**Serum Pancreatic Lipase Immunoreactivity Concentrations in Dogs Treated with Potassium Bromide and/or Phenobarbital**

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**CLINICAL RELEVANCE**

Potassium bromide, phenobarbital, or a combination of both is commonly used in the treatment of canine epilepsy. Several cases of clinical pancreatitis have been reported in dogs after treatment with potassium bromide, but the risk of elevated serum canine pancreatic lipase immunoreactivity concentrations in dogs treated with potassium bromide and/or phenobarbital has not previously been evaluated in a large group of dogs. This study suggests an increased risk for elevated serum canine pancreatic lipase immunoreactivity concentrations and possibly pancreatitis in dogs treated with potassium bromide or phenobarbital alone or in combination.

**INTRODUCTION**

Epilepsy is a common problem in dogs, with a reported incidence of 0.6% to 2.3%. For many years, phenobarbital was considered the treatment of choice for dogs with epilepsy. However, side effects such as hepatotoxicity and, more important, refractory epilepsy led to a search for new antiepileptic drugs and the rediscovery of potassium bromide (KBr) for the treatment of epilepsy in dogs.

KBr was first used in the treatment of epilepsy in humans by Locock in 1857. With the advent of newer antiepileptic drugs, KBr was used less frequently. Two decades ago, however...

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creatitis in dogs with epilepsy may be related to their treatment with antiepileptic drugs. Finally, in a recent study, 2 of 51 dogs (3.9%) treated with KBr and phenobarbital had histologically confirmed pancreatitis and others had a presumptive diagnosis of pancreatitis based on clinical signs and elevations of serum amylase and lipase activities.

Canine pancreatitis can have numerous etiologies or be associated with many risk factors, but it is often idiopathic. A large variety of pharmaceutical compounds have been implicated in causing pancreatitis in humans and dogs; more than 50 drugs and drug classes have been implicated in humans, although definitive proof for a causal relationship is often lacking. Suspected drugs commonly used in veterinary medicine include L-asparaginase, azathioprine, estrogen, furosemide, salicylates, sulfonamides, tetracyclines, thiazide diuretics, and vinca alkaloids. Corticosteroids are no longer believed to cause pancreatitis in humans, and there is little evidence that glucocorticoid administration causes pancreatitis in dogs.

A new diagnostic test, canine pancreatic lipase immunoreactivity (cPLI) concentration, has recently been developed and analytically validated. Serum cPLI concentration has been shown to be specific for being of exocrine pancreatic origin, and preliminary studies have suggested that it is also highly sensitive for the diagnosis of pancreatitis. The goal of this study was to measure serum cPLI concentrations in a large group of dogs treated with either KBr alone, phenobarbital alone, or a combination of KBr and phenobarbital and to

Exocrine pancreatic disease and pancreatitis have been reported in dogs treated with potassium bromide.
compare concentrations with those measured in healthy control dogs.

**MATERIALS AND METHODS**

Remnants of 337 serum samples submitted to the Texas Veterinary Medical Diagnostic Laboratory from October to December 2004 and from November 2005 to January 2006 for measurement of serum KBr and/or phenobarbital concentrations were collected. Serum phenobarbital and/or KBr concentrations are often tested repeatedly in dogs treated with antiepileptic medications, but only one sample per dog was entered into the study; repeat samples from the same patient were excluded from the study. All samples were stored at –80°C until analysis. The samples had been submitted by veterinary clinics throughout Texas and, thus, were thought to reflect the general population of dogs treated with antiepileptic drugs. KBr and phenobarbital concentrations were measured in serum using spectrophotometric methods (Shimadzu UV-160A, Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan, and Hitachi 911 Clinical Chemistry Analyzer, Roche Diagnostics Corporation, Indianapolis, IN, respectively).

Of the 337 samples, 219 were submitted for measurement of KBr concentration. Because KBr is most commonly used in combination with phenobarbital, these 219 serum samples were subsequently analyzed for phenobarbital concentration. For the remaining 118 samples, only measurement of serum phenobarbital concentration had been requested by the submitting veterinarian. The 337 samples were thus divided into three groups:

- **Group 1** \((n = 98)\): Samples from dogs classified as having been treated only with KBr based on undetectable serum phenobarbital concentrations \(< 5 \text{ ppm}\)
- **Group 2** \((n = 118)\): Samples from dogs classified as having been treated only with phenobarbital based on the submitting veterinarian’s request to assess only serum phenobarbital concentration
- **Group 3** \((n = 121)\): Samples from dogs classified as having been treated with both KBr and phenobarbital based on measurable serum concentrations of both analytes

An undetectable serum concentration of KBr could not be ascertained in all dogs in Group 2 because of the additional cost of assaying serum KBr concentrations in all serum samples used for this study. However, serum KBr concentrations were measured in all samples from Group 2 in which an increased serum cPLI concentration was detected, and all such samples analyzed had an undetectable serum KBr concentration.

In addition, serum samples from 74 healthy dogs previously used to calculate the reference range for the serum cPLI assay\(^{12}\) were used as control samples. The control dogs comprised 45 dogs belonging to several research colonies and 29 pet dogs; they were of different breeds and sexes, and all were adults (>1 year of age). None of the dogs had any clinical signs reported by the owner or animal caretaker, and all had an unremarkable physical examination.

Serum cPLI concentrations were measured using an in-house ELISA within 3 months after initial sample submission.\(^{12}\) The total number of dogs with a serum cPLI concentration greater than either the upper limit of the reference range \((102.1 \text{ µg/L})\)\(^{12}\) or the suggested diagnostic cutoff value for pancreatitis \((199.9 \text{ µg/L})\)\(^{13,17,18}\) was tabulated, and the number of dogs with concentrations above these limits was recorded for all three treatment groups.

**Statistical Analysis**

Serum cPLI and KBr concentrations were analyzed for possible correlation in Groups 1
and 3 individually and in all dogs treated with KBr. Similarly, serum cPLI and phenobarbital concentrations were analyzed for possible correlation in Groups 2 and 3 individually and in all dogs treated with phenobarbital.

Serum cPLI concentrations in the 74 healthy dogs and all three treatment groups were tested for normal distribution using the Kolmogorov–Smirnov test. Median serum cPLI concentrations were compared between the 74 healthy dogs previously described and the three treatment groups using a Kruskal–Wallis test. Median serum cPLI concentrations of each of the three treatment groups were individually compared with the median serum cPLI concentration of the healthy control group using a Mann–Whitney test. Additionally, the proportion of dogs in each group with serum cPLI concentrations above the upper limit of the reference range or above the diagnostic cutoff value for pancreatitis was compared with the proportion of control dogs with serum cPLI concentrations meeting the same criterion by use of Fisher’s exact test. Odds ratios, with their 95% CIs, for having serum cPLI concentrations either >102.1 µg/L or >199.9 µg/L were calculated for all dogs treated with an antiepileptic agent and for dogs belonging to Group 1, 2, or 3 compared with the controls. All statistical analyses were performed using a statistical software package (Prism4, GraphPad, San Diego, CA), and a P value of <.05 was considered statistically significant for all analyses.

RESULTS

Serum KBr, phenobarbital, and cPLI concentrations were measured in 219 samples originally submitted for assessment of serum KBr concentration. In 98 of these samples, serum phenobarbital concentrations were undetectable (<5.0 ppm; therapeutic range, 15 to 40 ppm), and the dogs from which these samples were collected were considered to have been treated with KBr alone (Group 1). In the remaining 121 of these 219 samples, serum phenobarbital concentrations were >5.0 ppm, and the dogs from which these samples were collected were considered to have been treated with a combination of KBr and phenobarbital (Group 3). Serum phenobarbital and cPLI concentrations were measured in the 118 samples for which only phenobarbital assessment had been requested by the submitting veterinarian (Group 2); in those samples in which serum cPLI concentration exceeded the upper limit of the reference range (n = 17), serum KBr concentrations were measured and found to be undetectable.

Serum cPLI concentration was above the upper limit of the reference range (102.1 µg/L) in 46 (13.6%) of 337 dogs (all dogs) and in 15 (15.3%) dogs in Group 1, 17 (14.4%) dogs in Group 2, and 14 (11.6%) dogs in Group 3 (Figure 1). Serum cPLI concentration was above the suggested diagnostic cutoff value for pancreatitis (199.9 µg/L) in 23 (6.8%) of 337 dogs and in 7 (7.1%) dogs in Group 1, 9 (7.6%) dogs in Group 2, and 7 (5.8%) dogs in Group 3 (Figure 1).

All four data sets (healthy dogs [controls], Group 1, Group 2, and Group 3) failed normality testing, and nonparametric methods were used for further analysis. There was no statistical difference in the median serum cPLI concentrations among the three treatment groups and the healthy control dogs using a Kruskal–Wallis test (P = .068). When using a Mann–Whitney test to compare the medians for two groups each, median serum cPLI concentration was not significantly different between 74 healthy control dogs (16.3 µg/L) and all 337 dogs evaluated (24.7 µg/L; P = .06) or dogs treated with phenobarbital alone (17.7 µg/L; P = .61). However, median serum cPLI concentration was significantly higher in dogs treated with KBr alone (31.6
µg/L; \( P = .03 \) and those treated with a combination of phenobarbital and KBr (26.2 µg/L; \( P = .02 \)) than in healthy controls.

There was no correlation between serum concentrations of cPLI and KBr in dogs treated with KBr alone (Group 1; Spearman \( r = 0.035; P = .73 \)) or all dogs treated with KBr (Groups 1 and 3; Spearman \( r = 0.1224; P = .07 \)). However, there was a weak positive correlation of serum cPLI and KBr concentrations in dogs treated with KBr and phenobarbital (Group 3; Spearman \( r = 0.2208; P = .02 \); Figure 2). There was no correlation between serum concentrations of cPLI and phenobarbital for dogs treated with phenobarbital alone (Group 2; Spearman \( r = 0.036; P = .70 \)), for dogs treated with KBr and phenobarbital (Group 3; Spearman \( r = 0.045; P = .63 \)), or for all dogs treated with phenobarbital (Groups 2 and 3; Spearman \( r = 0.059; P = .37 \)).

Odds ratios showed an increased risk for an elevated serum cPLI concentration above the upper limit of the reference range of 102.1 µg/L, which was statistically significant for all 337 dogs treated and for Groups 1 and 2 but not for Group 3 (Table 1). Odds ratios also showed an increased risk for an elevated serum cPLI concentration >199.9 µg/L in all three treatment groups compared with the healthy controls (Table 1). However, this increased risk did not meet the criterion for statistical significance.

**DISCUSSION**

Of the 337 dogs treated with KBr and/or phenobarbital, 23 (6.8%) showed a serum cPLI concentration above the suggested cutoff value of 199.9 µg/L for a diagnosis of pancreatitis. Unfortunately, because the samples were run blindly, further investigation as to whether the animals exhibited clinical signs of pancreatitis was not possible. We thus were unable to determine whether any of these dogs had clinical pancreatitis.

It has previously been speculated that KBr in combination with phenobarbital is a risk factor for pancreatitis in dogs. An increased risk for
pancreatitis in patients treated with phenobarbital alone has not been described previously. Therefore, it was interesting to note that the rates of elevated serum cPLI concentrations above the upper limit of the reference range and above the suggested cutoff value for pancreatitis were similar for the three treatment groups. Odds ratios for an elevated serum cPLI concentration were between 4.28 and 5.66 for the three treatment groups and the two levels of serum cPLI concentration, although statistical significance was reached only for serum cPLI concentrations above the upper limit of the reference range for all dogs treated and for dogs treated with KBr or phenobarbital alone. These findings would suggest an increased risk for elevated serum cPLI concentrations in patients treated with KBr and/or phenobarbital. The median serum cPLI concentration was higher in dogs treated with KBr, phenobarbital, or a combination of KBr and phenobarbital than in healthy dogs, but this increase reached statistical significance only for the dogs treated with KBr alone or in combination with phenobarbital. This can most likely be explained by an insufficient number of dogs to show a significant difference. However, it is also possible that there is no direct effect of phenobarbital therapy on serum cPLI concentration and that the elevated concentrations were caused by chance.

It should be noted that phenobarbital is known to induce the expression of some enzymes (e.g., UDP-glucuronosyltransferase or cytochrome P-450), and it is therefore possible that an elevation of serum cPLI concentration in dogs treated with phenobarbital and/or KBr could be related to such an induction of gene expression. However, we do not consider this possibility very likely because clinically relevant pancreatitis has previously been described in dogs that had been treated with KBr or a combination of KBr and phenobarbital. Further studies are necessary to exclude the in-

### TABLE 1. Odds Ratios for Elevated Serum cPLI Concentrations in Dogs Treated with Antiepileptic Drugs

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Odds Ratio</th>
<th>95% CI</th>
<th>Statistically Significant?</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Serum cPLI Concentration &gt;102.1 µg/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All dogs treated</td>
<td>5.05</td>
<td>1.20–21.27</td>
<td>Yes</td>
</tr>
<tr>
<td>Group 1: KBr only</td>
<td>5.66</td>
<td>1.26–25.53</td>
<td>Yes</td>
</tr>
<tr>
<td>Group 2: Phenobarbital only</td>
<td>5.33</td>
<td>1.20–23.74</td>
<td>Yes</td>
</tr>
<tr>
<td>Group 3: KBr and phenobarbital</td>
<td>4.28</td>
<td>0.95–19.37</td>
<td>No</td>
</tr>
<tr>
<td><strong>Serum cPLI Concentration &gt;199.9 µg/L</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All dogs treated</td>
<td>5.05</td>
<td>0.67–37.99</td>
<td>No</td>
</tr>
<tr>
<td>Group 1: KBr only</td>
<td>5.29</td>
<td>0.64–43.90</td>
<td>No</td>
</tr>
<tr>
<td>Group 2: Phenobarbital only</td>
<td>5.64</td>
<td>0.70–45.47</td>
<td>No</td>
</tr>
<tr>
<td>Group 3: KBr and phenobarbital</td>
<td>4.28</td>
<td>0.52–35.49</td>
<td>No</td>
</tr>
</tbody>
</table>

*This table shows the odds ratios and 95% CIs for elevated serum cPLI concentrations above the upper limit of the reference range (102.1 µg/L) and above the suggested cutoff value for pancreatitis (199.9 µg/L).
duction of gene expression of pancreatic lipase rather than pancreatic inflammation as a cause of elevations of serum cPLI concentration shown here.

Stability studies in our laboratory have shown that cPLI concentrations in serum samples stored at room temperature, refrigerated, or frozen at –20˚C or –80˚C are stable for at least 21 days. Based on the finding that serum cPLI concentrations were stable even at room temperature for at least 21 days, we believe that it is safe to assume that storage at –80˚C should not have affected the serum cPLI concentra-

One limitation of this study was the fact that samples could not be traced to the patient from which they originated. Because of this, the true disease status of the patients (i.e., whether they were appropriately treated for epilepsy) or any concurrent conditions or medications administered could not be excluded as factors contributing to the serum cPLI concentrations observed. It is important to note, however, that glucocorticoids, which are commonly administered to canine patients with epilepsy, have been shown not to affect serum cPLI concentrations in dogs.

Further studies are needed to assess serum cPLI concentrations in dogs with epilepsy that have not yet been treated with either KBr or phenobarbital. Also, further longitudinal studies are needed and are in progress to evaluate KBr and/or phenobarbital on the pancreas or associated with other factors (such as epilepsy itself). These findings would suggest that dogs treated with KBr and/or phenobarbital should be evaluated for pancreatitis, especially when compatible clinical signs are present.

### REFERENCES

7. March PA, Podell M, Sams RA. Pharmacokinetics and