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Short Communication

Proton NMR monitoring of the onset and recovery of experimental renal damage*

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Introduction

Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy is a powerful tool in uncovering novel biochemical markers of region-specific nephrotoxicity and monitoring the biochemical effects following nephrotoxic insult [1–5]. In particular, ^{1}H-NMR can be used to investigate the biochemical changes that occur at the onset and during the progression of the nephrotoxic lesion [1–5]. However, little ^{1}H-NMR work has been performed on the biochemical changes associated with recovery following nephrotoxic injury. In the present study we have investigated the biochemical changes associated with such recovery following challenge with $HgCl_2$ (a proximal tubular toxin) and bromoethanamine (BEA, a renal papillary toxin). $HgCl_2$ causes necrosis of the epithelial cells lining the *pars recta* of the proximal tubule and the primary mechanism of toxicity involves the combination with biological sulphydryl groups resulting in the inhibition of a number of key enzyme systems especially in Krebs cycle [1, 6]. BEA produces renal papillary necrosis (RPN) giving rise to several of the clinical features associated with analgesic nephropathy. Following a single injection of BEA, the percentage of filtering juxtamedullary nephrons is markedly reduced [7]. In the present study, conducted over 14 days, the changes in both endogenous and exogenous metabolites following single injections of BEA and $HgCl_2$ were examined. ^{1}H-NMR spectroscopy was used as the main analytical tool supported by conventional clinical chemistry methods.

Experimental

Animals and treatments

Twenty-four male Fischer 344 rats were allocated to three groups of eight rats each and received single i.p. injections of either 0.9% saline (control), 1 mg kg^{-1} $HgCl_2$ or 150 mg kg^{-1} BEA in saline. Rats were housed individually in metabolism cages for a period of 2 days prior to dosing to permit acclimatization, and urine was collected at the following time points: 0–8 h (day 1), 8–24 h (day 1), 0–8 h (day 2), 8–24 h (day 2), 8–24 h (day 3), and throughout 8–24 h on days 3, 6, 9 and 15. Rats were allowed free access to food and water throughout the experiment.

Clinical chemistry

Creatinine, lactate, alkaline phosphatase (ALP), γ-glutamate transpeptidase (GGT) and lactate dehydrogenase (LDH) were measured in urine using Baker assay kits. All urinary enzymes were measured following a desalt step using Sephadex G25M minicolumns (Pharmacia) in order to remove any low molecular weight inhibitors of enzyme activity.

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Urinary volumes and osmolalities were also determined.

^{1}H-NMR urinalysis

A calculated volume of urine [2] was lyophilized and redissolved in 0.5 ml ^2H_2O. ^{1}H-NMR spectra were recorded on Bruker WH400 and AM400 spectrometers at 400 MHz at ambient probe temperatures (298 ± 1K). For each sample 64 free induction decays (FIDs) were collected into 16,384 data points and zero-filled to 32,768 data points using a pulse width of 45°, an acquisition time of 1.7 s and a relaxation delay of 2–3 s to ensure that the spectra were fully T_1 relaxed. Water suppression was achieved using gated irradiation. An exponential line broadening function of 0.5 Hz was applied prior to Fourier transformation. Chemical shifts were referenced to internal sodium 3-trimethylsilyl-[2,2,3,3,-$^2H_4$]-1-propionate (TSP; $\delta = 0$ ppm). Resonance assignments were made by chemical shift, spin–spin coupling patterns, pH-dependencies of chemical shifts and ultimately by standard addition employing the method adopted by Bales *et al.* [8].

Results and Discussion

The recovery from mercuric chloride and bromoethanamine-induced nephrotoxic injury has been studied, employing both conventional clinical chemical and high resolution ^{1}H-NMR urinalysis techniques. Tables 1 and 2 demonstrate the overall patterns of change in urinary creatinine, lactate and selected enzymes following single i.p. injections of BEA or $HgCl_2$. These are region-specific nephrotoxins affecting different portions of the nephron and cause correspondingly different perturbations of urinary metabolites. Although the principal target of mercury-induced nephrotoxicity is the *pars recta* of the proximal tubule this chemical also affects the renal medulla causing a decrease in urine osmolality and an increase in urine flow rate [2]. Changes in ^{1}H-NMR spectra of urine following nephrotoxic insult with BEA and $HgCl_2$ over a 15 day time course are shown in Figs 1 and 2, respectively.

Bromoethanamine

Conventional urinalysis indicates that BEA produces a marked reduction in urine osmolality and a considerable, sustained increase in volume from 0 to 8 h on day 1 (Table 1). Modest enzyuria is also apparent [2, 7]. Metabolites of BEA appear in ^{1}H-NMR spectra of urine within 8 h after dosing (day 1) and are also present in minor quantities at 8–24 h (day 1). Bach *et al.* [9] observed as many

Table 1
Urinary creatinine, lactate, ALP, GGT and LDH from control and BEA-treated rats

Time	Creatinine	Lactate	ALP	LDH	GGT	UFR	OSM
Control	3.3 ± 0.5	0.3 ± 0.1	179 ± 38	21 ± 3	719 ± 142	0.4 ± 0.1	1608 ± 30
Day 1	2.9 ± 0.2	1.1 ± 0.1	193 ± 49	65 ± 8	626 ± 161	1.5 ± 0.2	379 ± 32
Day 2	3.6 ± 0.3	0.6 ± 0.1	208 ± 81	35 ± 7	824 ± 248	1.5 ± 0.2	448 ± 19
Day 3	3.8 ± 0.8	0.5 ± 0.1	198 ± 20	20 ± 4	599 ± 225	1.1 ± 0.2	594 ± 57
Day 6	3.9 ± 0.4	0.4 ± 0.0	178 ± 41	18 ± 3	647 ± 136	0.8 ± 0.1	805 ± 85
Day 9	3.3 ± 0.5	0.4 ± 0.1	181 ± 28	19 ± 5	550 ± 105	0.6 ± 0.1	939 ± 77

Values represent the mean ± the SD of the 8–24 h timepoints from the days listed; $n = 8$ rats for all groups. Units: creatinine, μmol h^{-1}; lactate, mmol h^{-1}; ALP (alkaline phosphatase), U h^{-1}; LDH (lactate dehydrogenase), U h^{-1}; GGT (γ-glutamyl transpeptidase), U $^{-1}$; UFR (urine flow rate), ml h^{-1}; OSM (urine osmolality), mOsm kg^{-1} H_2O.

Table 2
Urinary creatinine, lactate, ALP, GGT and LDH from control and $HgCl_2$-treated rats

Time	Creatinine	Lactate	ALP	LDH	GGT	UFR	OSM
Control	2.9 ± 0.1	0.33 ± 0.04	175 ± 45	20 ± 3	595 ± 85	0.4 ± 0.1	1796 ± 292
Day 1	3.4 ± 0.3	1.78 ± 1.2	1323 ± 437	1014 ± 489	9346 ± 894	0.7 ± 0.2	733 ± 124
Day 2	3.3 ± 0.3	8.9 ± 1.8	530 ± 255	801 ± 247	2299 ± 896	1.3 ± 0.2	704 ± 76
Day 3	4.0 ± 0.5	5.7 ± 1.9	118 ± 20	58 ± 14	359 ± 81	0.9 ± 0.2	905 ± 107
Day 6	3.6 ± 0.2	0.6 ± 0.06	77 ± 14	19 ± 2	199 ± 43	0.6 ± 0.2	1193 ± 246
Day 9	3.0 ± 0.2	0.4 ± 0.03	79 ± 20	13 ± 6	237 ± 45	0.4 ± 0.0	1493 ± 142

Values represent the mean ± the SD of the 8–24 h timepoints from the days listed; $n = 8$ rats for all groups. Units: creatinine, μmol h^{-1}; lactate, mmol h^{-1}; ALP (alkaline phosphatase), U h^{-1}; LDH (lactate dehydrogenase), U h^{-1}; GGT (γ-glutamyl transpeptidase), U h^{-1}; UFR (urine flow rate), ml h^{-1}; OSM (urine osmolality), mOsm kg^{-1} H_2O.

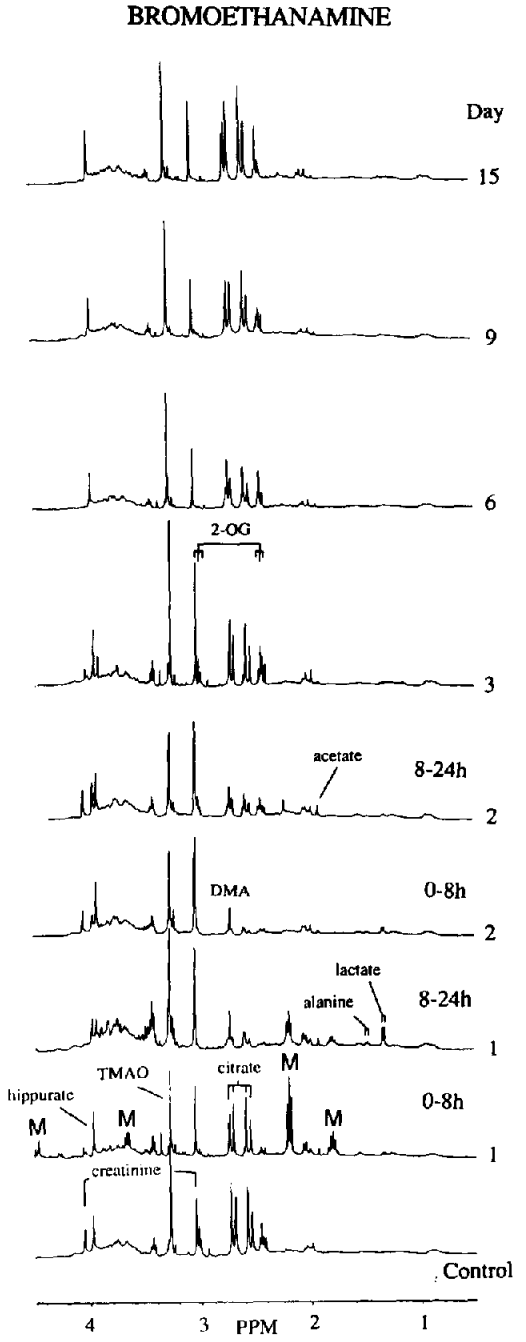

Figure 1
^{1}H-NMR spectra, 400 MHz (region to low frequency of water) of urine from rats before (control) and at various times up to 15 days following 150 mg kg^{-1} BEA. See text for experimental conditions. DMA, dimethylamine; DMG, *N*,*N*-dimethylglycine; TMAO, trimethylamine *N*-oxide; M, metabolites of BEA.

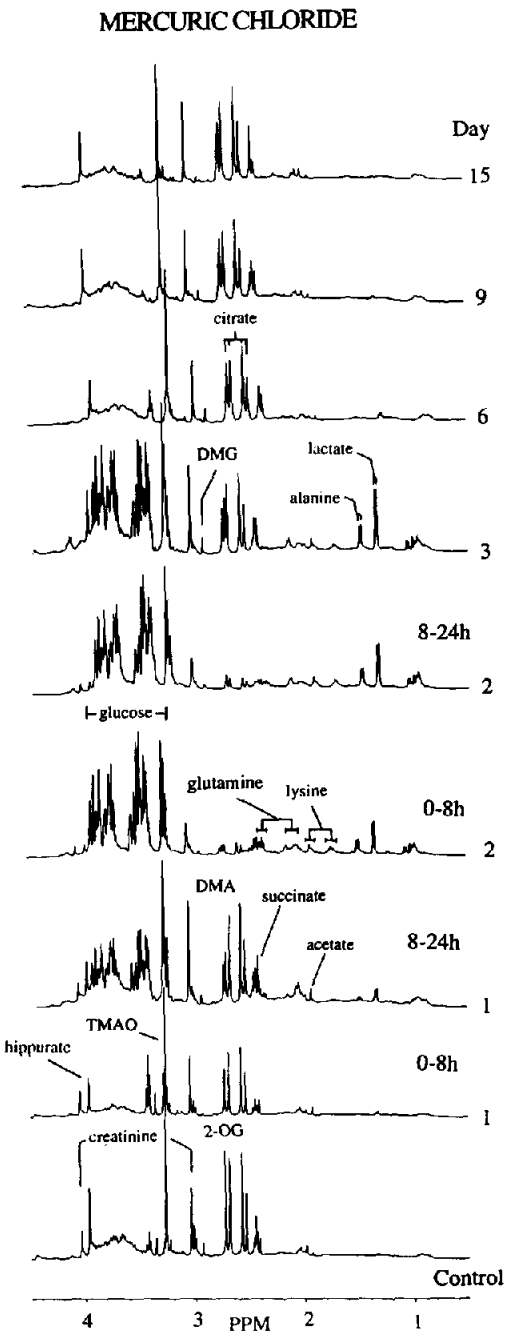

Figure 2
^{1}H-NMR spectra, 400 MHz (region to low frequency of water) of urine from rats before (control) and at various times up to 15 days following 1 mg kg^{-1} $HgCl_2$. See text for experimental conditions. DMA, dimethylamine; DMG, *N*-dimethylglycine; TMAO, trimethylamine *N*-oxide.

as 10 urinary metabolites using high-performance liquid chromatography (HPLC) but did not succeed in structurally identifying them. These metabolites remain at present unidentified.

At day 3 following BEA treatment, urinary levels of succinate and 2-OG increased. Succinic aciduria is also a feature of urine from rats dosed with propylene imine another toxin producing renal papillary necrosis [2]. Tri-

methylamine *N*-oxide (TMAO) and dimethylamine (DMA) are osmolytes in the renal papilla and have been shown to be present in control rat urine in significant quantities [2]. The findings of increased levels of both DMA and TMAO in urine following renal papillary insult support previous findings with BEA and also propylene imine [2]. Modest increases in lactate and glucose levels were seen by ^{1}H-NMR urinalysis following BEA suggesting secondary effects on the proximal tubule [2] (Fig. 1). These effects have been described previously and were not surprising since the toxic effects of BEA would result in the nephrons becoming no more than blind-ending "conduits". Indeed, in some cases, the tip of the renal papilla has been known to fall off leaving numerous such blind-ending nephrons. ^{1}H-NMR urinalysis clearly demonstrated that recovery of "normal" urinary metabolite levels following a single dose of BEA was achieved by day 6 post-dose. However, although the pattern of low molecular weight urine components had returned to normal, the rats still displayed increased urine flow rate at the latter timepoints following BEA.

Mercuric chloride

Considerable elevations in glucose were seen throughout the 8–24 h period (day 1) which were sustained until day 3, together with an elevation in taurine levels which occurred as early as 8 h post-dosing. Other features include aminoaciduria (alanine, glutamine, lysine and valine) beginning throughout the 0–8 h period (day 2), with alanine and lactate levels increasing during the earlier time point (Fig. 2). $HgCl_2$ causes a progressive hypocitraturia, the lowest level of urinary citrate seen throughout the 8–24 h period of day 2. After this time citrate levels recovered and approached those of the control by day 15. Succinate and 2-OG also decreased soon after dosing (Fig. 2). Conventional analyses revealed a slight increase in urinary volume and a pulse in enzyme levels throughout 0–8 h, which returned to control levels by day 3 (Table 2). The metabolite patterns discernible from ^{1}H-NMR urinalysis results suggested that the maximal perturbations in renal biochemistry occurred between 8–72 h after $HgCl_2$ treatment. Following exposure to 1 mg kg^{-1} $HgCl_2$, recovery was seen between days 3 and 6, a "normal" profile of low molecular weight urine components displayed at the latter time. UFR had returned to control levels by day 9, three days after the reappearance of the normal pattern of low molecular weight components. Urine spectra measured at day 6 were very similar to the controls, indicating that full tubular regeneration had occurred (Fig. 2).

Conclusion

These data show that ^{1}H-NMR urinalysis is clearly of value in following the time-course-related perturbations in renal biochemistry following nephrotoxic insult, and that information relevant to cellular regeneration and recovery of renal functional integrity can also be obtained.

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References

[1] J.K. Nicholson, J.A. Timbrell and P.J. Sadler, *Mol. Pharmacol.* **27**, 644–651 (1985).
[2] K.P.R. Gartland, F.W. Bonner and J.K. Nicholson, *Mol. Pharmacol.* **35**, 242–250 (1989).
[3] K.P.R. Gartland, F.W. Bonner and J.K. Nicholson, *Arch. Toxicol.* **63**, 97–106 (1989).
[4] J.K. Nicholson and K.P.R. Gartland, *Methodological Surveys in Biochemistry and Analysis, Vol. 17, Cells, Membranes and Disease, Including Renal* (E. Reed, G.M.W. Cook and J.P. Luzio, Eds), pp. 397–408. Plenum Press, New York (1987).
[5] K.P.R. Gartland, F.W. Bonner, J.K. Nicholson, P.H. Bach and E.A. Lock, *Nephrotoxicity: Extrapolation from* In Vitro *to* In Vivo *and from Animals to Man*. pp. 505–518. Plenum Press, New York (1989).
[6] T.U.L. Biber, M. Mylle, A.D. Baines *et al.*, *Am. J. Med.* **44**, 664–705 (1968).
[7] S. Sabatini, *Fund. Appl. Toxicol.* **4**, 909–921 (1984).
[8] J.R. Bales, D.P. Higham, I. Howe *et al.*, *Clin. Chem.* **30**, 426 (1984).
[9] P.H. Bach, J.W. Bridges, P. Grasso *et al.*, *Toxicol. Appl. Pharmacol.* **69**, 333–344 (1983).

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