

Plasma Bile Acids in Reptiles¹

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Abstract: To compare bile acids concentrations in blood of healthy green iguanas with blood of iguanas suffering from various types of liver diseases, plasma and serum samples were analyzed. Prior to blood collection 24 healthy green iguanas were fasting for 24 hours only, while 18 iguanas were fasting for 48 hours. The concentration of bile acids was determined by the enzymatic colorimetric method. Serum or plasma samples showing lipaemia or haemolysis were not analyzed. Although all samples of blood serum were handled properly, some of them clotted into a slimy mass and could not be examined. The samples of liver tissue received on necropsy of euthanized iguanas were prepared for histologic examination. Mean plasma bile acids concentration in samples from healthy green iguanas fasting 24 hours was $15.89 \pm 15.61 \mu\text{mol/L}$. Mean plasma bile acids concentration in samples from iguanas fasting 48 hours was $9.56 \pm 8.52 \mu\text{mol/L}$. 3α -hydroxy bile acids were significantly increased in plasma of green iguanas with chronic liver diseases. The highest concentrations ($>70 \mu\text{mol/L}$) were present in patients suffering from liver cirrhosis, liver lipidosis and liver neoplasma.

Key words: bile acids, serum, plasma, liver diseases, reptilian, lizards, *Iguana iguana*

Introduction



Fig 1. Chronic liverdisease in a iguana

While acute liver diseases in reptiles may be associated with elevations of several enzyme activities including aspartate aminotransferase, gamma glutamyltransferase, alkaline phosphatase, alanine aminotransferase and lactate dehydrogenase there is still a lack of information concerning the existence of a feasible method for monitoring the chronic liver failure in reptiles.¹ Studies that have been performed to characterize the bile acids in reptiles demonstrate that a variety of different bile acids are produced, nevertheless 3α bile acids appear to be conserved amongst all reptile groups.²⁻³ As the clinical symptoms of liver failure in reptiles are unspecific the attention is aimed at methods of indirect diagnostics, such as diagnostic imaging, clinical hematology and plasma chemistry.⁴⁻⁵ Monitoring of bile acids concentrations (BA) in peripheral blood of reptiles is currently the centre of attention, because elevated values are supposed to be the results of liver diseases.^{3,6-7} Determination of serum/plasma BA concentrations as the indirect method for monitoring the liver diseases should be used in research focused on reptilian metabolism. Therefore

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the first aim of this study was to compare BA in samples of blood serum and plasma in healthy green iguanas and/or in patients of the clinic, the second aim was to determine the range of plasma BA concentrations in clinical healthy green iguanas and the third aim was to determine plasma BA in a group of green iguanas with different types of liver diseases (Fig. 1).

Materials and Methods

The study was performed using a group of 24 healthy green iguanas (*Iguana iguana*) that had been capture bred and kept for two years under experimental conditions at the Avian and Exotic Animal Clinic, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences in Brno. The iguanas were housed in small groups (one male with one female) and maintained in a room with an ambient temperature of 27.9° C during the day and 24.5° C at night, general humidity was maintained at 70-80% by daily spraying. Special lamps in each enclosure provided a daytime basking area of 35.0° C and broad light spectrum (including UVB). The iguanas were exposed to a 12 hour light cycle. The diet consisted of dandelion flowers and leaves, lettuce, collard greens, cabbage and kale, supplemented with calcium powder and commercial iguana pellets (Premium Iguana Adult, Exo Terra, Germany). Water was available at all times.

The blood samples were taken from the ventral tail vein (*vena coccygea ventralis*). The one part of blood samples was taken without heparin, left to clot for four hours at 4°C, centrifuged, and the serum removed and stored at -20°C. The second part of blood samples was collected to tubes with heparin, centrifuged, and the plasma samples were stored at -20 °C. The range of BA in healthy green iguanas was determined in 5 repetitious examinations. The first samples were collected from iguanas at the age of 28 months, the last samples at the age of 42 months. To avoid erroneous interpretation of physiologically elevated bile acids concentrations, healthy green iguanas were fasting for 24 hours prior to blood collection. The plasma concentrations of BA were measured also in a group of 18 healthy green iguanas that were fasting 48 hours prior to blood collection. Samples showing lipaemia or haemolysis were omitted for calculating the mean values. All procedures were evaluated and approved by the University of Veterinary and Pharmaceutical Sciences Brno Animal Care and Use Committee (VFUB#11-207/2004).

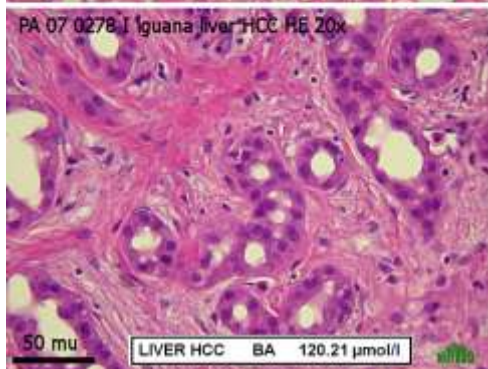
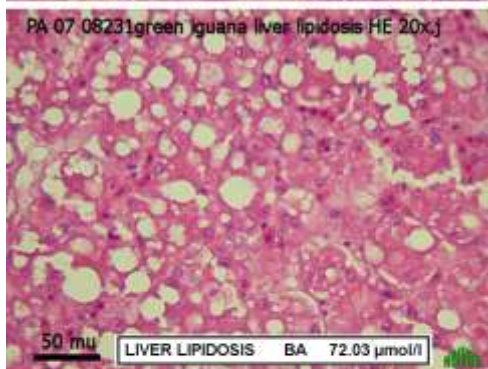
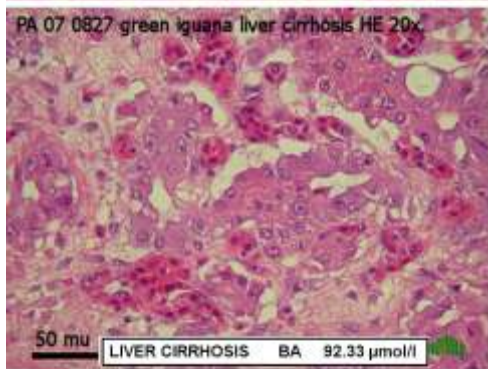
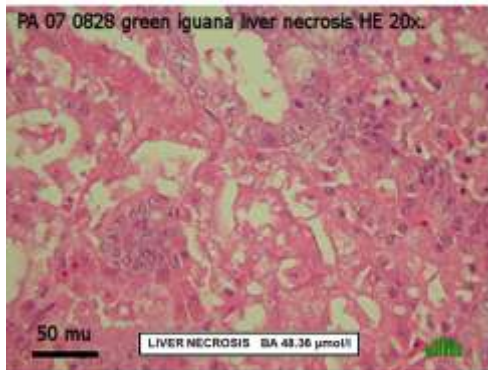
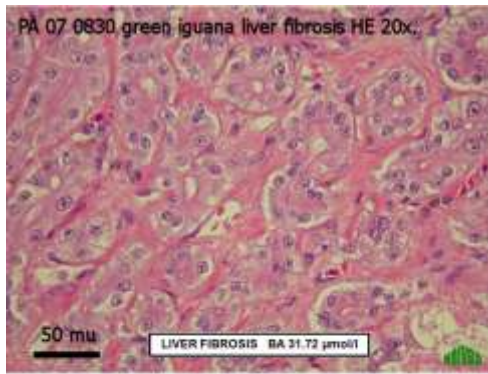
The plasma concentration of bile acids was determined by the enzymatic colorimetric method (Bile Acids, Randox Laboratories, UK). 3 α -hydroxy bile acids were converted to corresponding 3-keto-hydroxy bile acids in the presence of NAD⁺ by 3 α -hydroxysteroid dehydrogenase. The NADH formed reacted with nitrotetrazolium blue in a dehydrogenase catalysed reaction to form a formazan dye (blue color with an absorption maximum at 540 nm). The intensity of the colour produced, directly proportional to the bile acids concentration in the sample, was read colorimetrically on Cobas Mira Plus (Roche).

The liver tissue samples received on necropsy of iguanas that were euthanized were fixed in buffered 10% formalin and prepared for histological examination. Tissue blocks of the fixed liver sample were embedded in paraffin wax, sectioned at 3 μ m and routinely stained with hematoxylin and eosin (HE). Histopathologic examinations of liver samples revealed different forms of liver diseases.

The mean and standard deviation (SD) for plasma/serum BA concentrations were calculated. The significances of the differences between the BA concentrations in serum and plasma samples, healthy green iguanas fasting for 24 versus iguanas fasting for 48 hours, and a group of healthy iguanas versus iguanas suffering from liver diseases were analyzed by using Stat Plus software (Version 1.01, VUVeL Brno).⁸

Results

Comparing the plasma with the serum BA concentrations no significant difference has been registered (24.21 \pm 37.35 μ mol/L versus 27.68 \pm 33.91 μ mol/L). Concentrations significantly differed neither in healthy green iguanas nor in patients, including iguana with the highest values measured (plasma BA 120.21 μ mol/L, serum BA 113.73 μ mol/L). Although all samples of blood serum were handled properly, some of them clotted into a slimy mass and could not be examined. Because of these facts we decided to assess BA of green iguanas in blood plasma. Mean plasma BA concentration in 110 samples from healthy green iguanas that were fasting 24 hours was 15.89 \pm 15.61 μ mol/L (range 0.22



– 73.1761 μmol/L), mean BA concentration in 18 samples from healthy green iguanas that were fasting 48 hours was (9.56 ± 8.52 μmol/L). The high plasma BA concentrations were present in iguanas suffering from liver diseases. In these patients the BA values (59.52 ± 33.55 μmol/L) were markedly higher than BA of healthy iguanas. BA concentrations were significantly altered in patients suffering from liver lipidosis (72.03 μmol/L), liver cirrhosis (92.33 μmol/L), and liver neoplasma (120.21 μmol/L).

Discussion

Plasma 3α-hydroxy-bile-acids reference intervals for a group of healthy male green iguanas have been established recently.³ Pre-prandial bile acids concentrations (7.5 ± 7.8 μmol/L) significantly increased at 3 and 7.5 hours post-prandially to 33.3 ± 22.0 μmol/L and 32.5 ± 8.4 μmol/L, respectively.³ Fasting status must be considered when evaluating bile acid concentrations in the green iguana. In the present study the mean plasma bile acids concentration in 110 samples from healthy green iguanas (fasting for 24 hours only) was higher than BA observed in a previous study.³ In 18 samples from healthy green iguanas (fasting for 48 hours) the mean plasma bile acids concentration (9.56 ± 8.52 μmol/L) was very similar to the results of McBride et al.³ The data collected from their pilot study suggested that 3α-hydroxy bile acids concentrations would be significantly increased by feeding for at least 8 hours. Therefore these authors recommended to fast green iguanas for 48 hours prior to bile acids determination. It is in accordance with our present results. The broad spectrum of variance (mean ± SD) for bile acids concentrations has been observed accordingly in both of the studies. It is possible that the pressure exerted externally during restraint may result in contracture of the gall bladder and alteration of concentration of plasma bile acids. Gentle handling with the iguanas has to be recommended.³ Plasma bile acids concentrations in the present study were significantly altered in patients suffering from chronic liver diseases. The highest plasma BA concentrations were present in iguanas suffering from liver cirrhosis, liver lipidosis and liver neoplasma (>70 μmol/L). The data collected from this study suggest that 3α-hydroxy bile acids are significantly increased in green iguanas suffering from chronic liver diseases.

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