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Short Communication

Proton NMR studies on the effects of uranyl nitrate on the biochemical composition of rat urine and plasma*

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Introduction

Small doses of uranyl nitrate (UN) and other uranyl compounds cause reproducible acute nephronal damage and have been employed as models of experimental acute renal failure [1–4]. The site of the UN-induced lesion is the *pars recta* of the proximal tubule and is characterized by swelling, necrosis and shedding of epithelial cell cytoplasm into the tubular lumina [3]. The functional changes observed following a single nephrotoxic dose of UN include increases in blood urea nitrogen, plasma renin activity, urine flow rate and urinary sodium with decreases in glomerular filtration rate, renal blood flow and urine osmolality [1–4]. High resolution proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy is well suited to the study of toxicological events. The combination of non-selectivity with high chemical specificity provided by this technique is potentially of great value in the field of toxicology since a variety of metabolic effects can be investigated simultaneously without prior knowledge of the nature of the toxic lesion. We have previously shown that ^{1}H-NMR urinalysis studies can provide high order information on the biochemical effects of nephrotoxins [5–8]. Indeed, this technique highlighted the link between L-lactic aciduria and proximal tubular necrosis, and in addition, the association between increased urinary levels of dimethylamine (DMA), trimethylamine *N*-oxide (TMAO), *N*,*N*-dimethylglycine (DMG) and succinate, and experimentally induced renal papillary necrosis [6]. In the present study we have chosen to examine UN-induced nephrotoxicity employing high resolution ^{1}H-NMR urinalysis techniques in an attempt to evaluate the effects of this chemical on the profile of low molecular weight urine and plasma components, and hence generate new information on its biochemical effects and mechanism of toxicity within the kidney.

Experimental

Animals and treatments

Twelve male Fischer 344 rats were allocated to four groups of three rats each, placed individually in plastic metabolism cages and allowed free access to food and tap water. Rats were dosed with either 0.9% NaCl or doses of 5, 10 or 20 mg kg^{-1} $UO_2(NO_3)_2$. Urine was collected for 24 h prior to dosing and at 8, 24 and 48 h after dosing; plasma was taken at sacrifice (48 h).

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^{1}H-NMR analysis

Measurements were made on a Bruker WH400 spectrometer at 9.4 Tesla (400 MHz proton resonance frequency). A calculated volume of urine [5] was lyophilized and redissolved in an equal volume of ^2H_2O containing 3-trimethylsilyl-[2,2,3,3,-$^2H_4$]-1-propionate (TSP). Sixty-four free induction decays were collected into 16K points using 40° (4 μs) pulses, a spectral width of 5000 Hz and an acquisition time of 1.7 s. A delay of 3.0 s between pulses was added to allow full T_1 relaxation. Spin–echo spectra of lyophilized plasma in ^2H_2O were recorded using the Hahn sequence (90– τ–180–τ–collect FID) [9] with a τ value of 60 ms and a delay between cycles of 3 s. An exponential line broadening function of 1.0 Hz was applied prior to Fourier transformation.

Results

^{1}H-NMR urinalysis

Figures 1 and 2 show, respectively, the high field and low field portions of the ^{1}H-NMR spectra of control urine and urine collected 24–48 h following exposure to 5, 10 or 20 mg kg^{-1} UN. A gradual decrease in citrate, creatinine and 2-oxoglutarate excretion can be seen in addition to aminoaciduria and glycosuria after UN (Fig. 1). In addition, a dose-related increase in urinary 3-D-hydroxybutyrate can be seen (Fig. 1). Moreover, ^{1}H-NMR urinalysis uncovered changes in the pattern of metabolites in the low field region of the spectrum, principally a decreased excretion of hippurate and allantoin, with increased excretion of the aromatic amino acids histidine, phenylalanine and tyrosine (Fig. 2). Evidence of glycosuria can also be seen in Fig. 2 with increased intensity of the doublet resonance arising from the α-anomeric proton of glucose.

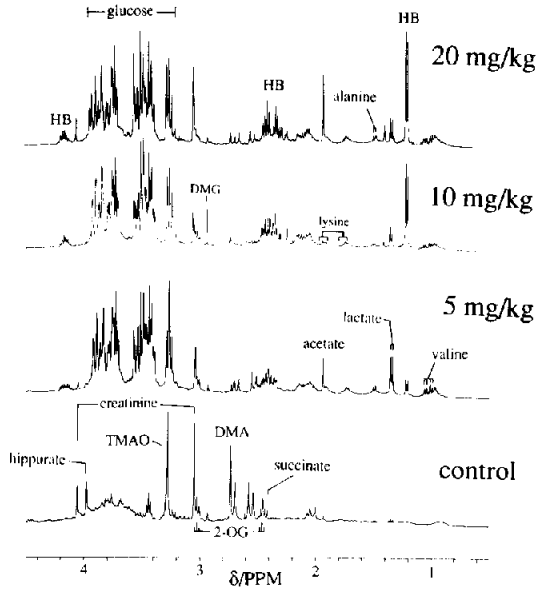


Figure 1. ^{1}H-NMR spectra, 400 MHz (high field region) of rat urine from control and 24–48 h after 5, 10 and 20 mg kg^{-1}UN. See text for experimental conditions. DMA, dimethylamine; DMG, *N*,*N*-dimethylglycine; HB, 3-D-hydroxybutyrate; 2-OG, 2-oxoglutarate; TMAO, trimethylamine *N*-oxide.

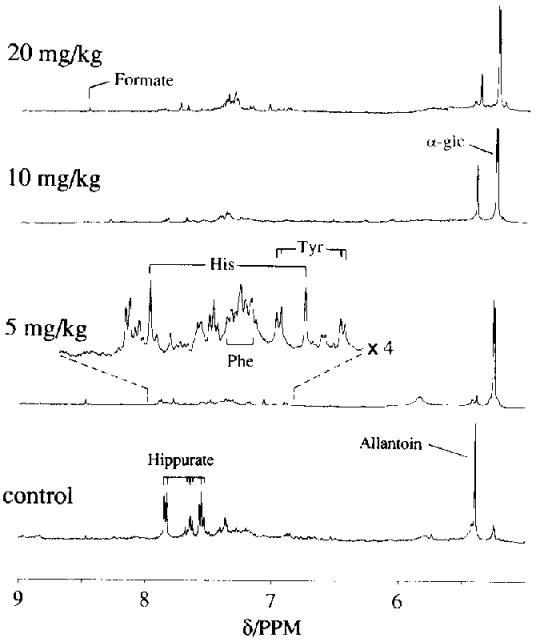


Figure 2
^{1}H-NMR spectra, 400 MHz (low field region) of rat urine from control and 48 h after 5, 10 and 20 mg kg ^{1} UN. See text for experimental conditions. α-glc, α-anomeric proton of glucose; His, histidine; Phe, phenylalanine; Tyr, tyrosine.

^{1}H-NMR plasma analysis

Hahn spin–echo ^{1}H-NMR spectra of plasma from UN-treated rats (Fig. 3) also showed differences from control. Dose-related increases in plasma creatinine and HB were seen in addition to a decrease in the resonances P_1 and P_2 arising, respectively, from CH_2 and CH_3 groups of mobile fatty acids. Examination of the plasma spectrum from the high dose group revealed an apparent decrease in the intensity of the alanine methyl doublet (Fig. 3). This was associated with the appearance of a singlet at δ = 1.5 ppm (Fig. 3). We believe that

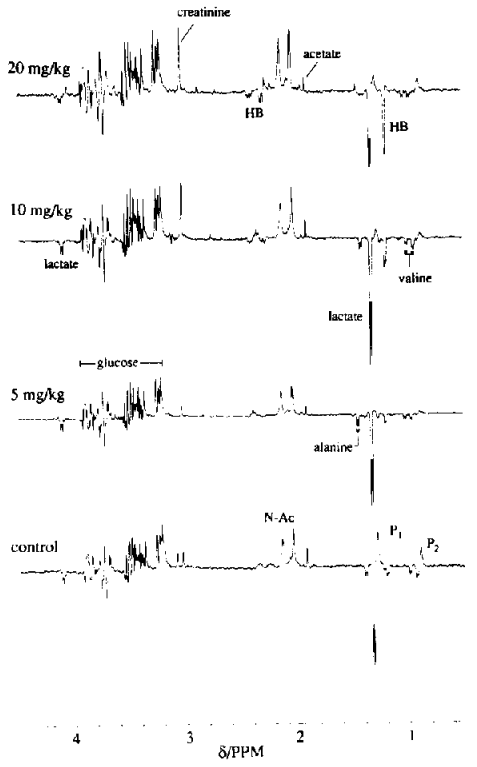
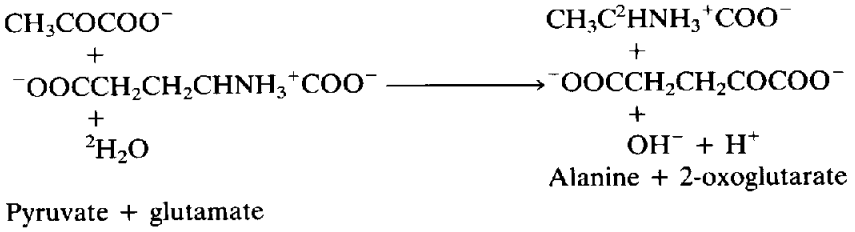


Figure 3
Hahn spin–echo PMR spectra, 400 MHz (high field region) of rat plasma from a control rat and 48 h after 5, 10 and 20 mg kg^{-1} UN. See text for experimental conditions. HB, 3-D-hydroxybutyrate; NAc, *N*-acetylated groups of plasma glycoproteins; P_2 and P_1, CH_3 and $(CH_2)_n$ groups of mobile fatty acids.

the singlet arises from the CH_3 protons of deuteralanine, i.e. alanine deuterated at the α-position arising as a result of elevated plasma alanine aminotransferase (ALT) activity, removing the coupling to the CH_3 group and appearing as an upright singlet. We are investigating this phenomenon further. The reaction showing the incorporation of a deuteron from deuterium oxide into α-alanine is as follows:



Pyruvate + glutamate

Alanine + 2-oxoglutarate

Discussion

Doses of 5, 10 and 20 mg kg^{-1} UN produced reproducible patterns of ^{1}H-NMR-detectable metabolic change in both the urine and plasma from treated rats. ^{1}H-NMR urinalysis uncovered changes in recognized markers of renal injury such as amino acids, creatinine and glucose as well as changes in more novel low molecular weight urine components like HB, citrate and L-lactate. The lactic aciduria seen following UN is modest in comparison to that seen following other toxins targeting the *pars recta* such as *p*-aminophenol, hexachlorobutadiene or $HgCl_2$ [5–7]. The reason for this is unclear although sodium chromate, a toxin causing necrosis in the *pars convoluta* of the proximal tubule, has also been shown to produce a modest lactic aciduria [6]. The lactic aciduria observed following UN was not dose-related, highest levels seen 24–48 h following the lowest dose (Fig. 1). Thus it would appear that proximal tubular necrosis and lactic aciduria are only associated in certain types of nephrotoxic injury. The hypocitraturia displayed after UN is in common with that seen following exposure to 2 mg kg^{-1} $HgCl_2$ [5, 6] but with differing timecourse. Whereas citrate in urine was undetectable by ^{1}H-NMR urinalysis 8–24 h after $HgCl_2$ [6], here this Krebs cycle intermediate was undetectable only 24–48 h after 10 mg kg^{-1} UN (Fig. 1). Such an effect of UN on urine citrate indicates an effect on renal acid–base status. In fact, UN may be precipitating a renal tubular acidosis similar to that induced by $HgCl_2$, possibly arising from inhibition of renal carbonic anhydrase activity [5]. A dose-related decrease in urinary 2-oxoglutarate was seen following UN (Fig. 1). This is a common effect following proximal tubular insult and has been observed following challenge with *p*-aminophenol (PAP), $HgCl_2$ and hexachlorobutadiene (HCBD) [7]. This is a further consequence of the impaired acid–base status following UN.

A novel and interesting finding is the apparent dose-related increase in urinary HB following UN. Although urinary HB has previously been shown to be increased following HCBD and PAP [6, 7] these changes were minor in comparison with those seen here. The HB-aciduria could arise from a number of causes including an effect of the toxin on food intake causing an appetite suppressant effect, overflow of increased plasma HB levels, or a biochemical effect within the kidney itself. The first of these possibilities can be eliminated immediately since in the fasting state plasma glucose is depressed [10] and in the present study UN produced no change in plasma glucose levels 48 h after dosing (data not shown). Furthermore, a dose-related increase in plasma HB was demonstrated following UN (Fig. 3). More considerable increases in plasma HB have been seen in fasting human subjects as well as in an insulin-dependent diabetic during insulin withdrawal [10]. The effect of UN on plasma HB combined with the disappearance of resonances from plasma mobile fatty acids following UN (Fig. 3) strongly suggests increased fatty acid catabolism. Disappearance of these resonances has been shown previously in plasma from normal fasting human subjects and an insulin-dependent diabetic following insulin withdrawal [10]. However, the situation seen following UN does not mimic either of these two examples insofar as no changes in either plasma acetoacetate, lactate or glucose were observed here.

At this stage the most likely explanation for the increased urinary HB would appear to be perturbed renal biochemistry. The kidney is well known to use as energy sources not only fatty acids [11] but also ketone bodies [12]. Indeed the highest activity of HB dehydrogenase in the kidney was recovered from the proximal and distal convoluted tubules [13]. Further work is required in order to establish whether UN is exhibiting an inhibitory effect on renal fatty acid catabolism. Such experiments would include incubating UN with selected enzymes of renal lipid metabolism such as HB dehydrogenase in order to test the hypothesis that UN is causing incomplete renal oxidation of HB. However, the possibility that the HB-uria arises as a result of decreased reabsorption in the proximal tubule secondary to toxic insult cannot be ruled out.

Conclusion

In the present study we have succeeded in characterizing several of the biochemical effects of UN on the kidney including changes in the excretion of novel low molecular weight urine components such as citrate, L-lactate and HB following toxic injury.

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