The Veterinary Journal 185 (2010) 152–156

Contents lists available at ScienceDirect

The Veterinary Journal

journal homepage: www.elsevier.com/locate/tvjl

Assessment of liver function in dogs using the ^{13}C-galactose breath test

S. Silva [a,*], C.A. Wyse [b], M.R. Goodfellow [a], P.S. Yam [c], T. Preston [d], K. Papasouliotis [a], E.J. Hall [a]

[a] Division of Companion Animal Studies, Department of Clinical Veterinary Science, University of Bristol, Langford House, Langford, Bristol, BS40 5DU, UK
[b] Department of Anatomy, University of Bristol, Southwell Street, Bristol, BS2 8EJ, UK
[c] Division of Companion Animal Studies, Institute of Comparative Medicine, University of Glasgow, Glasgow, G12 9JJ, UK
[d] Scottish Universities Environmental Research Centre, Kelvin Technology Park, East Kilbride, Glasgow G75 0QF, UK

ARTICLE INFO

Article history:
Accepted 23 May 2009

Keywords:
Liver function
Stable isotope
Breath test
Dog
Galactose

ABSTRACT

The aim of this study was to evaluate the application of the ^{13}C-galactose breath test (^{13}C-GBT) in assessing canine liver function by applying it to a group of healthy dogs, and to a group with clinicopathological evidence of liver dysfunction. Breath samples were collected 30 min before ingestion of ^{13}C-galactose, and then at regular intervals thereafter for 6 h. The proportion of $^{13}CO_2/^{12}CO_2$ in the breath samples was measured by isotope-ratio mass spectrometry. There was no significant difference in recovery of $^{13}CO_2$ in the diseased group, compared to the healthy controls, but there was considerable inter-subject variation in both groups, possibly due to differences in the rate of gastric emptying, which could preclude detection of alterations in hepatic metabolism of galactose. The results of this study do not support the application of the ^{13}C-GBT for assessment of canine liver function.

Introduction

Hepatic dysfunction is a commonly encountered problem in small animal practice and it is not infrequent for clinical signs to be mild and non-specific. The 'gold-standard' for assessing liver disease in dogs is the histological examination of tissue biopsy samples, but this method is invasive and may be inappropriate for screening or for monitoring disease progression. Radiography and abdominal palpation are only useful in indicating the presence of hepatomegaly, whilst ultrasonography may permit the detection of anatomical abnormalities, but cannot provide information on the functional capacity of the liver. Elevated serum liver enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP) and gamma glutamyltransferase (GGT) can indicate liver damage, but are not specific to primary hepatocyte damage and cannot be used to assess liver function. Similarly, assessment of serum total proteins, albumin and total bilirubin are of some use in establishing a diagnosis of liver disease, but are insensitive indicators of liver function.

The most reliable clinical test of canine hepatobiliary function is probably the measurement of pre- and post-prandial bile acids. However, the specificity of the bile acid stimulation test is affected by cholestasis, resulting in elevation of serum bile acids in the presence of normal hepatocyte function. The assessment of canine liver function using a labelled bile-acid (^{14}C-cholic acid) clearance test was recently described (Bosje et al., 2005), but did not offer any additional clinical information over the measurement of serum post-prandial bile acid concentrations. Assessment of plasma ammonia is a useful indicator of portosystemic shunting, had poor sensitivity in detecting primary hepatocellular disease and required a demanding sample collection procedure (Walker et al., 2001; Gerritzen-Bruning et al., 2006).

Other tests of liver function that have been described for use in the dog include the ^{13}C-aminopyrine blood test (Moeller et al., 2001, 2004, 2006), the galactose elimination test (Bernardini et al., 2005), serum phenylalanine test (Neumann et al., 2007a), L-carnitine test (Neumann et al., 2007b), and the bromosulphthalein (BSP) retention test (Flatland et al., 2000). However, these tests are not generally available in veterinary clinical practice, particularly since they require intravenous (IV) cannulation and some of the test substrates may be toxic (e.g. BSP). Measurement of plasma protein C was reported to improve differentiation of microvascular dysplasia from portosystemic vascular abnormalities, although the overall sensitivity and specificity of this test in differentiating hepatobiliary disease and portosystemic shunting was lower than that reported for the standard method of measurement of total bile acids (Toulza et al., 2006). There is currently no ideal method available for quantitative assessment of liver function in veterinary clinical practice.

In human medicine, the rates of metabolism of various isotope-labelled substrates have been used as quantitative probes of liver enzymatic function (Mion et al., 1999; Suzuki et al., 2001; Armuzzi et al., 2002; Saadeh et al., 2003; Giannini et al., 2005). ^{13}C is a stable isotope of carbon that may be used to label these substrates, with

* Corresponding author. Tel.: +44 117 9289447; fax: +44 117 9289628.
E-mail addresses: Susana.Silva@bristol.ac.uk, susanasrsilva@hotmail.com (S. Silva).

1090-0233/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.tvjl.2009.05.022

the advantage that recovery can be measured in either blood or breath using mass spectroscopy, since no ionising radiation is emitted. The substrates employed are preferentially metabolised in the liver and can be targeted specifically towards assessment of hepatic microsomal (methacetin, caffeine, aminopyrine), mitochondrial (ketoisocaproate, methionine) and cytosolic (galactose, phenylalanine) enzymatic activity (Armuzzi et al., 2002). In addition to being potentially useful diagnostic and prognostic indicators, these tests may provide insights into the pathophysiology of conditions that affect liver function, where specific metabolic pathways may be more susceptible to hepatocyte damage.

The ^{13}C-galactose breath test (^{13}C-GBT) uses a non-toxic substrate that can be delivered orally, rendering this test safe and non-invasive. Galactose is primarily metabolised by the liver and the rate of appearance of $^{13}CO_2$ in breath following ingestion of ^{13}C-galactose is used as a measure of hepatic enzymatic function (Armuzzi et al., 2002). The oxidation of ^{13}C-galactose to its end product, $^{13}CO_2$, occurs principally in the cytosol of the hepatocyte, and the rate-limiting step in this process is the phosphorylation of galactose to galactose-1-phosphate by the enzyme galactokinase (Armuzzi et al., 2002). Studies in humans have shown that the rate of metabolism of an oral dose of ^{13}C-galacose did not differ significantly from the same dose given IV, which suggested that gastrointestinal function did not affect the rate of metabolism of the test substrate and justified oral galactose administration in the ^{13}C-GBT (Mion et al., 1999).

Low doses of galactose are metabolised by the liver at flow-dependent rates, so doses sufficient to saturate the liver are usually administered during the ^{13}C-GBT. This ensures that the recovery of $^{13}CO_2$ in breath is dependent on functional hepatic mass, not on hepatic blood perfusion. Dosage rates of 500 mg/kg are used to ensure liver galactose saturation in human subjects, and unlabelled galactose is also administered due to the high cost of ^{13}C-galactose.

In human medicine, there is evidence to suggest that the ^{13}C-GBT may be a useful method for detecting mild, chronic liver disease, and for non-invasive monitoring of liver function. The results of the ^{13}C-GBT have shown good agreement with the degree of fibrosis in biopsy samples taken from patients with chronic hepatitis (Mion et al., 1999). The ^{13}C-GBT also showed good sensitivity (93%) and specificity (87%) in distinguishing patients with liver cirrhosis from healthy volunteers (Saadeh et al., 2003), but showed limited sensitivity in discriminating between degrees of disease severity within groups of patients with cirrhosis (Saadeh et al., 2003; Giannini et al., 2005). Stable isotope tests have been previously applied, and basal breath ^{13}C-enrichment established in dogs (Wyse et al., 2001), but the application of the ^{13}C-GBT in veterinary medicine has not been described.

The overall hypothesis of the present study was that the rate of metabolism of ^{13}C-galactose may be a useful index of hepatic function in dogs. The specific study objective was to measure the rate of ^{13}C-galactose metabolism in healthy dogs and in dogs with liver disease (portosystemic shunts and primary parenchymal hepatopathies) to assess the application of this test for assessment of liver function in dogs.

Materials and methods

Animals

Eighteen dogs diagnosed with liver dysfunction at the University of Bristol Veterinary School and 23 control dogs were employed in the study. The 18 dogs in the liver disease group were of various breeds, with an age range of 2 months to 13 years, and of bodyweight (BW) range 2.0–23.5 kg. All dogs showed clinical signs of liver dysfunction including, but not restricted to, hepatic encephalopathy, jaundice, vomiting, diarrhoea, and urate urolithiasis. Routine haematology and serum biochemistry including ALT, ALP, albumin, bilirubin and ammonia were measured at the primary clinician's discretion.

All animals had at least one severely abnormal bile acid stimulation test result. Imaging (radiographs and abdominal ultrasound) were used to try to obtain further information about liver size and echogenicity, presence of adequate portal vasculature, portal vein flow and turbulence and/or presence of a vascular abnormality. In dogs with elevated bilirubin concentration, abdominal ultrasound was used to rule out extra-hepatic bile duct obstruction, and those with evidence of extra-hepatic bile duct obstruction were excluded from the study. All animals with microhepatica and clinical or ultrasonographic suspicion of a primary vascular abnormality had portovenography performed. Liver biopsies were obtained in all the patients, either percutaneously (ultrasound and Tru-cut needle), laparoscopically or via laparotomy. Histology of the hepatic biopsies was assessed by at least one specialist pathologist following the WSAVA Liver Standardisation Group guidelines (Cullen et al., 2006).

The vascular disorders identified in this study subgroup were portal vein hypoplasia ($n = 2$) and congenital portosystemic shunts ($n = 7$). The patients with primary parenchymal liver disease were diagnosed with a multitude of conditions (copper-associated hepatitis ($n = 2$), idiopathic chronic hepatitis ($n = 6$), severe vacuolar hepatopathy secondary to hyperadrenocorticism ($n = 1$).

The control group consisted of 23 staff-owned dogs of various breeds, with an age range of 5 months to 10 years and with a BW range of 3.5–34.0 kg. These animals had not had any previous significant disease or no problems reported by their owners and had an unremarkable clinical examination performed by a veterinary surgeon. Additionally, no medication other than flea and worm treatments had been administered for a minimum of 3 weeks preceding the study day. It was not possible to collect blood samples to assess liver function (bile acid stimulation test) in the control animals since none of the dogs had clinical evidence of hepatic disease which would have contravened UK legislative guidelines.

Informed consent was obtained from the owners of all animals and the study was approved by the Animal Welfare and Ethics Committee of the University of Bristol.

Study design and breath collection

The ^{13}C-GBT was performed on each dog on one occasion. Food was withheld for 12 h before ingestion of the test meal. Breath samples were acquired 30 min prior to and immediately before ingestion of the ^{13}C-labelled test meal, and then every 15 min for 4 h, and every 30 min for a further 2 h. Exhaled breath samples were collected by allowing the dog to breathe through a plastic mask (German et al., 1998) connected to a non re-breathing valve and a 50 mL reservoir bag (QuinTron). Exhaled breath samples were transferred from the reservoir bag to sample tubes (Exetainer, Labco Systems) for analysis and stored at room temperature for a maximum period of 7 weeks.

Test meal

The test meal used in this study consisted of 50 mL (dogs < 5 kg) or 100 mL (dogs > 5 kg) of skimmed milk (146 kJ/100 mL) with 5 mg/kg ^{13}C-galactose (D-galactose, 1-^{13}C [minimum 99% atom % ^{13}C], Cambridge Isotope Laboratories Ltd) added to a carrier dose of 25 g/m^2 unlabelled galactose (Molekula).

Determination of ^{13}C excretion in breath

The ratio of $^{13}CO_2$:$^{12}CO_2$ was measured by isotope-ratio mass spectrometry (Preston and MacMillan, 1988). Results of ^{13}C-analysis were expressed as % dose administered recovered per hour (PDR/h), calculated as:

$$\text{PDR/h} = \frac{\frac{\text{ppm excess}^{13}C}{10^6} \times \dot{V}O_2}{\text{Dose } ^{13}C \text{ (mmol)}} \quad (1)$$

where VCO_2 is mmol CO_2 exhaled per hour, taken to remain stable at 0.194 L/m^2 body surface area/min (Mauderley, 1974). Body surface area was calculated as:

$$\text{Body surface area } (m^2) = \frac{10.1 \times \text{Bodyweight } (g)^{2/3}}{10,000} \quad (2)$$

(Altman and Dittmer, 1964)

The PDR/h values were plotted against time and non-linear regression analysis was used to model the data according to the formula described by Siegel et al. (1988) ad modified by Maes (1994):

$$y = m(1 - e^{-kt})^{\beta} \quad (3)$$

where y is the percentage of cumulative ^{13}C excretion in breath, t is time in hours and m, k, and β are constants predicted by non-linear regression analysis using the Solver function of a Microsoft Excel computer programme. The half dose recovery time ($t_{(½)}$), was defined as the area under the fitted cumulative $^{13}CO_2$ recovery curve (expressed as PDR/h) when half of the administered dose is excreted, when time is infinite, and calculated mathematically from (3) above, using the formula (Ghoos et al., 1993):