Abstract

Cholesterol 7-hydroxylase (CYP7A1) is the rate limiting enzyme of the classical pathway of bile acid (BA) synthesis, which catabolizes approximately half of cholesterol in man. Determination of CYP7A enzymatic activity is a key subject of lipid metabolism research. Direct determination of CYP7A1 activity in hepatic biopsy is mostly not allowed for ethical reasons, so indirect methods are used with serum markers such as 7α-hydroxy-4-cholestene-3-one (C4). The first, methodical aim of the work was to convert the introduced HPLC method for the determination of C4 to LC-MS in order to increase the sensitivity. We focused on the solid phase extraction step, adjusting the composition and volumes of the washing and elution solution. By converting the method from HPLC to LC-MS, the sensitivity was increased approximately 7 times (LD = 1.39 ng/ml). In the second, clinical part of our work, we attempted to confirm the preliminary results of our laboratory on the distribution of C4 in lipoprotein fractions (LPP) in order to find parameter that would correlate with CYP7A1 activity better than C4 level itself. Preliminary results (performed in healthy individuals) showed that most of C4 is carried on HDL, and that the C4 distribution within LPP fractions is similar among examined subjects. We repeated the experiment on several individuals with different cholesterol levels. The concentration of C4 was corrected to free cholesterol (FC), which served as a marker of LPP-surface size. Our results showed the highest C4 concentration in HDL, but the distribution ratios differed significantly among patients. We did not reproduced preliminary data and C4/FC ratio does not seem to be more accurate marker of CYP7A1 activity than C4 itself.

Keywords: CYP7A1, C4, free cholesterol, SPE extraction lipoprotein particles