Nuclear Magnetic Resonance Spectroscopy in Patients with Anion-Gap Acidosis

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ABSTRACT
Proton nuclear magnetic resonance spectroscopy was performed on blood or urine from five patients with an anion-gap metabolic acidosis. In all of these cases, this methodology allowed the rapid and specific diagnosis of the nature of the metabolic acidosis. In several of these patients, spectroscopic evidence for intoxication with toxic alcohols was obtained. On the basis of these preliminary data, proton nuclear magnetic resonance spectroscopy may be a useful technique in the evaluation of patients with anion-gap acidosis.

Key Words: Anion-gap acidosis, proton NMR, toxicology

Proton nuclear magnetic resonance (NMR) spectroscopy is an important analytical technique for chemical analysis (1). More recently, this technology has been applied to the study of human body fluids such as plasma and urine (2–4). This methodology allows for rapid, noninvasive measurement of the concentrations of many important normal components of these fluids as well as a powerful screening technique that might detect unexpected compounds. We therefore performed proton NMR analysis of urine or plasma specimens from patients who were referred to the renal consult service for the possibility of acute hemodialysis due to an intoxication.

EXPERIMENTAL METHODS

One-half milliliter of the urine or plasma specimen was diluted 1:1 with deuterium oxide (D2O) spiked with trimethylsilylpropionate (TSP). The TSP was used as both an internal concentration and a chemical shift standard. This mixture was placed in a standard 5-mm NMR tube. Spectra were collected in the Fourier transform mode with a high-resolution AM 300 (7.05 T), 10-cm vertical bore spectrometer (Bruker Instruments, Billerica, MA). The proton frequency in this instrument was 300.13 MHz. Spectra were collected with a sweep width of 12 ppm and an array size of 16 K. Samples were spun at 20 Hz, and an deuterium lock was employed during the acquisitions. Sixteen transients were obtained employing 90-degree pulses (4.5 μs) with an acquisition time of 2.25 and a total interpulse relaxation of 10 s. Water suppression was accomplished via gated decoupling of the water resonance. Identification of the chemical species were accomplished by analysis of the chemical shifts and coupling characteristics of the spectral peaks. Further validation of these peak assignments was achieved via “spiking” the samples with known compounds as well as confirmation with standard analytical techniques (1).

These samples were analyzed in a blinded fashion with the spectroscopist (D.M.) unaware of the clinical history of the patient.

PATIENTS AND RESULTS

All patients studied were referred to the University Hospital renal consultation service at University of Colorado Health Sciences Center for evaluation for possible intoxication. In all cases, NMR spectroscopic data was available within 10 to 15 min. The cases are summarized below.

Case 1

A young male was brought to the emergency department in a comatose state by his wife. She noted that the patient had been depressed for quite some time. She witnessed the ingestion of a can of paint thinner by the patient and subsequently brought the patient to the emergency department. The paint thinner was noted to contain methanol, toluene, and methylene chloride. The patient was intubated in order to protect his airway. A toxicology screen was sent. An ethanol infusion was also begun at this time. The serum creatinine was 1.8 mg/dL. The conventional toxicology screen revealed a methanol level of 80 mg/dL along with cocaine and marijuana metabolites. The conventional urinalysis was unrevealing. Hemodialysis was initiated. The patient ultimately recovered and was discharged from the hospital.
Case 2

A young male was brought to the emergency department in a comatose state. Before the development of the coma, the patient was noted to complain of visual disturbances. The patient was noted to have a profound metabolic acidosis with an elevated anion gap. The renal service was consulted, and a toxicol-

Figure 1. Proton NMR spectrum of the urine of Case 1. The resonances attributable to TSP (a chemical shift and concentration standard), ethanol, methanol, toluene, and various metabolic products are easily identified. The spectrum remaining upfield of the water resonance is shown in Figure 1a, and the spectrum remaining downfield of the water resonance is shown in Figure 1b.

The proton NMR spectrum of the urine from this patient before the initiation of hemodialysis is shown in Figure 1. The resonances attributable to the ethanol infusion, methanol, and to toluene and its metabolic products are easily identified and quantitated.

Figure 2. Proton NMR spectra of the urine of Case 2. The resonances due to methanol and formate are the dominant resonances of the spectra. The scale of from the region of the spectrum containing the formate resonance is one eighth compared with the rest of the spectra. The spectrum remaining upfield of the water resonance is shown in Figure 2a (the lower spectrum was obtained before the initiation of hemodialysis, and the upper spectrum was obtained from a urine sample at the completion of hemodialysis), and the spectrum remaining downfield of the water resonance is shown in Figure 2b.
ogy screen was sent. The serum creatinine was 2.0 mg/dL.

The proton NMR spectrum of this patient's urine is shown in Figure 2. The major resonances are attributable to methanol and its metabolic product, formate. The diagnosis of methanol intoxication was made, and hemodialysis was initiated. The results of the toxicology screen became available 1.5 h after the initiation of the hemodialysis. This individual died several days later.

Case 3

A middle-aged man in the medical intensive care unit was noted to be confused. Other than the slightly confused state, the physical examination was unrevealing. Routine laboratory tests revealed a serum creatine of 7.0 mg/dL and a mild anion-gap acidosis. An ethanol infusion was begun by the medical house officers for possible toxic alcohol intoxication. The conventional urinalysis as well as the proton NMR evaluation of the urine was unrevealing. The toxicology screen was also negative. The final diagnosis was chronic renal insufficiency. The mental state of the patient improved with the initiation of dialysis.

Case 4

A middle-aged woman was admitted to the hospital in a comatose state. She was noted to have a metabolic acidosis with an elevated anion gap (30 mEq/L). The renal service was consulted. The conventional urinalysis was unrevealing. A toxicology screen was also sent.

The proton NMR spectrum is shown in Figure 3. The major resonances are due to ethylene glycol and its metabolic product, glycolate. Oxalate, which is a further metabolic product of ethylene glycol metabolism, is not visible with proton NMR spectroscopy, because oxalate/oxalic acid does not contain any hydrogen atoms that are not rapidly exchanging with water.

The patient's serum was also evaluated with proton NMR spectroscopy in a similar manner. As noted in Figure 4, the major resonances are attributable to ethylene glycol and glycolate. The patient improved with therapy and was discharged from the hospital several days later.

Case 5

A 71-yr-old chronic hemodialysis patient was noted to be incoherent and to have a profound metabolic acidosis (pH 7.19; Pco₂ of 23) with an anion gap of 40 mEq/L. Because the patient did not make any urine, a plasma sample was analyzed with NMR spectroscopy. The proton NMR spectrum shown in Figure 5 is remarkable for being dominated by the two resonances of lactate, a doublet at 1.3 ppm and quartet at 4.05 ppm. Quantitation of these spectral peak areas, correcting for the number of protons contributing to each resonance, yielded a concentration of lactate of 27.0 mmol/L. This correlated quite well with the enzymatic measurement of lactate concentration of 27.4 mmol/L. The patient died the following day.

DISCUSSION

The differential diagnosis of the patient with an anion-gap metabolic acidosis includes intoxications due to any of a plethora of agents. The diagnosis of the offending toxin and specific therapy are often delayed for several hours until the results of a toxicology screen are available from a regional laboratory. NMR spectroscopy is a well-established method of chemical analysis. During the past 10 yr, technological advances have permitted construction of magnet large enough to accommodate the whole human body. As a result, magnetic resonance imaging has been established as a useful diagnostic tool. Furthermore, clinical studies of humans by NMR specros-
Figure 4. Proton NMR spectra (downfield of the water resonance) from the serum of Case 4. These spectra were obtained before (bottom spectrum), during (middle spectrum), and after (top spectrum) hemodialysis therapy. The resonances due to ethylene glycol and glycolate are identified and are shown to attenuate with hemodialysis therapy.

Figure 5. Proton NMR spectrum from the plasma of Case 5. The resonances due lactate are identified. The areas below the peaks when compared with the internal standard correspond to a concentration of about 27 mM.

copy are becoming a reality. In spite of the presence of NMR spectrometers in most medical schools and health sciences centers, the use of NMR spectroscopy in clinical chemistry and toxicology has not been explored.

The above cases demonstrate that proton NMR spectroscopy of the urine or plasma is a rapid and powerful technique by which to screen for various substances. The study of biological fluid was completed in approximately 10 to 15 min for all patients, and therefore, results of this study were considerably quicker than results from standard toxicology screening tests. Although proton NMR spectroscopy is relatively insensitive, it is possible to accurately assign resonances to compounds achieving concentrations in excess of 0.2 mM with the acquisition parameters described above. Certainly, virtually all toxic alcohol ingestions as well as any significant component of the anion gap will exceed this concentration threshold, making this an extremely versatile test. NMR spectroscopy may have other advantages in the diagnosis of increased anion-gap metabolic acidosis over conventional laboratory assays. In particular, the enzymatic evaluation of lactate is sensitive to the L form, whereas it is insensitive to the D form, of lactate. The D type of lactic acidosis, therefore, would not be detected with conventional enzymatic methods (5). Both the D and L forms of lactate may be observed with NMR spectroscopy. In addition, it should be stressed with proton NMR spectroscopy that one assay allows for the simultaneous screening for a variety of important compounds, including virtually all toxic alcohols requiring hemodialysis as part of the therapy of the patient (6).

In summary, we found proton NMR spectroscopy of urine or blood to be a sensitive, specific, and rapidly performed test in a pilot study of patients with anion-gap metabolic acidosis. If these preliminary results are confirmed in a larger group of patients, it may become desirable to have this methodology as part of the standard toxicology evaluation.

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