Screening for Cerebrotendinous Xanthomatosis by Using an Enzymatic Assay for 7α-Hydroxylated Steroids in Urine

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We used a commercial enzymatic kit for measuring 7α-hydroxylated bile acids to screen urines from normal subjects, liver-transplant recipients, and patients with various liver diseases, cerebro-hepato-renal syndrome, or cerebrotendinous xanthomatosis (CTX). Because of their high concentrations of 7α-hydroxylated compounds excreted, the CTX patients were clearly distinguished from all other groups except for a slight overlap with the patients with cerebro-hepato-renal syndrome and liver-transplant recipients. Gas chromatography for bile alcohols completed the differential diagnosis.

Cerebrotendinous xanthomatosis (CTX) is the manifestation of an inborn error in the metabolic breakdown of the cholesterol side-chain to bile acids (1). One of the consequences of this defect is the accumulation of cholesterol and cholestanol (the 5α-dihydro derivative of cholesterol) in many tissues. The deposition of these sterols brings about the characteristic symptoms of the disease, such as dermatitis, cataracts, and swelling of the tendons (especially of Achilles tendons; see also reference 1). Another consequence is the excretion of bile alcohol glucuronides in bile and feces (1) and in urine (2, 3).

For establishing CTX, urinary bile alcohols can be measured by gas chromatography (2, 3) or "high-performance" liquid chromatography (4). However, many laboratories do not have this special instrumentation, and these procedures are too time consuming to be of much use in routine clinical chemical analyses. In screening a population for CTX, a relatively simple test should be developed.

Recently, a kit for 7α-hydroxylated bile acids has become commercially available (Enzabille-T, Nyegaard Diagnostica, Oslo; see also ref. 5). This procedure includes a coupled enzyme reaction. First, 7α-hydroxysteroid dehydrogenase (EC 1.1.1.159) oxidizes 7α-hydroxylated compounds to their 7-keto derivatives, with concomitant reduction of NAD+ to NADH. In the second step, NAD+ is regenerated by the enzyme diaphorase (dihydrolipoamide reductase, EC 1.6.4.3), thereby reducing Nitro Blue Tetrazolium to a colored compound, with an absorbance maximum at 540 nm. Hedenborg et al. (6) demonstrated that this kit could also be used for urine samples (1 to 10 mL of urine).

Materials and Methods

Applying this kit to urine samples from CTX patients (200 μL of urine is sufficient) demonstrated the presence of substantial amounts of 7α-hydroxylated compounds. For repeated analyses of a urine sample from a CTX patient, we determined the within-day and day-to-day variation (CV) for the corresponding mean values to be 5.2% (n = 6) at 168 μmol/L and 10.8% (n = 6) at 172 μmol/L, respectively. A comparison with the results of a gas-chromatographic assay for urinary bile alcohols (2), measuring the main component 5β-cholestan-3α,7α,12α,23,25-pentol, in urine samples from 14 CTX patients, is depicted in Figure 1. Clearly, not all urinary bile alcohols were determined with the colorimetric assay, because the total amount of 7α-hydroxylated compounds was in the same order as the amount of its main component (5β-cholestan-3α,7α,12α,23,25-pentol), probably because some conjugated forms of the 7α-hydroxylated and (or) the 3α-hydroxy compounds were not measured in the colorimetric assay [e.g., 3α-sulfated compounds are not oxidized at all (5)].

After having established that this assay was reproducible, we assayed urine samples from 20 healthy volunteers, six patients suffering from various liver diseases, eight patients suffering from cerebro-hepato-renal syndrome (Zellweger), 13 patients who underwent an orthotopic liver transplantation, and 14 CTX patients.

Results and Discussion

Our results are given in Figure 2. The 7α-hydroxylated substances in urine of CTX patients ranged from 50 to 250 μmol/L, which is much greater than that found in the healthy state and in hepatobiliary diseases. In some cases, e.g., cirrhosis of the liver, highly increased concentrations of 7α-hydroxylated substances (bile acids and bile alcohols) were demonstrated, although not as great as in CTX patients (7, 8). Some values for patients undergoing liver transplantation were in the same range as for CTX patients.
When the disease is discovered in early childhood, treatment of the not-yet-affected patients with bile acids would prevent the occurrence of irreversible brain damage, cataracts, etc. seen in practically all CTX patients 20 years or more old. Similarly, as for phenylketonuria, the costs of postnatal screening tests on CTX could offset the costs of caring for affected patients. Although the incidence of the disease is presumed to be very low, it may be much higher than generally believed. For instance in The Netherlands, with 14 million inhabitants, 21 confirmed cases of CTX have been already found.

References