Etoposide (VP-16) is a semisynthetic derivative of podophyllotoxin and is widely used in the treatment of adults and children with various solid and hematopoietic cancers. Etoposide is 1 component of several standard regimens used to treat people with germ-cell tumors and testicular cancers. Etoposide is commercially available in 2 FDA-approved formulations (a parenteral formulation for intravenous [IV] or PO administration). Approved etoposide formulations are generally well tolerated and are administered at dosages that result in toxicological effects that are limited to hematologic and gastrointestinal toxicity. However, hypersensitivity reactions may be observed when etoposide is administered parenterally. These reactions may be related to the use of sodium chloride to reconstitute the parenteral formulation of etoposide. The use of PO formulations may provide a safe alternative for etoposide administration. Furthermore, the PO formulation may be more convenient for administration and allow for the use of a lower dose because of lower bioavailability after IV administration.

Objective—To characterize oral bioavailability and pharmacokinetic disposition of etoposide when the IV formulation was administered orally to dogs.

Animals—8 tumor-bearing dogs.

Procedures—An open-label, single-dose, 2-way crossover study was conducted. Dogs were randomly assigned to initially receive a single dose of etoposide (50 mg/m²) IV or PO. A second dose was administered via the alternate route 3 to 7 days later. Medications were administered before IV administration of etoposide to prevent hypersensitivity reactions. Oral administration of etoposide was prepared by reconstituting the parenteral formulation with 0.9% NaCl solution and further diluting the reconstituted mixture 1:1 with a sweetening agent. Plasma samples were obtained after both treatments. Etoposide concentrations were measured with a high-performance liquid chromatography assay, and plasma etoposide concentration–time profiles were analyzed by use of noncompartmental methods.

Results—4 dogs had hypersensitivity reactions during IV administration of etoposide. No adverse effects were detected after oral administration. Plasma etoposide concentrations were undetectable in 2 dogs after oral administration. Oral administration of etoposide resulted in significantly lower values for the maximum plasma concentration and the area under the plasma etoposide concentration-versus-time curve, compared with results for IV administration. Oral bioavailability of etoposide was low (median, 13.4%) and highly variable among dogs (range, 5.7% to 57.3%).

Conclusions and Clinical Relevance—Vehicle-related toxicosis can limit the IV administration of etoposide in dogs. The parenteral formulation of etoposide can be safely administered orally to dogs, but routine use was not supported because of low and variable oral bioavailability in this study. (Am J Vet Res 2008;69:1316–1322)
IV administration and capsules for oral administration. Capsules can be used for continuous, low-dose administration without the need for IV infusions and hospitalization. However, the oral formulation of etoposide is limited to 50-, 100-, and 500-mg gelatin capsules that cannot be divided, which precludes accurate dosing in many instances. The injectable formulation of etoposide can be diluted 1:2 with saline (0.9% NaCl) solution and will remain stable for at least 3 weeks, and diluted mixtures of the parenteral formulation of etoposide can be given orally to pediatric oncology patients. In a phase I study of children with refractory solid tumors, diluted injectable etoposide was suspended in juice or flavored syrup and then given orally 3 times daily for 21 consecutive days. Pharmacokinetic data revealed a dose-dependent increase in the duration of time that plasma etoposide concentrations remained > 1 µg/mL, which is a concentration that is purportedly associated with antitumor effects. Diarrhea was a dose-limiting effect, and myelosuppression was also detected.

The IV administration of etoposide to dogs causes cutaneous reactions and hypotension characteristic of an acute hypersensitivity reaction. Polysorbate-80 is the solvent in the etoposide formulation used for parenteral administration; severe acute hypersensitivity reactions caused by histamine release have been reported in dogs treated with polysorbate-80 alone or with drugs containing this solubilizing vehicle (eg, docetaxel). Limited data exist on the use and pharmacokinetics of etoposide when administered orally to dogs. Because commercially available capsules for oral administration would be difficult to use to achieve accurate doses in dogs of various body weights, the purpose of the study reported here was to characterize the bioavailability and pharmacokinetic disposition of etoposide when the same doses of the parenteral formulation were administered IV and orally to tumor-bearing dogs.

Materials and Methods

Animals—Eight tumor-bearing client-owned dogs were used in an open-label, single-dose, randomized crossover study performed at the Cornell University College of Veterinary Medicine. Dogs were considered eligible to receive etoposide when they had a recurrent or metastatic naturally developing neoplasm not amenable to surgical resection or refractory to conventional treatments; an expected survival time of at least 4 weeks; a history of no chemotherapy, immunotherapy, or radiotherapy for at least 2 weeks; and 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. 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—Etoposide was administered IV or orally 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 washout period of 3 to 7 days between administrations, which was based on the elimination half-life of etoposide in humans. The etoposide dose of 50 mg/m² was based on reports that dogs tolerate etoposide administered IV at doses of 25 to 100 mg/m² and on the clinical experience of one of the investigators (KMR) that dogs tolerate etoposide administered orally as capsules at a daily dose of 50 mg/m² for 21 days. Food and water were withheld for 12 hours before and 12 hours after drug administration. Clinical observations were recorded for at least 24 hours after administration, and toxic effects were graded in accordance with criteria reported in another study.

Oral administration of etoposide—A commercially available parenteral formulation of etoposide was used for oral administration. Each vial contained 20 mg of etoposide/ml and was diluted 1:2 to a final concentration of 6.67 mg/mL by the addition of saline solution. This concentration was selected to mimic oral administration of the parenteral formulation of etoposide to children. The dose for each dog (50 mg/m²) was then mixed 1:1 with a commercially available flavoring agent and administered via an oral dosing syringe. After oral administration of etoposide, each dog received 10 mL of drinking water administered with a syringe. Time of capsule administration was designated as time 0.

IV administration of etoposide—The commercially available parenteral formulation of etoposide was used for IV administration. The prescribed dose (50 mg/m²) was diluted with saline solution and then immediately administered as an IV infusion during a period of 60 minutes. Onset of the 60-minute infusion was designated as time 0. The volume of saline solution used for dilution was based on body weight (100 mL of saline solution for dogs weighing 10 to 35 kg and 250 mL of saline solution for dogs weighing > 35 kg). Injectable etoposide is complexed with polysorbate-80, and dogs can have hypersensitivity reactions to this vehicle. Therefore, beginning 24 hours before IV administration of etoposide, dogs were medicated with hydrocortisone (4.4 mg/kg, PO, q 12 h), diphenhydramine (2.2 mg/kg, PO, q 8 h), and famotidine (0.5 mg/kg, IV). Etoposide and other medications were administered IV via an indwelling catheter inserted into a cephalic vein.

Collection of pharmacokinetic blood samples—An indwelling catheter was inserted in a jugular vein of each dog, and blood samples (2 mL) were collected into lithium-heparin tubes after oral and IV administration of etoposide. Samples were immediately centrifuged at 1,500 × g for 10 minutes; plasma was harvested and stored at −70°C until analyzed. For oral administration of etoposide, samples were obtained before administration (time 0) and 0.5, 1, 2, 4, 6, 8, and 24 hours after administration. For IV administration of etoposide, samples were obtained before administration (time 0) and...
and 60, 65, 75, and 90 minutes after oral and IV administration.

Measurement of etoposide concentrations—The HPLC assay used to determine etoposide concentrations in canine plasma in the study reported here was a combined modification of 2 HPLC assays described elsewhere.16,17 Podophyllotoxin (10 µL of a 1 mg/mL solution in methanol) was added as an internal standard along with 300 µL of chloroform to each 200-µL plasma sample in a 1.5-mL polypropylene microcentrifuge vial. Contents were mixed, and vials were then centrifuged for 1 minute at 13,000 X g to separate the liquid phases. An aliquot of 150 µL was removed from the lower chloroform phase and transferred to a 12 X 75-mm borosilicate test tube. Tubes were evaporated to dryness under a nitrogen stream at 40°C. The residue was then resuspended in 200 µL of mobile phase and transferred to a 200-µL conical glass insert. To settle any remaining particulates, the glass insert was centrifuged inside a 500-µL microcentrifuge vial at 13,000 X g for 1 minute and then removed to an amber glass sample vial in an autosampler. The HPLC system consisted of dual-model pumps, an autosampler with a 300-µL sample loop, and a UV detector. Data acquisition, handling, and reporting were performed via HPLC software. Analyte separation was achieved by use of a reversed-phase octadecysilyl column (4.6 X 150 mm) protected with a guard column (3.2 X 15 mm). The mobile phase (50% methanol and 50% acetonitrile-to-water mixture [840:60]) was pumped at a flow rate of 1 mL/min at ambient temperature. Retention time for etoposide and podophyllotoxin was 4.1 and 7.9 minutes, respectively. Analyte peak areas were corrected for variations in sample volume by means of the internal standard and were compared with a known standard regression curve to calculate concentration.

The assay was conducted in accordance with the principles of good laboratory practice, which included standard curves at the beginning and end of the unknown samples and quality-assurance samples evaluated over the range of the standard curves provided at the beginning, mid point, and end of the unknown samples. The lower limit of quantification of the etoposide HPLC assay was 0.025 µg/mL. The etoposide standard curve was linear over the range of 25 to 10,000 ng/mL. Intraday assay variability ranged from 2.1% to 14.7%, and interday assay variability ranged from 6.6% to 12.1%. Mean accuracy at 0.25, 0.5, and 1 µg/mL was 116.6% (coefficient of variation, 12.0%), 114.6% (coefficient of variation, 12.1%), and 110.2% (coefficient of variation, 6.6%), respectively.

Pharmacokinetic analysis—Plasma etoposide concentrations after oral and IV administration were inspected on a semi-logarithmic plot of concentration versus time for each dog. The etoposide Cmax and Tmax were the values from the raw plasma etoposide concentration–time data. Pharmacokinetic parameters were estimated by use of a computer software program, and etoposide concentration-versus-time data were analyzed by use of an open noncompartmental model for both the oral and IV datasets. When adequate datum points yielded measurable etoposide concentrations, k was determined with a linear regression of the terminal 3 to 5 datum points of the logarithmic plasma etoposide concentration–time plot by use of a weighting paradigm of 1/Y, where Y is the observed concentration value. The t1/2 was estimated as...
The median dose of etoposide administered IV to the 8 dogs was 48 mg (range, 44.0 to 61.6 mg). During IV etoposide administration, 4 dogs had signs consistent with hypersensitivity reactions. Two dogs had intense, widespread pruritis with severe erythema (grade 3 hypersensitivity), and 2 dogs had mild pruritis with head shaking (grade 1 hypersensitivity). Duration of the etoposide infusion was extended to 2 hours in the dogs with grade 3 hypersensitivity reactions. None of the dogs had the administration of etoposide discontinued because of an infusion-related hypersensitivity reaction.

Pharmacokinetics—Plasma etoposide concentrations were less than the limit of detection (0.025 µg/mL) of the HPLC assay at all time points for samples obtained from 2 dogs (a mixed-breed dog and the Bernese Mountain Dog) after oral administration of etoposide. Plasma concentrations over time after oral and IV administration of etoposide were plotted (Figure 1). Standard pharmacokinetic variables after oral and IV administration of etoposide were tabulated (Table 1). There was no significant ($P = 0.16$) difference in $T_{\text{max}}$ after oral and IV administration of etoposide, which was not unexpected because the IV dose was infused over a period of at least 1 hour. The etoposide $C_{\text{max}}$ was >1 µg/mL in 7 of 8 dogs after IV administration and <1 µg/mL in all dogs after oral administration. Oral administration of etoposide resulted in significantly lower values for $C_{\text{max}}$ ($P = 0.031$) and AUC ($P = 0.031$), compared with values after IV administration. Median bioavailability after oral administration of etoposide, as determined on the basis of AUC, was 13.4% (range, 5.7% to 57.3%). Oral bioavailability was >30% in only 2 dogs (the Golden Retriever and the Shetland Sheepdog). Because the etoposide concentrations were less than the limit of detection of the assay after oral administration in 2 dogs, bioavailability was not estimated in those dogs. There was no significant ($P = 0.25$) difference in etoposide $T_{\text{max}}$ after oral or IV administration. Predictably, there was a strong relationship ($R^2 = 0.9995; P = 0.014; n = 3$) between the $T_{\text{max}}$ for oral and IV administration of etoposide in dogs.

**Discussion**

The results of the study reported here on the pharmacokinetics of etoposide after oral and IV administration indicated that the bioavailability of etoposide in dogs was low (median bioavailability was 13.4%) and that interdog variability was high (range, 5.7% to 57.3%). In addition, 2 dogs had undetectable plasma etoposide concentrations after oral administration, so bioavailability could not be quantitated in these dogs. Neither technical problems nor emesis were evident when the formulation was administered orally or during collection or analysis of the blood samples for determining pharmacokinetics in these dogs, but oral administration of etoposide was not repeated in these dogs to eliminate these possibilities. Numerous studies have been conducted to evaluate the bioavail-

**Table 1**—Median (range) values for pharmacokinetic parameters determined after oral and IV administration of a single dose (50 mg/m2) of the parenteral formulation of etoposide to 8 tumor-bearing dogs.

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Oral (n = 6)*</th>
<th>IV (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{\text{max}}$ (min)</td>
<td>60 (30–60)</td>
<td>65 (60–90)</td>
</tr>
<tr>
<td>$C_{\text{max}}$ (µg/mL)</td>
<td>0.22 (0.14–0.71)</td>
<td>1.53 (0.72–9.68)</td>
</tr>
<tr>
<td>$t_{\text{1/2}}$ (min)</td>
<td>91 (72–197)</td>
<td>74 (46–294)</td>
</tr>
<tr>
<td>AUC (µg min/L)</td>
<td>24.0 (5.6–89.6)</td>
<td>105.9 (77.3–374.4)</td>
</tr>
<tr>
<td>Bioavailability†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC method</td>
<td>13.4 (5.7–57.3)</td>
<td>NA</td>
</tr>
<tr>
<td>$C_{\text{max}}$ method</td>
<td>18.5 (2.9–34.5)</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Pharmacokinetic parameters are for the 6 dogs in which plasma etoposide concentrations were measurable; for 2 dogs, the plasma concentrations of etoposide at all time points after oral administration were less than the limit of quantification of the assay (0.025 pg/mL). †Adequate data points were available for only 3 dogs to estimate $t_{\text{1/2}}$ after oral administration. The equation for the AUC method is $\text{AUC}_{\text{oral}} = \text{AUC}_{\text{IV}} \times 100$, where $\text{AUC}_{\text{oral}}$ is the $\text{AUC}_{\text{oral}}$ for orally administered etoposide and $\text{AUC}_{\text{IV}}$ is the $\text{AUC}_{\text{IV}}$ for etoposide administered IV. The equation for the $C_{\text{max}}$ method is $\text{IC}_{\text{oral}} = \text{IC}_{\text{IV}} \times 100$, where $\text{IC}_{\text{oral}}$ is the $\text{IC}_{\text{oral}}$ for orally administered etoposide and $\text{IC}_{\text{IV}}$ is the $\text{IC}_{\text{IV}}$ for etoposide administered IV.

NA = Not applicable.
ability of oral administration of etoposide capsules in adult humans. Bioavailability in patients is considered to be approximately 50%, but mean values ranging between 17% and 76% have been reported. Oral absorption of etoposide capsules in humans is a dose-dependent event, with a greater percentage of drug being absorbed at lower absolute doses.

To our knowledge, information about the pharmacokinetic profile after IV administration of etoposide in dogs has not been published. However, Cmax, t1/2, and AUC values after IV administration of etoposide (50 mg/m²) obtained in this study are in agreement with the mean ± SD values of 5.46 ± 1.96 µg/mL, 1.43 ± 0.54 hours, and 2.58 ± 0.54 (h • µg)/mL, respectively, reported after IV administration of etoposide phosphate (57 µg/m²) to dogs. Etoposide phosphate is a water-soluble prodrug of etoposide and is rapidly and extensively converted to etoposide. Hypersensitivity reactions were common (4/8 dogs) during IV infusion of etoposide to the dogs in the study reported here, despite use of premedications to lessen adverse reactions. Hypersensitivity reactions during IV infusion of etoposide are the result of the solubilizing agent (ie, polysorbate) in which the parenteral formulation of etoposide is prepared. This finding and the findings in other reports confirm that IV administration of etoposide to dogs is neither practical nor safe.

All the dogs in our study readily accepted and tolerated the orally administered parenteral formulation of etoposide, which was delivered in a pleasant-tasting oral preparation, and no adverse effects were detected after administration. Pharmacokinetic data of etoposide after oral administration to dogs is limited. After oral administration of 2 mg of etoposide/kg, etoposide blood concentrations were < 0.3 µg/mL in 1 healthy dog; however, the etoposide formulation was not reported. In another study of 3 dogs with naturally developing hemangiosarcoma, serum etoposide concentrations of < 150 ng/mL were detected after oral administration of a single dose of a parenteral etoposide formulation at a dose of 50 mg/m², which is similar to the dose used in the study reported here.

Several preclinical findings suggest that the duration of exposure of neoplastic cells to etoposide is important for maximal antitumor activity. Etoposide's target (ie, DNA topoisomerase II) is substantially expressed only in dividing cells during selected mitotic phases of the cell cycle. Therefore, chronic dosing may be advantageous because it maximizes the likelihood of exposing malignant cells to etoposide during sensitive periods of the cell cycle. Cytotoxic effects of topoisomerase II–targeting drugs relate to the magnitude of formation of drug-induced, enzyme-mediated DNA strand breaks and to the intracellular persistence of these lesions. Therefore, antineoplastic agents or protracted dosing schemes that prolong the presence of DNA strand breaks in cells would be expected to result in superior efficacy.

Although the importance of the dosing schedule for drug administration on the antineoplastic activity of etoposide was suggested in early preclinical and clinical trials, the most informative study on the importance of schedule dependence included patients with small-cell lung cancer who received 500 mg of etoposide/m² as a 24-hour IV infusion or as a daily 2-hour infusion for 5 days. Although both groups received the same total dose of drug, differences in response rates were dramatic. For the 24-hour IV infusion, 10% of patients responded to treatment, compared with a response rate of 89% for the 5-day treatment group. Pharmacokinetic data from that study revealed prolonged maintenance of serum etoposide concentrations (≥ 1 µg/mL) was associated with superior efficacy in the 5-day treatment group. Clinical studies on the efficacy of etoposide in dogs with cancer are limited. Investigators in 1 study reported on the use of etoposide in dogs with lymphoma. Dogs received 100 mg of etoposide/m² as a 30-minute IV infusion or as a daily IV bolus at a fractionated dosage of 25 mg/m² each day for 4 days. Interestingly, 1 of the 3 dogs given the fractionated dose of etoposide had complete remission after treatment, whereas none of the 10 dogs given the drug as a 1-day treatment responded. These data suggest that a protracted duration of exposure to relatively low etoposide concentrations, instead of higher doses given over shorter periods, may also be important in dogs, similar to results in humans.

Currently, the FDA-approved soft-gelatin capsule formulation of etoposide provides a convenient means for protracted oral administration. However, in pediatric oncology, commercially available capsule sizes (50-, 100-, and 300-mg capsules) compromise the accurate delivery of smaller doses required for children. Thus, the parenteral formulation of etoposide is routinely given orally to circumvent this limitation. The bioavailability of etoposide when the parenteral formulation is given orally to people has not been reported, but pharmacokinetic data reveal that with the doses administered, most achieve plasma concentrations > 1 µg/mL and antitumor responses are common. Dose-dependent absorption of etoposide is not apparent when the parenteral formulation of etoposide is administered orally.

Oral bioavailability of etoposide is primarily limited by Pgp expression on the luminal surface of intestinal epithelial cells. The Pgp is a product of the MDRI gene, and it confers chemotherapy resistance when overexpressed on cancer cells. The functional orientation of Pgp throughout the intestinal tract results in the secretion of etoposide back into the intestinal lumen. Administration of cyclosporin, a modulator of Pgp, increases the plasma concentration of orally administered etoposide by at least 10-fold in rats. Coadministration of etoposide and cyclosporine in humans also results in dramatic improvement in oral uptake. Cyclosporine substantially improves the disposition of orally administered docetaxel (another Pgp substrate) in dogs, and it may be worthwhile to investigate such an approach in dogs treated by oral administration of etoposide.

A commercially available flavoring agent was mixed with the parenteral formulation of etoposide. We selected this product because it is commonly used in our clinic as a vehicle for oral administration of drugs and as a flavoring agent. Etoposide is chemically unstable in an acidic pH (pH < 2); simple syrups typically are acidic and can alter drug pH. The flavoring agent
is acidic (pH, 3.01), but bioavailability may still have been improved in the study reported here if etoposide had not been mixed with any vehicle. When children are orally administered injectable etoposide, it is typically suspended in juice (eg, apple juice, orange juice, or lemonade) or a flavored syrup.2 Finally, it is possible that the flavoring agent altered intestinal Pgp-mediated etoposide transport, as has been reported20 with coadministration of grapefruit juice. However, because the commercially available flavoring agent consisted of only sucrose, glycerin, sorbitol, and water, this alteration in Pgp-mediated transport was unlikely.

The duration of serum etoposide concentrations > 1 μg/mL is associated with superior efficacy in humans.17,20 Once absorbed, there is no pharmacologic difference between oral and IV administration of etoposide with respect to mechanism of action.2 The study reported here was not an efficacy trial, and serum concentrations needed in dogs with cancer are not known. Nonetheless, concentrations > 1 μg/mL were not achieved in any dog orally administered etoposide in other studies25,26 or in our study. The dosage of etoposide (50 mg/m2) used in the study reported here was based on reports9,10 that dogs tolerate IV administration of etoposide at dosages of 25 to 100 mg/m2 and on the clinical experience of one of the authors (KMR) that dogs tolerate etoposide administered as capsules at a dosage of 50 mg/m2/d for 21 days. Controlled studies to determine the maximum dosage of etoposide that is tolerated in dogs are not available. Increasing the dosage of orally administered etoposide in dogs may allow better systemic exposure; however, the wide interpatient variability in Cmax and AUC detected in our study may contribute to overexposure in some dogs.41

The parenteral formulation of etoposide administered orally to dogs was tolerated well and provided easy and accurate dosing. However, pharmacokinetic results of the study reported here revealed that the bioavailability was low and interpatient variability was wide. Methods to improve bioavailability and systemic exposure of this formulation of etoposide in dogs are warranted before routine use can be advocated. This could include oral administration of etoposide with an agent that inhibits intestinal Pgp or use of higher doses of etoposide.

References

20. Slevin ML, Joel SP, Whomsley R, et al. The effect of dose on the bioavailability of oral etoposide: confirmation of a clinically rel-

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