Identification of Urocanylglycine in Urocanase Deficiency

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Introduction
Urocanic aciduria (OMIM 276880) is caused by urocanase deficiency, a defect in the histidine pathway (Figure 1). Affected patients have an increased concentration of urocanic acid in their urine. HPLC analyses showed urocanic acid and an unknown metabolite (U1) in the urine of a girl with urocanic aciduria (Figure 2A).

Materials and Method
- Case report patient: Published in JMedGenet 2009;46.
- HPLC measurements for purines and pyrimidines were performed on a reversed-phase column and UV detection.
- 1H-NMR measurements were performed on a Bruker 500 MHz spectrometer.

Results (continued)

- Identification
  - “Urocanic acid peak” fraction (Figure 3B)
    Peak positions in the authentic standard of urocanic acid (Figure 3A) corresponded exactly with the observed positions in the fraction containing the urocanic acid peak.

- Peak U1 fraction (Figure 3C)
  The spectrum in Figure 3C shows similarities with the spectrum of urocanic acid (Figure 3A). Therefore, NMR spectra of urocanylcarcinitine, –glutamine, –taurine and –glycine were simulated. The simulation spectrum of urocanylglycine shows the same NMR pattern as the fraction containing peak U1. So, the unknown peak observed in the HPLC–chromatogram is in all likelihood urocanylglycine (structure and NMR characteristics are shown in Figure 4.). Formal proof is required but urocanylglycine is not available as model compound.

- Quantification
  The urocanic acid and urocanylglycine resonances can be observed directly in the urine spectrum of the patient. Urocanic acid and urocanylglycine concentrations were 200 and 180 µmol/mmol creatinine, respectively.

Conclusion
Urocanylglycine in urine is a novel and major hallmark in urocanic aciduria. As demonstrated here, the combination of HPLC with NMR spectroscopy provides a powerful tool for the elucidation of the structure of compounds present in urine samples.

Histidine
1
Urocanic acid
2
4-imidazolone
5-propionic acid

Figure 1. Deficiency in urocanase (black square) leads to the accumulation of urocanic acid.

Aim
Isolation, identification and quantification of the unknown metabolite (U1) in the urine from the patient with urocanic aciduria.

Results
- Isolation (Figure 2)

Figure 2. Fractions around the retention time in the chromatogram (A) of the urocanic acid peak and peak U1 were collected from a HPLC column (reversed-phase; buffer 0.025 M KH2PO4/0.5 % methanol). B: The fractions were dried under vacuum, redissolved in D2O (500 µl), and measured using NMR spectroscopy.

Figure 3. 500 MHz NMR spectra (pH 7.00) of model compound urocanic acid (A), the fraction containing the urocanic acid peak (B) and the fraction containing peak U1 (C). H-numbers refer to carbon numbers in the molecule (Figure 4).

Figure 4. Structure urocanylglycine and proton NMR assignments.