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Lomustine for treatment of canine transmissible venereal tumor

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ABSTRACT: Canine transmissible venereal tumor (TVTC) is a highly casuistic transmissible neoplasm in Brazil. Chemotherapy with vincristine sulfate is considered the treatment of choice, but the need for weekly applications and hematological monitoring, in addition to costs, are obstacles to owners' adhesion to the treatment. Lomustine is an alkylating class antineoplastic agent, and because it is administered orally, it is a more practical and less costly treatment option for the owners of animals with neoplasms sensitive to the drug. This study evaluated the therapeutic efficacy of lomustine in dogs affected by TVTC. Twelve dogs with cytopathological diagnosis of natural genital TVTC were selected. The dogs were submitted to the experimental protocol with lomustine administration at doses of 70 to 85 mg/m^2 orally every 21 days, totaling a maximum of two administration cycles. The animals were reevaluated every 7 days until a maximum of +49 days after the first dose of lomustine, to monitor the regression of neoplastic lesions through measurements. Among the 12 dogs submitted to the lomustine protocol, 8/12 achieved complete remission of the neoplasm and were considered cured (66.6%), 1/12 had partial response to treatment (8.33%) and 3/12 had stable disease (25%). Important adverse effects such as severe neutrophilic leukopenia were detected in 3/12 dogs (25%). The clinical study indicated that lomustine may be a treatment option for TVTC.
Key words: neoplasm, chemotherapy, alkylants, lomustine, venereal tumour, dogs.

Eficácia da lomustina no tratamento do tumor venéreo transmissível canino

RESUMO: O tumor venéreo transmissível canino (TVTC) é uma neoplasia transmissível de elevada casuística no Brasil. A quimioterapia com sulfato de vincristina é considerada o tratamento de escolha, mas a necessidade de aplicações semanais e acompanhamento hematológico, além dos custos, são obstáculos à adesão dos proprietários ao tratamento. A lomustina é um antineoplásico da classe dos agentes alquilantes e, por ser administrado por via oral, representa um opção de tratamento mais prática e menos onerosa para os proprietários de animais com neoplasias. O objetivo deste estudo foi avaliar a eficácia terapêutica da lomustina em cães acometidos por TVTC. Foram selecionados 12 cães com diagnóstico citopatológico de TVTC genital de ocorrência natural. Os cães foram submetidos ao protocolo experimental com administração de lomustina nas doses de 70 a 85 mg/m^2 por via oral a cada 21 dias, totalizando no máximo dois ciclos de administração. Os animais foram reavaliados a cada sete dias até um máximo de +49 dias após a primeira dose de lomustina, para monitorar a regressão das lesões neoplásicas por meio de mensuração das lesões. Entre os 12 cães submetidos ao protocolo, 8/12 obtiveram remissão completa da neoplasia e foram considerados curados (66,6%), 1/12 tiveram resposta parcial ao tratamento (8,33%) e 3/12 tiveram doença estável (25%). Efeitos adversos importantes, como leucopenia neutrofílica grave, foram detectados em 3/12 cães (25%). O estudo clínico indicou que a lomustina pode ser uma opção de tratamento para TVTC.
Palavras-chave: neoplasia, quimioterapia, agente alquilante, lomustina, tumor venéreo, caninos.

INTRODUCTION

Canine transmissible venereal tumor (CTVT) is a neoplasm with worldwide distribution, occurring more frequently in tropical and subtropical developing countries, such as Brazil. The dynamics of the geographic distribution of the disease is related to the population of stray dogs and the absence of birth control programs among the canine population of large cities (MURCHISON et al., 2014; ARAÚJO et al., 2016; PEIXOTO et al., 2016; ARCILA-VILLA et al., 2018).

The clinical manifestation occurs in the genital region of males (penis and foreskin) and females (vulva and vagina), as erythematous, multilobulated and friable, single or multiple, cauliflower-like masses, especially when in the penile region. The presence of bloody secretion is frequent in genital lesions of both sexes. When located in extragenital anatomical areas, the clinical signs depend on the organs affected, and can consist of cutaneous and subcutaneous nodules, facial deformation, epistaxis, exophthalmos, halitosis and

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oral masses with involvement of dental elements, among other symptoms (HUPPES et al., 2014; DALECK & DE NARDI, 2016; PEIXOTO et al., 2016; ABEKA, 2019).

Diagnostic suspicion is based on the observation of characteristic clinical lesions in the genitalia of dogs, however confirmation requires the performance of cytopathological and/or histopathological exams (VAIL et al., 2020). When in extragenital presentations, it is important to perform the differential diagnosis for other neoplasms such as mast cell tumors, lymphomas and plasmocytomas (DALECK & DE NARDI, 2016).

TVTCs often respond satisfactorily to treatment protocols. The surgical approach that used to be widely performed is currently in disuse, due to the considerable risk of local recurrences (GANGULY et al., 2013; ABEKA, 2019). Other treatment options such as radiotherapy and electrochemotherapy have shown good responses but are more often used in cases of chemotherapy resistance (SPUGNINI et al., 2008; GANGULY et al., 2013). The treatment of choice is chemotherapy with vincristine sulfate at doses ranging from 0.5 to 0.75 mg/m^2, administered intravenously every 7 days. On average, 4 to 6 cycles of chemotherapy are needed to obtain clinical cure (SILVA et al., 2007; RODASKI & DE NARDI, 2008; RAMADINHA et al., 2016).

In Brazil, the legislation that regulates the practice of biosafety in establishments that have antineoplastic therapy services specifies several safety items for the handling and application of drugs and disposal of waste used in anticancer therapies (ANVISA - RDC Nº 220). The compliance of veterinary clinics and hospitals to the current legislation causes increased costs of equipment and safety items, passed through to owners. The high costs mean that many animals affected by CTVT do not receive adequate treatment.

Lomustine is chemotherapeutic agent of the alkylating class and nitrosurea group, with highly lipid-soluble characteristic that gives it strong capacity for tissue diffusion and penetration into cells by passive diffusion (HEADING et al., 2011; DALECK & DE NARDI, 2016). Its mechanism of action requires the replication of cellular DNA to act. However, its activation can occur in different phases of the cell cycle. Cell death occurs mainly during the S phase of the cell cycle, in which its active metabolites act on DNA replication (DALECK & DE NARDI, 2016; VAIL et al, 2020). Lomustine is widely used in the treatment of neoplasms in dogs and cats, mainly in lymphomas (MOORE et al., 1999; RISBON et al., 2006), mast cell tumors (HOSOYA et al., 2009) and histiocytic sarcomas (CANNON et al., 2015). Despite its reported efficacy in other tumor types, lomustine has only recently been described as an option for the treatment of TVTC in a vincristine-resistant case (BARBOZA et al., 2021). Cyclophosphamide, also an alkylating agent, has already been evaluated with oral and intravenous administration; although, with unfavorable initial responses (AMBER et al.,1990).

The development of new effective treatment modalities that are less costly and more practical for owners is extremely necessary. The rate of treatment abandonment, or even no treatment at all, in dogs diagnosed with TVTC is considered high, and is usually related to treatment costs and/or the need for weekly follow-up of patients, as observed by HUPPES et al. (2014) in a study also carried out in Brazil. This studyevaluated the efficacy of lomustine for the treatment of naturally occurring TVTC.

MATERIALS AND METHODS

Twelve dogs meeting all the necessary criteria were selected and attended between 2017 and 2020 at the Oncology Service of the Veterinary Hospital of Federal Rural University of Rio de Janeiro. Patients were included in the clinical study with the authorization of their owners.

Dogs were included in the study regardless of breed, sex or age, with genital tumors that could be monitored by measurement. The other inclusion criterion was absence of comorbidities at the time of diagnosis of TVTC or in which comorbidities could be treated before the start of the study. The diagnosis of TVTC was obtained through cytopathological examination of genital tumor lesions and inguinal lymph nodes, when affected, using the fine needle puncture technique.

Evaluations and photographic records were performed on day 0 and later with an interval of seven days until clinical cure or until day +49 after the first drug administration. For the inspection of neoplasms, the lesions were exposed through preputial retraction in males and vaginal speculum in females. Then, measurements were taken in centimeters of the two largest perpendicular diameters of the neoplasm with the aid of a digital pachymeter. In addition, laboratory tests were performed at each return of the animal. Tumor surface area (TSA) was calculated using the formula TSA = largest tumor diameter x smallest tumor diameter. The measurement of inguinal lymph nodes of animals affected by regional metastasis

followed the same method. The photographs were always taken in the lateral decubitus position, maintaining standardization.

The experimental protocol was performed through the oral administration of lomustine in pharmaceutical presentation of 10 and 40 mg capsules at doses ranging between 70 to 85 mg/m^2 with an interval of 21 days, totaling a maximum of two cycles of administration. The doses administered to all animals could not be homogeneous due to the commercial presentation available and the option not to use the drug in presentations from compounding pharmacies.

The therapeutic response to the experimental protocol was classified as described by RODASKI & DE NARDI (2008) as: complete remission (when there was complete involution of clinical lesions); partial response (when there was a reduction equal to or greater than 50% of the total measureable tumor tissue); and stable disease (when no change in tumor tissue or less than 50% reduction was detected). To diagnose total remission and clinical cure of patients, parameters of complete involution of clinical lesions and cytological exams in the region of the tumor bed were observed. The absence of TVTC tumor cells in the samples was the parameter to diagnose complete remission of the neoplasm.

Blood counts were performed to assess the nadir for lomustine (5 to 7 days after oral drug administration) and serum biochemistry (ALT [alanine aminotransferase], ALP [alkaline phosphatase], urea and creatinine) serially throughout the experimental period, with an interval of 7 days. Serial laboratory tests were performed in order to detect possible myelotoxicity, hepatotoxicity and nephrotoxicity. We classified effects correlated with myelotoxicity as mild, moderate, severe or life-threatening, according to the consensus published in 2011 by the Veterinary Cooperative Oncology Group (VCOG, 2011).

Patients that did not present clinical cure with the use of the experimental protocol were later treated through a conventional chemotherapy protocol using vincristine sulfate at a dose of 0.75mg/m^2, administered intravenously, with intervals of 7 days, until obtaining clinical and cytological cure of the neoplasm.

RESULTS AND DISCUSSION

Among the dogs selected for the experimental protocol, the age ranged from 1 to 11 years. However, in some cases the owners did not know the correct age, making it impossible to observe the average age among the animals. Regarding breed, eleven dogs were considered mixed breed and only one was a pure breed (Pinscher). The distribution between sexes was 50% males and 50% females.

The epidemiological data results are in agreement with those described in the literature (CRUZ et al., 2010; HUPPES et al., 2014; PEIXOTO et al., 2016). The marked prevalence of mixed-breed dogs has often been reported in similar studies (SILVA et al., 2013; ARAÚJO et al., 2016; PEIXOTO et al., 2016; PAULINO et al., 2018), and can be related to the greater prevalence of mixed-breed dogs among the stray population in Brazilian cities. As for age, only one patient was classified a geriatric, while the others were young or middle-aged (of reproductive age). Regarding gender, the observed 50/50 distribution is not in line with other studies. Some have reported higher prevalence among females, based on the fact that a single male can transmit the disease to many females (HUPPES et al., 2014; ABEKA, 2019). However, a global study carried out by STRAKOVA & MURCHISON (2014) did not observe this difference.

Regarding the macroscopic presentation of the tumor, ten dogs had lesions exclusively in the genital region and two had genital tumors with bilateral metastases in inguinal lymph nodes, diagnosed through cytopathological examination. The life history of all dogs was compatible with the development of TVTC, with five dogs rescued from the street and adopted in the months prior to diagnosis, four with a history of recent escapes and three with semi-domicile habits, that is, they had owners but were allowed to wander in the streets during some periods. Table 1 reports the epidemiological and clinical data of the patients.

TVTC transmission mechanism is widely described in the literature, occurring mainly through the transplantation of tumor cells during coitus. Therefore, stray dogs or dogs with free access to the street are more susceptible to the disease (BRANDÃO et al., 2002; MURCHISON et al., 2014; ARAÚJO et al., 2016; PEIXOTO et al., 2016; ABEKA, 2019). The epidemiological data of the study regarding the history of the animals showed that all were at risk of the disease, since they had a history of free living or recent escapes.

TVTC can also affect anatomical regions other than the genitalia, such as oral and nasal cavities, skin and others (PEIXOTO et al., 2016; VAIL et al, 2020). Dogs that presented genital lesions and/or metastases in inguinal lymph nodes were selected to allow the measurement of neoplastic lesions and response to treatment.

The measurement criteria were adopted to standardize the assessments, since the neoplastic lesions in the different animals presented particularities that made it

Table 1 - Epidemiological and clinical characteristics of dogs diagnosed with canine transmissible venereal tumor submitted to the experimental protocol of treatment with lomustine.

Animal	Breed	Sex	Age	T.S.A at day 0 (cm^2)	L.N.M.	History
I	Mixed	M	8 years	2.96	Negative	Semi-domicile
II	Mixed	F	2 years	16.50	Negative	Rescued from the street
III	Mixed	F	5 years	14.40	Negative	Recent escape
IV	Mixed	M	7 years	31.18	Positive	Rescued from the street
V	Mixed	F	Adult	8.59	Negative	Recent escape
VI	Mixed	M	Adult	65.71	Negative	Semi-domicile
VII	Pinscher	F	1 year	5.14	Negative	Rescued from the street
VIII	Mixed	F	Adult	18.40	Positive	Rescued from the street
IX	Mixed	M	3 years	13.87	Negative	Recent escape
X	Mixed	M	6 years	9.51	Negative	Rescued from the street
XI	Mixed	F	11 years	31.61	Negative	Semi-domicile
XII	Mixed	M	7 years	13.88	Negative	Recent escape

Subtitle: Tumor surface area (TSA), regional lymph node metastasis (LNM).

impossible to obtain sufficient measurements to calculate the volume of each tumor mass. Among the particularities mentioned, there were intravaginal tumors with immeasurable depth of the neoplastic mass, and tumors in the penis and foreskin with dimensions that made it impossible to expose the penis and consequently the tumor lesions in their entirety.

Data regarding clinical response (table 2) showed that eight dogs (8/12 – 66.6%) had complete remission of tumor lesions and were considered cured of the neoplasm; one dog (1/12 - 8.33%) had a partial response to treatment, and three dogs (3/12 - 25%) had stable disease.

Two cycles of lomustine administration were required to achieve complete remission in six animals, and only one cycle in two of the animals. Dogs that presented vestigial lesions similar to the scarring process on day +42 were not submitted to a third cycle of lomustine administration and were followed up until day +49 for cytopathological examination to verify possible cure. Despite the observation of partial remission of the tumor mass in the genitalia, one of the animals did not receive the second cycle of lomustine administration, due to a slight increase in the surface area of the inguinal metastases. Tumor lesions before and after treatment with the experimental protocol of patients 2 and 9 are illustrated in figure 1.

The dogs that initially presented metastatic foci in inguinal lymph nodes (animals 4 and 8) showed distinct clinical responses. Animal 4 achieved complete remission of genital and metastatic lesions on day +35, while animal 8 showed partial response of genital lesions and stable disease, but with a slight increase in surface area in relation to nodal metastases.

A maximum of two administration cycles of lomustine were performed in each patient, totaling 42 days of treatment. This protocol was designed to observe the response to the drug in a period similar to that necessary to obtain the complete remission described with vincristine, which is based on 4 to 6 weeks of applications (HUPPES et al., 2014; RAMADINHA et al., 2016). Although, the protocols had a similar treatment time, the lomustine protocol had some advantages, among them the practicality of oral administration, which can be performed at home by the owner, in addition to the ease in treating agitated and/or aggressive dogs, behaviors that complicate intravenous administration, a fact that is aggravated by vincristine sulfate's vesicant effect, related to extensive tissue damage when vascular extravasation occurs (BILLER et al., 2016).

Among the 8 dogs that were cured, two achieved complete remission of the neoplasm with just one administration of lomustine. This result can be explained by the initial size of the neoplasm in these individuals, which were significantly smaller (2.96 and 5.14 cm^2) than the average of the others (22.36 cm^2). The other 6 dogs cured after two administrations of lomustine had initial lesions of varying sizes (13.87 to 31.61 cm^2). This result demonstrated that lomustine can be effective in controlling TVTC regardless of the size of the initial lesions.

Table 2 - Evaluation of tumor surface area (cm^2) regression in relation to the time to obtain clinical and cytological cure of animals affected by canine transmissible venereal tumor treated with lomustine.

Animal	Day 0	Day +7	Day +14	Day +21	Day +28	Day +35	Day +42	Day +49
I	2.96	1.16	0.94	0.0				
II	16.50	12.04	6.35	4.56	2.37	0.17	0.0	
III	14.40	13.94	9.18	8.06	C.P.			
IV	31.18	22.13	20.99	19.31	18.72	0.0		
V	8.59	8.38	5.68	7.86	7.88	7.01	6.97	C.P.
VI	65.71	64.60	62.48	50.40	40.70	50.80	33.98	C.P.
VII	5.14	2.49	1.12	0.81	0.0			
VIII	18.40	7.80	11.65	6.63	C.P.			
IX	13.87	12.00	18.09	M.	8.55	3.08	1.84	0.0
X	9.51	6.96	3.45	2.68	2.52	2.29	0.57	0.0
XI	31.61	30.00	12.33	3.89	1.67	M.	0.15	0.0
XII	13.88	12.00	2.60	2.15	0.62	0.61	0.0	

Missed the assessment (M), change of protocol (CP) to the administration of vincristine sulfate.

Stable disease was observed in three dogs, two of them even after the second administration of lomustine. In these cases, the lack of clinical response was attributed to intrinsic mechanisms of tumor resistance to the drug, which can occur in relation to practically all chemotherapy drugs (FLÓREZ et al., 2014; GERARDI et al., 2014; WICK & PLATTEN, 2014; KLOPFLEISCH et al., 2016; VAIL et al, 2020).

Two dogs had nodal metastases and only one achieved complete remission of genital and metastatic lesions. Another patient had stable disease and had its protocol modified on day +28 to the administration of vincristine sulfate. The option to change treatment before the second administration of lomustine was due to the slight increase in metastatic lesions and the priority given to the patient's well-being.

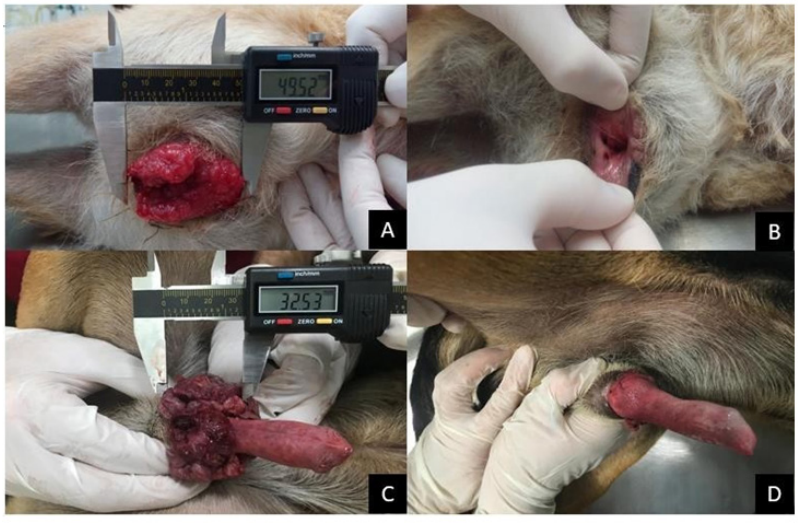

Figure 1 - TVT lesions in dogs, treated with the lomustine protocol. A) Animal 2, showing erythematous tumor lesion with irregular surface, located in the vaginal region on day 0. B) Animal 2, showing complete remission of tumor lesions on day +42. C) Animal 9, showing measurement of tumor lesions, with preputial retraction and exposure of a large erythematous multilobulated mass in the penile base region, on day 0. D) Animal 9, showing preputial retraction and small vestigial changes resulting from the healing process on day +42.

It was not possible to assess response to lomustine in vincristine sulfate-resistant TVTCs due to the absence of cases during the study period. However, since resistance to antineoplastics occurs through different pathways and the main known mechanisms may be different between alkylating drugs and antimicrobial agents (KLOPFLEISCH et al., 2016; VAIL et al, 2020), it is possible that lomustine was effective in part of these cases, as recently described by BARBOZA et al. (2021) in a case report.

Adverse effects related to lomustine myelotoxicity were evaluated for presence and intensity. The most frequent adverse effect was neutrophilic leukopenia, which ranged from mild (grade 1) to high risk of life for the patient (grade 4). Mild (grade 1) thrombocytopenia was also observed in some patients. Three dogs (3/12 - 25%) developed severe neutropenia (≤ 999 neutrophils/µL-1) (grade 3), noted at drug nadir. These were treated with recombinant human granulocytic colony stimulating factor (rhG-CSF) (Filgrastine - Laboratório Blau). The dose used was 5 µg/kg through subcutaneous injections for three consecutive days. Return to normal hematological parameters or similar values occurred in all three dogs. In no case was it necessary to discontinue treatment due to lack of medullary recovery.

Myelotoxicity is frequently observed in patients undergoing chemotherapy with lomustine (HEADING et al., 2011; BILLER et al., 2016). The three dogs that presented severe neutrophilic leukopenia (25% of cases) underwent treatment with rhG-CSF. Its use is one of the recommended therapeutic options in cases of severe neutropenia after chemotherapy treatment (LUCIDI & TAKAHIRA, 2007). The use in the cases observed in the study was important to avoid serious conditions related to infections and sepsis.

Another change observed was the elevation of serum ALT (580 U/L) in patient 3, which despite having a significant reduction in cancer severity, had its experimental treatment suspended before the second administration of lomustine. According to one report in the literature (RODASKI & DE NARDI, 2008), hepatotoxicity is considered rare. However, other researchers have described a high frequency of this adverse effect (HEADING et al., 2011; VAIL et al, 2020). Laboratory alterations of elevation of ALT, and less frequently of aspartate aminotransferase (AST) and ALP, are more often observed with chronic use and should be monitored due to the risk of severe and progressive drug hepatitis (HEADING et al., 2011; DALECK & DE NARDI, 2016; VAIL et al, 2020).

CONCLUSION

Lomustine can be considered a treatment option, having demonstrated efficacy in TVTC, regardless the size of the initial lesions. Monitoring of side effects, mainly through laboratory tests, is extremely important. More studies are needed to elucidate the mechanisms involved in cases of TVTC with intrinsic resistance to lomustine.

DECLARATION OF CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest of any kind.

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ETHICAL STATEMENT

The study was approved by the Animal Ethics and Use Committee of the Veterinary Institute of Federal Rural University of Rio de Janeiro (CEUA no. 1768060819). Patients were included in the clinical study with the authorization of their owners.

AUTHORS' CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved of the final version.

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