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ORIGINAL ARTICLE

A dose-escalation study of combretastatin A4-phosphate in healthy dogs

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Combretastatin A4-Phosphate (CA4P) is a vascular disrupting agent revealing promising results in cancer treatments for humans. The aim of this study was to investigate the safety and adverse events of CA4P in healthy dogs as a prerequisite to application of CA4P in dogs with cancer.

Ten healthy dogs were included. The effects of escalating doses of CA4P on physical, haematological and biochemical parameters, systolic arterial blood pressure, electrocardiogram, echocardiographic variables and general wellbeing were characterised. Three different doses were tested: 50, 75 and 100 mg m^{-2}.

At all 3 CA4P doses, nausea, abdominal discomfort as well as diarrhoea were observed for several hours following administration. Likewise, a low-grade neutropenia was observed in all dogs. Doses of 75 and 100 mg m^{-2} additionally induced vomiting and elevation of serum cardiac troponine I levels. At 100 mg m^{-2}, low-grade hypertension and high-grade neurotoxicity were also observed.

In healthy dogs, doses up to 75 mg m^{-2} seem to be well tolerated. The severity of the neurotoxicity observed at 100 mg m^{-2}, although transient, does not invite to use this dose in canine oncology patients.

KEYWORDS

anticancer therapy, combretastatin A4-phosphate, dogs, dose-escalation, vascular disrupting agent

1 | INTRODUCTION

The concepts behind antitumour therapeutics evolved from the conventional direct attack of the proliferating cells to windows of opportunities that rather influence of the tumour environment. Greater understanding of the mechanisms by which tumours grow has led to the identification of more selective oncology targets, such as the blood vessel support network of the tumour.[1,2] Blood flow inside the tumour is fundamental to guarantee adequate oxygen and nutrient delivery for continued growth.[3,4] Destruction of the existing tumour vasculature is a promising treatment modality in oncology patients with solid tumours.[5] Vascular disrupting agents (VDA) produce a rapid shutdown of tumour blood vessels by physical obstruction of their lumen and the resulting ischemia can lead to extensive cell death within tumours.[6] Combretastatin A4-phosphate, a microtubule-targeting agent, represents the lead-candidate VDA that recently (2016) became US FDA-approved in a treatment protocol for ovarian cancer in human patients and is currently evaluated in various Phase II and III clinical trials in combination with chemotherapy or radiation therapy.[7–10]

ABBREVIATIONS: ADL, activities of daily living; AE, adverse events; AIVR, accelerated idioventricular rhythm; BSA, body surface area; BPM, beats per minute; CA4P, combretastatin A4-phosphate; CBC, complete blood count; CINV, chemotherapy induced nausea and vomiting; CIPN, chemotherapy induced peripheral neuropathy; CTCAE, common terminology criteria for adverse events; cTnI, cardiac troponine I; DLT, dose limiting toxicity; ECG, electrocardiogram; EMG, electromyography; FN, female neutered; HR, heart rate; LLN, lower limit of normal; LMN, lower motor neuron; MN, male neutered; MRI, magnetic resonance imaging; MTD, maximum tolerated dose; NK-1, neurokinin-1; PBS, phosphate buffered saline; PW-TDI, pulsed-wave tissue Doppler imaging; RSA, respiratory sinus arrhythmia; SABP, systolic arterial blood pressure; TPN, total parental nutrition; UMN, upper motor neuron; VDA, vascular disrupting agents; VEB, ventricular ectopic beats

Because VDA exhibit high specificity for immature tumour vasculature, they should not share the side effects observed after conventional cancer therapy, that is, myelosuppression, stomatitis and alopecia.[11–17] In human cancer patients, 52 mg m^{-2} is the lowest dose at which tumour blood flow reduction is observed. Adverse events (AE) observed after CA4P administration are typically mild to moderate, with nausea and vomiting seen most commonly.[13–15] Other AE encountered sporadically in human patients are tumour pain, cardiovascular toxicity (hypertension) and neurotoxicity. In 2007, Kador and colleagues investigated intravenous administration of 32 mg m^{-2} CA4P in dogs as a possible therapy to regress retinal neovascularisation, and reported low-grade vomiting and loss of appetite as the only systemic side effects.[18] Administration of CA4P to dogs has not yet been studied for oncologic purposes[19]; however, the potential of CA4P in veterinary oncology warrants exploration as also pet dogs with cancer would be candidates for CA4P-treatment.

This research evaluates the intravenous administration of escalating doses of CA4P in healthy dogs. The aim of this study was to assess the safety, tolerability and the possible side effects of CA4P in healthy dogs, and the results will serve as a guide to establish an acceptable dose to be tested for efficacy in canine patients with solid cancer.

2 | MATERIAL AND METHODS

2.1 | Preparation of sterile CA4P solutions

Lyophilized CA4P (GMP quality) with a purity of > 99% was purchased from Selleck Chemicals LLC (Houston, Texas) and approval of the Belgian Federal Agency for Medicines and Health Products (FAMHP) for the use of CA4P was obtained (approval no. 0002588).

The drug was accurately weighed and reconstituted in sterile phosphate buffered saline (PBS) (Thermo Fisher Scientific, Erembodegem, Belgium) under sterile conditions in a biological safety cabinet. A stock solution of CA4P was prepared and diluted to a final concentration of 50, 75 or 100 mg m^2 CA4P in 10 mL PBS, based on the body surface area (BSA) of every dog.

2.2 | Experimental design

Ten healthy Beagles with a mean body weight of 12.5 kg (±5.5 kg) and 7.75 years old (±0.75 years) were enrolled into this study (Table 1). The protocol adhered to the European Communities Council Directive (86/609/EEC) and was approved by the local research ethical committee of the Faculty of Veterinary Medicine (Merelbeke, Belgium) (approval no. 2014/162). Adequate measures were taken to minimise pain and discomfort. Dogs were deemed healthy if no relevant abnormalities were found in a pre-treatment screening. This consisted of a physical and neurological examination, and a complete echocardiographic examination with additional pulsed-wave tissue Doppler imaging (PW-TDI), non-invasive Doppler measurements of the systolic arterial blood pressure (SABP) and electrocardiogram (ECG). Blood sample analysis included a complete blood count (CBC), serum biochemistry and cardiac troponine I (cTnI). Three-way radiographs of the thorax and an abdominal ultrasound concluded the pre-treatment screening.

The dose-escalation strategy was modelled in a 2 + 2 clinical trial design.[20] An intravenous catheter was inserted in the cephalic vein and the dogs were randomly divided into 5 groups. Each group received an intravenous infusion of dissolved CA4P (50, 75 or 100 mg m^{-2}). Combretastatin A4-phosphate was administered over a time span of 30 minutes using a volumetric pump in all groups but 1, in which CA4P was administered over a time span of 120 minutes. Two dogs (dogs no. 7 and 8) received prophylactic anti-emetic treatment with maropitant (2 mg kg^{-1} as oral tablets) 6 hours before CA4P administration and anti-diarrheic treatment with loperamide (0.08 mg kg^{-1} as oral tablets) 6 hours before and 2 hours after CA4P administration.

Physical and neurological examinations were performed hourly the first 6 hours after injection, every 2 hours the next 18 hours, followed by once daily examinations during 10 days. Blood samples were collected for CBC and biochemistry profile at 1, 3 and 14 days after CA4P administration.

To assess cardiovascular toxicity, blood pressure measurements were made using the Doppler method (Parks Medical Electronics Inc, Aloha, Oregon). Measurements were performed according to the American College of Veterinary Internal Medicine (ACVIM) consensus on blood pressure measurement[21] and were started 4 days in advance of CA4P administration to correctly recognise drug-induced

TABLE 1 Dog characteristics and allocation of dogs per dose level of combretastatin A4-phosphate (CA4P)

Dog no.	Sex	Age (years)	Body weight (kg)	CA4P dose (mg m^{-2})	Infusion time (min)
1	MN	7.5	15.5	50	30
2	MN	7.5	13.0	50	30
3	FN	8.0	6.5	75	30
4	FN	8.0	8.5	75	30
5	FN	8.5	7.2	100	30
6	MN	7.0	16.0	100	30
7	MN	7.5	18.0	75	30
8	MN	7.5	14.5	75	30
9	FN	7.0	12.5	100	120
10	MN	7.0	17.5	100	120

Abbreviations: FN, Female neutered; MN, male neutered.

hypertension and to establish a baseline SABP for every dog. After CA4P administration, SABP measurements were made every 15 minutes for the first hour, every hour during the next 5 hours, every 2 hours during the next 18 hours, and every 12 hours for the next 10 days.

During drug administration, ECG was monitored continuously using a standard 10-lead ECG. For a period of 5 hours following administration, 3-lead ECG monitoring was performed using a Holter ECG-recording device (Novacor Vista, Novacor UK Ltd., Swanley, UK). QT intervals, corrected for changes in heart rate (HR) with the Van de Water formula,[22] were measured and ventricular arrhythmias were determined. Blood samples for cTnI measurement were taken at 24 hours, and at 2 and 4 weeks after administration; and conventional echocardiography with additional PW-TDI was repeated 24 h after administration of CA4P.

Adverse events were recorded and graded using the Veterinary Cooperative Oncology Group - Common Terminology Criteria for Adverse Events (VCOG-CTCAE).[23]

3 | RESULTS

The doses of CA4P studied and the allocation of dogs per dose level are listed in Table 1. Abridgement of dose levels as a result of dose limiting toxicities (DLT) did not occur until the level of 100 mg m^{-2} was reached. Subsequent re-investigation of the lower dose level (75 mg m^{-2}) was pursued. Preventive administration of anti-emetic and anti-diarrheic drugs was successfully implemented in this group. To determine whether administration of CA4P over a longer time span diminished or eliminated the occurrence of side effects, 100 mg m^{-2} CA4P was administered over a time span of 120 minutes in 1 group; however, this did not result in either elimination or diminution of the side effects. Table 2 lists all AEs that were presumably drug-related.

3.1 | Hematologic toxicity

Transient neutropenia (neutrophil count lower than 2950 μL^{-1}) was the only hematologic toxicity observed, irrespective of the CA4P dose level. Neutropenia commenced 3 days after injection of CA4P and resolved spontaneously within 14 days after injection. Grade 1 neutropenia occurred at dose levels of 50, 75 and also at 100 mg m^{-2} when administered over 120 minutes, whereas grade 2 neutropenia was observed when 100 mg m^{-2} was administered over 30 minutes.

TABLE 2 Number of dogs per dose level that exhibited CA4P-related adverse events

CA4P Dose level	50 mg m^{-2} (n = 2)		75 mg m^{-2} (n = 4)		100 mg m^{-2} (n = 4)	
CTCAE grade	1-2	3-4	1-2	3-4	1-2	3-4
Neutropenia	2		4		4	
Hypertension					4	
Anorexia			2		4	
Diarrhoea	2		2		4	
Nausea	2		4		4	
Vomiting			2		4	
Ataxia						3
Motor neuropathy						3
cTnI elevation			2		4	

Abbreviations: CTCAE, common terminology criteria for adverse events; cTnI, cardiac troponine I.

3.2 | Gastro-intestinal toxicity

Across all doses and in all individuals, anorexia, diarrhoea, nausea and loss of appetite were observed, commencing 1 to 2 hours after the start of injection and resolving spontaneously after 3 to 4 hours. The severity of anorexia, nausea and diarrhoea appeared to be dose-dependent (grade 1 at dose levels of 50 and 75 mg m^{-2}, grade 2 at a dose level of 100 mg m^{-2}). Vomiting (grade 1) was only observed at dose levels of 75 and 100 mg m^{-2}. Preventive medical treatment with anti-emetic and anti-diarrheic drugs resulted in the absence of these AE, whereas reduction of the infusion rate of CA4P did not.

3.3 | Cardiovascular toxicity

All cardiac examinations were performed by a board-certified cardiologist. Table 3 lists all cardiovascular AE that were observed.

Concerning the SABP, at dose levels of 50 and 75 mg m^{-2} there was no significant increase compared to the baseline SABP throughout the infusion and a period of 24 h post injection. However, at a dose of 100 mg m^{-2} all dogs developed significant hypertension (SABP > 150 mmHg). The increase over baseline varied from 30% to 50%, commenced 2 hours after administration and returned to baseline values within 24 hours.

ECG recordings showed a mean baseline HR of 111 beats per minute (BPM) (range: 80-200 BPM) with 9 dogs displaying a physiologic respiratory sinus arrhythmia (RSA) and 1 dog with a sinus tachycardia of 200 BPM. During CA4P administration, no significant changes in HR were seen. The HR in the dog with baseline sinus tachycardia decreased from 200 BPM to 160 BPM at the start and 80 BPM (with RSA) at the end of injection. One dog with a baseline HR of 80 BPM (with RSA) showed sinus bradycardia during the injection (60 BPM during the first minute and 40 BPM at the end of CA4P administration). No significant changes were observed in the QTc-interval during administration when compared to baseline values. Three of 10 dogs had a baseline QTc > 250 ms. All had less than 10% change of their QTc during injection, except for 1 dog, that had an 11% increase in QTc.

Overall mean HR during Holter recordings was 104 BPM (range: 42-208 BPM) and recordings were unremarkable in 6 dogs. Four dogs showed an abnormally high number of ventricular ectopic beats (VEB) during monitoring. Three of these dogs showed phases of accelerated idioventricular rhythm (AIVR) and 1 dog showed 2 episodes of ventricular tachycardia. No other significant arrhythmias were observed.

Significantly increased serum cTnI concentrations were noted in 6 cases. At baseline, serum cTnI was below the upper limit of 0.06 ng mL^{-1} in all dogs. At dose levels of 75 and 100 mg m^{-2}, almost all dogs developed a mild elevation, irrespective of the CA4P infusion

TABLE 3 CA4P-related cardiovascular toxicities

Dog no	Dose (mg m^{-2})	SABP (mmHg)			HR (bpm)			QTc-interval (ms)					cTnI level (ng mL^{-1})	
		Base	Max	% increase	Base	Mean	% decrease	Base	Mean	% increase	VEB	AIVR phases	Base	24 h after injection
1	50	130	140	8	200	120	40	225	226	0	0	0	0.04	0.01
2	50	130	150	15	120	110	8	243	250	3	6	0	0.05	0.04
3	75	120	130	8	80	100	−25	263	251.5	−4	0	0	0.03	0.17
4	75	125	145	16	120	100	17	246	273.5	11	0	0	0.02	0.03
5	100	140	200	43	80	80	0	234	237	1	65	0	0.03	0.40
6	100	120	180	50	108	90	17	254	239	−6	3795	344	0.02	4.30
7	75	130	150	15	100	80	20	232	239.5	3	478	15	0.02	0.16
8	75	130	145	12	110	110	0	240	239	0	2	0	0.03	0.02
9	100	120	180	50	80	50	38	251	241	4	8	0	0.02	0.19
10	100	150	195	30	110	75	32	242	246	2	556	25	0.01	1.94

Abbreviations: AIVR, accelerated idioventricular rhythm; cTnI, cardiac troponine I; HR, heart rate; SABP, systolic arterial blood pressure; VEB, ventricular ectopic beats.

rate. Serum cTnI levels returned to baseline levels in all dogs within 4 weeks.

Echocardiographic examination after CA4P administration revealed no significant changes in cardiac dimensions, nor in systolic or diastolic functional parameters including PW-TDI, compared to baseline examinations.

3.4 | Neurotoxicity

Three cases of grade 3 ataxia and motor neuron toxicity of the hind limbs occurred at dose level 100 mg m^{-2}. Dog no. 6 developed ataxia and lower motor neuron (LMN) toxicity 24 h post-injection. A complete neurological examination performed by a board-certified neurologist revealed a bilaterally slightly crouched pelvic limb stance and gait with decreased ability to extend the stifle joints and a mild ataxia, and bilaterally absent patellar reflexes. Over the following 3 days, the ataxia did improve but the dog then mainly showed signs of a bilateral femoral neuropathy, presenting as difficulties in rising and supporting weight on the pelvic limbs, with complete inability to extend the stifle joints. Over the next 11 days, stifle extension and weight support improved markedly. However, patellar reflexes remained absent for another 12 weeks. Dogs no. 9 and 10 developed marked upper motor neuron (UMN) paraparesis and ataxia 20 to 24 hours post injection. Neurological signs remained stable for the following 24 h, after which dog no. 10 showed decreased patellar reflexes. After 3 days, both dogs ameliorated to a grade 2 ataxia but the patellar reflexes were now absent bilaterally in dog no. 10. Patellar reflexes recovered after 4 weeks. In dogs no. 6 and 10, a lumbar puncture was carried out, serum creatine kinase measured and electromyography (EMG) of the affected limbs and magnetic resonance imaging (MRI) of the entire thoracic and lumbar spinal cord were performed, revealing no abnormalities.

4 | DISCUSSION

This is the first report of the administration of CA4P in healthy dogs to identify possible AE at escalating doses. So far, the optimal dose in canine cancer patients has not yet been determined, but according to the manufacturer's (OXiGENE) guidelines, 100 mg m^{-2} is the single-dose maximum tolerated dose (MTD) of CA4P in healthy dogs (results unpublished). However, the results obtained in this study suggest that 75 mg m^{-2} is more likely to be the MTD in dogs. At 100 mg m^{-2}, several high-grade DLT occurred which, although transient, are unacceptable in a clinical setting.

In this study, we initially opted for an infusion schedule of 30 minutes for practical reasons. Infusing over 30 minutes avoided a high-drug peak plasma concentration of CA4P and continuous ECG monitoring was tolerated in the minimally restraint dogs. Dowlatti and colleagues found no demonstrable difference in the toxicity profile of a 10- or a 60-min CA4P-infusion schedule in human oncology patients.[13] Likewise, the results of our research did not demonstrate definite advantages of a longer infusion schedule in healthy dogs.

Low-grade nausea and diarrhoea developed in all individuals and at all CA4P doses, vomiting only at dose levels higher than 50 mg m^{-2}. Gastrointestinal signs are often-observed AE in CA4P-treated human cancer patients as well, especially at dose levels over 52 mg m^{-2}.[13–15] Nausea, vomiting and diarrhoea induced by CA4P may be attributable to circulatory changes within the small vessels due to increases in vascular resistance in the gastrointestinal tract, alterations in intestinal enzyme balance, or via efferent nervous stimulation of the chemoreceptor trigger zone.[13,24–26] The most effective anti-emetics for chemotherapy-induced nausea and vomiting (CINV) in human patients are the neurokinin-1 (NK-1) receptor antagonists.[25,26] In dogs, NK-1 receptor antagonists such as maropitant have also been demonstrated to have superior activity for prevention and treatment of acute CINV.[27–29] In this study, the combination of this prophylactic anti-emetic and anti-diarrheic treatment proved to be effective in preventing the gastrointestinal AE in both dogs.

Most appealing for CA4P administration in human clinical trials is the absence of traditional cytotoxic side effects common to conventional chemotherapeutic strategies, i.e. myelosuppression, stomatitis and alopecia.[13–17,30,31] Indeed, drug-related anaemia, lymphocytopenia or thrombocytopenia was not observed; however, a transient low-grade neutropenia did occur. This finding was not unexpected since it has previously been described in human patients that

neutropenia can be observed when administering microtubule-targeting agents such as CA4P.[32] in vitro studies have demonstrated that CA4P is non-toxic to neutrophil granulocytes,[33] so the observed neutropenia does not seem to result from immunosuppression. Studies revealed that CA4P may induce neutrophil adhesion and migration through the endothelial cell monolayer due to cellular adhesion molecule (CAM) expression and increased levels of neutrophil-recruiting cytokines.[34,35] A significant response of these cytokines was demonstrated in both tumour- and non-tumour-bearing mice treated with CA4P, suggesting non-tumour-specific recruitment of neutrophils.[33,35] This could explain the mild and transient neutropenia seen in our (non-tumour-bearing) dogs.

Signs of cardiovascular toxicity after infusion of CA4P observed in this study are similar to those reported in human medicine, including transient hypertension, increased serum cTnI concentration and electrocardiographic changes.[36] The exact mechanisms of CA4P-related cardiovascular toxicity remain to be elucidated, but some hypotheses have been suggested.[36] A clinically significant increase in SABP developed only in the 4 dogs receiving a dose of 100 mg m^{-2}. Compared to what has been described in human cancer patients,[13,14] the increase over baseline at this dose was notably higher and the onset and return to baseline were rather delayed. Although the increase was substantial, all dogs remained asymptomatic, intervention was not indicated, and SABP returned to baseline value within 24 hours. A CA4P-related increase in systemic vascular resistance has been suggested to explain the transient hypertension, since CA4P may cause vasoconstriction not only in tumours, but also in non-tumour vascular beds.[11,13]

Substantial increases in serum cTnI levels were observed in 6 dogs; 5 of them also had increased SABP. This increase in cTnI is therefore likely to be linked to hypertension, as shown in rats.[37] Another mechanism suggested in humans is vasospasm of normal coronary arteries after infusion of CA4P, leading to transient myocardial ischemia.[13,36] Recently, it has been shown by Tochinai and colleagues that CA4P may cause myocardial lesions in healthy rats, due to dysfunction of the myocardial microcirculation and possibly a direct toxic effect on cardiomyocytes.[38] Cardiac cTnI was not measured in the latter study, but if similar cardiac injury would occur in dogs, it could explain the observed increase in cTnI. Possible cardiac injury in the current study seems to be transient because follow-up of the serum cTnI concentrations showed normalisation within 4 weeks after CA4P administration.

The increase in cTnI did not seem to correlate to significant structural nor functional echocardiographic changes in this study. Although cases of myocardial stunning have been described, reports of echocardiographic myocardial dysfunction after CA4P administration are rare in humans.[39]

As for electrocardiographic changes in the CA4P-treated dogs, QTc prolongation did not seem to be clinically relevant. Similarly, CA4P administration can cause an increase in QTc-interval in humans that is statistically significant; however, this increase is often clinically irrelevant.[17] A remarkable finding in this study is the presence of a significant number of VEBs in 4 dogs, 3 of which received 100 mg m^{-2} and 1 which received 75 mg m^{-2}. These ventricular arrhythmias were considered "benign" and consisted mostly of single VPCs and accelerated idioventricular rhythms, although couplets and triplets were also present and 1 dog had 2 runs of ventricular tachycardia. Interestingly, the 2 highest cTnI concentrations were observed in the dogs with the highest number of VEBs. In humans, it has been proposed that CA4P-related QTc prolongation may exceptionally predispose to ventricular arrhythmia's.[17] Since no significant QTc prolongation was witnessed, other mechanisms are likely to contribute to the observed arrhythmias.

A vexing and often dose-limiting AE of the use of microtubule-targeting agents is the high rate of neuropathy induced by these compounds.[40] Chemotherapy-induced peripheral neuropathy (CIPN) is dose-cumulative and more frequently occurs in patients with pre-existing neuropathy, usually manifesting itself as a painful and debilitating peripheral axonal neuropathy consisting of paraesthesia, paresis and ataxia, often accompanied with diminished or absent deep tendon reflexes.[41–43] The preferential toxicity of microtubule-targeting agents for the nervous system is not understood at a mechanistic level but can be partially explained both by the relative abundance of tubulin in neurons, and the importance of an intact, functional microtubule cytoskeleton for adequate nerve conduction. Chemotherapy-induced peripheral neuropathy caused by microtubule-targeting agents is usually reversible after discontinuation of treatment and in general symptoms resolve within 3 to 4 months.[43]

Three completed dose-escalation studies in human cancer patients reported a range of neurological effects with neuromotor toxicity mostly taking the form of lower extremity weakness or ataxia.[13–15] These AE were transient, reversible and generally mild at lower doses, becoming more severe at higher doses. Interestingly, all these patients received prior platinum therapy,[15] which is also known to induce peripheral neurotoxicity to various degrees.[43]

The bilateral loss of patellar reflexes after CA4P administration has never been documented in human CA4P-treated cancer patients and was a surprising and unexpected finding. A possible explanation for these clinical signs is the similarity between combretastatines and colchicine. Combretastatines are structurally related to colchicine and interact with tubulin at or near the colchicine binding site.[44] In human patients, the long-term use of colchicine has been found to induce both myopathy and neuropathy,[45,46] and 1 case report of a woman treated with colchicine describes the absence of deep tendon reflexes in the legs.[46] However, the absence of abnormalities in the cerebrospinal fluid, the EMG and the MRI-images in the dogs makes the existence of a neuro- or myopathy less likely. More research is necessary to explain these clinical signs.

5 | CONCLUSION

This study is the first clinical trial conducted with escalating doses of the vascular targeting agent CA4P in healthy dogs. Although the data acquired in this study must be viewed with caution because of the small number of animals studied and because this study did not include a control or placebo group, the data do suggest that in healthy dogs, CA4P is well tolerated at doses up to 75 mg m^{-2}. Keeping in mind that 52 mg m^{-2} is the lowest dose at which changes in parameters associated with tumour blood flow reduction is seen in

human cancer patients, and on the basis of the toxicity criteria found in this research, the dose recommended for treating canine cancer patients would be 52 to 75 mg m^{-2}. The efficacy of this dose in canine cancer patients should be the subject of subsequent research. Based on the results of the current study it is advisable to perform screening for cardiovascular disease prior to administration of CA4P as well as to perform follow-up after treatment, using blood pressure measurement, cTnI concentration, ECG monitoring and possibly echocardiography. Preventive treatment for gastrointestinal side effects is recommended.

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Conflict of interest

The authors declare no potential conflict of interests.

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