential amino acid, synthesized in normal cells by enzyme asparagine synthase. *L*-asparaginase acts by reducing asparagine pool which is required for cellular proliferation and differentiation of tumor cells (Müller and Boos, 1998; Barton, 2001). However, the tumor was not regressed totally when the *L*-asparaginase was administered as single chemotherapeutic in canine cutaneous lymphoma whereas the total regression was observed when vincristine sulfate was accompanied (Theewasutrakul et al., 2007). Thus, these two chemotherapeutics might be worth a test also in non-responsive TVT cases in this study. The objective of this study was to reveal the combination treatment of *L*-asparaginase and vincristine sulfate in non-responsive TVT cases.

Case history

History of three dogs diagnosed cytologically as TVT is summarized in Table 1.

The dogs had been treated with vincristine sulfate at 0.025 mg/kg body weight once a week. Case I was an intact female mongrel dog presented with vaginal mass. She had been treated with vincristine sulfate intravenously once a week. After six months, the tumor recurred at the vulva area. A new treatment course was started, but the tumor regressed partially. Then, she was referred.

Case II was an intact female miniature pincher-cross breed dog. The TVT masses completely regressed after treatments with vincristine sulfate. Two years later, she presented with irregular vulval mass with ulcerative and deformed vulva. Treatment was started with vincristine sulfate once a week. The tumor regressed gradually, but the visit was skipped thereafter. Therefore, she was re-introduced but the treatment with vincristine sulfate alone could not cure. Thus, she enrolled in this program.

Case III was a spayed female mixed breed dog. She had been treated with vincristine sulfate intravenously once a week until the tumor regressed totally. After two months, a bloody vaginal discharge and vaginal mass were presented (Fig 1). A new treatment course was started, but leukopenia was noticed (2200 cells/µl). Therefore, she was referred to Small Animal Teaching Hospital, Chulalongkorn University.

Diagnosis and treatment

All three cases were referred to the Small Animal Teaching Hospital at Faculty of Veterinary Science, Chulalongkorn University. They were diagnosed as TVT by exfoliated cell cytology. The samples were smeared and stained with a commercial modified Giemsa staining (Diff-Quick®, SE Supply, Bangkok, Thailand). The cytology showed round-to-oval shaped cells with increased ration between nucleus and cytoplasm, dense nucleolus and intracytoplasmic vacuoles suggesting TVT (Fig 2). Hematological and blood chemistry profile including blood urea nitrogen, creatinine, alanine aminotransferase and alkaline phosphatase were analyzed and defined as in normal range before the treatment started. Blood samples were collected every two weeks for hematological and blood chemistry profile during treatment program.

Table 1 History of individual dogs prior to combination treatment submitted

Case Number	Gender	Genital TVT	Extra-genital TVT	Interval before previous course	Numbers of treatment	Results
1	Female	vagina	-	-	Course 1: treated with 4 injections of vincristine	Complete regression
		vulva	-	6 months	Course 2: treated with 10 injections of vincristine	Partial regression with bloody vaginal discharge was observed.
2	Female	vagina	orbit	-	Course 1: treated with 2 injections of vincristine	Complete regression of vaginal mass and ocular mass
		vagina	right upper eye lid	3 months	Course 2: treated with 5 injections of vincristine	Complete regression of vaginal mass after four injections and total regression of all tumors after five injections. Vaginal mass was observed two years after the last session of treatment.
3	Female	vagina	-	-		Partial regression of vaginal mass.
		dorsal	-	3 months	Course 1: treated with 5 injections of vincristine Course 2: treated with 8 injections of vincristine	Non-response was observed after 8 injections and side effects were presented as leucopenia. Then she was referred to CU-Vet Small Animal Hospital.

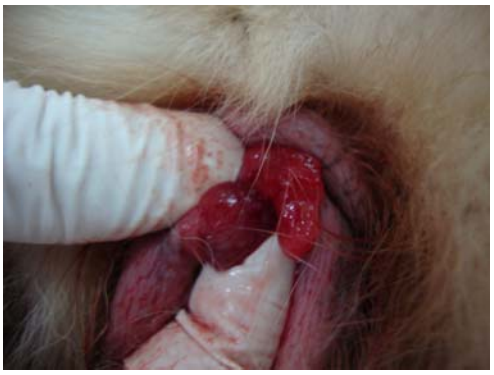

Figure 1 Venereal tumor mass covered with bloody discharge in the dorsal wall of the vagina of bitch case III (mixed breed dog) on the first day before treatment.

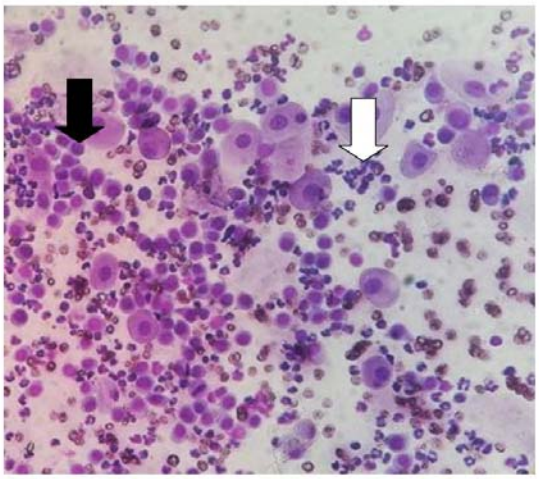

Figure 2 Plasmacytoid cell-type of the canine transmissible venereal tumor smeared from the vagina of a bitch (black arrow). Many of white blood cells were presented (white arrow).

In case I, the treatment started with vincristine sulfate (Vincristin®, Gedeon Richter, Hungary) at dosage of 0.025 mg/kg body weight (BW) intravenously and *L*-asparaginase (Leunase®, Kyowa Hakko Kogue, Japan) at dosage of 10,000 IU/m² body surface area (BSA) intravenously every two weeks for four treatments. Two weeks after the first treatment, the tumor size regressed more than 50% and blood profile did not show abnormality. Thereafter, 90% of the tumor mass regressed after the second treatment. After the third treatment, cytological finding revealed no round cell characterized TVT cells. The dog was followed up by physical examination and cytological method after two and six months after treatment. The tumor recurrence was not observed.

Case II was treated with four treatments of vincristine sulfate at dosage of 0.05 mg/kg BW intravenous injection and *L*-asparaginase at dosage of 10,000 IU/m² BSA intravenously once a week and continued with four treatments of vincristine sulfate at the same dosage once a week. Prior to giving *L*-asparaginase, chlorpheniramine maleate was injected intramuscularly at 4 mg/dog to reduce allergic sign Two weeks after the first treatment, the dog showed clinical signs of gastrointestinal aberrant as mild diarrhea and reduced appetite but the signs recovered before the next treatment. Tumor size regressed to more than 50% after three weeks from the first injection and regressed completely on the eighth week of treatment. There was no recurrence observed during six months monitoring since the last injection.

Case III started with with four treatments of vincristine sulfate at dosage of 0.025 mg/kg BW intravenously and *L*-asparaginase at dosage of 10,000 IU/m² BSA intravenously once weekly for four injections and followed by with four treatments of vincristine sulfate at 0.025 mg/kg BW once a week. Chlorpheniramine maleate was given at 4 mg/dog intramuscularly fifteen minutes before *L*-asparaginase administration. The tumor size regressed to 50% in one week after the first injection. Side effects such as soft feces, reduced appetite and leukopenia were observed at week 3 after the first injection. After the treatment course ended, the tumor size persisted at 0.3 cm in diameter and the tumor disappeared two months later. There was no recurrence observed at six months after the last treatment. All treatment courses and outcomes are summarized and presented in Table 2.

Results and Discussion

L-asparaginase hydrolyses asparagine to aspartic acid and reduces serum asparagine concentration. This mechanism causes depletion of asparagine which is necessary for protein biosynthesis (Müller and Boos, 1998). The restriction of asparaginase activity in tumor cells causes depletion of asparagines which is necessary for protein synthesis, leading to tumor regression (Capizzi et al., 1970, Müller and Boos, 1998). Side effects are the important concern for selecting type of chemotherapeutic usage. The side effects observed in every two-week treatment course were less than in every week treatment course. Previous study demonstrated the side effects of vincristine at the dosage of 0.5 mg/m²BSA, for example, decreasing in appetite, diarrhea and diffuse alopecia (Said ea al., 2009). However, the same side effects were not observed in the study of Kunakornsawat et al., 2009 in which the vincristine sulfate was given with a higher dosage at 0.7 mg/m² BSA. In this study, the side effects occurring in the two cases after the second treatment were similar to the study of Tuntivanich (1983) in which the vincristine sulfate was administered in combination with methotrexate for the TVT treatment.

In general, the vincristine sulfate yields a good response in TVT cases (Rogers, 1997; Rogers et al., 1998; Mello Martins et al., 2005; Said et al., 2009). With single chemotherapeutic use, the TVT regression occurs after four to six injections (Boscos and Ververidis, 2004; Said et al., 2009). A resistance may be implied if the regression is not achieved after the sixth injection (Said et al., 2009). In this study, all of the three cases had received up to six injections of the vincristine sulfate with failure of tumor regression;

therefore, they were postulated as resistance cases. Interruption of the treatment and duration of the development of the tumor mass likely were the causes of the resistance (Boscos and Ververidis, 2004). In this study, the dogs had the history of vincristine discontinuation during treatment which might contribute to a development of TVT resistance. Drug interruption inducing resistance was confirmed by previous studies demonstrating the lower administration of anti-neoplastic drug in TVT tumor cell culture, resulting in the survive cells and expand cell line (Rumjanek et al., 2001; Hirose, 2002; Sulova et al., 2009). Moreover, resistance to chemotherapy may be associated with failure of drug accumulation in neoplastic cells by increasing in drug efflux or decreasing in drug influx controlled by the cell transporters (Gottesman, 2002). P-glycoprotein (P-gp) and multidrug resistant associated protein (MRP) are transmembrane transporters causing drug elimination from normal and neoplastic cells. These two proteins play the major roles on neoplastic drug resistance in both human and animal. Recently, many researchers believed that the TVT resistance may be associated with these two proteins (Srisilapakorn et al., 2008; Gaspar et al., 2010), but the mechanism is still unclear.

The combination of L-asparaginase and vincristine is suggested as an alternative treatment for vincristine resistant TVT cases. The appropriate protocol is recommended as being given every two weeks in order to avoid side effects.

Table 2 Results after combination treatment

Case number	Previous treatment outcome	Interval from last treatment	Treatment course	Treatment outcome	Side effect
1	Partial response	3 months	Four treatments of vincristine 0.025 mg/kg BW with *L*-asparaginase 10,000 IU/m² BSA every 2 weeks	Complete remission	Not found
2	Non response	2 years	Four treatments of vincristine 0.05 mg/kg BW with *L*-asparaginase 10,000 IU/m² BSA once a week and followed by four treatments of vincristine 0.05 mg/kg BW once a week	Complete remission	Soft feces was found three days after treatment
3	Non response	4 months	Four treatments of vincristine 0.025 mg/kg BW with *L*-asparaginase 10,000 IU/m² BSA once a week and followed by four treatments of vincristine 0.025 mg/kg BW once a week	Remnant remission and disappearance after six months	Soft feces and inappetite was observed three days after treatment. Leukopenia (2400 cells/µl) was observed three weeks after treatment.

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