NMR Studies of Amniotic Fluid in Human Fetal Urogenital Tract Obstruction

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Introduction

NMR spectroscopy has been used extensively for the analysis of human and animal biofluids[1], in particular for the analysis of endogenous metabolites in urine[2]. Congenital urinary outflow obstruction caused by posterior urethral valves affects 1/5000 male births and is a common cause of end stage renal failure. If the obstruction is located between the kidney and the bladder a histologically abnormal kidney can result. If the obstruction is located posterior to the bladder then typically enlarged bladders are formed, with some bilateral kidney malformation. Here we are using high resolution ^{1}H NMR spectroscopy in a preliminary study to investigate the amniotic fluid from 3 patient groups, 'malformed kidneys', 'big bladders' and 'controls' with a view to better understanding of the biochemistry involved in these conditions.

Experimental

Amniotic fluid (1.00 cm^3) was freeze dried and made up to 0.75 cm^3 including 0.25cm^3 of sodium trimethylsilylproprionate (TSP, 1mg/cm^3) solution as a quantification and reference standard. The NMR system used in this preliminary study was an 11.7 Tesla Varian Unity Plus spectrometer (Varian, Palo Alto, Ca) operating at 500 MHz for protons. Spectra were obtained using 32 k data points, 6 kHz sweep width, with a 45^o pulse width and a relaxation delay of 5s using 128 scans at 25^{o}C. Spectrum quantification was carried out using SpecNMR (JEOL UK, Welwyn Garden City, UK). Principal component analysis (PCA) analysis was carried out using the software programme SIMCA (Umetrics Ltd, Windsor, UK). Amniotic fluid samples were analysed from subjects that presented with fetuses that showed malformed kidneys (n=4), big bladders (n=3) and were compared with 'control' amniotic fluid from patients with twin pregnancies where some of the amniotic fluid was removed as part of the patient care procedures (n=2). All samples were collected under ethical approval of Great Ormond Street Hospital for Children, London.

Results and discussion

Typical spectra for 'control' and 'malformed kidney' amniotic fluids are show in Figure 1. Full statistical analysis of the NMR data was not possible due to the small numbers of samples. However, a preliminary analysis shows a trend in the data where amniotic fluid from 'malformed kidneys' are characterised by increases in hippuric acid, trimethylamine N-oxide (TMAO), glycine, pyruvate and reductions in histidine, tyrosine, acetate and alanine, (see Figure 2). When these metabolite concentrations were used as input for PCA analysis complete separation of the 3 groups was achieved as shown in Figure 3. The 'malformed kidney' samples formed a much tighter group than the 'big bladder' as the severity of kidney damage in the latter group was not only less but also covered a greater range. Holmes *et al.*[3] showed the effect of selective nephrotoxins in model systems and how these toxins produced urine profiles indicative of the damaged region. For example, treatment with the medullary toxin bromoethanamine showed increases in TMAO, succinate and acetate as markers of toxicity. Bell *et al.*,[4] has previously shown that TMAO is an NMR marker for chronic renal failure in human subjects. We found here that TMAO as well as hippurate, glycine and pyruvate levels appear to be increased for 'malformed kidney' and 'big bladder' subjects compared with controls whilst the same subjects showed decreases in histidine, tyrosine, acetate and alanine concentrations. These data would indicate that there is kidney damage in both 'malformed kidney' and 'big bladder' groups compared with controls although the degree of damage appears to be different as shown by the PCA analysis and is reflected in histological studies[5].

Conclusion

NMR spectroscopic analysis of human amniotic fluid where ultrasound scans indicate the presence of fetal urinary tract problems appears to be useful in identifying possible markers of both severity and affected regions within the kidney. Further work is required with more subjects and controls as well as analysis of amniotic fluid from a sheep model[6] of this condition where the urogenital obstruction can be accurately controlled.

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References

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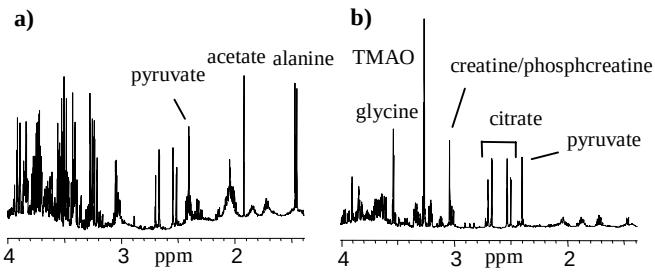

Figure 1: ^{1}H NMR spectra of a) 'control', and b) 'malformed kidney' amniotic fluid showing the aliphatic region of the spectra.

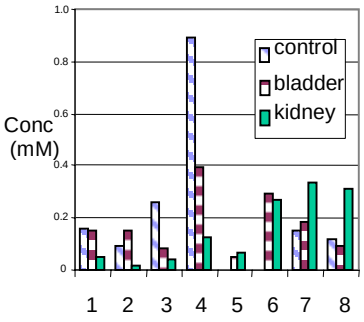

Figure 2: Metabolite levels of 1 histidine, 2 tyrosine, 3 acetate, 4 alanine, 5 hippurate, 6 glycine, 7 TMAO and 8 pyruvate.

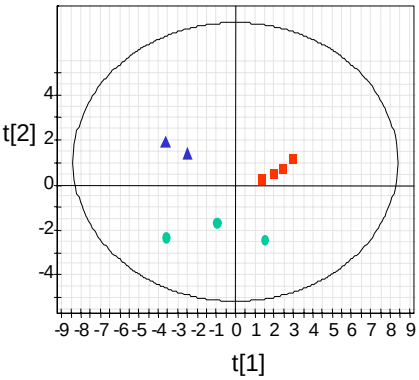

Figure 3: PCA of the 3 amniotic fluid groups using SIMCA. Triangles are 'controls', circles 'big bladder', and squares 'malformed kidney'.