Pharmacokinetics of thalidomide in dogs: can feeding affect it? A preliminary study

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ABSTRACT

Background: Tumor-associated neoangiogenesis is a crucial target for antitumor therapies. Thalidomide (TAL) is a promising anti-neoangiogenetic drug that has recently been used in the treatment of several malignancies in dogs.

Objectives: The aim of the study was to assess the pharmacokinetics of TAL after single oral administration in dogs. Additionally, the influence of feeding on the pharmacokinetic profile of TAL in dogs has been preliminarily investigated.

Methods: Six healthy adult female Labradors were enrolled according to a randomized single-dose, 2-treatment, 2-phase, paired 2 × 2 cross-over study design. The dogs were administered a single 400 mg capsule of TAL in fasted and fed conditions. Blood was collected from 15 min to 48 h after dosing, and TAL quantified in plasma by a validated high-performance liquid chromatography method. The pharmacokinetics of TAL were analyzed using a non-compartmental approach.

Results: TAL concentration was quantifiable up to 10 h and 24 h after fasted and fed conditions, respectively. C max (fasted, 1.34 ± 0.12 µg/mL; fed, 2.47 ± 0.19 µg/mL) and T max (fasted, 3 h; fed, 10 h) differed substantially between the 2 groups. AUC and t 1/2 λz were significantly higher in fed (42.46 ± 6.64 mg × h/L; 17.14 ± 4.68 h) compared to fasted (12.38 ± 1.13 mg × h/L; 6.55 ± 1.25 h) dogs. The relative oral bioavailability of TAL for the fasted group was low (36.92% ± 3.28%).

Conclusions: Feeding affects the pharmacokinetics of oral TAL in dogs, showing a delayed, but higher absorption with different rate of elimination. These findings are of importance in clinical veterinary settings, and represent a starting point for further related studies.

Keywords: Dogs; fasting; meals; pharmacokinetics; thalidomide
INTRODUCTION

Thalidomide (TAL) was first synthesised in 1954, and was used clinically in Europe as a non-barbiturate hypno-sedative and antiemetic drug for morning sickness. It was thought that the sedative effect of TAL was generated by a different mechanism of action than that of barbiturates. This led to the belief that TAL was a ‘safe’ drug, with little CNS and respiratory depression or muscle incoordination [1], and no deaths from overdose or attempted suicide have ever been recorded [2]. However, in 1961 TAL was found to have a teratogenic effect in humans, and so was withdrawn from market. Despite its known teratogenicity, by 1965 TAL was the drug of choice for erythema nodosum leprosum [3]. The safety profile of TAL was not completely determined until 1998 [4], and since then, several trials in inflammatory and oncologic conditions have been run. TAL has shown promising antitumour activity in several malignancies and has been proposed as a drug of choice in multiple myeloma [5-9].

Neoangiogenesis is a well recognized hallmark of cancer [10]. Today, tumor-associated neoangiogenesis is a crucial target for antitumoral therapy. Several studies have shown that the tumour microenvironment is able to induce and promote neoangiogenesis [10,11]. The potential anti-angiogenic effects of TAL were suspected in the early 1960s but were only confirmed in the 1990s [12,13]. To date, the precise mechanisms responsible for the clinical activity of TAL have not yet been established. However, TAL has been shown to inhibit angiogenesis induced by basic fibroblast growth factor in rabbit cornea or by vascular endothelial growth factor in a murine model of corneal vascularization [12,14]. TAL also reduced interleukin-6 (IL-6), 1b (IL-1b), 10 (IL-10) and tumour necrosis factor-α production in an in vitro model [15,16].

TAL has been used in canine chemotherapy for the treatment of hemangiosarcoma [17,18], pulmonary [19] and mammary carcinoma [20]. Equivalent or even longer survival times have been reported compared to traditional intensive-dose chemotherapy. Unlike many other chemotherapeutic drugs, TAL is relatively well tolerated by dogs. Experimental trials have not found significant toxicity in Beagles treated for up 53 weeks with a dose of up to 1,000 mg/kg/day [21]. To date, the dose of TAL proposed for the treatment of tumours in canine patients has been empirically selected, with studies using a wide range of doses. Indeed, dose in the range of 2 to 26 mg/kg/day or 100–400 mg/dog per day have been reported [17,19,22,23]. A dose regimen selected based on scientific data is thus necessary in order to optimise TAL therapy in canine patients.

To the best of the author’s knowledge, no studies on the pharmacokinetics of TAL in dogs have been reported in the literature. The aim of this study was therefore to assess the pharmacokinetics of TAL after single oral administration in dogs. Additionally, the likely influence of feeding on the pharmacokinetic profile of TAL in dogs has been preliminarily investigated.

MATERIALS AND METHODS

Drugs and chemicals
TAL for analytical testing (purity ≥ 99%) and phthalimide (purity ≥ 99%), used as internal standard (IS), were provided by Sigma-Aldrich (USA). Ammonium acetate, methanol (CH₃OH), acetonitrile (CH₃CN) and trifluoroacetic acid (98%) were purchased from VWR International (USA). Acetic acid 99–100% (CH₃COOH) was obtained from J.T. Baker (USA).
The water used was ultrapure grade, purified using a Milli-Q UV Purification System (Millipore Corporation, USA).

**Animals and experimental design**

Six adult female (2–7 years) Labradors with an average body weight of 34.6 ± 1.69 kg (median, 34.25 kg; range, 28.5–42.4 kg) were used. The experiment was approved by the University of Life Sciences, (Lublin, Poland) welfare ethics committee and carried out in accordance with the European law (2010/63/UE). The dogs were determined to be clinically healthy based on physical examination, serum chemistry and haematological analyses performed 48 h before the beginning of the study and were not treated with other therapeutic agents.

The dogs were randomly divided into 2 groups (each containing 3 animals) using Research Randomizer software, and participated in a single-dose, 2-treatment, 2-phase, paired 2 × 2 cross-over study.

The drug was prepared by a compounding pharmacy, and administered as capsules containing 400 mg of pure TAL. Since animals had different body weights, the dose administered was an average of 11.74 ± 0.56 mg/kg (median, 11.76 mg/kg; range, 9.4–14.0 mg/kg).

In the first phase, group 1 (n = 3) was administered with 400 mg/dog (one capsule) after overnight fasting and group 2 (n = 3) was fed prior to and after administration of the same dose. The capsule was placed on the back of the tongue and 5 mL of water was administered to ensure that the capsule was swallowed. Canned dog food (Nature’s Logic Canine Feast, USA) was provided as half the total amount 15 min before dosing, with the rest provided immediately after TAL administration. On each study day, in order to avoid the possibility of coprophagia impacting on the study, the dogs were kept in individual boxes for 48 h and observed closely during this period. A 2-week wash-out period was observed between the phases, then the treatment groups were inverted, and the experiment was repeated.

The dogs were checked daily for visible adverse effects for 7 days following completion of the study. To facilitate blood sampling, 1 h before the commencement of the study, an 18-gauge soft cannula (Delta Med, Italy) was inserted in the right medial saphenous vein. Blood samples (3 mL) were withdrawn into lithium heparin tubes (Aptaca Spa, Italy) at 15, 30, 45 min and 1, 1.5, 2, 4, 6, 8, 10, 16, 24, 34 and 48 h after administration of TAL. Blood samples were immediately centrifuged for 10 min at 1,500 × g at 4°C and the plasma was harvested. Since TAL is not stable in plasma [24], 2.0 mL of a stabilizer-solution (CH₃OH/CH₃CN, 1/1 (v/v) + CH₃COOH 2%) [25] was immediately added to each mL of plasma sample as soon as it was harvested. Samples were transferred into cryo-vials and immediately frozen and stored at −80°C. Samples were analysed within 2 weeks of collection.

**Sample extraction procedure**

Analysis was performed according to Saccomanni et al. [25], and slightly modified. In brief, an aliquot of 1.5 mL of sample (containing 0.5 mL of plasma and 1 mL of stabilizer-solution) was added to a 2.0 mL micro-centrifuge tube. After the addition of 100 µL of IS (50 µg/mL) and 2.0 µL of trifluoroacetic acid (deproteinizing agent), samples were vortexed for 30 sec, then sonicated and shaken at 60 oscillation/min for 10 min. Samples were then centrifuged (5,000 × g) for 10 min, and 1 mL of the organic layer was transferred into a clean tube and dried under a gentle stream of nitrogen at 30°C. The residue obtained was reconstituted with
100 µL of CH₃OH/CH₃CN, 1/1 (v/v) and after centrifugation (5,000 × g, 5 min) 20 µL of the upper layer was injected onto the high-performance liquid chromatography (HPLC).

**HPLC conditions**

TAL in dog plasma was determined using an HPLC coupled with diode array detector (Series 2000; Jasco Europe, Italy) according to a slightly modified version of the method described by Saccomanni et al. [25]. A Gemini C18 analytical column (250 × 4.6 mm, 5 µm particle size; Phenomenex, USA) maintained at 25°C by a Peltier System (LC-4000; Jasco Europe) was used for the chromatographic analysis. The mobile phase consisted of CH₃CN/10 mM acetate ammonium (pH 5.5) solution (25/75, v/v), which was freshly prepared each day before the analysis. The flow rate of the mobile phase was set at 1 mL/min. The wavelength was set at 220 nm.

**Method validation and quantification**

The analytical method was fully revalidated for dog plasma according to the European Medicines Agency guidelines [26] by examining the within-run precision, calculated from similar responses for 6 repeats of 3 control samples (0.1, 0.5, and 1 µg/mL) in one run. The between-run precision was determined by comparing the calculated response of the low (0.05 µg/mL), middle (1 µg/mL), and high (10 µg/mL) control samples over 3 consecutive daily runs (a total of 6 runs). The assay accuracy for within-run and between-runs was established by determining the ratio of calculated response to expected response for low (0.05 µg/mL), middle (1 µg/mL), and high (10 µg/mL) control samples over 6 runs. The limit of quantification (LOQ) was determined as signal-to-noise ratio of 10, and the limit of detection (LOD) as the signal-to-noise ratio of 3.

TAL and IS stock solutions were prepared in a mixture of CH₃OH/CH₃CN, 1/1 (v/v) and in water, respectively, at a concentration of 1,000 µg/mL and stored at −80°C. These solutions were freshly prepared every 2 weeks. TAL stock solution was then diluted to reach concentrations of 0.25, 2.5, 5, 25 and 50 µg/mL and stored at −20°C. These last concentrations were then diluted immediately prior to use to reach the final concentrations of 0.05, 0.5, 1, 5, 10 µg/mL. These final dilutions were then used in preparation of a 5-point calibration curve of TAL in plasma matrices.

Standard curves were constructed with standard TAL concentrations vs ratio of TAL/IS peak areas. The linearity of the regression curve was assessed based on the residual plot, the fit test and the back-calculation. Extraction recovery was evaluated by comparing the response (in area) of high, middle, and low standards and the IS, spiked into blank canine plasma (control), with the response of equivalent standards.

**Pharmacokinetic and statistical analysis**

The concentration of TAL vs. time was pharmacokinetically analyzed using a non-compartmental approach (ThothPro 4.3; ThothPro LLC, Poland). C_max was the peak plasma concentration, and T_max was the time at the peak plasma concentration. The elimination half-life (t_1/2) was calculated using linear least squares regression analysis of the concentration-time curve, and the area under the curve (AUC) was calculated by the linear-up log-down rule to the final concentration-time point (Ct). From these values, the apparent volume of distribution (V = dose × area under the first moment curve [AUMC]/AUC²), mean residence time (MRT = AUMC/AUC) and clearance (Cl = dose/AUC) were determined. The relative bioavailability (F) was calculated for each dog using the following equation:
\[
(%)F_{\text{fasted}} = \frac{\text{AUC}_{\text{fasted}}}{\text{AUC}_{\text{fed}}} \times 100
\]

Data were found to be normally distributed (Shapiro-Wilk test). Paired \( t \)-tests were used to investigate statistically significant changes in pharmacokinetic estimates between groups (GraphPad Software; GraphPad, USA). The pharmacokinetic parameters are presented as means \( \pm \) SE and \( T_{\text{max}} \) (categorical variable) is expressed as median and range. In all the experiments, differences were considered significant if \( p < 0.05 \).

**RESULTS**

The analytical method showed a good linearity in the range between 0.05 and 10 \( \mu g/mL \) with a determination coefficient (\( R^2 \)) above 0.994 (\( y = 0.0976x - 0.0456 \)). The intra- and inter-day precision resulted in coefficient of variation < 20%. The mean extraction recovery of TAL was 72.09\% \( \pm \) 5.04\%; the LOD and LOQ were 0.05 \( \mu g/mL \) and 0.5 \( \mu g/mL \), respectively.

In the first phase of the study one dog in group 2 (fed) showed some adverse effects 12 h after TAL administration. These included shaking, stiff walk, staggering and whining. However, the blood samples were still collected at each timepoint, and the dog completely recovered after a few hours. It was replaced in phase 2 with another dog. In all the other experimental animals no adverse effects and no behavioural or health alterations were observed during or after the study.

Plasma TAL concentration was quantifiable up to 10 h and 24 h after oral administration of 400 mg/dog in fasted and in fed conditions, respectively. The main pharmacokinetic estimates are reported in Table 1. One fed dog in phase 2 showed a short \( T_{\text{max}} \) and a higher \( C_{\text{max}} \) compared with other dogs in the same group, as well as a more similar pharmacokinetic profile to the fasting group. This individual data set was considered as an outlier, and was excluded from the pharmacokinetic analyses.

\( C_{\text{max}} \) normalized for the dose expressed in mg/kg, differed substantially between the 2 groups (fasted, 1.34 \( \pm \) 0.12 \( \mu g/mL \); fed, 2.47 \( \pm \) 0.19 \( \mu g/mL \)). \( T_{\text{max}} \) differed considerably between the fasted (3 h) and the fed (10 h) animals.

![Fig. 1. Mean TAL plasma concentration vs. time curve following single oral administration of 400 mg/dog in fasted (n = 6) and fed (n = 6) conditions.](https://vetsci.org)
The terminal half-life ($t_{1/2}$, λz) values were variable but significantly different between the groups (fasted, $6.55 \pm 1.25$ h; fed, $17.14 \pm 4.68$ h), in-line with a different λz (fasted, $0.12 \pm 0.02$ 1/h; fed, $0.05 \pm 0.01$ 1/h).

The AUC value was significantly higher in the fed group (normalized for the dose expressed in mg/kg: fasted, $12.38 \pm 1.13$ mg × h/L; fed, $42.46 \pm 6.64$ mg × h/L). As a result, the relative oral bioavailability of TAL for the fasted group was low ($36.92\% \pm 3.28\%$).

### DISCUSSION

The main aim of the present study was to evaluate the pharmacokinetic profile of TAL after oral administration in dogs and to determine whether this profile is affected by feeding.

The dose of TAL administered in the present study (400 mg/dog, average 11.7 mg/kg) was selected based on clinical efficacy/adverse effects previously reported in dogs. A dose of 8.7 mg/kg/day and a 3-month daily-dose of 20 mg/kg followed by a 3-month daily-dose of 10 mg/kg were successfully used in the management of stage II–III splenic hemangiosarcomas [18] and canine mammary carcinomas [20], respectively, in dogs. This latter study showed adverse sedative effects in some dogs when given the higher dose (20 mg/kg), with symptom improvement when the dose was reduced to 10 mg/kg. The dose administered in our study was found to be safe with no visible signs of toxicity in animals. This concurs with the findings of a previous study [21], which also reported no visible signs of toxicity associated with this dose in 56 dogs. However, a multiple-dose study is needed to confirm this finding.

The toxic signs showed by the subject in the fed group during the first phase of the animal study were transient (around 4 h). The causes of these signs are not clear but are unlikely to be due to TAL. A study into the effects of chronic TAL administration in dogs [21] found that TAL administered up to 1,000 mg/kg/day for 53 weeks did not induce any major systemic toxicity or tumours in dogs. There were no TAL-related changes in body weights, food consumption, electrocardiography, ophthalmoscopy, neurological function, or endocrine function. Some slight and/or transient variations observed in some hematology and blood chemistry values of dosed dogs were considered to be toxicologically insignificant, with these
conclusions being supported by the lack of histopathologic changes. The only gross finding attributable to TAL was a yellow-green discoloration of the femur, rib, and/or calvarium. This aspect was not assessed in the present study since animals were not euthanized. The estimated non observed adverse effect level in dogs was 200 mg/kg/day [21] which almost 20 times higher than the dose administered in the present study. However, adverse events such as sedation, dizziness, constipation, and headache have been reported in humans after multiple clinical doses [27-29] that do not match with the signs observed in the dog used in the present study.

Plasma concentrations of TAL after fasted and fed conditions varied widely in our study. Statistical analysis and inspection of the plasma concentration vs time curves indicated that feeding considerably affects both the pharmacokinetic parameters and profiles. This information could be of paramount importance in clinical settings. Food intake delayed (T\text{max}) but increased TAL absorption (C\text{max} and AUC), in line with the negligible hydrophilicity of the active compound [30,31]. Interestingly, the effect of food on TAL pharmacokinetics in humans are conflicting: some studies report no influences while others report minor effects on C\text{max} and AUC, with a significant delay to T\text{max} [2,28].

The type of food consumed can impact on the quality and quantity of the food effect. For example, fatty foods generally delay gastric emptying, thereby providing ample time for greater dissolution and absorption of drugs. This was seen with griseofulvin, a sparingly water-soluble drug, where coadministration with a fatty meal doubled its absorption relative to the fasted state. High-protein or carbohydrate-rich food had no effect on griseofulvin absorption [32]. The feed administered to dogs in our study was a fatty meal. TAL, which like griseofulvin is sparingly soluble in water, showed significantly higher absorption in 5 of the 6 fed dogs. However, some drugs’ bioavailability is increased with a high fat diet, while dietary fiber may reduce drug availability, thus diverse feed types may have different impacts on the pharmacokinetics of TAL [33-35]. Further studies investigating the impact of different types of feeds on TAL pharmacokinetics are warranted to investigate this issue.

One dog in the fed group was found to be a statistical outlier with a reduced T\text{max} similar to that reported for the fasting group. This could be explained by the contractile mechanism of the gallbladder emptying and filling in dogs [36]. In fact, the gallbladder alternates filling and emptying excursions even in fasted dogs. Alternatively, the dog may have had a reflux of duodenal fluid (containing bile) in the gastric lumen. Consequently, the production of an earlier emulsion may have led a higher C\text{max} and faster T\text{max} [37]. A statistical outlier was also described in a previous study that examined the effect of food on TAL pharmacokinetics in humans [28].

Half-life is a pharmacokinetic parameter used to compute the dose interval and the time to achieve the steady state concentration [38]. The half-life of TAL was statistically increased by feeding. This may be due to the feed acting as a drug reservoir, slowly releasing TAL during intestinal transit. If administered once-daily in fed dogs, TAL has an accumulation ratio (AUC\text{steady-state}/AUC\text{lat.adm}) of around 2.5, while the steady state plasma concentration would be attained in around 4 days [38]. Half-life is a hybrid parameter that incorporates both clearance and volume of distribution. The low water solubility of TAL has prevented the development of a commercial intravenous formulation [2], and consequently it is impossible to calculate absolute clearance and volume of distribution, making extensive discussion of this estimate too speculative.
Two uncontrolled multiple-dose studies in breast cancer and glioma have attempted to correlate TAL concentration with tumour response in humans. Steady-state plasma concentrations ranging from 5 to 7 μg/mL resulted in stable disease for up to 74 weeks in 12/31 glioma patients, however, similar concentrations (6.2 μg/mL) for 8 weeks in metastatic breast cancer patients showed no tumour response [39,40]. In a recent study in dogs, a similar TAL dose to that reported in the present study, was associated with equal or even longer survival times compared to intensive-dose chemotherapy in splenic hemangiosarcoma and mammary carcinomas [18,20]. The average plasma concentration computed at the steady state at 11.7 mg/kg TAL administration once-daily in fed dogs resulted in 3.7 μg/mL. Even though this concentration is theoretical it might be used as a target for the treatment of several malignancies in dogs, especially for splenic hemangiosarcoma and pulmonary/mammary carcinomas. Although it has been reported that the pharmacokinetics of TAL do not change significantly between healthy and cancer patients, further studies on canine patients are warranted to verify whether TAL pharmacokinetics are unchanged in healthy dogs versus dogs diagnosed with cancer [27,40,41].

The findings of this study should be interpreted while considering some limitations. Namely: the study used only female dogs of a single breed. It is well known that breed or sex-specific differences can lead to variances in drug absorption, distribution, metabolism, or elimination. In particular, differences in body weight and composition, animal size, P-450 enzyme isoforms or in plasma protein binding might occur in different breeds or in animals of different sex of the same breed [42-50]. For instance, Labradors have a higher percentage of body fat compared to other breed such as Greyhounds or Beagles, and this might lead to a larger volume of distribution of certain lipophilic compounds [43,51].

In conclusion, this is the first study to report the pharmacokinetics of TAL in dogs. Feeding significantly affects the pharmacokinetics, and this should be considered by veterinarians when using this drug in a clinical setting.

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REFERENCES

1. Somers GF. Pharmacological properties of thalidomide (α-phthalimido glutarimide), a new sedative hypnotic drug. Br J Pharmacol Chemother. 1960;15(1):111-116.
2. Teo SK, Colburn WA, Tracewell WG, Kook KA, Stirling DI, Jaworsky MS, et al. Clinical pharmacokinetics of thalidomide. Clin Pharmacokinet. 2004;43(5):311-327.
3. Sheskin J. Thalidomide in the treatment of lepra reaction. Clin Pharmacol Ther. 1965;6(3):303-306.
4. Calabrese L, Fleischer AB Jr. Thalidomide: current and potential clinical applications. Am J Med. 2000;108(6):487-495.
5. Singhal S, Mehta J, Desikan R, Ayers D, Roberson P, Eddlemon P, et al. Antitumor activity of thalidomide in refractory multiple myeloma. N Engl J Med. 1999;341(21):1565-1571.
PUBMED | CROSSREF

6. Govindarajan R, Heaton KM, Broadwater R, Zeitlin A, Lang NP, Hauer-Jensen M. Effect of thalidomide on gastrointestinal toxic effects of irinotecan. Lancet. 2000;356(9229):566-567.
PUBMED | CROSSREF

7. Marx GM, Pavlakis N, McCowatt S, Boyle FM, Levi JA, Bell DR, et al. Phase II study of thalidomide in the treatment of recurrent glioblastoma multiforme. J Neurooncol. 2001;54(1):31-38.
PUBMED | CROSSREF

8. Patt YZ, Hassan MM, Lozano RD, Nooka AK, Schnirer II, Zeldis JB, et al. Thalidomide in the treatment of patients with hepatocellular carcinoma: a phase II trial. Cancer. 2005;103(4):749-755.
PUBMED | CROSSREF

9. Palumbo A, Bringhen S, Caravita T, Merla E, Capparella V, Callea V, et al. Oral melphalan and prednisone chemotherapy plus thalidomide compared with melphalan and prednisone alone in elderly patients with multiple myeloma: randomised controlled trial. Lancet. 2006;367(9513):825-831.
PUBMED | CROSSREF

10. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-674.
PUBMED | CROSSREF

11. Murdoch C, Muthana M, Coffelt SB, Lewis CE. The role of myeloid cells in the promotion of tumour angiogenesis. Nat Rev Cancer. 2008;8(8):618-631.
PUBMED | CROSSREF

12. D’Amato RJ, Loughnan MS, Flynn E, Folkman J. Thalidomide is an inhibitor of angiogenesis. Proc Natl Acad Sci U S A. 1994;91(9):4082-4085.
PUBMED | CROSSREF

13. Verheul HM, Panigrahy D, Yuan J, D’Amato RJ. Combination oral antiangiogenic therapy with thalidomide and sulindac inhibits tumour growth in rabbits. Br J Cancer. 1999;79(1):114-118.
PUBMED | CROSSREF

14. Kenyon BM, Browne F, D’Amato RJ. Effects of thalidomide and related metabolites in a mouse corneal model of neovascularization. Exp Eye Res. 1997;64(6):971-978.
PUBMED | CROSSREF

15. Dunzendorfer S, Schratzberger P, Reinisch N, Kähler CM, Wiedermann CJ. Effects of thalidomide on neutrophil respiratory burst, chemotaxis, and transmigration of cytokine- and endotoxin-activated endothelium. Naunyn Schmiedebergs Arch Pharmacol. 1997;356(5):529-535.
PUBMED | CROSSREF

16. Corral LG, Haslett PA, Muller GW, Chen R, Wong LM, Ocampo CJ, et al. Differential cytokine modulation and T cell activation by two distinct classes of thalidomide analogues that are potent inhibitors of TNF-alpha. J Immunol. 1999;163(1):380-386.
PUBMED

17. Finotello R, Henríques I, Sabatini S, Stefanello D, Felisberto R, Pizzoni S, et al. A retrospective analysis of chemotherapy switch suggests improved outcome in surgically removed, biologically aggressive canine haemangiosarcoma. Vet Comp Oncol. 2017;15(2):493-503.
PUBMED | CROSSREF

18. Bray JP, Orbell G, Cave N, Munday JS. Does thalidomide prolong survival in dogs with splenic haemangiosarcoma? J Small Anim Pract. 2018;59(2):85-91.
PUBMED | CROSSREF

19. Polton G, Finotello R, Sabatini S, Rossi F, Laganga P, Vasconi ME, et al. Survival analysis of dogs with advanced primary lung carcinoma treated by metronomic cyclophosphamide, piroxicam and thalidomide. Vet Comp Oncol. 2018;16(3):399-408.
PUBMED | CROSSREF

20. De Campos CB, Lavalle GE, Monteiro LN, Pégas GR, Fialho SL, Balabram D, et al. Adjuvant thalidomide and metronomic chemotherapy for the treatment of canine malignant mammary gland neoplasms. In Vivo. 2018;32(6):1659-1666.
PUBMED | CROSSREF

21. Teo SK, Evans MG, Brockman MJ, Ehrhart J, Morgan JM, Stirling DI, et al. Safety profile of thalidomide after 53 weeks of oral administration in beagle dogs. Toxicol Sci. 2001;60(1):160-168.
PUBMED | CROSSREF

22. Jankowski M, Fulton L, Sheafor S, Prescott D, Khanna C. Ongoing Evaluation of Single Agent Thalidomide in Dogs with Measurable Cancer. Veterinary Cancer Society 19th Annual Meeting; 1999 Nov 13–Nov 16; Woods Hole, USA. Woods Hole: Veterinary Cancer Society Conference; 1999.
23. Woods JP, Mathews KA, Binnington AG. Thalidomide for the treatment of hemangiosarcoma in dogs. Vet Comp Oncol. 2004;2(2):108-109.

24. Toraño JS, Verbon A, Guchelaar HJ. Quantitative determination of thalidomide in human serum with high-performance liquid chromatography using protein precipitation with trichloroacetic acid and ultraviolet detection. J Chromatogr B Biomed Sci Appl. 1999;734(2):203-210.

25. Saccomanni G, Turini V, Manera C, Placanica G, Salè EO, Jemos C, et al. High performance liquid chromatographic determination of thalidomide in patients affected by hepatocellular carcinoma. J Pharm Biomed Anal. 2008;48(2):447-451.

26. Guideline on Bioanalytical Method Validation. EMEA/CHMP/EWP/192217/2009. EMA Committee for Medicinal Products for Human Use (CHMP) Rev. 1 Corr. 2** [Internet]. Amsterdam: European Medicines Agency; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2011/08/WC500109686.pdf. Updated 2011. Accessed 2017 Apr 18.

27. Teo SK, Colburn WA, Thomas SD. Single-dose oral pharmacokinetics of three formulations of thalidomide in healthy male volunteers. J Clin Pharmacol. 1999;39(11):1162-1168.

28. Teo SK, Scheffler MR, Kook KA, Tracewell WG, Colburn WA, Stirling DI, et al. Effect of a high-fat meal on thalidomide pharmacokinetics and the relative bioavailability of oral formulations in healthy men and women. Biopharm Drug Dispos. 2000;21(1):33-40.

29. Teo SK, Scheffler MR, Kook KA, Tracewell WG, Colburn WA, Stirling DI, et al. Thalidomide dose proportionality assessment following single doses to healthy subjects. J Clin Pharmacol. 2001;41(6):662-667.

30. Singh BN. Effects of food on clinical pharmacokinetics. Clin Pharmacokinet. 1999;37(3):213-255.

31. Chen LX, Ni XL, Zhang H, Wu M, Liu J, Xu S, et al. Preparation, characterization, in vitro and in vivo antitumor effect of thalidomide nanoparticles on lung cancer. Int J Nanomedicine. 2018;13:2463-2476.

32. Crousse RG. Human pharmacology of griseofulvin: the effect of fat intake on gastrointestinal absorption. J Invest Dermatol. 1961;37(6):529-533.

33. Walther FM, Allan MJ, Roepke RK, Nuernberger MC. The effect of food on the pharmacokinetics of oral fluralaner in dogs. Parasit Vectors. 2014;7(1):84.

34. Welling PG. Effects of food on drug absorption. Annu Rev Nutr. 1996;16(1):383-415.

35. Lebkowska-Wieruszewska B, Stefanielli F, Chericoni S, Owen H, Poapolathep A, Lisowski A, et al. Pharmacokinetics of Bedrocan®: a cannabis oil extract, in fasting and fed dogs: an explorative study. Res Vet Sci. 2019;123:26-28.

36. Abiru H, Sarna SK, Condon RE. Contractile mechanisms of gallbladder filling and emptying in dogs. Gastroenterology. 1994;106(6):1652-1661.

37. Ferguson L, Wennogle SA, Webb CB. Bilious vomiting syndrome in dogs: retrospective study of 20 cases (2002–2012). J Am Anim Hosp Assoc. 2016;52(3):157-161.

38. Toutain PL, Bousquet-Méolou A. Plasma terminal half-life. J Vet Pharmacol Ther. 2004;27(6):427-439.

39. Baidas SM, Winer EP, Fleming GF, Harris L, Pluda JM, Crawford JG, et al. Phase II evaluation of thalidomide in patients with metastatic breast cancer. J Clin Oncol. 2000;18(14):2710-2717.

40. Fine HA, Figg WD, Jacktle K, Wen PY, Kyritsis AP, Loeffler JS, et al. Phase II trial of the antiangiogenic agent thalidomide in patients with recurrent high-grade gliomas. J Clin Oncol. 2000;18(4):708-715.

41. Figg WD, Raje S, Bauer KS, Tompkins A, Venzon D, Bergan R, et al. Pharmacokinetics of thalidomide in an elderly prostate cancer population. J Pharm Sci. 1999;88(1):121-125.
42. Chen W, Xiao Y, Chen J, Liu J, Shao J, Li T, et al. Sex-related pharmacokinetic differences and mechanisms of metapristone (RU486 metabolite). Sci Rep. 2017;7(1):17190.
PUBMED | CROSSREF

43. Fleischer S, Sharkey M, Mealey K, Ostrander E, Martinez M. Pharmacogenetic and metabolic differences between dog breeds: their impact on canine medicine and the use of the dog as a preclinical animal model. AAPS J. 2008;10(1):110-119.
PUBMED | CROSSREF

44. Martinez M, Mahmood I, Hunter RP. Allometric scaling of clearance in dogs. J Vet Pharmacol Ther. 2009;32(5):411-416.
PUBMED | CROSSREF

45. Middleton RP, Lacroix S, Scott-Boyer MP, Dordevic N, Kennedy AD, Slusky AR, et al. Metabolic differences between dogs of different body sizes. J Nutr Metab. 2017;2017:4535710.
PUBMED | CROSSREF

46. Court MH, Hay-Kraus BL, Hill DW, Kind AJ, Greenblatt DJ. Propofol hydroxylation by dog liver microsomes: assay development and dog breed differences. Drug Metab Dispos. 1999;27(11):1293-1299.
PUBMED

47. Hay Kraus BL, Greenblatt DJ, Venkatakrishnan K, Court MH. Evidence for propofol hydroxylation by cytochrome P4502B11 in canine liver microsomes: breed and gender differences. Xenobiotica. 2000;30(6):575-588.
PUBMED | CROSSREF

48. Zoran DL, Riedesel DH, Dyer DC. Pharmacokinetics of propofol in mixed-breed dogs and greyhounds. Am J Vet Res. 1993;54(5):755-760.
PUBMED

49. KuKanich B, Lascelles BD, Papich MG. Use of a von Frey device for evaluation of pharmacokinetics and pharmacodynamics of morphine after intravenous administration as an infusion or multiple doses in dogs. Am J Vet Res. 2005;66(11):1968-1974.
PUBMED | CROSSREF

50. KuKanich B, Coetzee JF, Gehring R, Hubin M. Comparative disposition of pharmacologic markers for cytochrome P-450 mediated metabolism, glomerular filtration rate, and extracellular and total body fluid volume of Greyhound and Beagle dogs. J Vet Pharmacol Ther. 2007;30(4):314-319.
PUBMED | CROSSREF

51. Gunn HM. The proportions of muscle, bone and fat in two different types of dog. Res Vet Sci. 1978;24(3):277-282.
PUBMED | CROSSREF