BILE ACID FRACTIONATIONS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN EQUINE LIVER DISEASE

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ABSTRACT


Serum bile acids were fractionated by high-performance liquid chromatography (HPLC) in 13 control and 8 cases of liver disease in horses. The severity and type of liver injury was determined by histopathological examination of biopsy and/or necropsy specimens. The total serum bile acids (tSBA) were determined in these horses by an enzymatic method (SBA-EA) and by summation of the bile acids (SBA-LC) as fractionated by the HPLC. The SBA-LC were generally higher than the SBA-EA in both the controls and liver disease and they did not parallel each other. The primary bile acids, total cholates and total chenodeoxycholates accounted for most of the tSBA increases in liver disease. There was a shift in profile from taurocholate to free (unconjugated) cholate in direct relation to the severity of the liver injury. Among the secondary bile acids, total deoxycholates and total taurodeoxycholates increased at random. The pattern of the SBA profile in relation to the severity of the liver disease suggested that hepatocellular excretion is the most sensitive step in the enterohepatic circulation of the bile acids.

Keywords: bile acids, horses, liquid chromatography, liver disease

INTRODUCTION

The determination of the total serum bile acids (tSBA) is widely used (Balistreri and Shaw, 1987) as a liver function test because it is considered to have high sensitivity and specificity in humans (Kaplowitz and Kok, 1973; Campbell et al., 1978; Hofmann, 1988) and in animals (Anwer et al., 1976; Hauge and Abdelkader, 1984; Center et al., 1985; Parraga and Kaneko, 1985; West, 1989, 1991). In the horse, bile acid (BA) metabolism and serum bile acid (SBA) composition are not well known. About 90% of the BA pool appears to be confined to the enterohepatic circulation and in the hepatocytes (Anwer et al., 1975; Engelking and Gronwall, 1979). The primary bile acids are synthesized by the hepatocytes from cholesterol through two separate series of enzymatic pathways (McGilvery, 1983; Vlahcevic et al., 1990). The first pathway is focused on the cholesterol side-chain, oxidatively converting it to a carbonyl group with three fewer carbon atoms. The second is focused on the hydroxyl groups of the cholesterol nucleus. Through rearrangements, hydroxylations, dehydroxylations and epimerizations, a wide variety of derivatives are generated. Most of these derivatives are excreted into the gut via the biliary system and degradation of BA by gut bacteria forms the secondary BA. Additionally, there is an enterohepatic circulation of BA in all mammals. Therefore, after reabsorption of non-degraded (primary) or degraded
(secondary) BA, these may appear in the circulation depending upon the extraction efficiencies for these BA by the liver. In normal animals, only a few BA appear in the circulation and their patterns differ among species. It therefore follows that any change in the hepatocellular biochemical reaction pathways, interruption of the enterohepatic circulation or alterations in the gut flora can potentially alter the SBA pattern. Conversely, the SBA pattern might be expected to reflect the type and site of injury to the hepatic cell or to the enterohepatic circulation. The present study was undertaken to examine this hypothesis.

MATERIALS AND METHODS

Animals and diagnostic laboratory studies

Thirteen normal thoroughbred horses of both sexes, 3 to 12 years old and housed at the Equine Research Laboratory of the University of California at Davis, were used as controls. The horses were fed approximately 9 kg of alfalfa hay once a day in the morning and all the feed was consumed by late afternoon. Blood samples were taken in EDTA and in serum separator tubes in the morning after overnight fasting and before the morning feeding. Serum was obtained within 2 h after collection by centrifugation at 4°C at 1100g for 20 min and was used for (1) serum biochemical determinations (2) total serum bile acids and (3) SBA fractionations. Horses with liver disease were those presented at the Veterinary Medical Teaching Hospital (VMTH) of the School of Veterinary Medicine. Routine haematological tests and an equine serum biochemical liver screening panel of tests were performed initially and additional tests were performed when possible. Total SBA, SBA fractionations and liver biopsy and/or necropsy were performed within the 24 h period in which the sera were obtained. The livers were examined grossly and by light microscopy after sectioning and then staining with haematoxylin and eosin.

Routine haematological studies were performed in the diagnostic laboratories of the VMTH using standard techniques. The serum biochemical analyses were performed by an automated system (Dacos II, Coulter Electronics, Hialeah, FL, USA) and included aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), urea nitrogen (UN), glucose (Gluc), total protein (tProt), albumin (Alb) and total bilirubin (tB). Iditol (sorbitol) dehydrogenase (ID), conjugated bilirubin (CB), ammonia (NH₃), prothrombin time (PT) and partial thromboplastin time (PTT) determinations were by standard manual methods. Globulin (Glob) and unconjugated bilirubin (UCB) were by appropriate calculations. Total SBA were assessed by enzyme assay (SBA-EA) using 3α-hydroxysteroid dehydrogenase (3HSD) (Nycomed, Oslo, Norway).

High-performance liquid chromatography (HPLC)

A Sep-Pak C18 cartridge (Waters, Milford, MA, USA) (Whitney and Thaler, 1980) was first washed with 5 ml methanol followed by 20 ml double-distilled water at a flow rate of 10 ml/min in a flow syringe pump. The SBA were extracted by first diluting 1 ml of serum with 9 ml of double-distilled water containing 50 μl of 50 μmol/L