Evaluation of the effects of dietary n-3 fatty acid supplementation on the pharmacokinetics of doxorubicin in dogs with lymphoma

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Objective—To determine the effect of dietary n-3 fatty acids on the pharmacokinetics of doxorubicin in dogs with lymphoma.

Animals—23 dogs with lymphoma in stages IIIa, IVa, and Va.

Procedure—Dogs receiving doxorubicin chemotherapy were randomly allocated to receive food with a high (test group) or low (control group) content of n-3 fatty acids. Serum doxorubicin and doxorubicinol concentrations were measured via high-performance liquid chromatography before and 6 to 9 weeks after initiation of the diets. Lymph node concentrations of doxorubicin were assessed 6 hours after the initial treatment. Dogs' body composition was assessed by means of dual-energy x-ray absorptiometry scans.

Results—No significant differences in doxorubicin pharmacokinetics were detected between treatment groups. Significant differences existed between the first and second sampling times among all dogs for area under the curve, maximum serum concentration, and clearance. Differences in body composition did not affect measured pharmacokinetic variables. The terminal elimination half-life was longer in dogs in which a long-term remission was achieved than in dogs that did not have remission.

Conclusions and Clinical Relevance—Dietary supplementation of n-3 fatty acids is common in veterinary patients with neoplasia, but supplementation did not affect doxorubicin pharmacokinetics in this population of dogs. Explanations for the beneficial effects of n-3 fatty acids other than alterations in the pharmacokinetics of chemotherapy drugs should be investigated. Dogs may metabolize drugs differently prior to remission of lymphoma than when in remission. The pharmacokinetics of doxorubicin at the time of the first administration may predict response to treatment. (Am J Vet Res 2006;67:145–151)

Doxorubicin is an anthracycline chemotherapeutic agent widely used in humans and companion animals with neoplastic disease. The drug is used alone and in multidrug combinations for treatment of dogs with lymphoma. Although reports of doxorubicin pharmacokinetics in dogs are sparse and almost exclusively limited to data from studies of healthy dogs, more is known about the drug in humans.

The disposition of doxorubicin in humans is highly variable, with coefficients of variation for inter- and intra-individual variability ranging from 37% to 93% and 6% to 59%, respectively, for most pharmacokinetic variables. This variability is not fully understood, but serum concentrations of doxorubicin can be correlated with both toxicity and efficacy, so alterations in the pharmacokinetics of the drug may affect treatment success. Although AUC is independent of the doxorubicin administration schedule, peak plasma or serum concentrations are inversely proportional to the duration of infusion. Pharmacokinetic variables may also predict the concentration of the drug achieved in the target tissue.

Factors that may affect the pharmacokinetics of doxorubicin include body composition, liver dysfunction, enterohepatic recirculation, gender, and dietary intake. Obesity has been reported to decrease clearance by approximately half. In a study that used a linked rat model, 22% of a dose of doxorubicin administered IV was passed to another rat via a bile duct-to-duodenum catheter, providing evidence that enterohepatic recirculation occurs after biliary excretion of the drug. In a study of humans, males had significantly higher doxorubicin clearance than females. It is plausible that dietary intake would influence the pharmacokinetic variability in humans because...

AUC Area under the curve
PUFA Polyunsaturated fatty acids
HPLC High-performance liquid chromatography
DEXA Dual-energy x-ray absorptiometry
CL Clearance
\( t_{1/2} \) Terminal half-life
\( C_{max} \) Maximum plasma concentration
\( V_{ss} \) Volume of distribution at steady state
humans consume widely varying diets. There is also evidence that diet influences the pharmacokinetics of doxorubicin in animals; doxorubicin clearance was decreased in rabbits fed a low-protein food.  

The pharmacokinetics of drugs other than doxorubicin are affected by food composition. A clinical study in dogs receiving phenobarbital revealed that the volume of distribution, mean residence time, and $t_{1/2}$ decreased and clearance increased over time and that the changes were greater in dogs fed diets restricted in fat or protein.  

Polyunsaturated fatty acids of the n-3 series may play a role in prevention and treatment of neoplastic disease. A study in dogs with spontaneously occurring lymphoma revealed an improved survival rate and decreased toxicity in patients consuming food supplemented with fish oils (a natural source of n-3 fatty acids), compared with patients consuming the same food with soy oil supplementation. The mechanism of this beneficial effect is unknown.

The potential effects of n-3 fatty acids on the pharmacokinetics and pharmacodynamics of a given drug may be attributable to enzyme synthesis, alterations in cell membrane fluidity or composition, or facilitation of oxidative damage and lipid peroxidation. In a study in rats, cytochrome P-450 enzyme activity and the resulting metabolism of toxic chemicals were increased in rats fed diets rich in n-3 fatty acids, compared with rats fed diets rich in other oils such as soy and coconut oil or lard. In addition, fatty acids may alter the P-glycoprotein-mediated drug resistance of cultured neoplastic cells to the effects of doxorubicin. The purpose of the study reported here was to test our hypothesis that n-3 fatty acids would alter the pharmacokinetic profile of doxorubicin in a population of dogs with spontaneously occurring lymphoma.

**Materials and Methods**

**Animals**—Client-owned dogs evaluated at the Colorado State University Animal Cancer Center for spontaneously occurring lymphoma were enrolled between October 2000 and June 2001 and entered into a prospective, randomized, double-blinded clinical study (neither the investigator nor the owner was aware of a given dog's assigned food group) study. Informed owner consent was obtained for each dog, and the disease was staged by means of a CBC, serum biochemistry analyses, thoracic and abdominal radiography, and a bone marrow aspirate. Twenty-three dogs with disease in World Health Organization stages IIIa, IVa, and Va were initially enrolled. Values for pharmacokinetic variables and lymph node concentrations of doxorubicin were compared between dogs that were initially enrolled but in which clinical remission was not achieved or maintained (n = 4) and dogs that completed the study as planned (19).

**Food and feeding protocol**—Dogs were fed 1 of 2 foods that were identical in composition except that the experimental food (n = 9 dogs) was supplemented with n-3 fatty acids, whereas the control food (10) was not. The foods were isocaloric, providing 6.1 kg of metabolizable energy/g (Table 1). Dogs were assigned 1 of the 2 specialized foods to be fed at a calculated amount (maintenance energy requirement) until recurrence of disease. The amount fed was adjusted in 10% increments at each evaluation period as necessary to maintain the dogs at a stable, ideal body weight. The experimental food was supplemented with feed-grade menhaden fish oil, whereas the control food was supplemented with soybean oil. The food containing the fish oil was manufactured such that the oil was incorporated into the product during the manufacturing process. Fish oil remains stable in the oxygen-limited environment of the canned product. Foods were analyzed for nutrient content by a commercial laboratory by means of proximate analysis. Fatty acid profiles of the foods were determined via gas chromatography. Stability of the fish oil in the food was determined by analyzing the fatty acid content at various times (up to 18 months of storage of the food at room temperature [21°C]). Both foods contained identical amounts (500 U/kg of dry matter) of vitamin E as $\alpha$-tocopherol. Both foods were formulated and determined to be isonitrogenous, equivalent in metabolizable and digestible energy, and nutritionally adequate in that the vitamin and mineral content met or exceeded National Research Council requirements. The foods were also tested for palatability and stability. Each was labeled in code prior to initiation of the study, and neither owners nor investigators were aware of which food dogs received. Owners maintained detailed records of daily food consumption, signs of toxicosis associated with treatment (chemotherapy), clinical signs associated with the tumor, and quality of life.

Dogs were fed the specified diet for a minimum of 6 weeks to allow for fatty acid washout. Fatty acid washout represents the time necessary for elution and metabolism of fatty acids from body lipids (such as cell membranes) as determined by the quantification of fatty acid concentrations in particular compartments (such as blood plasma) and the waning of biological effects associated with those fatty acids. In lieu of direct measurements of tissue-specific cell membrane or eicosanoid concentrations, blood plasma concentrations of fatty acids are frequently used as a gross measure of fatty acid turnover after a dietary change. Serum PUFA concentrations in dogs were assessed at the time of each pharmacokinetic evaluation to confirm owner compliance with feeding the prescribed diet.

**Treatment and assessment protocol**—Dogs received doxorubicin every 3 weeks for 5 treatments. For each treatment, doxorubicin (30 mg/m²) was diluted in 150 mL of saline (0.9% NaCl) solution and administered IV at a constant rate over 20 minutes. Pharmacokinetic variables were assessed twice, once at the first treatment and again 6 to 9 weeks later to permit fatty acid washout. On each occasion, serum samples were collected through a separate central venous catheter at 0, 10, 20, 25, 35, 50, 65, 80, 180, 240, and 360 minutes after initiation of the doxorubicin infusion. At the first treatment, 360 minutes after the beginning of the infusion, three 18-gauge needle core biopsy specimens were collected from an affected peripheral lymph node. Serum and tissue samples were frozen in cryovials at −20°C until assayed via HPLC for doxorubicin and doxorubicinol concentrations.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Soy oil</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>378</td>
<td>378</td>
</tr>
<tr>
<td>Fat</td>
<td>329</td>
<td>326</td>
</tr>
<tr>
<td>CHO</td>
<td>207</td>
<td>216</td>
</tr>
<tr>
<td>C20:5</td>
<td>0.1</td>
<td>29</td>
</tr>
<tr>
<td>C22:6</td>
<td>0.2</td>
<td>24</td>
</tr>
<tr>
<td>Linoleic acid</td>
<td>123</td>
<td>16</td>
</tr>
<tr>
<td>α-Linolenic acid</td>
<td>4.2</td>
<td>0.3</td>
</tr>
<tr>
<td>n-6/n-3 ratio</td>
<td>7.1</td>
<td>0.3:1</td>
</tr>
<tr>
<td>Total n-6</td>
<td>125</td>
<td>23</td>
</tr>
<tr>
<td>Total n-3</td>
<td>16</td>
<td>73</td>
</tr>
</tbody>
</table>

**Ingredients**—beef by-products, water, liver, rice, soybean or menhaden fish oil, chicken, cellulose, amino acids, minerals, and vitamins.

$\text{CHO} = \text{Carbohydrate}$. 

Table 1—Nutrient content (g/kg of dry matter) of diets that contained soy or fish oil and were fed to 19 dogs with lymphoma.
After collection of the biopsy specimens at the time of the first treatment, each dog received a single dose of l-asparaginase (10,000 U/m²) IM to increase remission rate.21 One week after the initial treatment, dogs were returned and a CBC was performed to assess toxicosis from chemotherapy; during that visit, dogs were anesthetized with propofol and a DEXA scan was performed to determine body fat. Dogs were positioned in sternal recumbency and scanned in single-beam mode, a technique that has been used for dogs in other pharmacokinetic studies.22 To minimize distortion of body composition values by lymphoma tissue, scans were performed when the disease was in clinical remission. Dogs were removed from the study or treatment protocol if clinical remission was not attained or maintained. At the time of the third or fourth scheduled chemotherapy treatment for dogs in which disease was still in remission, a second DEXA scan was performed in addition to collection of blood samples for the second pharmacokinetic assessment.

Pharmacokinetics—Standard pharmacokinetic variables were assessed and included AUC (ng/mL·h); CL (L/h/m²); t₁/₂α,β,γ (hours); Cmax (ng/mL); and Vss (L/m²). Dogs were analyzed for differences in those values between food groups and between the first and second pharmacokinetic assessments. Pharmacokinetic modeling and parameter estimation were performed with commercially available software.4

Doxorubicin extraction and HPLC assay—An extraction protocol modified from DeJong et al22 was used for determining serum and lymph node doxorubicin concentrations. Lymph node tissue was homogenized in water, and an aliquot of 100 µL of the homogenate or 100 µL of serum was transferred to a 1.5-mL polypropylene microcentrifuge tube; 30 µL of 1 M daunorubicin was added as an internal standard, and 0.6 mL of methanol was added. The samples were mixed (vortexed) for 15 minutes, 0.25 mL of 12 mM phosphoric acid was added, and samples were centrifuged at 10,000 × g for 8 minutes. The resulting supernatant was collected, the volume adjusted to 1.0 mL, and 100 µL was injected on the HPLC system.4

The HPLC system consisted of a pump module, autosampler, and fluorescence detector with excitation and emission wavelengths set at 480 and 580 nm, respectively. The mobile phase consisted of 15mM NaH₂PO₄ (pH, 4) and acetonitrile in a 2:1 volume-to-volume ratio at a flow rate of 1 mL/min. Separation was performed on a carbon 18 (5-µm), 250 × 4.6-mm column fitted with a carbon 18 cartridge. A standard curve was calculated with good linearity (R² = 0.9964 for doxorubicin and 0.9871 for doxorubicinol). Extraction efficiency was 0.73 ± 0.13 (mean ± SD).

Statistical analysis—Data were evaluated for normal distribution by means of Kolmogorov-Smirnov analysis. Groups were analyzed by use of the Fisher protected least significant difference test and ANOVA. For comparison of dogs in which remission was not achieved or maintained versus the study group, Mann-Whitney statistics were applied. Although those data were normally distributed, values for variance were not similar enough to qualify for parametric analyses. Values of P < 0.05 were considered significant. Data were analyzed with statistical software.4

Results

Nineteen dogs were enrolled in the study, 5 of which were of mixed breeding. Breeds represented more than once included Australian Cattle Dog (n = 2), Labrador Retriever (2), and Australian Shepherd (2). One dog from each of the following breeds comprised the rest of the group: Bull Terrier, Weimaraner, Bernese Mountain Dog, English Springer Spaniel, Golden Retriever, Cocker Spaniel, Rottweiler, and Miniature Schnauzer. There were 9 castrated male dogs, 8 spayed female dogs, and 2 sexually intact male dogs. Mean age was 7.3 years (range, 1.8 to 14 years), and mean body weight was 28.2 kg (range, 10.8 to 49.1 kg). There was no significant difference between groups with regard to weight (compared at baseline and second pharmacokinetic evaluation) or age.

All data were normally distributed. The model that best described the individual serum drug concentration-versus–time profiles was a 3-compartment open model with first-order drug elimination from the central compartment and a correlation coefficient > 0.9 for all profiles (Figure 1). Doxorubicinol, the predominant active metabolite of doxorubicin, was also evaluated, and no significant differences between groups were detected. Among all dogs, there were significant differences in the AUC, Cmax, and CL between the first and second analyses (Table 2). There was no difference in pharmacokinetic values between the treatment groups at baseline except Vss, which was greater in dogs that consumed the fatty acid-supplemented food than in control dogs (P = 0.019; power, 0.696). Pharmacokinetic values were not different between groups after ≥ 6 weeks on the diets (Table 3). There was a significant (for both PUFAs, P < 0.001; power, 1.00) difference between groups in blood PUFA concentrations (eicosapentaenoic acid [C20:5] and docosahexaenoic acid [C22:6]) after ≥ 6 weeks on the diet, confirming owner compliance in feeding the prescribed food.

In 7 dogs (3 fed the control food and 4 fed the test food), long-term remission was not achieved; of those dogs, 4 had complete data sets for comparison with dogs that did have long-term remission. Because an approximately equal number of test and control dogs failed to respond favorably to chemotherapy, it is unlikely that the food had a substantial impact on the absence of a sustained remission. Of the 7 dogs in this group, remission was never achieved in 1, whereas in the rest, remission had been achieved but there was progressive disease at the time of treatments 2 (1 dog), 3 (3), and 4 (2) prior

Figure 1—Serum concentrations (mean ± SD) of doxorubicin for the 6 hours after administration of the first and second treatments in 19 dogs with lymphoma that were fed a control or an n-3 fatty acid-supplemented diet. Because there was no difference in value for dogs fed the test and control diets, values from both groups were combined to indicate the difference between values from the first and second treatments in all dogs.
to the second pharmacokinetic analysis. Of the 4 dogs with complete data for comparison, 3 had relapsed at the third treatment and 1 was the dog in which clinical remission was never attained. The remaining dogs had continued remission throughout the rest of the treatment protocol. The mean ± SD t½ in dogs that had long-term complete remission (8.7 ± 4.2 hours) was significantly (*P = 0.006; power, 0.318) longer than in dogs that did not have remission (4.4 ± 0.7 hours).

Values for AUC and Cmax were greater and for CL were less in dogs that had long-term remission (a finding that was consistent with increased drug exposure), but the differences were not significant. Lymph node concentrations of doxorubicin after 6 hours were not different between groups (dogs that had remission, 6.04 ± 4.08 µg of doxorubicin/g of tissue; dogs that did not have remission, 8.70 ± 4.79 µg/g of tissue), and there was no difference between dogs with remission and those without remission in the serum concentration-to-lymph node concentration ratio at the 6-hour time point (*P = 0.264; power, 0.175). No correlation was detected between lymph node concentrations of doxorubicin at 6 hours after treatment and AUC or Cmax (*P = 0.4 and 0.7, respectively). Additional comparisons of serum versus lymph node concentration at various time points were not performed because the values for concentration in the node were not correlated with treatment success. Because the goal of our study was to evaluate alterations in pharmacokinetics and not to correlate drug pharmacokinetics with outcome, remission duration and survival time were not recorded. Additionally, dogs did not receive the study food after the second pharmacokinetic assessment, and owners pursued a variety of rescue protocols to reinforce remission; survival analysis was therefore not meaningful under the conditions of the study.

Data obtained by means of DEXA analysis included percentage of body fat and lean body mass; there were no differences between treatment groups or among all dogs between values from the first and second doses (Table 4). There was no significant change in lean body mass or percentage of body fat among all dogs over time. When dosages were converted to milligram per kilogram of body weight and to milligram per kilogram of lean body mass and compared, there was a strong correlation (r, 0.782; *P < 0.001), indicating that there was no advantage in assessing pharmacokinetic values on the basis of lean body mass. Drug dosages with either calculation were not correlated with measured pharmacokinetic variables. Therefore, differences in drug pharmacokinetics between the first and second doses were not a result of changes in body composition or dosing. Percentage of body fat was not correlated with pharmacokinetic variables.

Table 2—Mean ± SE Pharmacokinetic variables assessed after first administration of doxorubicin and 6 to 9 weeks later in 19 dogs with lymphoma.

<table>
<thead>
<tr>
<th>Analysis time</th>
<th>AUC (ng/mL-h)</th>
<th>Cmax (ng/mL)</th>
<th>Terminal t½ (h)</th>
<th>Vss (L/kg or L/m²)</th>
<th>CL (L/h/kg or L/h/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After first treatment</td>
<td>427 ± 44</td>
<td>879 ± 83</td>
<td>8.7 ± 3</td>
<td>703 ± 50</td>
<td>83 ± 8</td>
</tr>
<tr>
<td>9 weeks</td>
<td>621 ± 46</td>
<td>1067 ± 72</td>
<td>10.8 ± 1.2</td>
<td>639 ± 42</td>
<td>52 ± 3</td>
</tr>
</tbody>
</table>

Values are normalized to body surface area.

Table 3—Mean ± SD pharmacokinetic variables of doxorubicin in dogs with lymphoma that received a control diet that contained soy oil or a diet supplemented with n-3 fatty acids that contained fish oil.

<table>
<thead>
<tr>
<th>Food</th>
<th>AUC (ng/mL-h)</th>
<th>Cmax (ng/mL)</th>
<th>t½ (h)</th>
<th>Vss (L/m²)</th>
<th>CL (L/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soy oil</td>
<td>599.6 ± 139.5</td>
<td>1,160.4 ± 365.7</td>
<td>9.2 ± 3.3</td>
<td>565.4 ± 192.4</td>
<td>51.8 ± 8.7</td>
</tr>
<tr>
<td>Fish oil</td>
<td>657.5 ± 241.8</td>
<td>951.1 ± 159.5</td>
<td>12.6 ± 6.2</td>
<td>720.8 ± 112.8</td>
<td>52.1 ± 17.9</td>
</tr>
</tbody>
</table>

Table 4—Variables (mean ± SE) associated with dose of doxorubicin and body composition in dogs with lymphoma that were fed a diet supplemented with soy oil or fish oil.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Soy oil PK 1</th>
<th>Soy oil PK 2</th>
<th>Fish oil PK 1</th>
<th>Fish oil PK 2</th>
<th>All dogs PK 1</th>
<th>All dogs PK 2</th>
<th>P value (power)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose (mg/kg)</td>
<td>1.05 ± 0.05</td>
<td>1.04 ± 0.04</td>
<td>1.03 ± 0.07</td>
<td>1.08 ± 0.08</td>
<td>1.04 ± 0.04</td>
<td>1.06 ± 0.04</td>
<td>0.48 (0.10)</td>
</tr>
<tr>
<td>Lean body mass (kg)</td>
<td>17.8 ± 1.3</td>
<td>17.9 ± 2.1</td>
<td>17.4 ± 3.1</td>
<td>18.6 ± 3.6</td>
<td>17.4 ± 1.9</td>
<td>18.2 ± 2.0</td>
<td>0.79 (0.08)</td>
</tr>
<tr>
<td>Dose (mg/kg)</td>
<td>1.6 ± 0.03</td>
<td>1.42 ± 0.08</td>
<td>1.54 ± 0.12</td>
<td>1.51 ± 0.15</td>
<td>1.57 ± 0.07</td>
<td>1.46 ± 0.07</td>
<td>0.19 (0.23)</td>
</tr>
<tr>
<td>Lean body mass (%)</td>
<td>21.7 ± 2.4</td>
<td>21.3 ± 2.5</td>
<td>21.3 ± 3.0</td>
<td>21.8 ± 3.0</td>
<td>21.5 ± 1.7</td>
<td>21.4 ± 1.9</td>
<td>0.92 (0.05)</td>
</tr>
</tbody>
</table>

*P value (power) for all dogs regarding PK 1 versus PK 2. PK 1 = Pharmacokinetic data obtained at the time of the first doxorubicin treatment. PK 2 = Pharmacokinetic data obtained at the time of the third or fourth doxorubicin treatment.
Discussion

In the study reported here, the pharmacokinetics of doxorubicin in dogs with spontaneously occurring lymphoma were significantly different at baseline from the pharmacokinetics after induction of remission. Once the disease was in remission, AUC and $t_{1/2}$ were increased and CL was decreased, consistent with increased drug exposure in the tissues. Also, dogs in which remission was achieved and maintained had significantly longer $t_{1/2}$ than did dogs without remission, suggesting improved response to chemotherapy with increased drug exposure in tissues. This underscores the importance of ascertaining which patient and disease characteristics affect doxorubicin pharmacokinetics so that dosage regimens can be derived that will result in more uniform tissue concentrations and treatment response and permit avoidance of adverse effects.

The $t_{1/2}$ was longer in dogs in which complete remission was achieved and maintained than in those in which long-term remission did not result, suggesting a role for drug pharmacokinetics in predicting treatment response for dogs with lymphoma. In theory, therapeutic drug monitoring is most helpful with drugs that have substantial interindividual variability. This could be achieved with the use of pharmacokinetic computer models known to have strong correlation with actual patient data. Alternatively, limited sampling times (multilinear regression) or Bayesian estimation could be investigated to determine whether 1 or 2 blood samples could be used to predict a patient’s drug exposure and subsequent response to chemotherapy and help determine the future dosage of a given drug. Therapeutic drug monitoring of chemotherapy and help determine the future dosage of a given drug. Therapeutic drug monitoring of chemotherapy drugs is not commonly used in clinical patients but may be useful in treatment planning. If therapeutic blood concentrations of drugs are not achieved, patients may fail to respond to treatment because of pharmacokinetic variation rather than because of drug resistance.

Models exist for modification of drug dosing. In our dogs, the concentration of doxorubicin achieved in the lymph node 6 hours after injection was not correlated with whether remission was achieved or maintained. This finding suggests that serum or plasma drug concentrations may be more important than tissue drug concentrations in the use of pharmacokinetics for treatment planning.

The pharmacokinetics of doxorubicin in dogs have been described with 2-compartment and 3-compartment models. The number of compartments used in a model should reflect the lowest number required to explain experimental data. Use of too few compartments may underestimate patient drug exposure, whereas use of too many may subject patients to unnecessary sampling without a substantial change in results. Three-compartment modeling, as was used in our study, more accurately depicts the pharmacokinetics of doxorubicin, a conclusion that is supported by results of other published studies and makes comparison with studies that used 2-compartment models impossible.

One problem with the calculated terminal half-life ($t_{1/2}$ for a 3-compartment model) was that our final sampling point (6 hours postinfusion) was inside the model-derived terminal half-life. It has been recommended that samples should be collected at time points 3 to 5 times the estimated $t_{1/2}$ for accurate estimation of this variable. With that guideline, we would have collected samples between 24 and 48 hours after dosing; although such sampling is common in pharmacokinetic studies in humans, we felt case enrollment would be affected if prolonged hospital stays and the collection of additional blood samples were required. Previous studies have revealed benefits and limitations of extended sampling times on calculation of $t_{1/2}$ for doxorubicin and docetaxel. Furthermore, for the purpose of comparison, in most previous studies, sampling times of 2 to 8 hours after drug infusion were used to estimate doxorubicin pharmacokinetics. Although the values we calculated for terminal half-life may be imperfect, the fact that the values were significantly different between dogs with remission and dogs without remission suggests that the calculation is useful. Our study underscores the importance of using 3-compartment modeling in the determination of doxorubicin pharmacokinetics.

Metabolism of doxorubicin varies among patients, and endogenous factors such as enzyme induction may be related to this variability. Doxorubicin is distributed from blood to tissues, reaches equilibrium concentrations with the tissue, and is cleared from tissues via liver metabolism and biliary excretion. Changes in enzyme activity or other metabolic changes as a result of serial dosing are not likely to be the cause of the significant changes in pharmacokinetic variables that we observed. In a study of healthy dogs, there was no change in pharmacokinetic variables measured between 2 doses separated by 3 weeks. In another study in which healthy dogs received doxorubicin every 3 weeks for 3 treatments, there were no differences in pharmacokinetic values among the 3 infusions. Concurrent administration of phenobarbital, an inducer of cytochrome P-450 enzymes, decreases doxorubicin plasma concentration and increases metabolism, suggesting that enzyme induction may affect pharmacokinetic measurements.

In addition to inherent interindividual variability in doxorubicin pharmacokinetics, our data may also have been influenced by the administration protocol. It is logical that the longer infusion time resulted in a lower $C_{max}$ and higher AUC than in previous studies. In our study, dogs were treated over a 20-minute period to allow standardization of blood sampling times for analysis. Furthermore, the method of dosing (ie, on the basis of square meters of body area vs kilogram of body weight) may have influenced pharmacokinetic calculations. In this case series, there was no difference between groups when final dosages were evaluated in terms of milligram per square meter, milligram per kilogram, or milligram per kilogram of lean body mass as determined by DEXA scans. Furthermore, there were no correlations between any pharmacokinetic variables and the dosages expressed in milligram per kilogram or milligram per kilogram of lean body mass, a finding that contrasted with results of a previous study.
in which there were strong correlations between all pharmacokinetic variables and the milligram per kilogram dosage. We did not examine the effect of rate of drug infusion in our dogs because all dogs received the drug over the same duration.

The initial intent of the DEXA scan was to determine whether body composition (specifically body fat) would change significantly in response to a new diet and whether body fat content would have an impact on the pharmacokinetics of doxorubicin. Neither body fat content at the first treatment nor change in body composition significantly affected our results. This is an important finding because obesity in humans may double the half-life of doxorubicin.6

Doxorubicin dose is determined on the basis of body surface area as an estimation of metabolically active body size, but the calculation in dogs was made almost exclusively on the basis of body weight. Fat is not metabolically active, so an overweight dog may be at risk of relative overdose if the traditional meter-squared dosage system is used. Our data indicated that the changes in body composition that occurred between pharmacokinetic assessments were not a confounding variable for the results reported here. Also, either the dogs in this series were not obese enough to have altered doxorubicin pharmacokinetics, the sample size was too small to detect differences in pharmacokinetics on the basis of body fat content, or obesity is not as important in dogs as it is in humans.

The lack of significant differences in the comparisons in our study may be related to the variables discussed. Furthermore, the power of the study for insignificant findings was low. It is possible that with a larger sample size, more robust associations would have been detected.

Analysis of PUFAs in the blood confirmed that there was owner compliance with feeding. Food rich in both docosahexaenoic acid and eicosapentaenoic acid was used in this study. Results of recent research suggest that docosahexaenoic acid may be the more important of the 2 types of fatty acids (eg, it is a more potent vasorelaxant, induces apoptosis, and has greater effects on nitric oxide synthase translation and cyclooxygenase inhibition activity); therefore, future investigations into the beneficial effects of n-3 fatty acids should include isolation and separate analysis of these subtypes of fatty acids.38

In our study, only the fatty acid content of the food was manipulated. Additional investigations into the variability of doxorubicin pharmacokinetics should include assessment of diets with various amounts of protein, carbohydrate, and fat. Mechanisms associated with n-3 fatty acids other than alterations in drug pharmacokinetics should be investigated, such as modulation of pharmacodynamics. There is some evidence that the beneficial effects are related to pro-oxidant action; in 1 study,28 benefit was reversed with addition of antioxidants such as vitamin E. The foods used in our study were identical in vitamin E content, but future studies should focus on antioxidant content of the food.

References


