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A ^{1}H NMR spectroscopic study of the biochemical effects of ifosfamide in the rat: evaluation of potential biomarkers

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Ifosfamide (55 mg kg^{-1} and 110 mg kg^{-1}) was administered via single i.p. injections to Sprague–Dawley rats and urine samples were collected for the periods of –24–0, 0–8 h, 8–24 h, 24–48 h and 48–72 h post-dose. Quantitative changes in the excretion pattern of small organic molecules in the urine of rats treated with ifosfamide were studied using high frequency ^{1}H NMR spectroscopy. The kidneys and livers of the animals were also examined, but showed no marked histopathological changes. ^{1}H NMR urinalysis showed significant changes in metabolite pattern, the maximal effects being observed at 8–24 h with (partial) recovery of the 'normal' urinary metabolite profile by 72 h. A marked reduction in the urinary excretion concentrations of citrate, α-ketoglutarate (2-oxoglutarate) and succinate was observed, consistent with inhibition of renal tubular carbonic anhydrase. The decrease of the citric acid cycle intermediates was accompanied by a marked reduction in urinary hippurate concentrations. The marked elevation in urinary creatine level following ifosfamide dosing, confirmed in a second study, may be indicative of testicular damage/dysfunction although there were no changes detectable by histopathology. The significance of these biochemical findings is discussed.

Keywords: ifosfamide, ^{1}H NMR spectroscopy, rat, creatine, testicular toxicity.

Introduction

Ifosfamide [*N*,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorine-2-oxide] is the drug of choice in the treatment of tumours of childhood and the treatment of lung cancer, sarcomas and cancer of the cervix in adults. Ifosfamide is a pro-drug which requires extensive metabolic transformation to produce the cytotoxic DNA cross-linking metabolite, ifosfamide mustard (e.g. Lind *et al*. 1990, Brade *et al*. 1986).

The principal metabolic pathways of ifosfamide are well established (e.g. Lind *et al*. 1990, Foxall *et al*. 1996). However, both clinical activity and toxicity are a consequence of the presence of ifosfamide metabolites, some unidentified or partially characterized. Despite the high response rate there are also considerable toxicological problems associated with ifosfamide anti-tumour therapy/

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chemotherapy and the dosage regime and schedule remain to be optimized (Klein *et al*. 1984, Skinner *et al*. 1990, 1993, Foxall *et al*. 1997). Thus, ifosfamide therapy is associated with neurotoxicity and severe nephrotoxicity, the precise mechanisms of which are unknown (Skinner *et al*. 1990, 1993, Sood and O'Brien 1993). Bladder toxicity, resulting in haemorrhagic cystitis, has also been reported to be a major side effect of ifosfamide treatment and has been attributed to the metabolic by-product, acrolein (2-propenal) (Lind *et al*. 1990).

One metabolite, chloroacetaldehyde, has been shown to be neurotoxic in rats (Lind *et al*. 1990) and was also reported to target the liver after oral administration to mice (Sood and O'Brien 1993). Chloroacetaldehyde was found to induce hepatocellular necrosis, hyperplasia and chronic active inflammation, increases in absolute liver weights and liver tumours. Renal toxicity involves glomerular, proximal tubular and distal tubular dysfunction or any combination of these. The onset and severity of nephrotoxicity varies widely between studies and individual patients, ranging from subclinical toxicity to chronic renal tubular impairment (Fanconi Syndrome) (Skinner *et al*. 1990, 1993, Foxall *et al*. 1996, 1997).

Considerable interindividual variability in the nature and extent of ifosfamide metabolism has also been observed in both children and adults, but there is limited information concerning the relationship between the metabolism of ifosfamide and its nephrotoxicity.

^{1}H NMR spectroscopy is now a well established technique for the study of toxicological events (Gartland *et al*. 1988, Anthony *et al*. 1994, Beckwith-Hall *et al*. 1998, Holmes and Shockcor 2000) and has been previously applied to the study of ifosfamide toxicity in patients (Foxall *et al*. 1996, 1997). NMR allows multi-component analyses on biological materials to be carried out simultaneously, without the need for pre-selection of the analysis conditions. ^{1}H NMR spectroscopy of urine has successfully uncovered several potential biomarkers of toxic effect (Gartland *et al*. 1988, Nicholson and Wilson 1989, Nicholson *et al*. 1989, Timbrell *et al*. 1994). In patients, ^{1}H NMR urinalysis detected subclinical metabolic changes as early as 24 h after the first dose of ifosfamide (administered i.v. over 3–5 days). Thus increased urinary excretion of glycine and histidine and decreased secretion of hippurate preceded the onset of maximal tubular dysfunction. These observations suggest that ifosfamide, or one of its metabolites, altered selectively the proximal-tubule handling of these low molecular mass (LMM) solutes (Foxall *et al*. 1996, 1997).

In clinical studies, distal tubular damage (including raised levels of trimethylamine *N*-oxide, dimethylalanine, dimethylglycine, acetate and succinate) and proximal tubular damage (including raised levels of lactate, glycine and glucose) were reported. The aim of this study was to assess the biochemical changes after single high doses of ifosfamide in the rat in order to determine potential biomarkers and evaluate the toxicity. Despite the long clinical use of the drug little is known about the toxic and biochemical effects of ifosfamide in the rat.

Experimental

Two separate studies were carried out, the first utilizing NMR spectroscopy and the second biochemical and pathological measurements.

Chemicals

Ifosfamide (Mitoxana^{TM}, for i.v. use) was obtained from Asta Medica_Cambridge, UK. 3-(Trimethylsilyl)propionic 2,2,3,3-d_4 acid sodium salt (TSP) was obtained from Aldrich, UK. D_2O (v/v,

Table 1. ^{1}H NMR signals used for metabolite quantification.

Signal	TSP	Succinate	α-KG	Citrate	Creatine	Creatinine	TMAO	Hippurate
Multiplicities	s	s	t	AB	s, s	s	s	d
$\delta_{^1H}$	0	2.43	2.46	2.72	3.04, 3.94	3.05	3.29	7.84
Proton value of peak	9	2	1	1	3 or 2	3	3	0.5
MW (Daltons)	172.6	118.1	146.1	192.1	131.1	113.1	75.1	179.2

Note: The peak height values were corrected for the number of protons contributing to the signal height. It was assumed that the line-widths of the peaks of interest were approximately equal and baseline distortions were taken into account in areas of severe chemical noise.

$\delta_{^1H}$ = chemical shift values of the resonances/signals of interest. s = singlet, d = doublet, t = triplet, AB = second order spin system consisting of two sets of doublets. α-KG = α-ketoglutarate, TMAO = trimethylamine-*N*-oxide.

99.9% purity) was obtained from Fluorochem Ltd, UK. Taurine, Dowex and *o*-phthalaldehyde were obtained from Sigma Chemical Co., Poole, UK. Creatine/creatine kit was obtained from Boehringer UK. Multistix SG diagnostic reagent strips were obtained from Bayer Diagnostics UK Ltd. Hypnorm was obtained from Janssen Animal Health (High Wycombe, UK) and hypnovel was obtained from Roche (Welwyn Garden City, UK).

Rats

Male Sprague–Dawley rats (NMR study: average weight 200 g, UCL Biological, London, UK; second study: average weight 208 g, GlaxoWellcome, R&D UK) were used. Animals were housed in metabolism cages throughout in a 12 h dark–light cycle and food and water were available *ad libitum*. Rats were allowed to acclimatize in metabolism cages for 24 h and were weighed immediately before dosing.

First study (NMR)

Dosing and sample collection. The dose solution was administered i.p. dissolved in saline (0.9%) at pH 7 (1 ml kg^{-1}). Groups of rats (n = 3) received single doses of ifosfamide (55 mg kg^{-1}, or 110 mg kg^{-1}). Control rats (n = 3) received a dose of 0.2 ml of saline (0.9%). The doses (55 and 110 mg kg^{-1}) were based on the acute i.p. LD_{50} value of 220 mg kg^{-1} reported in the literature (Barnett 1982). One rat given the higher dose of ifosfamide died at the start of the study. Urine samples were collected over solid CO_2 after –24–0, 0–8 h, 8–24 h, 24–48 h and 48–72 h. The samples were centrifuged in order to rid them of food, hair and faecal contaminants. The sample volumes and the pH values were recorded. The urine was stored at –20 °C until NMR analysis. Urine samples were also analysed for blood traces with Multistix.

Histopathology. At the end of the NMR study the rats were sacrificed by exposure to a rising concentration of CO_2 and livers and kidneys were removed immediately. The tissues (liver, renal cortex and renal medulla) were fixed in 10% formaldehyde in 0.9% saline and after dehydration embedded in paraffin wax and sectioned. Sections were stained with haematoxylin and eosin.

NMR spectroscopy. Single pulse ^{1}H NMR spectra of urine were acquired on a Bruker AMX 600 spectrometer operating at 600.13 MHz. Sixty-four scans were collected into 32 K data points over a spectral width of 8474.58 Hz. A pulse width of 7 μs and a relaxation delay of 2.5 s were used. The water resonance was suppressed via the NOESYPRESAT pulse sequence (Bruker Spectrospin, Germany) which involves irradiation at the water frequency during the relaxation delay and during the mixing time (100 ms).

Spectra were referenced to TSP at $\delta_{^1H}$ 0.0.

D_2O (10%) was added to each urine sample prior to NMR analysis in order to provide a field frequency lock. All spectra were scaled to the same signal-to-noise ratio so that resonance intensities were directly comparable between urine samples.

Determination of urinary metabolite excretion rates from NMR data. ^{1}H NMR peak height measurements were performed to establish the concentrations of endogenous metabolites with respect to TSP (1 mM final concentration) which was added as internal chemical shift reference ($\delta 1_H$ 0.0) and standard for quantification. Integral values could not be determined reliably as many peaks were partially overlapped in the ^{1}H NMR spectra. The chemical shift values of the signals of interest are given in table 1.

Corrections for the urinary volumes excreted during the period of collection were made and the results are expressed as mg kg^{-1} h^{-1}. The calculation method used is as follows:

$$\frac{\text{peak height}_{\text{metabolite}}}{\text{peak height}_{\text{TSP}}} \times \frac{\text{no. of protons}_{\text{TSP}}}{\text{no. of protons}_{\text{metabolite}}} = A$$

$$A \times \frac{\text{total Vol.}_{\text{excreted}}}{\text{Vol.}_{\text{NMR-tube}}} \times \text{no. of moles}_{\text{TSP}} = B$$

$$B \times \frac{1000}{\text{weight of rat (g)}} = C \text{ in moles kg}^{-1}$$

$$\frac{C \times \text{RMM}}{\text{time interval (h)}} = \text{mg kg}^{-1}\ \text{h}^{-1}$$

Second study: dosing and sample collection

Rats ($n = 4$) were dosed as previously with ifosfamide in saline (0.9%, i.p.) at a dose level of 110 mg kg^{-1}. Controls ($n = 4$) received saline only. Urine was collected over ice for 24 h before dosing and for 0–24 h and 24–48 h after dosing and food, water, water consumption and body weight were monitored daily. Exsanguination was carried out under anaesthesia (Hypnorm:hypnoval:water; 1:1:2; 3.33 ml kg^{-1}) 48 h after dosing. Blood was taken for the preparation of serum. Samples of liver and kidney and the testes were taken for histology. Urine was centrifuged and diluted to 20 ml with distilled water and stored at –80 °C before analysis.

Creatine and creatinine determinations. Creatine and creatinine in urine were measured by the enzymatic method of Siedel *et al*. (1984) using a kit as previously described (Gray *et al*. 1990).

Taurine determination. Taurine in urine and serum was measured after separation with Dowex resin and derivatization with *o*-phthalaldehyde by HPLC as previously described (Waterfield 1994).

Results and discussion

^1H NMR spectroscopic studies on urine: first study

Typical ^{1}H NMR spectra for a control animal are shown in figure 1(a and b). Slight variations in the levels of endogenous urinary metabolites were observed between the spectra of the control animals. Dosing with ifosfamide caused distinct changes in the urinary ^{1}H NMR spectral profiles/urinary excretion patterns compared to predose profiles (figure 2 (a and b)). Thus, marked increases in the signal intensity of creatine and decreases in the signal intensities of the citric acid cycle intermediates, succinate, α-ketoglutarate (α-KG), and citrate were observed in the 0–8 and 8–24 h samples. Hippurate signals were also decreased in intensity at these time-points. A return towards the control metabolite profile was observed 72 h post-dosage. Ifosfamide metabolites were also present in the samples taken at earlier time-points (figure 2 (a and b)).

On the basis of these results, a series of metabolite excretion rates was calculated for individual rats. The mean values for each dose group, plotted as histograms, are shown in figure 3. Maximum changes in urinary excretion patterns were observed at 8–24 h (figure 3). At later time points (48–72 h), the excretion rates of the citric acid cycle intermediates increased and the creatine excretion rate returned to control levels. It should also be noted that for the dosed animals, there was an increase in urinary volume at all three collection times, but especially at 0–24 h and the increase was statistically significant in the low dose group at 48–72 h (table 2).

In the 55 mg kg^{-1} and 110 mg kg^{-1} dose group, marked decreases in the

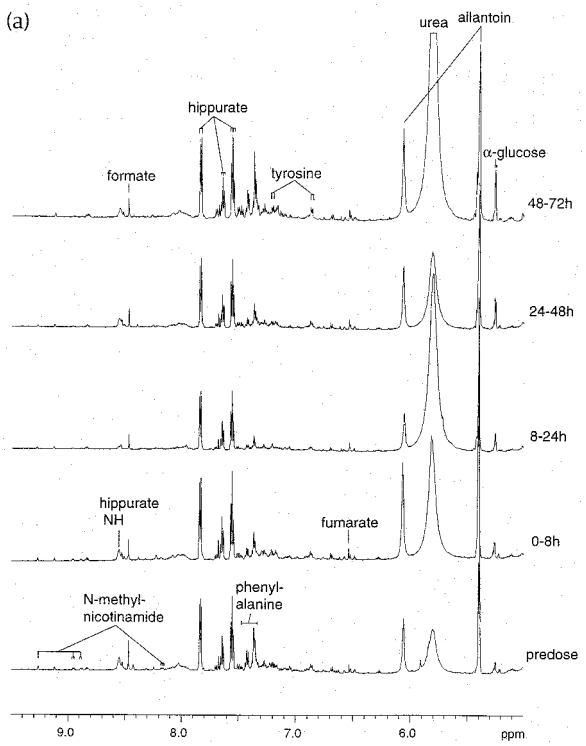

Figure 1. 600 MHz ^{1}H NMR spectra of the aromatic regions (a) and the aliphatic regions (b) of the urine samples collected at different time-points from a control rat dosed with saline (64 scans, no window function, internally referenced to TSP at δ_{1H} 0.0). Key: DMG = dimethylglycine, TMAO = trimethylamine-*N*-oxide, DMA = dimethylamine, αα-CH = methine groups of amino acids, HOD = residual partially deuterated water signal.

concentrations of succinate, α-KG and citrate, and hippurate, were observed in the 0–8h, 8–24 h and 24–48 h urine samples. These were statistically significant in the 55 mg kg^{-1} dose group (figure 3). The level of citrate then became significantly raised in the 48–72 h urine samples. However, there was no change in urinary creatinine over the period of the study. The citric acid cycle intermediates are normally abundant (up to 20 mM) in rat urine (Nicholson *et al*. 1989). The reduction in excretion may be indicative of renal tubular acidosis attributed to the inhibition of carbonic anhydrase (Nicholson *et al*. 1989, Windhager 1992). Alternatively, direct effects on the citric acid cycle by the drug may also decrease the levels of the intermediates. The decreases seen in the citric acid cycle intermediates and hippurate were also observed after dosing with cadmium (Nicholson *et al*. 1989) and therefore the changes might also be related to testicular damage.

In contrast to the decreases in citric acid cycle metabolites, creatine was markedly and significantly elevated in both 8–24 and 24–48 h urine samples (figure 3); the maximum level was observed in the 8–24 h urine (figure 3). The rise in creatine level was statistically significant at the 55 mg kg^{-1} dose and at the higher dose it was approximately double that at the lower dose (figure 3). Signals from the

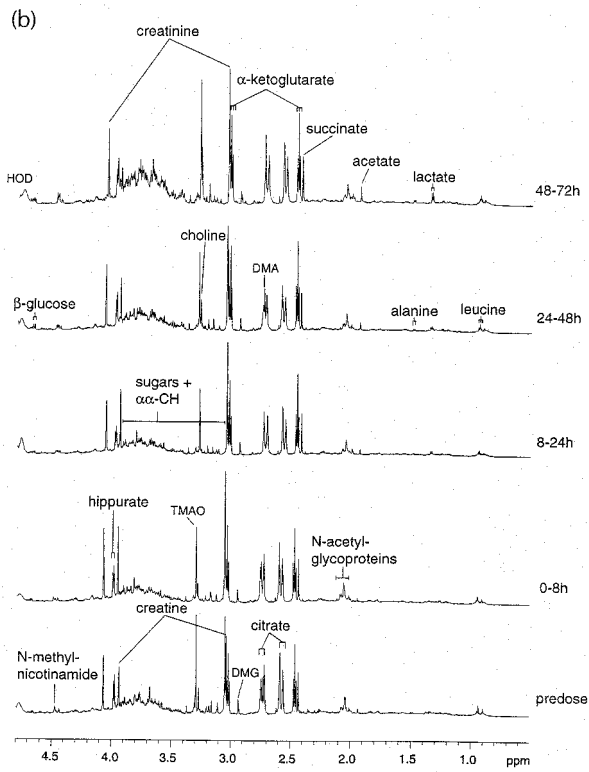

Figure 1. For legend see facing page.

mixture of ifosfamide metabolites in the 0–8 h urine fractions were detectable. However, these signals were not readily assigned because of their low concentrations and the overlapped splitting patterns due to ^{1}H–^{31}P couplings which resulted in complex multiplets of low intensity. The identification of these metabolites is described elsewhere (Foxall *et al*. 1996). The relationship between drug-related material and toxicity is not clear, although the presence of the metabolites predominantly in the 0–8 h samples, appeared to precede (55 mg kg^{-1} dose) or coincide with (110 mg kg^{-1} dose; figure 2) the changes observed in the endogenous metabolite profiles.

The increases in lactate (δ 1.34 ppm), acetate (δ 1.93 ppm) and benzoic acid (a doublet at δ 7.89 ppm, the residual resonances being overlapped with endogenous hippurate) observed for these samples were attributed to slight bacterial contamination in the 48–72 h urine fractions (Sweatman *et al*. 1993).

Histopathological studies. Sections of liver and kidney (renal cortex and papilla) from control and ifosfamide treated rats were examined by light microscopy (data not shown). Comparison of the liver and kidney sections obtained from animals following treatment with ifosfamide at 55 mg kg^{-1} with the control sections confirmed that no major pathological lesions were induced at this dose level. In the rats treated with 110 mg kg^{-1} ifosfamide, slight thickening of the tip of the papilla was observed together with a dense protein precipitate. In this study, the livers and kidneys showed little evidence of cellular necrosis or inflammatory cell infiltration,

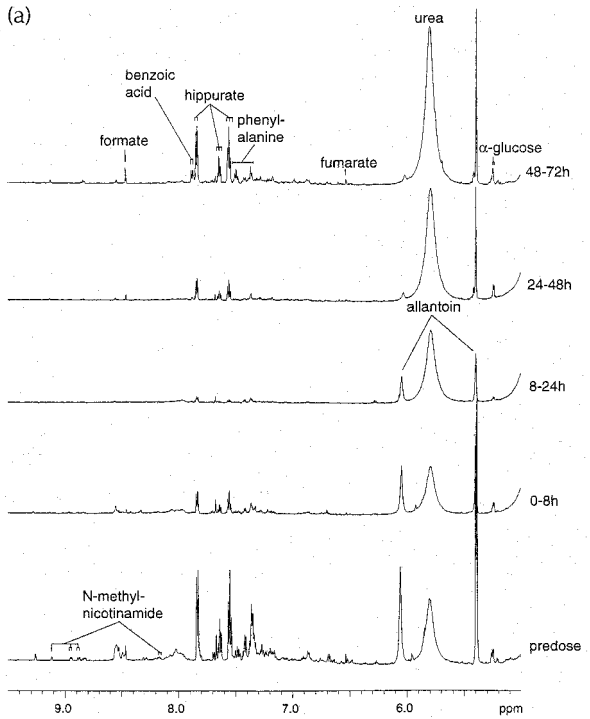

Figure 2. 600MHz ^{1}H NMR spectra of the aromatic regions (a) and the aliphatic regions (b) of the urine samples collected at different time-points from a control rat before and after dosing with 110mg kg^{-1} of ifosfamide. (64 scans, no window function, internally referenced to TSP at δ_{1H} 0.0). Key: DMG = dimethylglycine, TMAO = trimethylamine-*N*-oxide, DMA = dimethylamine.

although ifosfamide therapy (after multiple dosing) is associated with severe nephrotoxicity in man (Skinner *et al*. 1990, 1993, Foxall *et al*. 1996, 1997).

The changes in the urinary metabolite patterns observed here (i.e. increased creatine and decreases in citric acid cycle intermediates and hippurate excretion) were similar to those previously observed in rats after single doses of cadmium chloride were administered (Nicholson *et al*. 1989). The kidneys in these previous studies showed no evidence of structural abnormalities, and creatinine excretion rates were reported to be constant, suggesting little effect of cadmium on renal blood flow or glomerular filtration rates. However the rise in urinary creatine level observed was related to the dose of cadmium and the levels of creatine in the urine corresponded well with the occurrence of severe testicular damage (Nicholson *et al*. 1989, Gray *et al*. 1990, Draper and Timbrell 1998). The changes in the citric acid cycle intermediates and hippurate may reflect testicular damage but could also reflect the general catabolic state of the rat.

Second study

As the changes observed by NMR in the first study were similar to those previously observed after dosing with cadmium which acutely causes testicular damage, the study was repeated. This was in order to quantitate urinary creatine

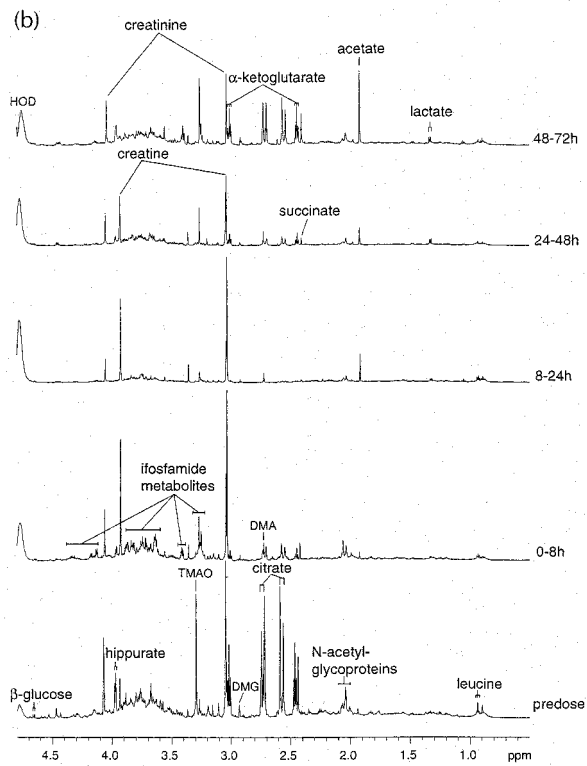

Figure 2. For legend see facing page.

and other parameters (creatinine, taurine, body weight, kidney weight and testis weight) and evaluate testicular pathology in male rats given a single dose of ifosfamide (110 mg kg^{-1}). In this study there was a significant reduction in body weight and food consumption 24 h after dosing in the ifosfamide-treated group (table 3). Conversely, water consumption was increased at 48 h after dosing in the treated group but although urine volume was increased the difference was not statistically significant (table 3). As in the first NMR study, there was a marked and significant increase in urinary creatine 24–48 h after dosing but the small increase earlier (0–24 h after dosing) was not significant (table 4). There was a small rise in serum creatine also but this was not statistically significant (0.17 ± 0.02 vs 0.15 ± 0.004). There was also a slight but non-significant decrease in creatinine on day 2 (consistent with the decreased food intake and body weight decrease). However testis weights were not significantly increased and histological examination of the testes did not reveal any damage.

Neither liver nor kidney weights were significantly altered in the treated group (table 3) and liver and kidney histology was normal. Also taurine levels in urine (table 4) and serum were unchanged in the ifosfamide-treated group. These data are consistent with a lack of liver damage which might otherwise have accounted for the increase in urinary creatine (Timbrell *et al*. 1995).

One of the metabolites of ifosfamide is allyl aldehyde (acrolein) which is toxic to the liver and cardiovascular systems when generated *in situ*. However, creatine

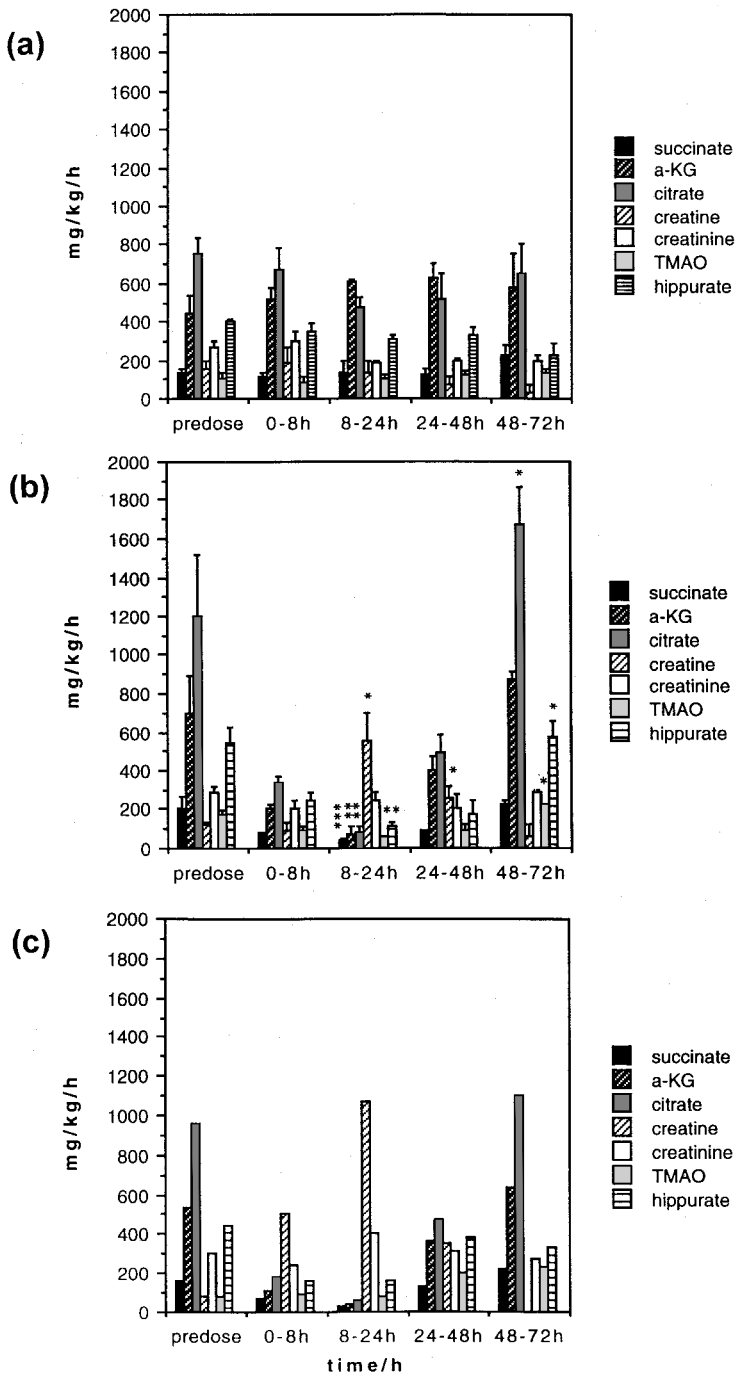

Figure 3. Time course of excretion of some of the endogenous metabolites (succinate, α-KG, creatine and hippurate) derived from peak height measurements from the 600 MHz NMR data. (a) from the control group dosed with saline, (b) following ifosfamide treatment of 55 mg kg^{-1} and (c) 110 mg kg^{-1}. Results are means ± SEM, $n = 3$ for a and b ($n = 2$ for c). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ vs control, respectively.

Table 2. Urine volumes in rats treated with ifosfamide: study 1(NMR study).

	Predose	0–24 h	24–48 h	48–72 h
Control	9.0 ± 3.2	8.2 ± 1.7	8.1 ± 2.1	6.8 ± 1.6
55 mg kg^{-1}	7.2 ± 1.7	18.9 ± 5.3	10.3 ± 1.5	11.9 ± 0.9*
110 mg kg^{-1}	5.4; 15.2	48; 51.4	35.9; 31.4	14.0; 19.2

Means ± SEM, $n = 3$.
$p < 0.05$ unpaired t-test.

kinase and transaminases were not measured in this study, although there was no histopathological evidence of liver damage. However in a previous study (Waterfield *et al*. 1993) when allylamine was administered to rats causing cardiac damage, urinary creatine was not elevated.

Previous studies have shown that ischaemic necrosis of the testis caused by ligation of the testicular arterial supply also caused marked creatinuria (Gray *et al*. 1990). Urinary creatine has been compared with several other markers of testicular damage and was found to correlate well with histopathological assessment and was reported to be more sensitive than biochemical assays such as serum LDHC4 and testosterone in detecting testicular damage (Timbrell *et al*. 1995, Draper and Timbrell 1998). Urinary creatine is also significantly raised in rats with acute liver necrosis but not fatty liver (Timbrell *et al*. 1995). However, there are other markers available to discount this as a cause, e.g, urinary taurine, bile acids, bilirubin and plasma transaminases. Therefore, combined measurement of urinary taurine and creatine can be used to detect and discriminate between testicular damage and liver dysfunction (Timbrell *et al*. 1995).

Thus, the biochemical observations may be consistent with ifosfamide-induced testicular damage. However, in the second study testicular damage could not be confirmed histopathologically 48 h post-dose. This may reflect the type of testicular damage which occurred in this study. Dosing of male rats with another alkylating agent, ethane methane sulphonate, resulted in raised urinary creatine in male but not in female rats. However, no damage to the testes was detected by histopathology (Draper and Timbrell, unpublished observations). This may reflect the fact that these agents cause damage to late stage spermatids. Thus, histological assessment of testes later than 48 h post-dosing might be a more suitable time point to examine. These preliminary data suggest that urinary creatine should be further investigated as a sensitive marker of late stage testicular damage.

Chronic testicular toxicity was reported to have been observed in dogs after multiple ifosfamide administration involving testicular atrophy with wasting of the epithelium of the seminiferous tubules (Barnett 1982).

In comparison with studies in patients whose urines were subjected to ^{1}H NMR analyses, distinctly different toxicological effects have been observed in this study by ^{1}H NMR and histological investigations. In addition, the Fanconi Syndrome, which includes renal tubular damage and a failure to reabsorb solutes such as amino acids, phosphate, sugars and low molecular weight proteins, has not been evident in the ^{1}H NMR spectroscopic urinary profiles in this study.

The acquisition of a Fanconi Syndrome is characteristic of chronic cadmium exposure and chemotherapy with ifosfamide (Nicholson *et al*. 1989, Skinner *et al*. 1990, 1993, Foxall *et al*. 1996, 1997). However, these differences can possibly be explained considering the different routes of administration, the extent of exposure

Table 3. Effect of ifosfamide on body weights and other parameters: study 2.

Parameter	Control	Ifosfamide
Body weight	216 ± 1.0	203.9 ± 2.9*
Food consumption	18.5 ±	9.0 ± 1.3***
Water consumption	20.7 ± 0.8	32.7 ± 2.3**
Urine volume	9.3 ± 0.2	13.9 ± 2.3
Kidney weight (% body wt)	0.75 ± 0.02	0.81 ± 0.02

Results are means ± SEM, $n = 4$. * $p<0.05$; ** $p<0.001$; *** $p<0.0001$.

Table 4. Effect of ifosfamide on urinary creatine, creatinine, and taurine: study 2.

Parameter	Control, predose urine	Ifosfamide-treated, predose urine	Control, 0–24 h urine	Ifosfamide-treated, 0–24 h urine	Control, 24–48 h urine	Ifosfamide-treated, 24–48 h urine
Urinary creatine (μmol h^{-1} kg^{-1})	0.80 ± 0.1	0.83 ± 0.01	0.82 ± 0.01	0.90 ± 0.05	0.84 ± 0.01	1.48 ± 0.07**
Urinary creatinine (μmol h^{-1} kg^{-1})	15.7 ± 2.7	13.2 ± 0.6	12.0 ± 0.8	12.0 ± 2.4	13.3 ± 0.6	11.2 ± 0.2
Urinary taurine (μmol h^{-1} kg^{-1})	43.2 ± 4.3	98.3 ± 62.7	78.0 ± 19.1	91.3 ± 52.1	144.2 ± 9.0	128.9 ± 16.5

Results are means ± SEM, $n = 4$.
** $p<0.01$; unpaired t-test compared with control value.

to the drug, especially in clinical studies where infusions are administered over a period of days, and species differences. In addition, changes in the general metabolism due to the presence of a tumour may be important.

Conclusions

Sublethal doses of ifosfamide produced a pattern of characteristic changes in urinary metabolite concentrations similar to that previously observed after single doses of cadmium. There was no significant liver or kidney damage but the markedly raised creatine is consistent with testicular damage as previously described for cadmium. This conclusion is also supported by evidence in the literature from studies in the dog. Thus, this study has shown that, following a single dose of ifosfamide, ^{1}H NMR was able to detect distinct disturbances in the urinary fingerprint of endogenous components consistent with testicular toxicity and metabolic dysfunction. The rat has been used as a model species in this study as it allows the use of high doses and seems to be susceptible to the toxic effects of ifosfamide .

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