Kinetic analysis of demethylation of 13C-aminopyrine in healthy dogs

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Objective—To describe the kinetics of demethylation of ^{13}C-aminopyrine in healthy dogs for use in determining the most appropriate time for collection of blood samples for a ^{13}C-aminopyrine demethylation blood test for evaluation of hepatic function.

Animals—9 healthy dogs.

Procedures—A 2-mL baseline blood sample was collected into an evacuated heparinized tube, and ^{13}C-aminopyrine was administered to each dog (2 mg/kg, IV). Additional 2-mL blood samples were collected 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 240, 300, and 360 minutes after ^{13}C-aminopyrine administration. The CO_2 was extracted from blood samples by addition of a strong acid, and the percentage dose of $^{13}CO_2$ (PCD) in the extracted gas was determined by fractional mass spectrometry.

Results—No dogs had gross evidence of adverse effects, and all had an increase in PCD after IV administration of ^{13}C-aminopyrine. The PCD had the least variability among 5 variables used to evaluate hepatic demethylating capacity. Peak PCD was detected at 30 minutes in 1 dog, 45 minutes in 5 dogs, 60 minutes in 2 dogs, and 75 minutes in 1 dog. The mean PCD for the 9 dogs peaked at 45 minutes after ^{13}C-aminopyrine administration.

Conclusions and Clinical Relevance—PCD appears to be the preferable variable for evaluation of hepatic demethylating capacity. Intravenous administration of ^{13}C-aminopyrine leads to a consistent increase in PCD. Mean PCD peaked 45 minutes after administration, suggesting that blood sample collection 45 minutes after ^{13}C-aminopyrine administration may be appropriate for use in estimating hepatic demethylating capacity. (*Am J Vet Res* 2004;65:159–162)

Hepatic disorders are common in dogs and cats. However, arriving at a definitive diagnosis is often difficult without performing examination of a hepatic biopsy specimen. Several diagnostic tools are available to evaluate the liver. Currently, no single diagnostic tool is available that is sensitive and specific for hepatic function.[1] Results of abdominal palpation and abdominal radiography may suggest hepatomegaly or, less commonly, microhepatica. However, an enlarged liver is not always associated with loss of hepatic function because nonhepatic disorders, such as hyperadrenocorticism, can lead to hepatomegaly without impairing hepatic function.[2] Although abdominal ultrasonography may reveal morphologic changes in hepatic structure, it does not give any indication of hepatic function.[3]

Serum activities of hepatic enzymes are often analyzed as markers for hepatocellular damage. However, some of these enzymes are also expressed in tissues other than the liver, and abnormalities in serum activities can arise from nonhepatic disorders without an effect on hepatic function.[4]

Several other tests have been developed to assess hepatic function. Indocyanine green and sulfobromophthalein have been used to evaluate hepatic perfusion and hepatobiliary function.[5] Tests that use these compounds have several disadvantages and are rarely used in dogs and cats. Another test, the ammonia tolerance test, measures the ability of the liver to extract and detoxify ammonia from the portal circulation.[6] This test requires the ability to measure serum ammonia concentration shortly after sample collection and is limited by its insensitivity in detecting hepatic insufficiency.

The only clinically useful hepatic function test that is currently available is the measurement of pre- and postprandial serum bile acid concentrations. However, this test is neither extremely sensitive or specific for identification of a particular liver disease.[7] For example, dogs with mild hepatic dysfunction may have serum bile acid concentrations within the reference range. In addition, dogs with cholestasis may have increased serum bile acid concentrations with initially unaltered hepatic function.[7] Furthermore, serum bile acid concentrations cannot be used to estimate the severity of hepatic disease.[7] Clearly, a hepatic function test that is sensitive and specific is needed for the clinical evaluation of hepatic function in dogs and cats.

In human beings, the **aminopyrine breath test (ABT)** is useful in quantifying hepatic microsomal enzyme function.[8-12] In a number of studies,[10-13] investigators have documented that the ABT is a useful diagnostic test to evaluate patients with hepatic cirrhosis and chronic hepatitis by use of histopathologic findings to correlate ABT results with the severity of hepatic disease.

A recent preliminary study revealed that a ^{13}C-aminopyrine demethylation blood test is technically feasible in dogs.[14] In that study, a dose of ^{13}C-aminopyrine (2 mg/kg) was administered orally to healthy dogs, and a detectable increase in the **percentage dose of ^{13}C administered as ^{13}C-aminopyrine and recovered in gas**

extracted from blood samples (PCD) was observed in each of those dogs. Thus, further development of a ^{13}C-aminopyrine demethylation test appears warranted. The next step in the evaluation of such a test is the study of the kinetic behavior of ^{13}C-aminopyrine to determine a suitable time point for collection of blood samples.

Studies[11,15,16] of the ^{13}C-ABT in human beings have revealed that oral and IV administration of ^{13}C-aminopyrine yield essentially the same results. In human beings, PCD and the summation of PCD values up to a given time point (ie, **cumulative PCD [CUMPCD]**) have traditionally been measured when assessing hepatic demethylating capacity.[14,17] Other variables that could be used to estimate demethylation of ^{13}C-aminopyrine are **dose over baseline (DOB)**, which is the difference between δ of a particular time point and δ at baseline (δ is the difference in the amount of ^{13}C in a gas sample compared to the amount of ^{13}C in a standard gas[17]), **DOB per kilogram of body weight (DOB/kg)**, and **DOB per kilogram$^{0.75}$ (DOB/kg$^{0.75}$**; ie, DOB per metabolic body weight).

The objectives of the study reported here were to determine whether IV administration of ^{13}C-aminopyrine causes an increase in PCD similar to that seen with oral administration of ^{13}C-aminopyrine, describe the kinetics for demethylation of ^{13}C-aminopyrine after IV administration in healthy dogs, and determine the variable for estimation of ^{13}C-aminopyrine demethylation that would vary the least among healthy dogs.

Materials and Methods

Animals—Nine young healthy adult dogs (4 males and 5 females) were enrolled in the study. Dogs comprised 3 Labrador Retrievers, 2 Brittany Spaniels, 1 Pointer, 1 German Shepherd Dog, and 2 Siberian Husky crossbred dogs. The dogs were part of a research colony owned by a pet food company,[a] and dogs were housed there throughout the study. All dogs were healthy as determined on the basis of results of physical examination, CBC counts, and serum biochemical analyses. Dogs were observed during the period when blood samples were collected and for several hours thereafter. The animal care team of the pet food company closely observed the dogs for several days after the study for gross evidence of adverse effects. The protocol was approved by the animal care and use committee of The IAMS Company (No. 990022).

Procedure—Food was withheld from all dogs for a 12-hour period. Then, a 2-mL baseline blood sample was collected into an evacuated tube that contained sodium heparin.[b] The ^{13}C-aminopyrine[c] was dissolved in deionized water, sterilized by filtration through a 0.1-μm pore-size syringe filter,[d] and stored in an amber glass bottle at 4°C. Each dog received an injection of ^{13}C-aminopyrine (2 mg/kg, IV; time 0). Additional 2-mL blood samples were collected 15, 30, 45, 60, 75, 90, 105, 120, 135, 150, 180, 240, 300, and 360 minutes after administration of ^{13}C-aminopyrine. Each sample was collected into an evacuated tube containing sodium heparin. Blood samples were stored at 22°C and shipped overnight to our laboratory at Texas A&M University. The CO_2 was extracted from each sample by addition of 1 mL of 6 N hydrochloric acid.[e] After addition of the acid, samples were immediately vortexed to prevent acid coagulation and promote maximum CO_2 release. Gas samples were then analyzed by fractional mass spectrometry by use of an automated breath-carbon analyzer[f] to determine the fraction of $^{13}CO_2$ in the extracted CO_2.

Data analysis—The PCD and CUMPCD were calculated as described elsewhere.[14,17] In addition, DOB, DOB/kg, and DOB/kg$^{0.75}$ were also calculated. Mean ± SD for all 5 measures of hepatic demethylation of ^{13}C-aminopyrine was calculated. **Coefficients of variation (CVs)** were also determined for all 5 variables.

The PCD values for each time point were compared with the value for the baseline sample by use of a repeated-measures ANOVA, performed by use of a statistical software package,[g] to determine whether there was a significant difference between the PCD for each time point and the baseline value. The Dunnett multiple-comparison test was used as a post hoc test to compare values for each time point with the baseline value. The CVs for the 5 variables were also analyzed by use of a repeated-measures ANOVA to determine whether there was a significant difference among variables. The CV for each variable was compared separately with the CVs for other variables by use of the Bonferroni multiple-comparison test. Significance for all tests was set at a value of $P < 0.05$.

Results

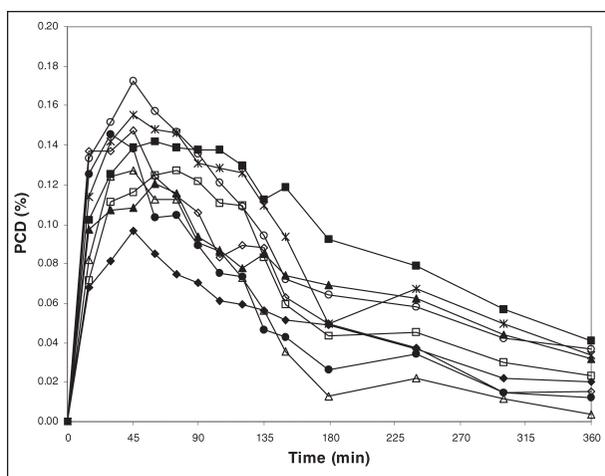None of the 9 dogs had gross evidence of adverse effects during the course of the study. Furthermore,

Figure 1—Percentage dose of ^{13}C administered as ^{13}C-aminopyrine and recovered in gas extracted from blood samples (PCD) after administration of ^{13}C-aminopyrine (2 mg/kg, IV) to 9 healthy dogs. Each symbol represents results for 1 dog. Time 0 = Time of administration of ^{13}C-aminopyrine.
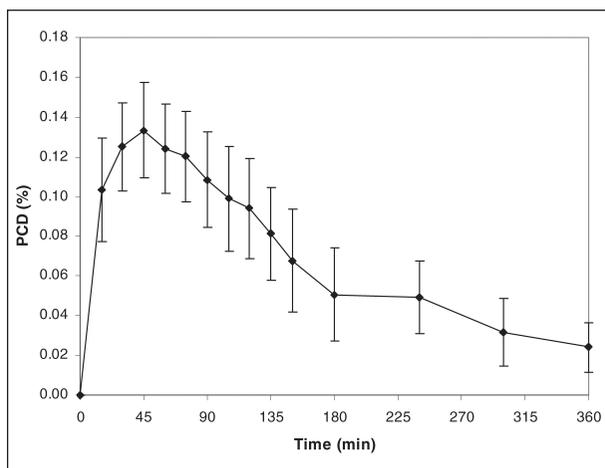

Figure 2—Mean ± SD PCD after IV administration of ^{13}C-aminopyrine to 9 healthy dogs.

monitoring revealed that the dogs did not have evidence of adverse effects for several days after the study.

The PCD in gas extracted from blood samples after IV administration of ^{13}C-aminopyrine increased in all dogs (**Fig 1**). Demethylation of ^{13}C-aminopyrine had a sharp peak followed by a slow tapered reduction in all 9 dogs. Peak PCD measured in gas extracted from blood samples was detected 30 minutes (1 dog), 45 minutes (5 dogs), 60 minutes (2 dogs), and 75 minutes (1 dog) after administration of ^{13}C-aminopyrine. Mean PCD reached a peak 45 minutes after IV administration of ^{13}C-aminopyrine (**Fig 2**). The CUMPCD continuously increased in each dog over time (**Fig 3**). Mean CUMPCD also increased over time (**Fig 4**).

The PCD differed significantly ($P < 0.001$) over time, as determined from results of the repeated-measures ANOVA. The PCD values at all time points after ^{13}C-aminopyrine administration differed significantly ($P = 0.01$) from the baseline value when compared separately by use of the Dunnett multiple-comparison test. Mean ± SD for PCD, CUMPCD, DOB, DOB/kg, and DOB/kg$^{0.75}$ were calculated for each time point (data not shown).

The CVs for the 5 variables differed significantly ($P < 0.001$), as determined from results of the repeated-measures ANOVA (**Table 1**). When compared separately, the CV for PCD did not differ significantly from those for CUMPCD, DOB, or DOB/kg but did differ significantly ($P < 0.001$) from the CV for DOB/kg$^{0.75}$, as determined by results of the Bonferroni multiple-comparison test. Mean CV for PCD at the peak time points (ie, 30, 45, 60, and 75 minutes) was lower than the mean CV for CUMPCD, DOB, DOB/kg, and DOB/kg$^{0.75}$ at those peak time points. The CV for CUMPCD differed significantly from the CV for DOB ($P < 0.001$), DOB/kg ($P < 0.05$), and DOB/kg$^{0.75}$ ($P < 0.001$). Also, the CV for DOB/kg$^{0.75}$ differed significantly ($P < 0.001$) from those for DOB and DOB/kg. The CV for DOB did not differ significantly from the CV for DOB/kg.
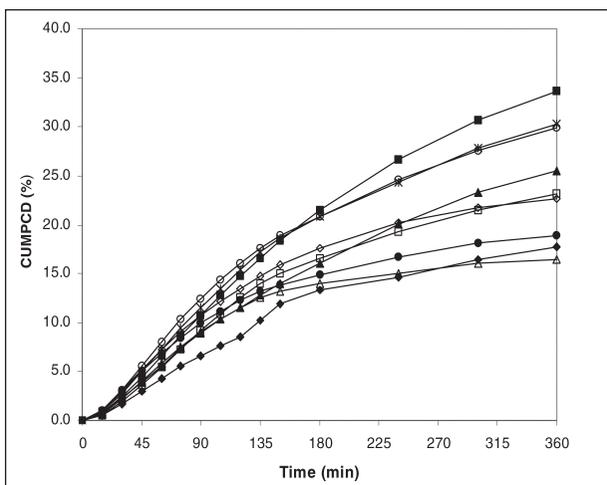

Figure 3—Cumulative PCD values up to a given time point (CUMPCD) after IV administration of ^{13}C-aminopyrine to 9 healthy dogs. Each symbol represents results for 1 dog.
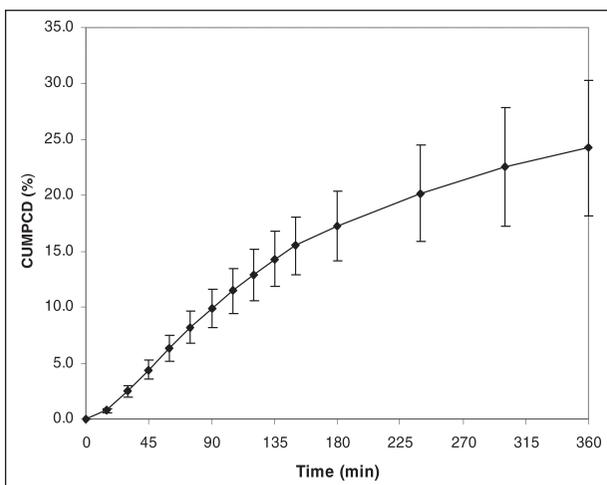

Figure 4—Mean ± SD CUMPCD after IV administration of ^{13}C-aminopyrine to 9 healthy dogs.

Discussion

We did not observe gross evidence of adverse effects during the course of the study and for several days after the study. Although subclinical effects, such as subclinical organ damage, may have been evident during this time, our observations give an indication that IV administration of ^{13}C-aminopyrine appears to be safe in healthy dogs. Thus, at least in healthy dogs, IV administration of ^{13}C-aminopyrine appears to be an acceptable route of administration of the drug. Drug safety needs to be further evaluated in dogs with altered hepatic function.

Intravenous administration of ^{13}C-aminopyrine led to an increase in PCD in gas extracted from blood samples in all 9 dogs evaluated. The mean PCD at all time

Table 1—Comparison of the coefficients of variation for 5 variables that can be used to estimate hepatic demethylation of ^{13}C-aminopyrine

Variable	Time (min)													
	15	30	45	60	75	90	105	120	135	150	180	240	300	360
PCD	25.2	17.8	17.9	18.2	19.1	22.2	26.6	26.9	28.9	38.1	45.9	37.4	53.5	51.5
CUMPCD	25.1	21.7	19.5	18.2	17.4	17.3	17.6	18.1	17.1	16.7	18.0	21.4	23.3	25.0
DOB	21.7	17.1	19.6	23.3	24.6	28.3	33.6	34.4	35.2	45.2	50.0	43.4	58.5	55.3
DOB/kg	61.2	55.4	49.5	38.4	39.7	37.7	34.2	34.6	30.2	34.1	45.6	39.6	48.1	51.0
DOB/kg$^{0.75}$	113.0	109.9	101.0	84.7	87.9	84.7	79.4	81.5	64.0	69.6	68.2	77.5	66.5	71.9

Values reported are percentages. Time 0 = Time of IV administration of ^{13}C-aminopyrine. PCD = Percentage dose of ^{13}C administered as ^{13}C-aminopyrine and recovered in gas extracted from blood samples. CUMPCD = Cumulative PCD values up to a given time point. DOB = Dose over baseline, which is the difference between δ of a particular time point and δ at baseline (δ is the difference of the amount of ^{13}C in a gas sample compared to the amount of ^{13}C in a standard gas). DOB/kg = DOB per kilogram of body weight. DOB/kg$^{0.75}$ = DOB per kilogram$^{0.75}$ (ie, DOB per metabolic body weight).

points after ^{13}C-aminopyrine administration was significantly greater than mean PCD at baseline. Kinetic behavior was similar for all 9 dogs, with an initial peak followed by a slow reduction in PCD for each dog (Fig 1). The PCD for all dogs reached a peak by 75 minutes after administration (mean, 45 minutes). Analysis of these findings suggests that IV administration of ^{13}C-aminopyrine leads to an increase of PCD in gas extracted from blood samples.

To determine the variable with the least between-individual variability among dogs, the CVs for 5 measures of hepatic demethylation of ^{13}C-aminopyrine (ie, PCD, CUMPCD, DOB, DOB/kg, and DOB/kg[0.75]) were compared. Because blood samples will be collected at a time point when values for the variables are at their peak, the CVs for the peak time points (ie, 30, 45, 60, and 75 minutes) were considered. Mean CV for PCD at the peak time points was lower than the mean CV for CUMPCD, DOB, DOB/kg, or DOB/kg[0.75], with CUMPCD being the next closest value. The higher CV for PCD, compared with the CV for CUMPCD, at later time points is of little clinical concern because peak recovery of [13]CO$_2$ was during the earlier time points (all dogs had peak PCD values by 75 minutes after administration; mean, 45 minutes). Determination of CUMPCD requires the collection of several blood samples, whereas determination of PCD requires only 2 blood samples and, thus, would be preferable in a clinical setting. Therefore, determination of PCD would appear to be the preferred variable for assessment of hepatic demethylating capacity in future studies. Because mean PCD peaked at 45 minutes in the 9 dogs examined, 45 minutes appears to be a suitable time point for collection of a blood sample after ^{13}C-aminopyrine administration.

Several additional studies need to be conducted to assess the clinical usefulness of the ^{13}C-aminopyrine demethylation blood test. Assuming that results of those studies indicate that quantification of ^{13}C-aminopyrine demethylation is clinically useful, such a test could easily be performed by veterinarians in private clinical practice. Blood samples collected into tubes containing sodium heparin for use in the ^{13}C-aminopyrine demethylation test remain stable at room temperature for several weeks; thus, shipment of samples to a laboratory with a fractional mass spectrometer for analysis would not be prohibitive. Furthermore, as the clinical use of the test increases, more laboratories, including commercial laboratories, would invest in the equipment necessary to perform the analysis.

Intravenous administration of ^{13}C-aminopyrine appears to be a safe procedure in healthy dogs. Also, IV administration of ^{13}C-aminopyrine leads to a consistent increase of PCD in gas extracted from blood samples. Furthermore, the between-individual variability of PCD and CUMPCD are comparable during the first 75 minutes after IV administration of ^{13}C-aminopyrine, making the determination of PCD the most practical measure of ^{13}C-aminopyrine demethylation. Finally,

collection of a blood sample at 45 minutes after IV administration of ^{13}C-aminopyrine appears to be a suitable time point. Additional studies are needed to determine the clinical use of this hepatic function test in dogs with hepatic disease.

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