
Curcumin and piperazin in association with chemotherapy protocol for cutaneous lymphoma: animal clinical report

Curcumina e piperazina em associação ao protocolo de quimioterapia para linfoma cutâneo: relato clínico animal

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ABSTRACT

The anticancer properties of curcumin were associated with its ability to promote apoptosis, decrease metastatic potential, and proliferation inhibition of distinct tumor types. The objective of the present study was to describe an animal clinical case, reporting the combination of curcumin (450mg *Curcuma longa* L. rhizome extract), and piperine (15mg *Piper nigrum* fruit extract) associated with chemotherapy for cutaneous lymphoma treatment. The phytotherapeutic treatment was continued every 12 hours, and the chemotherapy protocol was a combination of doxorubicin, cyclophosphamide, vincristine, and prednisone. The patient demonstrated a partial response, identified by more than 50% of tumor diameter regression. However, the lesions relapsed in the sixth week, and the lomustine was used. The partial remission was again achieved, and the 19-week maintenance protocol with metronomic therapy using cyclophosphamide was introduced for 10 consecutive months. It was observed that these treatments provided quality survival for the cancer patient, demonstrating the benefit of this co-adjuvant therapy for the chemotherapy protocol cancer treatment. However, the total cure could not be achieved for this patient.

Keywords: Cancer; Medicinal Plants; Natural Treatment; Saffron.

RESUMO

As propriedades anticancerígenas da curcumina foram associadas à sua capacidade de promover apoptose, diminuir o potencial metastático e inibir a proliferação de distintos tipos de tumores. O objetivo do presente estudo foi descrever um caso clínico animal, relatando a combinação de curcumina (450mg de extrato do rizoma de *Curcuma longa* L.) e piperina (15mg de extrato da fruta *Piper nigrum*) associada à quimioterapia para tratamento de linfoma cutâneo. O tratamento fitoterápico foi continuado a cada 12 horas, com curcumina associado a piperina, e o protocolo quimioterápico constituiu-se da combinação de doxorubicina, ciclofosfamida, vincristina e prednisona. O paciente demonstrou resposta parcial, identificada pela regressão em mais de 50%, do diâmetro tumoral. Entretanto, as lesões recidivaram na sexta semana de protocolo, e utilizando-se lomustina, obteve-se novamente remissão parcial. Assim, foi introduzido protocolo de manutenção de 19 semanas com terapia metronômica de ciclofosfamida por 10 meses consecutivos. Nesse período, observou-se que esses tratamentos foram capazes de proporcionar sobrevida de qualidade ao paciente oncológico, demonstrando o benefício desta terapia coadjuvante para o protocolo quimioterápico, embora não tenha sido possível alcançar a cura total do paciente.

Palavras-chave: Cancer, Plantas Medicinal, Tratamentos Naturais, Açafrão.

INTRODUCTION

Cancer corresponds to a relevant public health issue, and its prevalence has increasingly been observed both in human and veterinary medicine (Kitsoulis *et al.*, 2020; Cray; Selmic; Ruple, 2020). A variety of natural medicine modalities have been used as complementary therapy in oncological treatment, emphasizing the use of medicinal plants (Twilley, Rademan, Lall, 2020). The recognition of these natural therapies among patients is mainly related to the dissatisfaction with the outcomes of conventional protocols, as well as the ability to minimize the usual side effects of chemotherapy and radiotherapy (Almeida *et al.*, 2020).

Curcuma longa L. is an herbaceous plant with ovoid rhizomes that belongs to the family Zingiberaceae. It is native to South and Southeast Asia, but, has spread to Europe and America. In Latin-speaking countries, it is known as curcuma or saffron.

From the saffron curcuminoid root, chemical compounds such as demethoxycurcumin, bisdemethoxycurcumin and tetrahydrocurcumin have been isolated. Curcumin is the most abundant compound, noting that 13 curcuminoids with anticancer potential have been described (Sultana *et al.*, 2021). In addition, saffron has been associated with other cancer therapies.

The saffron anticancer activity was evidenced by its capacity of inducing apoptosis, inhibiting different tumor types proliferation, and favoring metastasis reduction, besides its inhibitory effect against angiogenesis, growth factor receptors, and cell adhesion molecules involved in tumor growth. Thus, it has been associated with metastasis reduction (Mello-Peixoto *et al.*, 2022).

Despite all these benefits, the bioavailability of curcumin represents an important limiting factor for its use. The high metabolism and low half-life of curcumin impairs its absorption when administered orally. Therefore, several associations have been proposed, mainly with piperine, to increase the solubility, bioavailability, and anticancer activity of curcumin (Facina *et al.*, 2021). So, the association of curcumin with piperine, a polyphenol isolated from *Piper nigrum* fruit (black pepper), increased the curcumin oral bioavailability (Heidari *et al.*, 2023). Moreover, it is essential to consider that some piperine compounds may be able to determine some mechanism of synergistic action with curcumin. Its association with piperine was able to potentiate the antigenotoxic effect, revealed by the decline of polychromatic erythrocytes and acrosomal aberrations (Heidari *et al.*, 2023).

Canine lymphoma is classified according to its anatomical location, and it can be non-epitheliotropic or epitheliotropic when originating from B or T cells, respectively. Lymphomas predominantly affect animals between 6.3 and 7.7 years of age, and the most common form of lymphoma consists of T cells (CD8) that determine pruritic, desquamative, and erythematous eruptions (Mazaro *et al.*, 2023). Survival times and responses to therapy are variable, and indicators to predict outcomes are lacking (Dettwiler *et al.*, 2023). The chemotherapy effect is frequently insufficient to inhibit the disease's signs of progress (Chan *et al.*, 2018), and the survival varies from a few days or weeks to up to years. The median survival time corresponds to approximately six months (Fontaine *et al.*, 2010; Chan *et al.*, 2018).

Thus, considering the antitumor potential of saffron, this study aims to present an animal clinical case reporting the association of curcumin with piperine to conventional chemotherapy protocol combination of doxorubicin, cyclophosphamide, vincristine, and prednisone (CHOP) for cutaneous lymphoma treatment.

CASE REPORT

A seven-year-old black Newfoundland dog presented cutaneous nodules near the tibia-fibula-metatarsal joint of the pelvic limb. The animal was submitted to surgical excision (Figure 1), and the nodule was forwarded to a histopathologic exam.

Figure 1 – The nodule presented a central blackened coloration and white margins, measuring 7.2 x 5.9 cm of diameter.



Fonte: Sanches *et al.*, 2024.

Regarding microscopic appearance, it was possible to observe neoplastic tissue with high cell content composed of cells with an elongated nucleus, loose nuclear chromatin, evident nucleoli, sparse cytoplasm, and no defined limits. Also, the cells were infiltrated into the superficial, medium, and deep dermal tissues with neutrophils and amorphous acidophilus secretion, being associated with ulcerated areas of the skin. Considering that three to four mitoses were seen per high power field, this exam suggested soft tissue sarcoma.

While it had not been possible to establish a definitive diagnosis, the natural therapy was immediately initiated. The curcumin oral tablet was constituted of 450mg of *Curcuma longa* L. rhizome extract (95% curcuminoids), 15mg *Piper nigrum* fruit extract (95% piperine), and 300mg of olive leaf extract (20% oleuropein), as a vehicle, were administered every 12 hours.

Additionally, the patient underwent a chest X-ray. Right and left lateral and ventrodorsal projections revealed bronchial and interstitial lung patterns in the caudal lung lobes and, cardiac silhouette with no marked changes. Although this patient presented good general conditions without any clinical signs such as discomfort, pain, apathy, or changes in appetite, other skin nodules appeared, so another surgical excision was performed. The nodules were again sent for histopathologic evaluation. Three fragments were sent to a new histological evaluation. The first cutaneous fragment was

from the right pelvic limb, measuring 4,5 x 3,0 x 1,8 cm, containing a node measuring 3 cm high in its largest axis and presenting brown, soft, and smooth surface cutting. The second cutaneous fragment was from the neck measuring 7,0 x 6,0 x 2,5 cm, containing an elevated and ulcerated node measuring 3,2 cm in its largest axis, also presenting brown, soft and smooth surface cutting. The last cutaneous fragment was from the left flank, measuring 9,8 x 9,5 x 3,0 cm, containing an elevated and ulcerated node measuring 6,0 cm in its largest axis, presenting pinkish-brown, soft and smooth surface cutting. These three fragments presented the same microscopic appearance, with round cells with a large nucleus, evident nucleoli, and sparse cytoplasm invading the deep dermal tissues and panniculus adjacent. Also, all the surgical margins (deep and lateral) were taken as free of malignant cells. The final diagnosis was established as a round cell neoplasia with appearance suggestive of cutaneous lymphoma, or poorly differentiated mast cell tumor. Therefore, to provide an adequate characterization of the histogenesis of the lesion, immunohistochemical examination was recommended.

The tissue fragment was analyzed by immunohistochemistry in order to better establish the low-differentiate neoplasia histogenesis. The tissue cuts processed routinely for histology and included in paraffin were placed on previously signaled blades. The antigenic recovery by moist heat method was held in a steamer for 20-30 minutes. The incubation with primary antibodies was held at 4 °C (39.2 °F) overnight. The Advance system was used for revelation. The staining and counterstaining were performed using 3,3-diaminobenzidine and hematoxylin, respectively. Internal and external controls were used to validate the reaction. The results were compatible with CD3 neoplastic cells immunoexpressed. No CD79a, MUM1, C-Kit, Tryptase, or E-Cadherin were expressed. Therefore, the immunohistochemical and morphologic profile indicated the diagnosis of non-epitheliotropic cutaneous lymphoma (immune phenotype T). Considering the histopathology and immunohistochemistry characterization, the CHOP protocol was elected as a treatment, using a combination of doxorubicin, cyclophosphamide, vincristine, and prednisone.

All nodules that were surgically excised did not develop recurrence at the lesion site, and the patient continued to present excellent clinical conditions. However, these treatments were not able to prevent the appearance of metastasis in different other cutaneous regions. Partial response was observed for these nodules, identified by more than 50% of tumor diameter regression. Nonetheless, the lesions relapsed at the sixth

week of protocol. Then, lomustine was used for rescue purposes and partial remission was again achieved. The 19 weeks protocol was concluded, but full remission was not achieved. A maintenance protocol with metronomic therapy was given after the CHOP protocol, using cyclophosphamide at a dose of 12.5mg/m², every 24 hours daily for a total of 10 mouths.

The patient was submitted to periodic complementary exams, including a hemogram and biochemistry analysis during the entire treatment period. All the measures were executed at the Veterinary Hospital from State University of Northern Paraná (UENP). In each exam, the animal was contained physically and the blood was collected. One fraction of the blood collected was stored in a tube of 3mL containing anticoagulant (EDTA). The other fraction was stored in a tube without any anticoagulant in view to obtain the animal serum. After that, both samples were submitted for analysis.

The hemogram analyses were held by hematology analyzer BC-2800 Vet (MINDRAY®) and by leukocytes and platelets differential count in blood smear. Hematocrit (Ht), red blood cells (RBC) count, total serum hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelets, white blood cells (WBC), neutrophils, rod cells, lymphocytes, monocytes, and eosinophils count were measured.

The biochemistry parameters were made using automatic clinical chemistry analyzer PKL Biochemistry®. The total blood collected was submitted to centrifugation during 5 minutes and 5000 revolutions per minute to obtain the serum. The biochemistry parameters analyzed were: serum albumin (SA), alanine aminotransferase (ALT), alkaline phosphatase (AP) creatinine and blood urea nitrogen (BUN).

RESULTS AND DISCUSSION

Regarding the results of hemogram and biochemical parameters, even though some variations have been observed in specific treatment periods, no one variation was relevant that could be considered clinically in any parameter analyzed during the entire treatment period (Table 1, 2).

Table 1. The mean values of the parameters evaluated by the erythrogram and leukogram during 11 months of treatment.

Parameter	Erythrogram		Leukogram		
	Average	Standard	Parameter (μ l)	Average	Standard
Hematocrit (%)	38.56	37-55	White Blood Cells (WBC)	7586.67	6000-17000
Red Blood Cells count ($\times 10^6/\mu$ L)	5.38	5.5-8.5	Rod cells	84.13	0-300
Hemoglobin (g/dL)	12.90	12-18	Neutrophils	6131.47	3000-11500
Mean Corpuscular Volume - MCV (fL)	71.74	60-77	Lymphocytes	1037.20	1000-4800
Mean Corpuscular Hemoglobin Concentration MCHC (%)	33.42	32-36	Eosinophils	247.73	150-1250

Although monocytopenia and low rod cell counts were observed, this was not considerable due to the physiological variation of these cells in healthy dogs (Sarkozy; Salles; Falandry, 2015) and by the absence of any other alteration in hemogram.

Table 2. The mean values of the biochemical parameters, during 11 months of treatment.

Parameter	Average	Standard
Serum Albumin (g/dL)	2.35	2.6-3.3
Alanine aminotransferase (UI/L)	41.88	21-86
Alkaline phosphatase (UI/L)	95.00	20-156
Creatinine (mg/dL)	0.90	0.5-1.5
Blood Urea Nitrogen (mg/dL)	30.00	21.4-59.92

Regarding the mean values observed in platelet count ($229.87 \times 10^3/\mu$ L) and in the levels of total serum protein (7.89g/dL), both corresponded to values consistent with a normal range ($200-500 \times 10^3$ and 6.0-8.0).

Curcumin and cisplatin association for the treatment of lung adenocarcinoma cells (A549) was beneficial due to reversing tumor resistance. It was observed the *in vitro* inhibition of factor induced by hypoxia-1 (FIH- α) and decrease of P-glycoprotein, which

are proteins linked to tumor resistance (Jiang *et al.*, 2012). In addition, curcumin reduced neurotoxicity (Liao *et al.*, 2023) and cisplatin nephrotoxicity.

The effect of curcumin as a sensitizing agent to enhance the apoptotic potential of doxorubicin has been studied (Yag *et al.*, 2020). Additionally, since some tumor cells develop multidrug pharmacological resistance (MDR), it is valuable to highlight the contribution of curcumin to drug resistance control. The mechanisms of MDR may be connected to the overexpression of efflux pumps of the chemotherapy drug used, such as the P-glycoprotein, which is often found to be overexpressed in cases of acquired MDR. However, there are no P-glycoprotein inhibitors used in current clinical practice due to toxicity issues, drug interactions, or pharmacokinetic disturbances. In this context, curcumin has been emerging as a potential drug for the antitumor treatment response (Attia *et al.*, 2020).

Furthermore, the anti-inflammatory and immunomodulatory properties promoted by curcumin can represent a consistent adjuvant mechanism of control for neoplastic pathogenesis (Chamani *et al.*, 2023). Therefore, the results demonstrated by *in vitro*, *in vivo* tests, pre-clinical and clinical trials showed that curcumin presents evidences of prophylactic and curative effects on the treatment of different types of cancer (Myazaki *et al.*, 2023; Gahtori, *et al.*, 2020; Kumar *et al.*, 2023).

Canine cutaneous epitheliotropic T-cell lymphoma (CETCL) is a spontaneous neoplastic skin disease with a variety of clinical presentations (Dettwiler *et al.*, 2023). In the present case, we observe a different presentation because the lymphoma is a T-cell phenotype but non-epitheliotropic histologically. That may justify not only the long-term survival but also the continuing evolution of cutaneous lesions. Even so, we can infer that the use of phytotherapeutic treatment may not only have prolonged survival time, but may also have favored a better quality of that survival period, since the results using only conventional chemotherapy are often frustrating (Montaner-Angoiti; Marín-García; Llobat, 2023). Only when the clinical condition was significantly compromised by the difficulty of locomotion, due to claudication caused by ulcerated nodules in the thoracic limb, euthanasia was performed at 19 months after starting the phytotherapy.

CONCLUSIONS

Although the treatment in this reported case was not able to inhibit the occurrence of metastasis, it was observed that the patient presented good general conditions without

any clinical signs such as discomfort, pain, apathy, or changes in appetite. Consequently, the results of the combination of phytotherapy and chemotherapy pointed to an important option for better therapeutic results.

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