

Lomustine therapy for vincristine-resistant canine transmissible venereal tumor: a case report

Terapia com lomustina para tumor venéreo transmissível canino resistente à vincristina: relato de caso

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Abstract

Canine transmissible venereal tumor (CTVT) is a transmissible cancer that affects the external genitalia of dogs. In this study, a female canine with CTVT in the vagina was treated with vincristine (0.75 mg/m²; intravenous (IV); weekly for eight cycles), the currently preferred drug for CTVT, but without any progress. Therefore, this case was considered resistant to vincristine, and the preferred alternative chemotherapy, doxorubicin, was suggested. However, based on echocardiographic evidence, the patient could not be administered doxorubicin. Thus, the administration of lomustine was proposed. Although there are no studies to support this decision, the authors based their decision on the fact that lomustine is effective for round cell tumors, and that CTVT belongs to this tumor group. After three doses (60 mg/m²; every 3 weeks) complete remission was achieved. The use of lomustine at a dose of 60 mg/m² every 3 weeks for vincristine-resistant CTVT proved to be effective, without any harmful side effects. The treatment is cost-effective and simple to manage.

Keywords: vaginal neoplasia, vincristine-resistant tumor, veterinary oncology, oral chemotherapy.

Resumo

O tumor venéreo transmissível canino (TVTC) é um câncer transmissível que afeta a genitália externa dos cães. Neste estudo, uma cadela com TVTC na vagina foi tratada com vincristina (0,75 mg/m²; intravenosa (IV); semanalmente por oito ciclos), o medicamento atualmente preferido para TVTC, mas sem qualquer progresso. Portanto, este caso foi considerado resistente à vincristina, e a quimioterapia alternativa preferida, a doxorubicina, foi sugerida. No entanto, com base em evidências ecocardiográficas, o paciente não pôde receber doxorubicina. Assim, foi proposta a administração de lomustina. Embora não existam estudos que apoiem esta decisão, os autores basearam a sua decisão no facto de a lomustina ser eficaz para tumores de células redondas e de o TVTC pertencer a este grupo de tumores. Após três doses (60 mg/m²; a cada 3 semanas), a remissão completa foi alcançada. O uso de lomustina na dose de 60 mg/m² a cada 3 semanas para o TVTC resistente à vincristina mostrou-se eficaz, sem efeitos colaterais prejudiciais. O tratamento é econômico e simples de gerenciar.

Palavras-chave: neoplasia vaginal, tumor resistente à vincristina, oncologia veterinária, quimioterapia oral.

Introduction

Canine transmissible venereal tumor (CTVT) is a transmissible neoplasm that affects the external genitalia of dogs (Ganguly et al., 2013). CTVT is transmitted by inoculation of intact neoplastic cells in the vaginal or penile epithelium, nasal or oral mucosa, conjunctiva, anal region, and/or skin wounds. Inoculation generally occurs through copulation or social behaviors, such as sniffing and licking (Gurel et al., 2002). Adult canines are the most affected, and the disease has no sexual or breed-specific predisposition (Rogers et al., 1998). The tumor has a cauliflower-shaped appearance, pedunculated, nodular, papillar or multinodular, with



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variable size. The tumors have soft and friable consistency that easily bleeds. The surface is usually ulcerated, inflamed, hemorrhagic, and infected. In female dogs, CTVT is most frequently found in the vestibule and less commonly in the vagina (Ganguly et al., 2013).

Definitive diagnosis may be performed by cytology (Lorimier & Fan, 2007). Vincristine sulfate is generally considered to be the most effective treatment for canine CTVT (Gonzalez et al., 2000; Martins et al., 2005; Nak et al., 2005; Scarpelli et al., 2010; Reis Filho et al., 2020); however, some tumors are resistant to this drug. Vincristine is preferred because of its high effectiveness (complete remission in more than 90% cases), low cost, and mild toxicity (Woods, 2020). The drug is a *Vinca* alkaloid that interrupts microtubule assembly required for mitotic spindle formation during metaphase. It is strictly to be administered intravenously with saline (Ogilvie & Moore, 2008). Its secondary effects include neurotoxicity, paresthesia, constipation, and paralytic ileus. If accidentally extravasated, it can cause significant tissue irritation and necrosis. Myelosuppression is a less-frequent, dose-dependent effect. Vincristine is used to treat lymphomas, sarcomas, and immune-mediated thrombocytopenia (Das & Das, 2000; Nak et al., 2005; Ogilvie & Moore, 2008). Other treatments have been developed with varying success rates (Da Silva et al., 2014; Kunakornsawat et al., 2010; Rogers et al., 1998; Spugnini et al., 2008; Sudjaidee et al., 2012).

In this study, the authors treated a vincristine-resistant case of CTVT with lomustine, an alkylating agent that inhibits the replication of tumor cells. Lomustine is effective for treating round cell tumors, and CTVT belongs to this group.

Historical findings

An approximately 5-year-old female Pitbull was presented to the Veterinary School Hospital. It was found on the street 4 months ago (Figure 1), with a tumor (20 × 10 × 8 cm; measured using a caliper) in the perineal region and vulva (Figure 2). The tumor was hard to palpation, with small, irregular, and bleeding ulcers on the skin surface. As a rescued animal, she had no anamnesis. CTVT was diagnosed by cytology, and IV vincristine (0.75 mg/m²) was administered weekly for 8 weeks. On the day of the medical appointment, the patient's general condition had improved (Figure 3), but the owner stated that although the bleeding had decreased, the treatment did not reduce the tumor size. The lack of improvement



Figure 1. Patient on the day she was found on the street. She had poor general health condition, with a conspicuous CTVT in the perineal region.

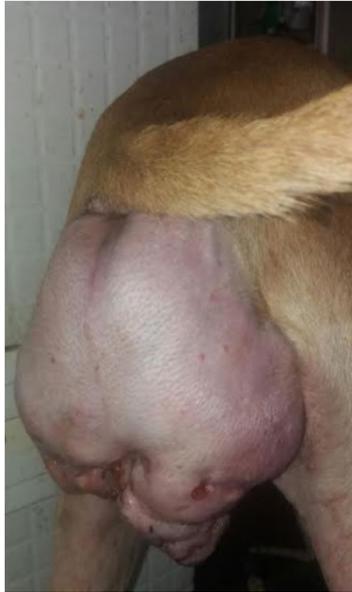


Figure 2. Patient during initial presentation at the Veterinary School Hospital. The tumor is has a large size and shows bleeding ulcers on its surface.



Figure 3. Patient at first visit to the Veterinary School Hospital. The patient's general condition shows improvement as compared to that on the day she was found on the street.

prompted the veterinarian to switch treatment to doxorubicin. After further evaluation, including echocardiography, doxorubicin was found to be contraindicated in this patient because the patient's fractional shortening was 21%. Thus, the administration of lomustine was suggested at a dose of 60 mg/m², three doses every three weeks. Cytological valuation confirmed the clinical diagnosis (Figure 4), and a full biochemical profile was completed before treatment, including evaluation of transaminase enzymes, to monitor the liver's response to treatment. After the first dose of lomustine, the tumor greatly decreased in size and the bleeding completely stopped. A second dose was administered 3 weeks later, and blood was collected for biochemical study. After the second treatment, the tumor was

nearing complete reduction; thus, a third dose was administered for complete remission (Figure 5). Two weeks after the third dose, complete remission was achieved. The patient did not manifest any secondary effects to the medication and was tumor-free for 24 months after the treatment.

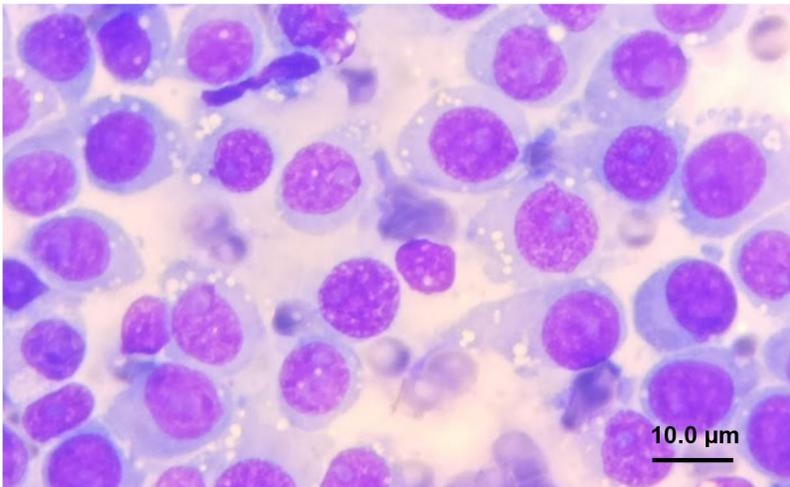


Figure 4. Cytological image of the vulvovaginal tumor. The image shows abundant isolated round cells, medium-sized cells, eccentric and reduced nuclei, uniform patterns of granular chromatin, single round, and prominent nucleolus. A moderate amount of clear basophilic cytoplasm and small clear intracytoplasmic vacuoles are seen, arranged in a linear form on the internal surface of the cell membrane, characteristic of CTVT (100 \times , Diff-quick).



Figure 5. The tumor after the third dose of lomustine. The patient shows complete tumor remission.

Discussion

In this study, CTVT was diagnosed by cytology, widely accepted as the gold-standard diagnostic method. Cytological examination provides better evidence of CTVT than biopsy due to the complex histological form of CTVT that differentiates it from other round cell tumors such as mast cell tumors, histiocytoma, and lymphosarcoma. Furthermore, cytology is cheaper, easier, and less painful than biopsy (Birhan and Chanie, 2015; Das et al., 1990; Ganguly et al., 2013; Santos do Amaral et al., 2007). On cytology, CTVT shows distinct cells with nuclei that contain chromatin clumping and one or two prominent nucleoli. The cytoplasm is pale blue or colorless, with distinctive light vacuoles, and often contain many mitotic figures. It is classified as a round cell tumor (Ganguly et al., 2013; Ogilvie & Moore, 2008).

CTVT mainly affects the external genitalia, as in our case; however, there are reports of the tumor affecting extra genital and intra-abdominal organs (Bastan et al., 2008; Peixoto et al., 2016; Rogers et al., 1998). There are various clinical signs associated with tumor size and location (Dobson & Lascelles, 2014). The patient had a prominent perineum deformation due to large tumor size but did not present evidence of clinical, ultrasound, or radiographic metastases. When metastases are present, they most frequently occur in the regional lymph nodes, kidneys, spleen, liver, eye, tonsils, brain, and subcutaneous tissue (Ganguly et al., 2013). In addition, Salamanca et al. (2008) described a CTVT case with pulmonary metastasis.

The most useful treatment is chemotherapy with vincristine as the primary drug of choice, although vincristine-resistant CTVT may be encountered, as in our case. Calvert et al. (1982) reported a complete remission of CTVT in a dog after the administration of 30 mg/m² of doxorubicin (IV) doses three times a week, which initially not respond to vincristine therapy. This drug is an antitumor antibiotic that inhibits DNA and RNA synthesis. In another retrospective study of 29 dogs with CTVT (Rogers et al., 1998), one of the patients had a partial response to vincristine; therefore, doxorubicin (30 mg/m² IV; three times a week for five treatments) was administered, which resulted in complete remission, but with recurrence after 2 months. Cumulative doses of this drug has cardiotoxic effects, which manifests as decreased systolic function and/or arrhythmias (Gustafson & Bailey, 2020; Hallman et al., 2019). Therefore, an echocardiography should be performed before its application. In our patient, after cardiology consultation, doxorubicin was strongly contraindicated because of a low fractional shortening of 21%. Fractional shortening is a contractility index and it is considered normal at above 25% (Boon, 2011).

Surgery for CTVT is not a good approach because it is impractical in some regions of the body and might be invasive in cases where the tumor size is large (Das & Das 2000), with risks of reinfection during removal. It is estimated that 50-68% of relapses occur due to the transplantation of tumor cells into the surgical wound during traditional surgery (Ganguly et al., 2013). The recurrence rate with the surgery is frequent and ranges from 12% (Dass & Sahay, 1989) to 68% (Idowu, 1984). Using electrosurgery or cryosurgery may be a better alternative to conventional surgery, as CTVT can be easily transplanted into the surgical margins when conventional surgical methods are used (Ganguly et al., 2013).

Kunakornsawat et al. (2010) surgically treated three dogs with vincristine-resistant CTVT, and all patients remained tumor-free for 18 months. They performed episiotomy and vulvovaginoplasty in two female dogs and subtotal penile amputation and scrotal urethrostomy in a male dog. These techniques require expertise since it can cause postoperative urinary diseases. Da Silva et al. (2014) used a combination of L-asparagine, prednisone, and surgery in a clinical case, wherein the dog remained tumor-free for 12 months. The authors suggest that it is possible that the use of any of the treatments alone can cause the response seen in this case. Spugnini et al. (2008) treated three patients (male canines) with CTVT, which was resistant to the combination of vincristine and doxorubicin therapy, using two sessions of electrochemotherapy and local bleomycin therapy, with complete remission in 24 and 48 months. This technique is considered effective with no harmful side effects; however, it is expensive, and the patient needs to be anesthetized, which can be risky for animals that previously received multiple doses of chemotherapy. The use of vinblastine has been suggested as an option to treat vincristine-resistant CTVT (Varela et al., 2013).

Resistance is partially due to the high expression of p-glycoprotein (Pgp) by tumor cells, which is produced by the gene MDR-1. Pgp leads to the expulsion of the chemotherapeutic agent from tumor cells (Gaspar et al., 2009; Gaspar et al., 2011; Gerardi et al., 2014). Thus,

it reduces intracellular drug concentration to non-lethal levels (Binkhathlan & Lavasanifar, 2013). Flórez et al. (2017) worked with cultured CTVT cells and found that vincristine-treated cells exhibited a variable expression of the MDR-1 gene. This demonstrates the variability of CTVT cells response to therapy and indicates the modulatory effect of vincristine on MDR-1 gene expression. Another protein that could contribute to greater survival of tumor cells is P53, encoded by the TP53 gene. Mutations in TP53 are observed in human tumors and are related to the poor response to chemotherapy in lymphomas (Veldhoen & Milner, 1998) and reduced prognosis in breast cancer (Katapodi et al, 2004). TP53 overexpression is considered a marker for the presence of mutations, and gene mutations for CTVT have already been described (Choi & Kim, 2002; Sánchez-Servín et al., 2009). Gerardi et al. (2014) concluded that Pgp is significantly expressed in CTVT, which occurs naturally either before or after vincristine exposure, but no significant difference was found in the P53 expression. Future studies are needed to understand the molecular basis of CTVT resistance to vincristine, but regardless of the cause, there have been several descriptions of alternative treatments.

In this case, the authors use lomustine, an alkylating agent. The antitumor effect of lomustine is based on its chemical reaction with DNA, thereby inhibiting cell division (Sauerbrey et al., 2007). It is used at a dose of 60-90 mg/m² every 3 or 4 weeks orally. In veterinary medicine, this drug has been recently reported as the only therapy for canine and feline mastocytoma, lymphoma, sarcoma, malignant histiocytosis, and probably brain canine tumors, because it crosses the blood-brain barrier (Sauerbrey et al., 2007; Maina et al., 2014; Thamm, 2019). To the best of our knowledge, there are no publications on the used of lomustine to treat dogs with vincristine-resistant CTVT. In this case, there were no changes in complete blood cell counts and comprehensive metabolic panel, including electrolytes and liver function tests. According to the study by Hosoya et al. (2009), high alanine aminotransferase (ALT) levels are observed in dogs treated with lomustine, especially in younger dogs. ALT elevation usually appears after the first few doses and, thereafter, remains unaffected by cumulative doses. In most cases, these changes are reversible, resulting in a low prevalence of liver diseases. Tripp et al. (2011) used a metronomic therapy protocol with lomustine at an oral dose of 2.84 mg/m² daily in patients with different types of tumors (osteosarcoma, hemangiosarcoma, oral melanoma, nasal adenocarcinoma, among others). They concluded that it was well-tolerated in dogs with metastatic or terminal tumors without renal involvement, and could be a good strategy for patients who do not have a standardized treatment.

Conclusions

The use of lomustine at an oral dose of 60 mg/m² every 3 weeks proved to be effective in a case of vincristine-resistant CTVT, without showing any marked adverse effects. The benefits of this treatment include the simplicity of its management as it is administered orally every 3 weeks, with a low cost and no side effects.

As the results obtained in this case were from one animal, further studies using lomustine as the treatment of vincristine-resistant CTVT should be conducted in a large number of animals to establish the findings reported here.

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Ethics statement

The dog's owner consented formally to the treatment proposed in this study.

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Conflict of interests

No conflict of interests declared concerning the publication.

Authors' contributions

ADB Member of the oncology team. Primary attention of the patient, treatment and followship; NRA Bibliographic research and writer of the article; CMC Member of the oncology team. Followship of the patient; NBV Member of the oncology team. Followship of the patient; CAO Member of the oncology team. Followship of the patient; SB Translation and writing corrections in English; ABG Hospital service coordinator. Writing correction

Availability of complementary results

All the information obtained as a result of the study is included in the manuscript.

The study was carried out at Veterinary School, UdelaR, Montevideo, Uruguay.

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