CLINICAL SIGNIFICANCE OF SERUM BILE ACID MEASUREMENT IN LIVER DISEASES

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Summary

To determine the clinical significance of serum bile acid measurements, changes in the serum bile acid composition in liver diseases and endogenous bile acid clearance due to test meal loads were investigated. In the case of changes in the serum bile acid composition, a characteristic pattern of a remarkable increase of chenodeoxycholic acid (CDCA) was found in fulminant hepatitis. In patients with acute hepatitis, increases in CDCA were somewhat greater than those of cholic acid (CA) and there was tendency for these changes to precede changes in other liver function tests. In cases of extrahepatic obstructive jaundice, the CA/CDCA ratio was a large value exceeding 1.0. In investigations of endogenous bile acid clearance, serum bile acid concentration two hours after the test meal load clearly reflected the hepatic disorder and it was useful in differentiating between active and inactive form in chronic hepatitis and compensation and decompensation in liver cirrhosis.

Key Words: serum bile acids, endogenous bile acid clearance, liver function test.

Introduction

Since the healthy liver efficiently ingests bile acids from the portal vein, the bile acid pool under normal conditions is present mainly in the enterohepatic circulation. However, in hepato-biliary diseases, the metabolic mechanism of bile acids is disrupted, the bile acid pool distribution is changed and the bile acid concentration in the systemic circulating blood, urine, skin, etc. increases. To determine the metabolic conditions of bile acids in patients with hepato-biliary diseases, it is necessary to determine the quantitative and qualitative changes of bile acids in the blood, bile, small intestine contents, urine, feces, skin, etc. However, because of the difficulties in collecting materials and the measuring methods, it is not easy to obtain information from the all angles.

Since the materials were easy to obtain and the bile acid composition was comparatively uniform, the authors measured the serum bile acids and investigated the significance of the measurement of serum bile acids in liver diseases to evaluate the status of the liver.

Methods

The significance of serum bile acid measurements in liver diseases was investigated from the following two standpoints:
1) Pathological conditions of the liver and changes of serum bile acid composition.

2) Total serum bile acid concentrations before and after a test meal load in liver diseases.

The first standpoint is concerned mainly with investigations of qualitative changes in the composition of bile acids in the blood, while the second standpoint involves observation of the endogenous bile acid clearance by means of the test meal load test to investigate its usefulness as a liver function test.

1. Pathological conditions of the liver and changes of serum bile acid composition

The diseases examined were acute hepatitis, fulminant hepatitis, acute intrahepatic cholestasis and extrahepatic obstructive jaundice (carcinoma of the head of the pancreas). Blood samples were obtained from the antecubital veins before breakfast after an overnight fast, and the separated sera were kept at -20°C. Serum bile acid composition were estimated by the procedure described by Sandberg et al.1) with some modification. Briefly, the procedure were as follows. A sample of serum (2-10 ml) was diluted 1:1 with distilled water and passed through a 5ml column of the anion exchanger Amberlyst A26. The absorbed bile acids were eluted with 150ml of 0.2 M \( (\text{NH}_4)_2\text{CO}_3 \) in 80% ethanol. The eluate was evaporated to dryness and then hydrolyzed in 5 ml of 1 N NaOH for 4 hours in an autoclave at a pressure of 1 Kg per cm\(^2\) at 120°C. After ether extraction of the acidified hydrolysate, the bile acids were methyrated with diazomethane. Bile acid methyl esters were transferred to an aluminium oxide column with about 10 ml of benzene-hexane (1:9, v/v) and washed with about 40 ml of the same solvent mixture. Lithocholic acid methyl ester was eluted with 70 ml of benzene and all the other bile acid methyl esters with 40 ml of methanol-acetone (1:9, v/v). The bile acids were converted to their methyl ester trifluoroacetates and dissolved in carbone disulfide and an aliquot was injected into the gas-liquid chromatography (GLC). A Shimazu GC4BMPF hydrogen flame ionization detector was used for GLC. The column was a 2% OV-210 glass column 3 mm in diameter. The column temperature was 230°C and the detector temperature 258°C. Nitrogen was used as the carrier gas, the flow rate was 60 ml/min. and the sensitivity 10\(^3\) range 0.04 V.

2. Total serum bile acid concentrations before and after a test meal in various status of liver

The diseases examined were acute hepatitis, chronic hepatitis and liver cirrhosis. In all cases, the diagnosis had been confirmed by liver biopsy with Laparoscopy. Chronic hepatitis was divided into the active and inactive forms. The histological definition was as follows: active form showed enlargement of portal area with fibrosis and inflammatory cells infiltration, piecemeal necrosis due to destruction of limiting plate in mesenchymal region and focal necrosis and remarkable mobilization of Kupffer's cell in parenchymal region. While inactive form showed no destruction of limiting plate and minimal change of parenchymal region. Liver cirrhosis was divided into compensated and decompensated types. For measurement of the bile acids, blood samples were obtained in the morning after an overnight fasting and one, two and three hours after the test meal load and the measurements were performed by simple and sensitive assay2) using 3a-hydroxysteroid dehydrogenase (3a-HSD), NAD, diaphorase and Resazurin. The test meal consisted of 200 g of milk, one egg, 5 g of sugar and a small amount of vanilla essence, mixed by shaking and given as a liquid meal. Other liver function tests were performed at the same