Effects of diet on pharmacokinetics of phenobarbital in healthy dogs

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Objective—To determine effects of various diets on the pharmacokinetics of phenobarbital and the interactive effects of changes in body composition and metabolic rate.

Design—Prospective study.

Animals—27 healthy sexually intact adult female Beagles.

Procedure—Pharmacokinetic studies of phenobarbital were performed before and 2 months after dogs were fed 1 of 3 diets (group 1, maintenance diet; group 2, protein-restricted diet; group 3, fat- and protein-restricted diet) and treated with phenobarbital (approx 3 mg/kg [1.4 mg/lb] of body weight, PO, q 12 h). Pharmacokinetic studies involved administering phenobarbital (15 mg/kg [6.8 mg/lb], IV) and collecting blood samples at specific intervals for 240 hours. Effects of diet and time were determined by repeated-measures ANOVA.

Results—Volume of distribution, mean residence time, and half-life (t 1/2) of phenobarbital significantly decreased, whereas clearance rate and elimination rate significantly increased with time in all groups. Dietary protein or fat restriction induced significantly greater changes: t 1/2 (hours) was lower in groups 2 (192.4 ± 47.5) and 3 (202.0 ± 98.2) than in group 1 (125.0 ± 47.5). Phenobarbital clearance rate (ml/kg/min) was significantly higher in group 3 (0.22 ± 0.05 ml/kg/min) than in groups 1 (0.17 ± 0.03) or 2 (0.18 ± 0.03). Induction of serum alkaline phosphatase activity (U/L) was greater in groups 2 (192.4 ± 475 U/L) and 3 (202.0 ± 98.2) than in group 1 (125.0 ± 475).

Conclusions and Clinical Relevance—Clinically important differences between diet groups were observed regarding pharmacokinetics of phenobarbital, changes in CBC and serum biochemical variables, and body composition. Drug dosage must be reevaluated if a dog’s diet, body weight, or body composition changes during treatment. Changes in blood variables that may indicate liver toxicity caused by phenobarbital may be amplified by diet-drug interactions. (J Am Vet Med Assoc 2000;217:847–852)

Epilepsy constitutes 3 to 5% of all diseases seen in dogs.15 Because a specific cause for epilepsy is often not identified (idiopathic epilepsy), treatment is directed at controlling recurrent seizures. Phenobarbital is considered the drug of choice for the treatment of epilepsy because of its efficacy, convenient dosing regimen, low cost, and relative safety.1 It is routinely administered daily for long periods, often for the life of the dog.

Pharmacokinetics of phenobarbital may be difficult to predict in individual dogs because of the induction by phenobarbital of the enzymes responsible for its own hepatic metabolism.5–3 Although serum concentrations of phenobarbital correlate with therapeutic benefit in some studies, phenobarbital dosage often does not.16 Therefore, the dosage of phenobarbital administered is often not predictive of serum concentrations. Variability and unpredictability of serum phenobarbital concentrations can have serious clinical consequences such as increased frequency or severity of seizures when serum concentrations decrease to subtherapeutic values. Loss of seizure control can lead to owner frustration and euthanasia of the dog or augmentation of the dose to toxic values in an attempt to regain control. Chronic adverse effects of high serum phenobarbital concentrations include increased serum activities of hepatic enzymes in 50 to 80% of cases,17 altered response to adrenocortical function testing in 100% of cases studied,12 decreased serum concentrations of total and free thyroxine,1 and overt hepatotoxicosis in up to 15% of cases.19

Many studies have demonstrated the effects of dietary manipulation on hepatic metabolism, drug disposition, and hepatic microsomal enzyme activity in humans and rodents.10–17 Likewise, it is well-known that body composition and metabolic rate influence drug distribution and metabolism.16,19 Although it is recognized that many factors may affect the pharmacokinetics of phenobarbital,1,5,19 to our knowledge, dietary interactions (apart from the effects of feeding and fasting16) have not been examined in dogs. Diet formulation, body composition, and metabolic rate may have considerable effects on phenobarbital distribution, metabolism, and, subsequently, clinical efficacy and toxicosis in dogs with epilepsy. The purposes of the study reported here were to ascertain the effects of various diets on the pharmacokinetics of phenobarbital in healthy dogs and to determine potential interactive effects of changes in body composition and metabolic rate induced by various diets.

Materials and Methods

Dogs—Twenty-seven clinically normal sexually intact adult female Beagles weighing between 7.7 and 11.6 kg (3.5...
to 5.3 lb) were acquired from a commercial supplier and were housed in groups of 3 at the Colorado State University Veterinary Teaching Hospital. Dogs were acclimated for 2 weeks during which they were fed a commercially available maintenance dry food. Water was provided ad libitum. During this period and throughout the subsequent study period, body weights were recorded weekly. Food consumption was estimated weekly per pen of 3 dogs by weighing full feeders at the start of each week and then reweighing each feeder prior to being refilled at the end of the week. The difference represented the weight in kilograms of food consumed per week in each pen of 3 dogs. Dogs were determined to be healthy by physical examination, results of CBC, analyses of serum concentrations of glucose, urea nitrogen, creatinine, phosphorus, calcium, total protein, albumin, globulin, cholesterol, total bilirubin, sodium, potassium, chloride, and analyses of serum activities of alanine transaminase, aspartate transaminase, alkaline phosphatase (ALP), and γ-glutamyltransferase.

**Study design**—Baseline pharmacokinetics studies of phenobarbital were performed after the 2-week acclimation period. Following a 24-hour period in which food was withheld, dogs were given a single bolus of phenobarbital sodium (130 mg/ml, IV). Blood samples were collected by jugular or cephalic venipuncture at 0, 0.25, 0.50, 0.75, 1, 2, 4, 6, 12, 24, 48, 96, 144, 192, and 240 hours after phenobarbital administration. Blood samples obtained at 24 hours and beyond were collected after a 24-hour period in which food was withheld. Serum for phenobarbital analysis was obtained from blood collected after a 24-hour period in which food was withheld. Serum phenobarbital concentrations were obtained from blood collected into sterile collection tubes and allowed to clot at room temperature; the whole blood was centrifuged, and serum was removed and stored at −20°C until it was assayed for phenobarbital concentration.

Dogs were randomly assigned to 1 of 3 dietary treatment groups and fed 1 of 3 commercially available diets for 2 months: maintenance (diet 1/group 1; n = 9); low protein (diet 2/group 2; n = 9); or low fat and low protein (diet 3/group 3; n = 9) [Table 1]. Dogs in each pen (n = 3) were fed ad libitum from a group feeder and allowed to clot at room temperature; the whole blood was centrifuged, and serum was removed and stored at −20°C until it was assayed for phenobarbital concentration.

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**Table 1**—Nutrient content of 3 diets fed to healthy adult female Beagles

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Diet 1*</th>
<th>Diet 2*</th>
<th>Diet 3*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>g/100g DM*</td>
<td>g/100 kcal</td>
<td>g/100g DM*</td>
</tr>
<tr>
<td></td>
<td>21.8</td>
<td>4.8</td>
<td>14.6</td>
</tr>
<tr>
<td>Creatine</td>
<td>21.1</td>
<td>4.6</td>
<td>19.0</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>50.4</td>
<td>11.1</td>
<td>60.9</td>
</tr>
<tr>
<td>Crude fiber</td>
<td>2.50</td>
<td>0.5</td>
<td>1.40</td>
</tr>
<tr>
<td>Energy density (kcal/kg)</td>
<td>4195</td>
<td>2938</td>
<td>2927</td>
</tr>
</tbody>
</table>

*Dry Matter basis.
of butorphanol (10 mg/ml), 0.5 mg of acepromazine\textsuperscript{c} (10 mg/ml), and 0.4 mg of atropine sulfate\textsuperscript{c} by subcutaneous injection. A catheter\textsuperscript{f} was placed in the cephalic vein, anesthesia was induced with propofol\textsuperscript{a} (5 mg/kg, IV, titrated to effect), and anesthesia was maintained with isoflurane (1 to 2\%). Dogs were positioned in sternal recumbency with hind limbs extended caudally, and DEXA measurements were performed by use of a whole body scanner\textsuperscript{a} operated in single beam mode, as described.\textsuperscript{24} Calibration of the instrument was verified by scanning a calibration phantom. Commercially available software\textsuperscript{a} was used to analyze scans. Scans took approximately 10 minutes and were performed in duplicate if any gross movement was detected. These data were obtained: mean coefficients of variation for DEXA scanning in quadruplicate measurements from each of 2 dogs, total bone mineral density, total bone mineral content, body fat content (g), and lean-tissue content (g).

Statistical analyses—Pharmacokinetics data (such as AUC, mean residence time [MRT], Ke, Cl, Vd\textsubscript{ss}, and half-life \(t_{1/2}\))\textsuperscript{d} for phenobarbital, values derived from CBC and serum biochemical analyses as well as indirect calorimetry and DEXA data (such as REE and percentage of body fat) were subject to statistical analysis. A modified Kolmogorov-Smirnov test was used to assess normality of distribution of data, and a Bartlett test of homogeneity was used to assess equality of variances among groups to determine the distribution characteristics and whether the actual data could be used for parametric analysis. All parameters were normally distributed and were compared between groups by 2-way ANOVA for animal and treatment effects with repeated-measures for time effects. The Fisher least significance difference test was used to identify individual group and time differences. Values of \(P < 0.05\) were considered significant.

**Results**

Feed intake was 26.7 ± 4.3, 25.9 ± 2.1, and 32.9 ± 4.4 g/kg/24 h in groups 1, 2, and 3, respectively, and was significantly higher in dogs fed diet 3 than in those fed the other 2 diets. Caloric intake was 196.6 ± 31.5, 180.8 ± 13.9, and 174.4 ± 22.8 kcal/kg\textsuperscript{0.75}/24 h in groups 1, 2, and 3, respectively, and was not significantly different among the 3 groups of dogs.

Mean (± SD) values for all CBC, serum biochemical analyses, and pharmacokinetic variables prior to phenobarbital treatment (when dogs were consuming the maintenance diet) were not significantly different between dogs. Following the 2-month treatment period, significant differences were detected in a number of CBC, serum biochemical, and pharmacokinetic values. These differences were observed within dietary treatment groups as a function of time (comparing values obtained before and after treatment) and between dietary treatment groups in the posttreatment period.

Group 3 dogs had significant decreases between the pre- and posttreatment RBC count, PCV, and hemoglobin concentration. Significant differences were detected in PCV and RBC counts between groups 1 and 3 and groups 2 and 3, and in hemoglobin values between groups 1 and 3 after treatment with phenobarbital (Table 2).

Cholesterol concentrations in group 2 increased significantly in the posttreatment period. Posttreatment cholesterol concentrations were significantly different between groups 2 and 3, and groups 1 and 3 in the posttreatment period. A significant decrease in serum albumin concentration was observed in all groups over time, and significant differences were detected in the posttreatment period between groups 1 and 3 and groups 2 and 3 (Table 2).
Mean values for Vdss, MRT, and \( t_{1/2} \) decreased significantly with time in all groups (Table 3). Percentage decreases for Vdss were 17.3, 25.9, and 17.2% for groups 1, 2, and 3, respectively. The \( t_{1/2} \) values decreased by 32.6, 45.2, and 49.0% for groups 1, 2, and 3, respectively. The MRT values decreased by 32.6, 45.2, and 49.1% in groups 1, 2, and 3, respectively. Total body clearance and Ke increased significantly with time in each group; CI increased by 15.7, 25.6, and 40.3% in groups 1, 2, and 3, respectively, whereas Ke increased by 33.2, 50.6, and 49.8% in groups 1, 2, and 3, respectively.

Coefficient of variation for DEXA analyses was 3.61%. Although significant differences were not detected between groups in mean body weight at the time of DEXA analysis, percentage of body fat in group 3 was significantly lower than that for groups 1 and 2.

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Coefficient of variation for DEXA analyses was 3.61%. Although significant differences were not detected between groups in mean body weight at the time of DEXA analysis, percentage of body fat in group 3 was significantly lower than that for groups 1 and 2. Increased clearance of phenobarbital resulted in a decreased \( V_d \), and \( A_{UC} \) were also most notable in group 3 dogs, and changes in body composition caused by obesity and its associated metabolic effects or restricted food intake during weight loss can have considerable effects on drug metabolism in animals and humans. Changes in body composition caused by obesity and its associated metabolic effects or restricted food intake during weight loss can have considerable effects on drug metabolism in animals and humans. Changes in body composition caused by obesity and its associated metabolic effects or restricted food intake during weight loss can have considerable effects on drug metabolism in animals and humans. Changes in body composition caused by obesity and its associated metabolic effects or restricted food intake during weight loss can have considerable effects on drug metabolism in animals and humans.

Discussion

Epilepsy is an important disease affecting dogs, in which treatment is directed at controlling recurrent seizures. Phenobarbital has been the drug most commonly chosen for management of seizures. One of the problems encountered with phenobarbital use over time is that the amount of drug administered is often not predictive of serum concentration, which can lead to unexpectedly low serum phenobarbital concentrations. This may result in loss of seizure control or high serum phenobarbital concentrations that may contribute to toxicity or biochemical alterations that confound the practitioner's ability to monitor hepatic function.

All dogs in our study had significant changes in \( t_{1/2} \), MRT, Ke, and CI between the pre- and posttreatment periods during which phenobarbital was administered orally. These changes were likely an effect of induction of hepatic biotransformation enzyme activities by phenobarbital, as has been reported. This effect appeared to be exacerbated in groups 2 and 3, as evidenced by the greater magnitude of changes in \( t_{1/2} \), MRT, and Ke values, and may be attributed to dietary differences or diet-induced differences in body composition. Studies in rodents and humans demonstrate that manipulation of dietary nutrients such as protein and fat considerably alter hepatic microsomal enzyme activity, as well as the disposition of drugs metabolized hepatically. Although there is some variability in the specific effects reported, high-protein diets appear to enhance rates of cytochrome P450-catalyzed oxidative reactions in rodents and humans, whereas protein-deficient diets cause cytochrome P450-associated enzyme activities to decrease. Dietary lipids modulate activity of cytochrome P450 enzymes in rats and are reported to be essential for the optimal induction of cytochrome P450 enzymes by phenobarbital. However, the changes we observed suggested that in dogs, the low protein diet, as well as the low fat and low protein diet, may have increased hepatic enzyme induction and subsequent metabolism of the drug relative to the effects of the maintenance diet.

Dogs in our study that were fed the low-fat and low-protein diet consumed significantly more food of a lower energy density, which resulted in approximately the same caloric intake as dogs fed the maintenance diet or low-protein diet. Although our dogs were near or below ideal body weight, dogs fed the low-fat and low-protein diet appeared to lose body fat, compared with those fed the other diets. This change in body composition may have contributed to the differences we observed in pharmacokinetic variables. Changes in body composition caused by obesity and its associated metabolic effects or restricted food intake during weight loss can have considerable effects on drug metabolism in animals and humans. Changes in CI and AUC were also most notable in group 3 dogs, and these changes were significantly different from those for groups 1 and 2. Increased clearance of phenobarbital in the dogs fed the low-fat and low-protein diet also may reflect changes in the activity of the hepatic microsomal enzymes. Another hypothetical explanation might be that the significantly decreased body fat and lower serum albumin concentrations in group 3 relative to groups 1 and 2 resulted in a decreased \( V_d \), thereby accelerating the rate of clearance. If this explanation was valid, we would have expected the calculated \( V_d \) for group 3 to be lower than that for group 2, but this was not the case.

Because feeding the low-fat and low-protein diet resulted in a significant decrease in body fat, we hypothesized that group 3 dogs might also have an
altered resting metabolic rate. If so, this altered metabolic rate might result in a change in the pharmacokinetics of phenobarbital through associated changes in drug distribution or metabolism. However, no significant diet-induced differences were detected in REE; therefore, changes in pharmacokinetics variables cannot be attributed to alterations in metabolic rate.

It is possible that the different diets may have affected the bioavailability of phenobarbital when administered orally. Phenobarbital was administered in the morning, approximately 3 to 4 hours after the daily meal, and again following a fasting period, approximately 12 hours later. Diet 3 was lower in fat and protein and higher in dietary fiber content, which may have altered gastric emptying time or gastrointestinal transit time. Meals containing cellulose or wheat bran increase the frequency of postprandial contractions in dogs and may decrease duodenal flow and prolong gastrointestinal transit time. Fat content of a meal changes intragastric distribution of solid material, and larger amounts may retard gastric emptying. The higher fiber content of diet 3 may have impaired the absorption of phenobarbital by binding to it, increasing the luminal unstirred water layer, or promoting goblet cell mucus secretion. To our knowledge, interprandial effects of meals or diet composition on phenobarbital absorption in dogs have not been reported. However, unless prolonged differences in bioavailability cumulatively altered hepatic metabolism or whole body distribution of phenobarbital when administered orally, it is unlikely that this would play a role in diet-related differences in the pharmacokinetics of when phenobarbital was administered intravenously.

The net result of the changes in pharmacokinetics in the low-protein and low-fat diet group was serum phenobarbital concentrations that were significantly lower than the other two groups. Mean serum phenobarbital concentration in group 3 also was below reported therapeutic concentrations. Decreases in serum albumin and cholesterol concentrations are associated with phenobarbital administration in epileptic dogs; it is hypothesized that these decreases may be the result of phenobarbital-induced hepatic dysfunction. Serum albumin concentrations were decreased in all diet groups in our study, and this change was exacerbated in the low-protein and low-fat diet group. In this group, posttreatment serum albumin concentrations were significantly lower than those of the other groups. Possible explanations include diet- or drug-induced changes in hepatic metabolism or lower protein intake associated with the diet itself. Serum cholesterol concentrations in the low-protein diet group were significantly increased in the posttreatment period. This finding is in contrast to previously reported findings and may represent differences in dietary cholesterol intake, induction of cholesterol synthesis, or decreased hepatic removal of cholesterol. Previous studies have demonstrated this effect of increased dietary cholesterol intake on serum cholesterol concentrations. Altered hepatic metabolism, decreased protein intake, or both also may explain the significantly lower PCV, RBC, and hemoglobin concentrations in the posttreatment period in the low-protein and low-fat diet group, compared with the other diet groups. Increases in serum ALP activities that were detected in the posttreatment period were anticipated and are likely attributable to phenobarbital induction, as has been reported. Increases in ALP activity for the low-protein and low-fat diet group were greater than that of the maintenance group, which suggests a diet-associated effect on hepatocellular enzyme induction. Other studies have demonstrated this effect of restricted dietary protein intake on serum ALP activities.

Management of seizures in epileptic dogs with phenobarbital is largely based in part on empirical dose adjustments. Adjustments in dose are made with the goal of achieving target therapeutic serum concentrations of phenobarbital while minimizing toxic effects that are more commonly associated with high serum concentrations of the drug. This effort is easily confounded when factors that influence the pharmacokinetics of phenobarbital, such as diet, are not recognized by the clinician. Findings of the present study revealed that differences in diet or body composition do not substantially alter the pharmacokinetics of phenobarbital, and there is a significant effect of diet-drug interactions on CBC and serum biochemical concentrations commonly used to monitor general health. Consequently, diet and body composition changes (obesity or weight loss) should be taken into consideration by clinicians when contemplating phenobarbital dose adjustments, and serum phenobarbital concentrations should be monitored closely when changes in diet or body composition develop.

References


