Tandem Mass Spectrometric Determination of Atypical 3β-Hydroxy-Δ^5-bile acids in Patients with 3β-Hydroxy-Δ^5-C_{27}-steroid oxidoreductase (HSD3B7) Deficiency – Application to Diagnosis and Monitoring Bile Acid Therapeutic Response

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Abstract

BACKGROUND: 3β-Hydroxy-Δ^5-C_{27}-steroid oxidoreductase (HSD3B7) deficiency, a progressive cholestatic liver disease, is the most common genetic defect in bile acid synthesis. Early diagnosis is important because patients respond to oral primary bile acid therapy, which targets the negative feedback regulation for bile acid synthesis to reduce the production of hepatotoxic 3β-hydroxy-Δ^5-bile acids. These atypical bile acids are highly labile and difficult to accurately measure yet accurate determination of 3β-hydroxy-Δ^5-bile acid sulfates is critical to monitoring response to therapy and for dose titration.
METHODS: A new robust and accurate HPLC tandem mass spectrometric method was developed for the direct measurement of atypical 3β-hydroxy-Δ^5-bile acid sulfates in urine from patients with HSD3B7 deficiency. Quantification was performed in negative ion mode with electrospray ionization and the limitations of GC-MS and FAB-MS are discussed.
RESULTS/DISCUSSION:
Separation of sulfated 3β-hydroxy-Δ^5-bile acids was achieved by reverse-phase HPLC in a 12 min analytical run and by application of this method, the mean±SEM urinary concentration of the total 3β-sulfated-Δ^5-cholenoic acids in patients with HSD3B7 deficiency was 4,650 ± 1711 μmol/L, approximately 1000-fold higher than in non-cholestatic and cholestastic patients with intact primary bile acid synthesis. Evidence is presented that GC-MS is not a reliable method for measuring 3β-hydroxy-Δ^5-bile acid sulfates, however, direct analysis of urine by FAB-MS yields meaningful semiquantitative assessments of urinary excretion.
CONCLUSIONS: A tandem mass spectrometry method is described for the measurement of 3β-hydroxy-Δ^5-bile acid sulfates in urine applicable to the diagnosis and accurate monitoring of responses to primary bile acid therapy in HSD3B7 patients.