Assessment of beta-hydroxybutyric acid (BHBA) as a plasma biomarker for hepatic lipidosis in bearded dragons (Pogona vitticeps)

Lisa P. Pucumio, Danielle K. Tarbert, Kevin Keel, Melanie Ammersbach, Hugues Beaufreure

Department of Medicine and Epidemiology (Pucumio, Beaufreure), Veterinary Medical Teaching Hospital (Tarbert), Pathology, Microbiology, and Immunology (Keel, Ammersbach), School of Veterinary Medicine, University of California Davis, Davis, CA.

Introduction

Hepatic lipidosis (or fatty liver disease) is characterized by the pathologic accumulation of triglycerolys in hepatocytes leading to impaired liver function. Hepatic lipidosis is one of the most common non-infectious diseases of captive bearded dragons, affecting approximately 38.3% of pet dragons, one of the most common species of pet reptiles. Routine hematologic, biochemical, radiographs, and coelomic ultrasonics have low sensitivity for diagnosing this disease. Current diagnosis mainly relies on advanced imaging techniques, such as CT-scanning, or invasive techniques, such as liver biopsy and histopathology. A previous pilot study using plasma metabolomics on a small cohort of 14 bearded dragons, with varying degrees of spontaneously occurring hepatic lipidosis, identified β-hydroxybutyric acid (BHBA) as a candidate biomarker for hepatic lipidosis. The main confounding factor was the varying ages between disease groups.

Methods (continued)

- Forty-eight bearded dragons originally selected to be culled were collected from a large breeding facility in Chico, CA. Twenty-four dragons were between 1.5-3 years of age and another twenty-four were between 4-7 years old. There were twenty-five females and twenty-three males.
- Dragons were fasted for 48 hours prior to sample collection. A 2-3 ml of blood was collected from caudal tail vein or right jugular vein for measurement of plasma BHBA concentration using a point-of-care analyzer on whole blood (Nowflex™ ketone/glucometer) immediately following blood collection and using a reference analyzer (Vitros 5600 Analyser, University of Miami Avian and Wildlife Laboratory) on heparinized plasma.
- A hepatic biochemistry panel (AST, ALT, ALP, GGT, GLOH, LDH, Bile acids) was also performed.
- Remaining heparinized plasma was stored at -80°C for additional analyses.
- Drages were sedated/anesthetized using 10-15 mg/kg alfalone SC.
- Whole body CT scans were performed in groups of 8 bearded dragons (Figure 1).
- Hepatic density in Hounsfield units (HU) was determined from standardized ROI from CT images (Figure 2).
- Drages were humanely euthanized via intracardiac injection of 0.3-0.5 ml of KCl.
- Necropsy and sample collection were performed as well as histopathology and hepatic lipidosis grading using a validated histopathology scoring system.

Results

- There was no significant association between plasma BHBA concentrations and fat liver content (R2=0.05, p=0.12) (Figure 3).

Discussion

Our findings regarding BHBA's relationship to hepatic lipidosis are not consistent with the results of the pilot study, as we did not find any significant association. There were several potential factors that may have contributed to this difference, including:
- Captive bearded dragon husbandry practices, diet, or breeding schedule may have predisposed animals to hepatic lipidosis and impaired liver function.
- Routine hematologic, biochemical, radiographs, and coelomic ultrasonics have low sensitivity for diagnosing this disease.
- Our a priori study using plasma metabolomics on a small cohort of 14 bearded dragons, with varying degrees of spontaneously occurring hepatic lipidosis, identified β-hydroxybutyric acid (BHBA) as a candidate biomarker for hepatic lipidosis. The main confounding factor was the varying ages between disease groups.

Conclusion and Future Direction

Our data does not support the use of BHBA as a plasma biomarker for the diagnosis of severe hepatic lipid accumulation. The POC BHBA meter may be used in clinics to assess fatty acid metabolism when access to a reference analyzer is limited, but caution is advised when interpreting high BHBA values. To further explore metabolic pathway disruption with hepatic lipidosis in bearded dragons and continue to screen for novel plasma biomarkers, metabolomics data are pending for this cohort.

Acknowledgements

Support for this project was granted by Boehringer Ingelheim Veterinary Scholarship Fellowship and the Students Training in Advanced Research (STAR) Fellowship. I would like to also give special thanks to Paula Rodriguez for her technical support, and Animal Specialty Inc. for generously providing the animals needed for this study.

Cited Literature