The herbicide paraquat (1,1′-dimethyl-4,4′-bipyridylium dichloride; PQ), is a poison known to cause delayed mortality due to lung and kidney injuries. High-resolution proton nuclear magnetic resonance (1H NMR) spectroscopy has been extensively applied in evaluating nephrotoxicity by the characteristic perturbations in the excretion pattern of low molecular weight endogenous metabolites. The application of the method allows the rapid localization of the renal injury noninvasively. In this study, we report 1H NMR and conventional clinical chemistry urinalysis in two patients suffering from paraquat intoxication after overdose with suicidal intent. The alterations in the urine NMR spectrum suggest necrosis of the pars recta of the proximal renal tubules. The molecule of paraquat is also clearly detected in the same spectrum. In conclusion, the rapid screening of urine by NMR spectroscopy provides information about both the identity of the poison and the abnormal pattern of endogenous metabolites that characterize the location of the injury in renal tubules and reveals alterations in unusual metabolites that are not commonly measured.

Paraoquat (1,1′-dimethyl-4,4′-bipyridylium dichloride; PQ) is a widely used and effective herbicide with a broad spectrum of activity. However, PQ is quite toxic; the toxicity on animals and humans has been well documented (1–3). Experimental studies have shown that it is accumulated in the lung and kidney epithelial cells, leading eventually to pulmonary fibrosis and acute renal failure.

PQ-related primary lesions, from both acute and chronic exposure, occur in the lung. The ability of the lung to accumulate this herbicide probably causes the selectivity of the PQ-related toxicity. A mouthful of the herbicidal compound usually results in death from caustic burns, renal tubular necrosis, and circulatory failure due to pulmonary fibrosis. Intoxication with PQ still has a low prognosis because of no efficient treatment.

High-resolution proton nuclear magnetic resonance (1H NMR) spectroscopy has been extensively applied for the analysis of the composition of biological fluids in endogenous and drug metabolites (4–7). NMR provides quantitative information of the low molecular weight metabolites present in the specimen studied and allows the detection of unexpected constituents related to disease or tissue damage.

Urinalysis by NMR spectroscopy has led to the detailed investigation of the excretion pattern in various physiological and pathological situations (8–12). Renal damage as a consequence of acute toxic exposure in drugs or other xenobiotics has been studied extensively in experimental animals by Nicholson and co-workers (13–17). These studies showed that the NMR pattern appears to be dependent stringently on the type of toxin to which an animal has been exposed. Each toxic compound or class of compounds produces characteristic changes in the concentrations and patterns of endogenous metabolites in biofluids that provide information on the sites and basic mechanisms of the toxic process. Although there are many and detailed NMR studies in experimental animals concerning renal damage, only a few studies have been reported in humans (18–20).

In the present study, we used 1H NMR spectroscopy and conventional clinical chemistry methods to explore the changes in low molecular weight metabolites in urine and localize the renal injury in two patients suffering from paraquat intoxication after overdose with suicidal intent.

**Materials and Methods**

Two patients (28 and 60 years of age, respectively), who had taken paraquat with suicidal intent, were admitted to our hospital on different occasions; the time of admission after ingestion was estimated at 12 h and 7 h for the younger and the older patient, respectively.
The first patient had taken ~150 mL of “Gramoxone” (200 g/L paraquat), whereas for the second patient, it was reported that he had taken a large amount of the same herbicidal compound.

To eliminate the poison, the patients were immediately submitted to sequential sessions of hemoperfusion, because, in both of them, an adequate gastric lavage had been performed at a local hospital. The first patient received a total of 11 sessions of hemoperfusion, and the second patient received one. In addition, supportive measures, including maintenance of water, electrolytes, and acid-base balance, were taken. In addition to this treatment, 1 g of methylprednisolone was administered intravenously to the second patient. Urine and serum samples were collected until death (80 h after poisoning for the first patient due to gastrointestinal hemorrhage and 12 h for the second, who succumbed to heart failure).

Samples were centrifuged and stored at –35 °C until NMR analysis. Standard clinical chemistry routine analysis of serum and urine was made the same day of the collection. For urine protein analysis, the samples were stored at 4 °C, and the measurements were performed within 3 days.

**NMR Analysis**

In a 0.5-mL volume of crude urine, 50 μL of 2H2O was added containing sodium-3-trimethylsilyl-[2,2,3,3-2H4]-1-propionate (TSP) as a chemical shift reference (δ = 0.0). The final concentration of TSP in the working solution was 0.193 mmol/L. 1H NMR measurements were made on a Bruker AMX400 spectrometer (Bruker Analytische Messtechnik), operating at a field strength of 9.4 Tesla (400 MHz 1H frequency), at 22 °C (NMR Center, University of Ioannina). A continuous secondary irradiation field of 2H2O was applied to suppress the intense H2O signal. Typically for each sample, 64 free induction decays were collected into 16,384 computer points. The assignments of resonances were confirmed by consideration of chemical shifts and the addition of standards. NMR analysis of the urine of healthy individuals was used for comparison. Quantitation of the metabolites was made against internal TSP. For method comparison, we related the creatinine results obtained by NMR spectroscopy with those of conventional clinical chemistry assay. The intramethod correlation ranged between 0.89 and 1.08 (Table 1).

**Results and Discussion**

A typical spectrum of healthy human urine is shown in Fig. 1 (aliphatic region, δ: 0.5–4.5 ppm). The main constituents of the spectrum in the aliphatic region are creatinine, which gives rise to two intense peaks at 3.05 and 4.12 ppm, hippurate, glycine, N-methylated metabolites, citrate, small amounts of alanine, lactate, and other metabolites in lower concentrations (9, 18).

In the NMR urinalysis performed by Nicholson and co-workers after renal damage in experimental animals (13–17), the excretion of altered metabolite concentrations has been described in detail. Proximal tubular injury is associated with glucosuria, aminoaciduria, lactic aciduria, and 3-n-hydroxybutyric aciduria, along with reduced excretion of citric acid cycle intermediates, such as citrate and succinate (indicative of impaired oxidative metabolism), whereas medullary damage leads to the early appearance of

<table>
<thead>
<tr>
<th>Time after poisoning</th>
<th>Creatininea</th>
<th>Creatinine</th>
<th>Lactate</th>
<th>Citrate</th>
<th>Hippurate</th>
<th>Alanine</th>
<th>Valine</th>
<th>Acetone</th>
<th>Carnitine</th>
<th>Parquat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subject</td>
<td>9.02</td>
<td>9.70</td>
<td>0.47</td>
<td>4.57</td>
<td>12.72</td>
<td>0.20</td>
<td>0.18</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>First patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12–18 h</td>
<td>3.78</td>
<td>3.71</td>
<td>7.35</td>
<td>0.17</td>
<td>1.34</td>
<td>1.07</td>
<td>0.44</td>
<td>0.70</td>
<td>0.19</td>
<td>ND</td>
</tr>
<tr>
<td>25–30 h</td>
<td>1.80</td>
<td>1.64</td>
<td>6.46</td>
<td>ND</td>
<td>ND</td>
<td>1.23</td>
<td>0.52</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>44–50 h</td>
<td>1.19</td>
<td>1.28</td>
<td>6.25</td>
<td>ND</td>
<td>ND</td>
<td>1.18</td>
<td>0.52</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Second patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–12 h</td>
<td>1.06</td>
<td>0.94</td>
<td>7.24</td>
<td>0.16</td>
<td>ND</td>
<td>0.75</td>
<td>0.28</td>
<td>ND</td>
<td>ND</td>
<td>3.5</td>
</tr>
</tbody>
</table>

* Conventional clinical chemistry assay.

* ND, not detectable.
Fig. 1. Spectra (400 MHz) of urine (δ 0.5–4.5) from a healthy subject and from the two patients suffering from paraquat intoxication (sample from urine collection: 12–18 h after ingestion for the first patient and 8–12 h for the second patient).
N-Me, N-methylated metabolites.
trimethylamine-N-oxide and dimethylamine, followed by increased excretion of acetate and succinate.

The urine spectrum of the two patients, after paraquat intoxication, was markedly altered compared with the unaffected urine (Fig. 1). Renal damage was manifested in the first urine collection after poisoning (12–18 h for the first and 8–12 h for the second patient, respectively). The excretions of glucose, lactate, alanine, valine, and glutamine were markedly increased. The excretion of creatinine was reduced, whereas the resonances of hippurate were completely suppressed and those of citrate were detected only in traces (Table 1).

![Spectra (400 MHz) of urine (pH 6.5–9.5) from a healthy subject and from two patients suffering from paraquat intoxication (sample from urine collection: 12–18 h after ingestion for the first patient and 8–12 h for the second patient).](image)

![Spectra (400 MHz) of urine (pH 6.5–9.5) from a healthy subject and from two patients suffering from paraquat intoxication (sample from urine collection: 12–18 h after ingestion for the first patient and 8–12 h for the second patient).](image)

**Table 2. Conventional clinical chemistry analysis of urine from the paraquat-intoxicated patients.**

<table>
<thead>
<tr>
<th>Time after poisoning</th>
<th>Creatinine, mg/L</th>
<th>Glucose, mg/L</th>
<th>Total proteins</th>
<th>Albumin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Serum</td>
<td>Urine</td>
<td>Serum</td>
<td>Urine</td>
</tr>
<tr>
<td>First patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12–18 h</td>
<td>26</td>
<td>428</td>
<td>1110</td>
<td>2770</td>
</tr>
<tr>
<td>25–30 h</td>
<td>46</td>
<td>204</td>
<td>1360</td>
<td>2620</td>
</tr>
<tr>
<td>44–50 h</td>
<td>48</td>
<td>135</td>
<td>1410</td>
<td>3250</td>
</tr>
<tr>
<td>Second patient</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8–12 h</td>
<td>36</td>
<td>120</td>
<td>1240</td>
<td>1360</td>
</tr>
</tbody>
</table>

*α₂-m, α₂-microglobulin.
This NMR pattern is similar to that observed after exposure of the experimental animals to proximal tubule toxins and closer to that observed after mercury chloride intoxication, which is well known to result in necrosis of the $S_3$ region of renal tubules (pars recta) (13, 14).

Increased excretions of the lipid peroxidation products, formaldehyde, acetaldehyde, malondialdehyde, and mainly acetone detected by gas chromatography–mass spectroscopy and HPLC in rats, has been associated with paraquat intoxication (21). In the NMR spectrum of the first patient, an intense signal from acetone was detected in the urine collection 12–18 h after intoxication (Fig. 1). In addition, in this urine collection, an intense signal at 3.23 ppm was noticed, derived from the $N$-trimethyl group of the molecule of carnitine, which is a carrier molecule in the transport of fatty acids from the...
cytoplasm into the mitochondria across the membranes for β-oxidation. This signal at 3.23 ppm is hidden by the double-double resonance of the β-C2 proton of glucose at 3.2–3.3 ppm. We confirmed the presence of carnitine and re-recording the spectrum; and (b) by using temperature variation to separate the resonances of glucose and carnitine (unpublished data). The increased concentrations of carnitine in urine after paraquat intoxication have never been reported previously.

Conventional clinical chemistry analysis has further supported the NMR findings (Table 2). Reduced creatinine clearance and glucose reabsorption are indicative of progressive renal failure. Mild proteinuria was measured (~1.5 g/L) with a IgG/albumin ratio of 0.11, suggesting a selective proteinuria due to glomerular damage. Furthermore, the excretion of α1-microglobulin, a specific marker for tubular damage, was progressively increased.

In addition, in the urine of the second patient, a high concentration of unmodified toxic compound (1,1′-dimethyl-4,4′-bipyridylium dichloride) was detected. Fig. 2 shows the NMR spectrum of the aromatic region in the urine of healthy subject and the two patients. The resonances of hippurate present in the healthy urine spectrum were suppressed in the two patients, as mentioned above. Two well-resolved doublets due to the pyridyl ring of paraquat were detected in the urine of the second patient, who was admitted in the hospital soon after the episode. This spectrum was identical to the spectrum of the commercial product Gramoxone (data not shown).

The concentrations of lactate, glucose, and amino acids were maintained high in all urine collections during the 3 days of the first patient’s life, whereas the creatinine concentration was further reduced, also confirmed by conventional clinical chemistry methods (Fig. 3). The NMR pattern of the urine sample at 44–50 h of the first patient was similar to that of the second patient (Fig. 1), whose survival after intoxication was shorter. This is probably the pattern of the end stage of paraquat injury.

Based on the above findings, we can conclude that the rapid screening of urine by NMR spectroscopy provides information about both the identity of the poison and the resulting abnormal pattern of endogenous metabolites. This pattern is related to the site and severity of toxicity within the kidney, reveals alterations in unusual metabolites that are not commonly measured, and can be used as a noninvasive identification procedure for paraquat poisoning.

References