Comparison of various doses of carbon 13-labeled aminopyrine for a carbon 13-labeled aminopyrine demethylation blood test in healthy dogs

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Objective—To determine an optimal dose of carbon 13 (13C)-labeled aminopyrine for use in a 13C-aminopyrine demethylation blood test in healthy dogs.

Animals—9 adult dogs.

Procedures—Food was withheld from each dog for 12 hours. A 2-mL baseline blood sample was obtained from each dog and placed into an evacuated tube containing sodium heparin. Carbon 13-labeled aminopyrine was administered IV at doses of 1, 2, 5, or 10 mg/kg. Additional blood samples (2 mL) were obtained and placed into evacuated tubes containing sodium heparin 30, 45, 60, and 75 minutes after 13C-aminopyrine administration. Hydrochloric acid was used to extract CO2 from blood samples. The extracted gas was analyzed by fractional mass spectrometry to determine the percentage dose of 13C administered as 13C-aminopyrine and recovered in extracted gas (PCD).

Results—Gross evidence of clinical adverse effects were not detected in any dog after administration of 13C-aminopyrine. The mean coefficient of variation (CV) for PCD was significantly lower than the mean CV for the summation of PCD values up to a given sampling time (CUMPCD). Mean PCD values among the 4 doses for each sample time were not significantly different. Administration of 13C-aminopyrine at a dose of 2 mg/kg resulted in the lowest interindividual variability.

Conclusions and Clinical Relevance—The PCD is superior to CUMPCD for the quantification of 13C-aminopyrine demethylation. Administration of 13C-aminopyrine at a dose of 2 mg/kg is appropriate for use in the 13C-aminopyrine demethylation blood test in healthy dogs. (Am J Vet Res 2006;67:1110–1114)
function. Results of numerous studies indicate that this test is clinically useful and that test results correlate with disease severity as assessed by histopathologic evaluation of biopsy specimens obtained from patients with hepatic cirrhosis and chronic hepatitis.

In contrast to humans, breath tests are difficult to perform in dogs and cats, and thus a blood-based test would be preferable for use in these animals. The principles of the carbon 13 (13C)-labeled aminopyrine demethylation blood test have previously been reported and are similar to those of the breath test used in humans. Aminopyrine is administered orally or IV and is demethylated in the liver by microsomal enzymes. As a result, the methyl groups produced are then oxidized to CO2. The CO2 diffuses into the vascular space, is eventually carried to the pulmonary alveoli, and is released into the expiratory air. By use of aminopyrine labeled with various carbon isotopes, either 13C or carbon 14 (14C), the amount of 13C or 14C derived from the labeled aminopyrine can be measured in the expired air (breath test) as either 13CO2 or 14CO2, respectively. For the breath test, the amount of 13CO2 or 14CO2 is determined as a percentage of the oral or IV dose of 13C-aminopyrine or 14C-aminopyrine administered that is recovered in expiratory air. Similarly, the blood test involves measuring the amount of 13CO2 extracted from blood samples.

Results of a preliminary study indicated that a 13C-aminopyrine demethylation blood test is technically feasible in dogs. In that study, 13C-aminopyrine administered orally at a dose of 2 mg/kg to healthy dogs resulted in a detectible increase in the PCD in all dogs. Another study performed to evaluate the demethylation kinetics of 13C-aminopyrine administered IV in healthy dogs and determine an appropriate parameter for quantification of aminopyrine demethylation. Results of that study indicated that IV administration of 13C-aminopyrine, as previously detected for oral administration, does not result in any gross clinical adverse effects. In addition, PCD was found to be an appropriate parameter for the quantification of aminopyrine demethylation. Results of that study also indicated that a single blood sample collected 45 minutes after IV administration of 13C-aminopyrine is sufficient for assessment of hepatic demethylating capacity in dogs.

The purpose of the study reported here was to determine the optimal dose of 13C-aminopyrine for use in a 13C-aminopyrine demethylation blood test in healthy dogs. We used a dose of 2 mg/kg as an arbitrary starting dose because this dose is commonly used for the aminopyrine breath test in humans. The ideal dose would be one that gives the lowest variability of PCD values in healthy dogs and is cost-effective for use in a clinical setting.

**Materials and Methods**

**Dogs**—Nine young healthy adult dogs (4 males and 5 females) were enrolled in the study. Breeds of dogs included Labrador Retriever (n = 3), Brittany (2), Pointer (1), German Shepherd Dog (1), and Siberian Husky cross (2). Dogs were owned by and housed at an animal research facility and remained in the care of this facility for the duration of the study. All dogs were determined to be healthy on the basis of results of physical examination, CBC, and serum biochemistry analyses. None of the dogs had a history of receiving drugs known to alter hepatic enzyme function. Dogs were closely monitored during and for several hours after each experimental period. The animal care staff monitored dogs for development of gross evidence of clinically adverse effects for several days after each experimental period. The study protocol was approved by the Animal Care and Use Committee at The IAMS Company (protocol No. 990022).

**Procedures**—The study was divided into 4 experimental periods in which 13C-aminopyrine was administered at doses of 1, 2, 5, or 10 mg/kg. During the first experimental period, 13C-aminopyrine administered at a dose of 2 mg/kg was evaluated, followed by evaluation of 13C-aminopyrine administered at doses of 5, 1, and 10 mg/kg. Each dog was given the same dose of 13C-aminopyrine during each experimental period. There was a 2-week resting period between each experimental period.

Food was withheld from each dog for 12 hours prior to the initiation of each experimental period. A 2-ml baseline blood sample was collected from each dog and placed into an evacuated glass tube containing sodium heparin. The 13C-aminopyrine was dissolved in deionized water, and the solution was sterilized by passage through a 0.1-μm pore-size syringe filter and stored in an amber glass bottle at 4°C until administered. The 13C-aminopyrine was administered IV at the dose that had been predetermined for each experimental period. Additional blood samples (2 ml each) were collected from each dog 30, 45, 60, and 75 minutes after 13C-aminopyrine administration and stored in evacuated tubes containing sodium heparin. Samples were stored at 22°C and shipped overnight to the Gastrointestinal Laboratory at Texas A&M University for analysis.

The CO2 was extracted from each blood sample by addition of 1 ml of 6N hydrochloric acid. Immediately after addition of the acid, samples were vortexed to prevent acid coagulation and to maximize CO2 release. Gas samples were then analyzed by use of fractional mass spectrometry using an automated breath-carbon analyzer to measure the fraction of 13CO2 in the CO2 extracted from blood samples. Mean ± SD and CV values were calculated as previously described. The PCD values for each sample time in each experimental period were compared by use of repeated-measures ANOVA and Dunnett multiple comparison tests. A statistical analysis package was used for data analysis; values of P < 0.05 were considered significant.

**Results**

Gross evidence of clinically adverse effects was not observed in dogs during, or for a period of several days after, each experimental period. The PCD values increased initially and then began to decrease with time for all dogs and all doses. Consequently, the mean PCD values for each dose also increased with time and reached a maximum at 30 minutes. The maximum observed PCD values remained constant for at least 60 minutes after administration. The CV values were calculated for PCD and CUMPCD for each dose. Mean CV values for PCD and CUMPCD for all doses and sample times were compared by use of a t test. The PCD values for the various doses were compared by use of repeated-measures ANOVA and Bonferroni's multiple comparison tests. A statistical analysis package was used for data analysis; values of P < 0.05 were considered significant.
peaked 45 minutes after administration of \(^{13}\)C-aminopyrine at doses of 1, 2, and 10 mg/kg and 30 minutes after administration of \(^{13}\)C-aminopyrine at a dose of 5 mg/kg (Figure 1). The CUMPCD values for all dogs and doses increased with time, and the mean CUMPCD values for each dose also increased with time (Figure 2).

As determined by repeated-measures ANOVA, the mean PCD values for all sample times differed significantly \((P < 0.001\) for all doses) with time for all 4 doses. When compared separately by use of the Dunnett multiple comparison test, mean PCD values after \(^{13}\)C-aminopyrine administration differed significantly \((P < 0.01\) for all 4 doses) from the baseline sample.

The mean ± SD CV \((21.5 ± 4.4\%)\) for the PCD value from all doses was significantly \((P < 0.01)\) lower than the mean ± SD CV \((23.8 ± 4.6\%)\) for the CUMPCD value for all the doses and sample times (Table 1). Therefore, the PCD value was used as an estimate of hepatic demethylation of \(^{13}\)C-aminopyrine for the remainder of the study.

No significant \((P = 0.41, 0.73, 0.58, \text{ and } 0.70)\) differences were detected in mean PCD values among the 4 doses (1, 2, 5, and 10 mg/kg, respectively) when compared by use of repeated-measures ANOVA. No dose was significantly \((P > 0.05\) for all comparisons) different from any other dose.

To compare interindividual variabilities, CV values were calculated for each dose. No significant \((P = 0.07)\) differences in the mean CV values for PCD between doses were detected. There were also no significant (all values of \(P > 0.05\)) differences when the mean CVs for PCD for individual doses were compared.

### Discussion

In the study reported here, gross evidence of clinically adverse effects was not observed in any dog during any experimental period. Although gross evidence of clinically adverse effects was not seen, subclinical adverse effects, such as subclinical organ damage, could not be definitively excluded. The safety of \(^{13}\)C-aminopyrine needs to be further evaluated in dogs with altered hepatic function.

A 2-week resting period was provided to dogs between experimental periods to eliminate any possible induction of hepatic demethylating enzymes caused by \(^{13}\)C-aminopyrine administration. We assumed that any microsomal enzyme induction that may have occurred after administration of a single dose of \(^{13}\)C-aminopyrine would have returned to baseline values during this 2-week resting period. To prove or
disprove this contention, measurement of microsomal enzyme activities in hepatic biopsy specimens would have been required. However, obtaining hepatic biopsy specimens in dogs used in our study was not possible because of animal welfare guidelines at the facility in which dogs were housed. Additionally, assays for hepatic microsomal enzyme function in dogs were not available.

Although the mean PCD value for $^{13}$C-aminopyrine administered at a dose of 5 mg/kg peaked earlier than that for $^{13}$C-aminopyrine administered at a dose of 2 mg/kg (as well as at the other 2 doses), we do not believe that this was caused by induction of hepatic microsomal enzyme function. If this were the case, we would have expected the mean PCD value for $^{13}$C-aminopyrine administered at a dose of 1 mg/kg to peak earlier than that for $^{13}$C-aminopyrine administered at a dose of 2 mg/kg and perhaps at a dose of 5 mg/kg. Because mean PCD values for $^{13}$C-aminopyrine administered at doses of 1 and 10 mg/kg did not peak earlier than or at the same time as that for $^{13}$C-aminopyrine administered at a dose of 2 mg/kg, we are confident in our assumption that hepatic microsomal induction did not lead to the delayed peak time for the PCD value detected after administration of $^{13}$C-aminopyrine at a dose of 5 mg/kg.

Data for $^{13}$C-aminopyrine administered at a dose of 2 mg/kg were collected during a kinetic study performed previously. The remaining 3 doses of $^{13}$C-aminopyrine were evaluated during various experimental periods so that doses were actually evaluated in the following order: 2, 5, 1, and 10 mg/kg. All dogs received the same dose during each experimental period to enhance the possibility of identifying potential adverse effects of repeated $^{13}$C-aminopyrine administration.

Intravenous administration of $^{13}$C-aminopyrine resulted in an increase in the PCD value of gas extracted from blood samples in all 9 dogs and for all experimental periods. For all 4 doses, the mean PCD value at each sample time after $^{13}$C-aminopyrine administration was significantly greater than the mean PCD value at baseline.

One of the parameters used to assess the potential clinical usefulness of a new diagnostic test is interindividual variability of that test in a group of healthy animals. This is based on the assumption that the lower the interindividual variability in healthy dogs, the easier it would be to differentiate between clinically healthy dogs and dogs with disease. Interindividual variability can be assessed by calculating CV. In the study reported here, the CV values for PCD and CUMPCD were calculated and compared. Results of our study indicated that the mean CV value for PCD was significantly ($P < 0.01$) lower than the mean CV value for CUMPCD. Taking into consideration the relative ease of collecting a single blood sample after administration of $^{13}$C-aminopyrine for determination of the PCD value, compared with collection of multiple samples as necessary for determination of CUMPCD, this finding indicates that determination of PCD is preferable to CUMPCD for assessment of hepatic $^{13}$C-aminopyrine demethylation. These findings are consistent with that of another study. However, whether determination of CUMPCD would be clinically more useful than determination of PCD in dogs with altered hepatic function cannot be definitively determined on the basis of results of the study reported here.

Significant differences in CV values among the 4 doses were not detected. However, $^{13}$C-aminopyrine administered at a dose of 2 mg/kg had the lowest CV value, compared with the other doses. As previously mentioned, a low interindividual variability in healthy dogs is desirable. Use of $^{13}$C-aminopyrine at a dose of 2 mg/kg would also be more cost-effective than use of $^{13}$C-aminopyrine at doses of 5 and 10 mg/kg because less $^{13}$C-aminopyrine is needed for the test. Thus, we concluded that administration of $^{13}$C-aminopyrine at a dose of 2 mg/kg is appropriate for use in the $^{13}$C-aminopyrine demethylation blood test in healthy dogs. Unfortunately, in the study reported here, dogs with hepatic dysfunction were not evaluated. Demethylation kinetics may be severely altered in dogs with hepatic dysfunction; therefore, administration of $^{13}$C-aminopyrine at a dose of 2 mg/kg may not be optimal for use in dogs with hepatic dysfunction. Additional studies are required to fully evaluate the clinical usefulness of a $^{13}$C-aminopyrine demethylation blood test in dogs with hepatic disease. An initial clinical study$^{15}$ has been reported since the completion of the study reported here, but further studies are required.

References


