Cancer Chemother Pharmacol (2011) 67:165–171
DOI 10.1007/s00280-010-1304-2

ORIGINAL ARTICLE

# Oral bioavailability of DN101, a concentrated formulation of calcitriol, in tumor-bearing dogs

**Kenneth M. Rassnick · Josephia R. Muindi · Candace S. Johnson · Dennis B. Bailey · Donald L. Trump**

Received: 16 November 2009 / Accepted: 2 March 2010 / Published online: 21 March 2010


**Abstract**
*Purpose* High-dose calcitriol (1,25-dihydroxyvitamin $D_3$) has antineoplastic activity against a range of tumors and potentiates chemotherapeutic agents. In an earlier canine study, the MTD of intravenous (i.v.) calcitriol was 3.75 μg/kg, but polysorbate-associated hypersensitivity reactions were common. Use of commercially available oral calcitriol is limited by the absence of a formulation of suitable strength to allow administration of a reasonable number of caplets. This study evaluated the bioavailability of DN101, a concentrated oral calcitriol formulation specifically developed for anticancer applications.
*Methods* An open-label, single-dose, 2-way crossover study was conducted. Dogs randomly received a single 3.75 μg/kg dose of calcitriol either i.v. or oral (as DN101), followed by cisplatin (60 mg/m$^2$). Three weeks later, the alternate form of calcitriol was given prior to another dose of cisplatin. Dogs received antihistamines and corticosteroids prior to both treatments. Food was withheld for 12 h before and after therapy. Serum calcitriol concentrations were measured by radioimmunoassay.
*Results* Ten tumor-bearing dogs received both i.v. and oral calcitriol. Six dogs experienced hypersensitivity reactions during i.v. calcitriol. Sequence of calcitriol administration (day-1 vs. day-21) by either i.v. or oral routes had no effect on the major calcitriol pharmacokinetic parameters. Oral calcitriol resulted in significantly lower values for AUC ($P = 0.05$) and prolonged $T_{1/2}$ ($P = 0.003$) when compared to i.v. Calcitriol oral bioavailability was highly variable among dogs (mean ± SEM, 71 ± 12.6%).
*Conclusions* This study demonstrates that a high-dose formulation of calcitriol has a moderate bioavailability in dogs, but inter-individual variability in PK parameters is similar to that observed in people. With this bioavailability, serum concentrations of calcitriol that exhibit antitumor activity in a preclinical murine model were achieved in some dogs. Exploration of methods to minimize variation in calcitriol systemic exposure is warranted.

**Keywords** Vitamin D · 1,25-Dihydroxyvitamin $D_3$ · Platinum · Canine · Cancer · Pharmacokinetics

This article was presented in part at the 28th Annual Conference of the Veterinary Cancer Society, Seattle, Washington, October 18–21, 2008.

K. M. Rassnick (✉) · D. B. Bailey
Department of Clinical Sciences, Cornell University, College of Veterinary Medicine, Box 31, Ithaca, NY 14853, USA
e-mail: kmr32@cornell.edu

J. R. Muindi · D. L. Trump
Department of Medicine, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

C. S. Johnson
Department of Pharmacology and Therapeutics, Roswell Park Cancer Institute, Buffalo, NY 14263, USA

## Introduction

Calcitriol, (1,25-dihydroxyvitamin $D_3$; 1α25-dihydroxycholecalciferol) the most active form of vitamin D, has antineoplastic activity in vitro and in vivo in a broad range of murine, human, and canine tumor models.[9, 27] Preclinical data indicate that antitumor effects are related to the

dose of calcitriol, and hence serum calcitriol area under the concentration–time curve (AUC) and/or peak calcitriol concentration ($C_{max}$) would be expected to be important pharmacokinetic (PK) determinants of antineoplastic activity [23]. The evaluation of calcitriol as an anticancer agent alone or in combination with dexamethasone and antitumor agents is ongoing [6, 22].

Calcitriol is commercially available in three FDA-approved formulations: a parenteral formulation for intravenous (i.v.) administration as well as liquid and capsule formulations for oral administration. The i.v. administration of calcitriol to dogs causes cutaneous reactions (pruritus and urticaria), characteristic of an acute hypersensitivity reaction [27]. Polysorbate is the solvent in the i.v. calcitriol formulation; severe hypersensitivity reactions caused by histamine release are common in dogs treated with polysorbate [19] or with drugs containing this solubilizing vehicle [12]. Administration of calcitriol by mouth would circumvent this adverse effect; however, in people, PK evaluation of commercial oral preparations (liquid and caplet) shows substantial limitations: calcitriol AUC and $C_{max}$ do not increase proportionally with increasing calcitriol dose, and substantial inter-patient variability exists for both AUC and $C_{max}$ [4, 24, 25]. DN101 (Novacea, Inc., San Francisco, CA, USA) is a new formulation of oral calcitriol designed specifically for cancer therapy. Studies in people show linear relationships between dosage and AUC and $C_{max}$; however, substantial inter-individual variability was observed in people treated with high doses of DN101 [2, 3].

A study by our group showed that the maximum-tolerated dosage (MTD) of i.v. calcitriol in dogs was 3.75 μg/kg, and as anticipated, hypercalcemia was the dose-limiting toxicity [27]. Neither the toxicity nor PK of oral calcitriol in dogs has been studied. Trials to evaluate commercially available calcitriol capsules in dogs are not reasonable, because the largest capsule size is 0.5 μg, and administering the MTD 3.75 μg/kg dosage to an average-sized dog would require approximately 250 capsules. Also, unfavorable pharmacological conditions in the intestines associated with ingestion of such a large number of capsules might decrease calcitriol absorption, as clearly occurs in people [4, 24, 30]. DN101 is a concentrated formulation (15 or 45 μg per capsule) of calcitriol; DN101 would allow treatment with an acceptable number of capsules and affords the opportunity to study the PK characteristics of this formulation in dogs.

Formal bioavailability studies of calcitriol formulations have not been done, and no data exist on the use and PK of high-dose calcitriol when administered orally to dogs. The purpose of this study is to characterize the bioavailability and PK disposition of calcitriol when administered orally (DN101) and i.v. to tumor-bearing dogs.

## Materials and methods

### Animals

Ten tumor-bearing, client-owned dogs were used in an open-label, randomized crossover study performed at the Cornell University Hospital for Animals at the Cornell University College of Veterinary Medicine. Dogs weighing >10 kg were considered eligible to receive calcitriol combined with cisplatin if they met the following criteria: (1) a resected, recurrent, or metastatic spontaneously occurring neoplasm that had been confirmed histologically; (2) an expected survival of at least 4 weeks; (3) no chemotherapy, immunotherapy, or radiotherapy within 4 weeks of study entry; and (4) adequate bone marrow (absolute neutrophil count ≥3,000 cells/μl and ≥100,000 platelets/μl), renal (serum creatinine concentration ≤1.3 mg/dl), hepatic (serum bilirubin concentration ≤0.3 mg/dl and serum alanine transaminase and aspartate transaminase activities ≤2 times the upper limit of the respective reference ranges), and cardiac function. Total serum calcium was used to assess (and grade) hypercalcemia. Dogs with a pretreatment total serum calcium ≥12.0 mg/dl (reference range, 9.3–11.6 mg/dl) were excluded from the study. Written informed consent was obtained from all clients. The study protocol and consent form were approved by the Institutional Animal Care and Use Committee at Cornell University.

### Experimental design

Dogs received 2 treatments of calcitriol combined with cisplatin; there was a washout period of 21 days between administrations. Calcitriol was administered i.v. or orally (DN101) in accordance with a randomized (by coin toss) crossover design such that each dog received the drug by both routes of administration in a random order. Food and water were withheld for at least 12 h before and 12 h after drug administration. Medical history, physical examination, CBC, serum biochemical analysis, and urinalysis were performed on days 7, 14, and 21 after calcitriol/cisplatin administration. Evaluation of toxic effects of calcitriol/cisplatin was monitored by evaluation of the medical histories obtained from dog owners and results of laboratory data. Toxic effects were graded in accordance with the Veterinary Co-operative Oncology Group Common Terminology Criteria for Adverse Events 1.0 [1].

### Intravenous administration of calcitriol

The calcitriol dosage, 3.75 μg/kg body weight, was the MTD defined in our previous phase I trial [27]. The prescribed dose of calcitriol (Sicor Pharmaceuticals Inc.,

Irvine, CA) was diluted in 0.9% NaCl solution, protected from light, and given i.v. over 60 min simultaneously with initiation of the pre-cisplatin fluid diuresis. The end of the i.v. calcitriol infusion was designated as time 0. To combat hypersensitivity reactions, 24 h before treatment with calcitriol, dogs received prednisone (Lloyd Inc., Shenandoah, IA; 1 mg/kg, p.o.), and 30 min before treatment dogs were premedicated with dexamethasone sodium phosphate (American Reagent Laboratories Inc., Shirley, NY; 2 mg/kg, i.v.), cimetidine (Hospira Inc., Lake Forest, IL; 4 mg/kg, i.v.), and diphenhydramine (Baxter Healthcare Corp, Deerfield, IL; 4 mg/kg, i.m.). Following treatment with calcitriol/cisplatin, dogs received dexamethasone (0.1 mg/kg, p.o., q 12 h for 4 days), to reduce calcitriol-induced hypercalcemia, metoclopramide (Pliva, East Hanover, NJ; 0.5 mg/kg, p.o., q 8 h for 7 days), and maropitant citrate (Pfizer Animal Health, NY; 2 mg/kg p.o. q 24 h for 5 days) as prophylactic antiemetics for cisplatin-induced emesis.

### Oral administration of calcitriol

Calcitriol as DN101 (Novacea, Inc., San Francisco, CA, USA) was used for oral administration. The dosage of oral calcitriol (DN101) was 3.75 μg/kg, the same as that administered i.v. However, DN101 was available as 15- or 45-μg capsule, so doses were rounded to the closest 15 μg. Oral calcitriol was given to the dogs at the initiation of the pre-cisplatin fluid diuresis, and time of capsule administration was designated as time 0. Although hypersensitivity reactions after oral treatment were not expected, premedications and posttreatment drugs were given as described for i.v. calcitriol to standardize treatment conditions.

### Administration of cisplatin

The dosage of cisplatin (Sicor Pharmaceuticals Inc., Irvine, CA) was 60 mg/m$^2$ body surface area. Body surface area was calculated by use of the equation: $(10.1 \times [\text{body weight (g)}^{0.67}])/10^4$. Cisplatin was given through an indwelling cephalic vein catheter following a standard antiemetic and fluid diuresis protocol. Specifically, dogs received 0.9% NaCl solution (18.3 ml/kg/h, i.v., for 4 h) followed by a bolus of dolasetron (Aventis Pharmaceuticals Inc., Kansas City, MO; 0.6 mg/kg, i.v.). The calculated dose of cisplatin (60 mg/m$^2$ body surface area) was then infused over a 20-min period. A dose of butorphanol (Fort Dodge Animal Health, Fort Dodge, IA; 0.4 mg/kg, i.m.) was given immediately after completion of the cisplatin administration. Diuresis with 0.9% NaCl solution was continued for another 2 h.

### Pharmacokinetic analysis

An indwelling catheter was inserted in the jugular vein of each dog, and blood samples (3 ml) were collected into non-heparinized tubes before, immediately after (0 min), and at 0.5, 1, 1.5, 2, 3, 4, 5, 10, 15, and 24 h after the calcitriol treatment. Samples were protected from light, centrifuged for 10 min at 2,000×$g$, and serum was stored in 1–2 ml aliquots at −70°C until analyzed. Serum calcitriol levels were determined using the 1,25-dihydroxyvitamin $D_3$-[$I^{125}$] radioimmunoassay kit from DiaSorin Co (Stillwater, MN). The analytic characteristics of this assay have previously been described [30]. Non-compartmental analysis of pharmacokinetic data was done using WinNonlin® Version 5.1, Pharmasight (Mountain View, CA). The pharmacokinetic parameters estimated were peak levels ($C_{max}$), time at which maximum serum concentration was achieved ($T_{max}$), area under the concentration–time curve from time 0 to 24 h ($AUC_{0-24h}$), terminal half life ($T_{1/2}$), volume of distribution ($V_z$), and total body clearance ($Cl_{tb}$).

Calcitriol bioavailability was estimated on the basis of AUC by use of the equation $(AUC_{(0-24h)\ p.o.}/AUC_{(0-24h)\ i.v.}) \times 100$, where $AUC_{(0-24h)\ p.o}$ is the $AUC_{(0-24h)}$ for orally administered calcitriol and $AUC_{(0-24h)\ i.v.}$ is the $AUC_{(0-24h)}$ for calcitriol administered i.v.

### Statistical analysis

Mean and standard error of the mean (SEM) values were computed for serum calcitriol concentrations and for pharmacokinetic parameters. Values for $T_{max}$, $C_{max}$, $T_{1/2}$, and AUC were compared for each route of administration by use of Wilcoxon signed-rank tests. The relationship between $T_{1/2}$ for the oral and i.v. routes was examined by use of linear regression analysis. Analyses were performed with a computer software program (SAS software; Cary, NC), and two-sided values of $P \leq 0.05$ were considered significant.

## Results

### Animals

The ten dogs enrolled in the study ranged from 8 to 14 years of age (median, 10) and weighed 18–48.5 kg (median, 26). Five dogs were mixed-breed dogs, and five were purebred including two Golden Retrievers, two Labrador Retrievers, and one German Shepherd. Four dogs had osteosarcoma, one hemangiosarcoma, one mammary carcinoma, one bronchoalveolar carcinoma, one oral squamous cell carcinoma, one salivary gland adenocarcinoma, and

one undifferentiated cutaneous carcinoma. The dogs with osteosarcoma were treated adjunctively following limb amputation, while the remaining dogs had gross, non-resectable local or metastatic disease.

### Intravenous administration of calcitriol

The median dose of calcitriol administered i.v to the ten dogs was 97 μg (range, 68–182 μg). During i.v. calcitriol administration, six dogs had signs consistent with hypersensitivity reactions. Four dogs showed signs of intense, widespread pruritus with severe erythema (grade 3 hypersensitivity), and two dogs had mild pruritus with head shaking (grade 1 hypersensitivity). Duration of the calcitriol infusion was extended to 2 and 4 h, respectively, in two of the dogs with grade 3 hypersensitivity reactions. All dogs completed their calcitriol infusions despite any related hypersensitivity reactions. One dog had severe hypercalcemia (serum calcium 16 mg/dL; calcitriol $C_{max}$ 23.83 ng/ml; calcitriol AUC 108.34 [ng h]/ml) with adverse gastrointestinal signs including grade 3 vomiting and grade 3 diarrhea 3 days after treatment. Supportive treatments were given (i.v fluids), and clinical signs and hypercalcemia resolved within 48 h.

### Oral administration of calcitriol

The median dose of calcitriol administered orally to the ten dogs was 97 μg (range, 60–180 μg). Due to capsule size and rounding of the calcitriol dose, four dogs received a final calcitriol dose that was 3–9% lower, and four dogs received a final dose that was 1–15% higher than the planned 3.75 μg/kg dosage. Oral calcitriol dose was exactly 3.75 μg/kg in the remaining two dogs. All dogs readily swallowed the calcitriol capsules, and no immediate adverse effects were detected in any dog. Two dogs had hypercalcemia day 1 after treatment: one dog had total serum calcium of 13 mg/dl (calcitriol $C_{max}$ 7.94 ng/ml, AUC 69.6 [ng h]/ml) with grade 3 vomiting and grade 3 anorexia and the other dog had total serum calcium of 13.2 mg/dl (calcitriol $C_{max}$ 14.42; AUC 98.7 [ng h]/ml) with grade 2 vomiting and grade 2 diarrhea. Clinical signs and hypercalcemia resolved within 72 h in both dogs.

### Pharmacokinetics

Standard pharmacokinetic parameters after i.v. and oral administration of calcitriol were tabulated. Sequence of calcitriol administration (day 1 or day 21) by either i.v. or oral route had no significant effect on the major serum calcitriol PK parameters (all $P > 0.05$); Tables 1 and 2). Since sequence of administration did not impact PK parameters, day 1 and day 21 pharmacokinetic data for each route of administration were pooled to examine the effect of route of administration (Table 3). Serum calcitriol $T_{max}$ was seen at the end of the i.v. infusion and was delayed after oral administration of calcitriol ($P = 0.005$). Calcitriol $T_{1/2}$ was longer after oral administration ($P = 0.003$). The calcitriol $C_{max}$ was >10 ng/ml in all dogs after i.v. administration and in five of the ten dogs after oral administration. Calcitriol systemic exposure ($AUC_{(0–24h)}$) was >40 (ng h)/ml in all dogs after i.v. administration and in eight of the ten dogs after oral administration. Oral administration of calcitriol resulted in significantly lower values for $C_{max}$ ($P = 0.009$) and $AUC_{(0–24h)}$ ($P = 0.05$) compared with values after i.v. administration. Mean bioavailability, as determined on the basis of AUC, was 71% (SEM, ±12.6%, range, 17–126%). Serum concentrations over time after i.v. and oral administration of calcitriol are plotted in Fig. 1.

**Table 1** Mean (±SEM) values of the pharmacokinetic parameters after i.v. administration of a single dose (3.75 μg/kg) of calcitriol to ten tumor-bearing dogs

| Pharmacokinetic parameters | I.V. route (day 1, $n = 5$) | I.V. route (day 21, $n = 5$) | $P$ value[a] |
|---|---|---|---|
| $C_{max}$ (ng/ml) | 20.16 ± 1.85 | 19.32 ± 3.53 | 0.838 |
| $AUC_{0–24h}$ ([ng h]/ml) | 128.98 ± 15.15 | 99.01 ± 13.56 | 0.179 |
| $T_{1/2}$ (h) | 5.61 ± 1.24 | 6.02 ± 0.84 | 0.792 |

The initial route of administration (i.v. or p.o) was randomly assigned, and the second dose was administered via the alternate route 21 days after the first dose

[a] The exclusion of two dogs that had calcitriol intravenous infusion durations extended because of hypersensitivity reactions does not change the conclusions

**Table 2** Mean (±SEM) values of the pharmacokinetic parameters after p.o. administration of a single dose (3.75 μg/kg) of calcitriol to ten tumor-bearing dogs

| Pharmacokinetic parameters | P.O. route (day 1, $n = 5$) | P.O. route (day 21, $n = 5$) | $P$ value |
|---|---|---|---|
| $C_{max}$ (ng/ml) | 12.39 ± 2.73 | 6.58 ± 2.29 | 0.167 |
| $AUC_{0–24h}$ ([ng h]/ml) | 92.74 ± 15.11 | 57.03 ± 14.31 | 0.125 |
| $T_{1/2}$ (h) | 7.75 ± 1.27 | 10.60 ± 1.21 | 0.142 |

The initial route of administration (i.v. or p.o) was randomly assigned, and the second dose was administered via the alternate route 21 days after the first dose

## Discussion

Calcitriol has antineoplastic activity through induction of differentiation, inhibition of cancer cell proliferation, and sensitization of neoplastic cells to other antitumor agents [9, 14]. Antineoplastic effects of calcitriol are dose related,

**Table 3** Mean (±SEM) values of the pharmacokinetic parameters after oral and intravenous administration of a single dose (3.75 μg/kg) of calcitriol to tumor-bearing dogs

| Pharmacokinetic parameters | P.O. route ($n$ = 10) | I.V. route ($n$ = 10) | $P$ value[b] |
|---|---|---|---|
| $T_{max}$ (h) | 3.35 ± 0.83 | 0.21 ± 0.11 | 0.005 |
| $C_{max}$ (ng/ml) | 9.68 ± 1.91 | 19.74 ± 1.88 | 0.009 |
| $AUC_{0-24h}$ ([ng h]/ml) | 74.89 ± 11.47 | 114 ± 10.81 | 0.05 |
| $T_{1/2}$ (h) | 9.18 ± 0.95 | 5.82 ± 0.71 | 0.003 |
| % Bioavailability (mean)[a,b] | 71 | NA | NA |

The initial route of administration (i.v. or p.o) was randomly assigned, and the second dose was administered via the alternate route 21 days after the first dose. Day 1 and day 21 pharmacokinetic data for each route of administration are pooled

*NA* not applicable

[a] The equation for calculating percentage bioavailability is $(AUC_{(0-24h)\ p.o.}/AUC_{(0-24h)\ i.v.}) \times 100$, where $AUC_{(0-24h)\ p.o.}$ is the $AUC_{(0-24h)}$ for orally administered calcitriol and $AUC_{(0-24h)\ i.v.}$ is the $AUC_{(0-24h)}$ for calcitriol administered intravenously

[b] The exclusion of two dogs that had calcitriol intravenous infusion durations extended because of hypersensitivity reactions does not change the conclusions; however, the mean bioavailability decreases to 55.6%

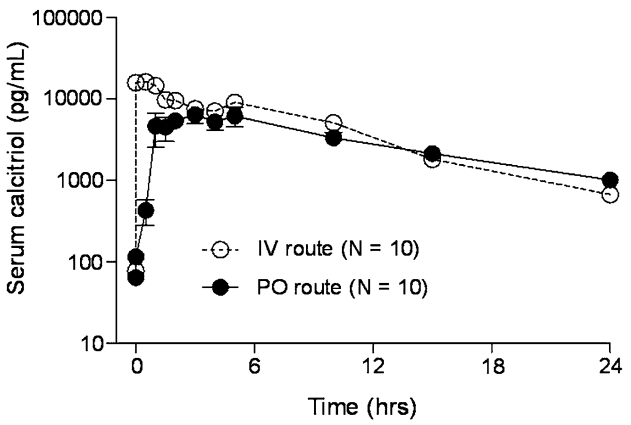


**Fig. 1** Mean serum concentration (±SEM) over time plots after oral and intravenous administration of a single dose (3.75 μg/kg) of calcitriol to ten tumor-bearing dogs. Calcitriol was administered i.v. or p.o. in accordance with a randomized crossover design such that each dog received the drug by both routes of administration in a random order (there was a 21-day period between treatments). Data = day 1 + day 21 for each route

and high-dose pulse administration of calcitriol improves its therapeutic index [23]. There are no definitive studies on the bioavailability of oral calcitriol. Some authors have claimed, however, that only 50% of a dose of a calcitriol is absorbed after oral administration [20]. In contrast, others have suggested that oral bioavailability of calcitriol is very efficient (e.g. 80–90%) [9]. All studies of oral calcitriol PK to date, however, suffer from the absence of i.v. data and therefore true determination of bioavailability [2, 3, 5]. Furthermore, given the high inter-subject PK variability found in some studies [2, 3], inferences made from parallel studies are difficult to interpret. To our knowledge, this is the first bioavailability study of calcitriol; the PK of calcitriol after i.v. and oral administration to dogs indicates a moderate bioavailability (mean bioavailability was 71%). However, bioavailability is greatly dependent on aspects of drug formulation including release of the drug from an oral dosage form, solubility and stability of the released drug in the gastrointestinal contents, and drug permeability at the site of absorption, among other factors [33]. Therefore, we recommend that the bioavailability of all new high-dose calcitriol formulations be determined prior to widespread use in oncology practices.

Inter-individual variability in PK parameters was high; bioavailability ranged from 17 to 126%. First-pass metabolism is a common cause of incomplete and variable bioavailability of orally administered drugs. Since the study reported here was performed in dogs of various breeds, the observed variability could reflect polymorphic breed differences in 25-hydroxyvitamin $D_3$-hydroxylase (CYP24A1, the predominant calcitriol catabolizing enzyme), breed differences in serum levels of vitamin D-binding proteins (VDBP, the major transporter of vitamin $D_3$ in circulation), and/or VDBP polymorphisms exhibiting different binding affinities for vitamin $D_3$ metabolites. Furthermore, in addition to calcitriol, dogs in this study received other medications. Concurrent medications might have contributed to the high inter-patient variability in calcitriol bioavailability via drug–drug interaction and/or drug-induced up-regulation or down-regulation of other drug metabolizing enzymes in the liver and gastrointestinal mucosa. Finally, since the dogs studied were of different ages and had different types of cancers, age- and disease-specific influence on drug metabolism profiles could also attribute to variable calcitriol bioavailability.

The site in the gastrointestinal tract where calcitriol absorption takes place is uncertain. The absorption of all fat-soluble vitamins such as vitamin D occurs in association with fats and bile salts via chylomicrons into the lymphatic system [20]. Evaluation of calcitriol as an anticancer agent is ongoing, and rodent studies are frequently used as preclinical models to human trials [23]. However, for oral administration, extrapolating rodent data of lymphatically transported lipophilic drugs to humans is complicated by differences between humans and rodents; unlike in humans, bile flow in rodents is continuous and independent of food intake [16]. Unlike the rodent, the canine intestinal environment is similar to that of humans, and the dog is an appropriate in vivo model for assessing the absorptive characteristics of lymphatically transported compounds [16, 17]. For example, lycopene, a fat-soluble carotenoid

with antioxidant and potential chemopreventative properties, has PK parameters in dogs that are comparable with those in people [18]. Since the anticancer effects of calcitriol are dose related, continued exploration of ways in which higher doses of calcitriol might be orally administered is warranted, and we propose use of dogs as a relevant large animal model.

High inter-patient variability as demonstrated here can lead to inconsistent clinical responses; so methods to minimize variation in systemic exposure are needed. Dogs were fasted before receiving oral calcitriol, and both meals and their composition are known to positively affect the PK of orally administered lipophilic drugs. For many lipophilic drugs, meals enhance gastrointestinal absorption and bioavailability by increasing drug dissolution and solubility, increasing bile output, slowing gastric emptying, increasing intestinal motility, and providing for chylomicron synthesis to maximize intestinal lymphatic transport [11, 13, 26]. People given fenretinide, a synthetic oral retinoid, for example, along with a standard meal show improved bioavailability compared to those that are fasted [10]. Coadministration with lipids or high-fat meals significantly enhances bioavailability of other retinoids and importantly and reduces the overall variability between patients [7, 8, 21, 31]. Serum lycopene AUC is higher in dogs when the dose is administered as a solution in triglycerides compared with lycopene alone [32]. A similar approach should be considered in dogs treated with oral calcitriol. Finally, as mentioned previously, inter-patient variability can be related to differences in the expression CYP24A1. Oral or i.v. administration of selective inhibitors of CYP24A1 could represent a novel strategy to decrease the first-pass effect that might contribute to PK variability [15, 28, 29].

The PK of oral calcitriol in humans has been extensively studied using standard, commercial formulations. Importantly, these PK studies revealed that dose escalation did not result in the escalation of systemic exposure (a linear relationship was lost at doses >16 μg) [4, 24]. PK studies of concentrated calcitriol as DN101 revealed a linear relationship between dose and exposure at oral doses up to 168 μg, indicating that the apparent "saturable" absorption of commercial preparations of calcitriol is a product of the pharmaceutical characteristics of the compounds when given at high doses rather than reduced absorption or increased catabolism [5]. Marked variation (five to tenfold) in AUC and $C_{max}$ occurs in people receiving the same doses of commercial calcitriol formulations [4, 24]. Despite the improved relationship between dose and systemic exposure in people treated with concentrated calcitriol (DN101), substantial inter-patient variability, as observed in the dogs in this study, still exists [2, 3].

$C_{max}$, $T_{1/2}$, and AUC values after i.v. administration of 3.75 μg/kg calcitriol obtained in this study were in agreement with the mean ± standard deviation values of 29 ± 3.81 ng/ml, 7.7 ± 1.3 h, and 119.3 ± 16.9 ng h/ml, respectively, reported in our previous study [27]. Hypersensitivity reactions were common (six of ten dogs), however, during the i.v. infusion of calcitriol to the dogs in the study reported here, despite use of premedications to lessen adverse reactions. Hypersensitivity reactions during the i.v. infusion are the result of the solubilizing agent (polysorbate) in which the parenteral formulation of calcitriol is prepared. This finding and the findings of our previous study confirm that i.v. administration of calcitriol to dogs is problematic, making oral administration highly preferable.

A randomized crossover study design was used, and sequence of calcitriol administration did not effect the PK results. As expected, $T_{max}$ was seen at the end of the i.v. infusion and was delayed after oral administration. Calcitriol $T_{1/2}$ was longer after oral administration, but PK samples were only collected for 24 h after the calcitriol treatment, and data might have been insufficient to capture the true terminal elimination phase. Extended PK sampling would be needed to more accurately delineate calcitriol elimination $T_{1/2}$ after administration.

Three dogs developed dose-limiting hypercalcemia (one after i.v. calcitriol and two after oral calcitriol). Based on the study design, total serum calcium was measured beginning day 7 after treatment. All dogs with dose-limiting hypercalcemia had severe gastroenteritis that prompted laboratory evaluation before day 7. It is likely that elevated serum calcium values would have been seen in other dogs that were asymptomatic had evaluation been conducted within 2–5 days following calcitriol administration. A larger sample size would be needed to determine whether the incidence of adverse reactions is different in dogs given oral calcitriol compared to i.v.

Preclinical findings suggest PK parameters are important determinants of calcitriol antitumor activity. In a murine squamous cell carcinoma model, doses of calcitriol that result in $C_{max} > 10.0$ ng/ml and AUC > 40.0 ng h/ml are effective at suppressing tumor growth [23]. Calcitriol $C_{max}$ and AUC exceeded these values in all dogs after i.v. administration. Despite significantly lower values for both $C_{max}$ and AUC in dogs treated orally when compared to i.v., $C_{max}$ still was >10 ng/ml in five of the ten dogs and AUC was >40 ng h/ml in eight of the ten dogs after oral administration. Although squamous cell carcinoma is a sensitive model, this datum supports high doses of oral calcitriol might have clinical utility in treatment of cancer-bearing dogs.

In summary, this study demonstrates that a high-dose formulation of calcitriol has a moderate bioavailability in dogs. With this bioavailability, serum concentrations of calcitriol that exhibit antitumor activity in a preclinical murine model were achieved in some dogs, but exploration of ways

to minimize inter-individual variation in calcitriol systemic exposure is warranted.

**Acknowledgments** This work was supported by grants (NCI and DOD Grants CA67267, CA95045, and PCRP050202) and The Cornell University Hospital for Animals, Cornell University College of Veterinary Medicine. The authors thank Dr. Gerald Fetterly, Associate Director, Pharmacokinetics/Pharmacodynamics, Roswell Park Cancer Institute, for assistance with the pharmacokinetic analysis.

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