8. Smith IE. A randomized comparative trial of mitoxantrone, methotrexate, minomycin C (MMM) and cyclophosphamide, methotrexate, 5-FU (CMF) in the treatment of advanced breast cancer. *Third Nordic Novantrone Symposium Helsingør, Denmark*, 1990.
9. Judson IR, Powles TJ, Ashley SE, Callagher CJ, O'Keefe A. Mitomycin C, mitozantrone and methotrexate (3M) for advanced breast cancer. A randomised crossover trial versus vincristine, adriamycin and cyclophosphamide (VAC). 3M is equally effective with reduced toxicity. *ASCO New Orleans*, 1988.
10. Powles TJ. A randomised trial of mitomycin C, mitoxantrone and methotrexate (3M) compared to vincristine, anthracycline and cyclophosphamide (VAC) for treatment of advanced breast cancer. *Third Nordic Novantrone Symposium Helsingør, Denmark*, 1990.
11. *WHO Handbook for Reporting of Results of Cancer Treatment*. Geneva, World Health Organisation, 1979.
12. Blomqvist C, Elomaa I, Rissanen P, Hietanen P, Nevasaari K, Helle L. FEC monthly versus FEC weekly in metastatic breast cancer. A randomized trial. *Acta Oncol* 1992, in press.
13. Stein RC, Dougherty L, Gazet J-C, Ford HT, Coombes RC. Comparison of methotrexate and mitozantrone with and without mitomycin C in the treatment of advanced breast cancer. *5th European Conference on Clin Oncol London*, 1989.

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Uroepithelial and Nephrotubular Toxicity in Patients Receiving Ifosfamide/Mesna: Measurement of Urinary *N*-Acetyl-β-D-glucosaminidase and β-2-Microglobulin

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The effect of three ifosfamide/mesna regimens on urinary *N*-acetyl-β-D-glucosaminidase (NAG) activity and β-2-microglobulin (β_2M) was studied. All regimens produced significant increases in these urinary proteins, indicating nephrotubular damage. In regimen A ($n = 15$), plasma nitrobenzylpyridine (NBP) alkylating activity area under the curve (AUC) on day 1 correlated with the percentage increase above baseline of maximum urinary NAG activity ($r^2 = 0.538$, $P = 0.0022$) and maximum β_2M concentration ($r^2 = 0.413$, $P = 0.0097$). In regimen B ($n = 5$), plasma NBP alkylating activity AUC correlated with the percentage increase above baseline of maximum NAG activity ($r^2 = 0.843$, $P = 0.03$) and β_2M ($r^2 = 0.78$, $P = 0.046$). In these two regimens the renal exposure to ifosfamide metabolites correlated with the increases in urinary NAG and β_2M. The relation of these urinary protein abnormalities to longer term effects on renal function with different ifosfamide/mesna schedules requires further study.
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INTRODUCTION

IFOSFAMIDE IS an effective antineoplastic agent and has a toxicity profile typical of oxazaphosphorine alkylating agents [1, 2]. When first used as chemotherapy in the early 1970s the dose-limiting side-effect of ifosfamide was haemorrhagic cystitis. This was reported in 18–40% of courses [3, 4] and was attributed to acrolein and possibly other ifosfamide metabolites [5, 6]. The co-administration of thiol compounds, e.g. mesna (sodium-2-mercaptoethane sulphonate) with ifosfamide has substantially reduced the frequency of ifosfamide-induced haemorrhagic cystitis [7–11].

The administration of high-dose ifosfamide as a bolus or by short infusion has been associated with severe renal toxicity, despite concomitant mesna administration [12–16]. The optimal mesna regimen and duration of therapy for different ifosfamide regimens has yet to be determined. A lower frequency of nephrotoxicity was noted if ifosfamide/mesna was administered by fractionated doses rather than by bolus, or mesna given as a continuous infusion [9]. Studies in children have shown significant reductions in glomerular filtration rate (GFR) following ifosfamide/mesna chemotherapy [14, 15]. Researchers treating children with fractionated 5-day ifosfamide/mesna in combination chemotherapy have reported the development of significant subclinical renal tubular damage despite mesna administration [17, 18]. In these studies there was no evidence of permanent renal impairment, even after several courses of chemotherapy [18].

The objectives of our observational study were to quantify the changes in urine content of *N*-acetyl-β-D-glucosaminidase (NAG) and β-2-microglobulin (β_2M) produced by different ifosfamide/mesna regimens. We intended to correlate these changes in urinary proteins with the degree of haematuria and with the pharmacokinetic parameters of ifosfamide (or ifosfamide metabolites). We studied three ifosfamide/mesna

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chemotherapy regimens and monitored uroepithelial toxicity by dipstick urinalysis. Nephrotubular toxicity was monitored by measurement of urinary NAG activity (an enzyme released from the lysomes of damaged proximal tubular cells [19]) and urinary β_2M concentration (a low molecular weight peptide, normally reabsorbed by functional proximal tubule cells [20]).

PATIENTS AND METHODS

Patients' selection

The patients in this study were subgroups of individuals who were taking part in pharmacokinetic studies of three different ifosfamide administration regimes (the pharmacokinetic data have been published elsewhere [21, 22]). All patients gave informed verbal consent, and the study protocol was agreed by the local ethics committee.

Inclusion criteria were: histologically proven inoperable carcinoma; malignant disease responsive to oxazaphosphorine therapy; normal haematological profile (Hb > 10 g/dl; WBC> 3500/μl and platelets > 150 000/μl); normal renal function (serum creatinine, urea, electrolytes and urinalysis); no known hypersensitivity to oxazaphosphorines; not pregnant or lactating; no previous treatment with cisplatin or other platinum compounds and no concurrent administration of potentially nephrotoxic agents (e.g. aminoglycosides) during study; agreement to collect a timed sample of urine from each micturition immediately before, during and in several cases for several days after the completion of the ifosfamide/mesna course.

Before entry into this study all patients underwent full physical examination, electrocardiography and had routine laboratory haematological and biochemical profiles measured. 1 week after chemotherapy and before receiving a further cycle of ifosfamide/mesna, the patients' haematological and biochemical profiles were repeated.

Ifosfamide/mesna regimens

In this study patients received one of the following ifosfamide/mesna treatment regimens. Treatment A: patients received intravenous ifosfamide 1.5 g/m^2 given over half an hour in 250 ml of 0.9% saline, daily for 5 days. Prior to each ifosfamide infusion and at 3, 6 and 9 h postifosfamide, patients received an intravenous bolus of mesna of 400 mg/m^2. The total mesna dose of 1.6 g/m^2 was equivalent to 107% of the total ifosfamide dose. Treatment B: patients received 5 g/m^2 of intravenous ifosfamide infused over 24 h in 2 l of 0.9% saline. Mesna was given as a 400 mg/m^2 intravenous bolus immediately preceding the ifosfamide infusion, and then as a 5 g/m^2 infusion over 24 h with the ifosfamide. This was followed by a 3 g/m^2 infusion of mesna over 12 h in 1 l of 0.9% saline. The total mesna dose of 8.4 g/m^2 is equivalent to 168% of the total ifosfamide dose. Treatment C: patients received 5 g/m^2 of intravenous ifosfamide as a short infusion over half an hour in 500 ml of 0.9% saline. Mesna was administered as an intravenous bolus of 5 g/m^2 preifosfamide, followed by intravenous boluses of 1 g/m^2 at 4, 8 and 12 h postifosfamide. The total mesna dose of 8 g/m^2 is equivalent to 160% of the total ifosfamide dose. During all treatment regimes patients were encouraged to drink sufficient fluid to ensure an intake of at least 3 l per day.

Urine collection and storage

All patients collected timed samples of urine before ifosfamide/mesna therapy commenced and then from each micturition during the course. Several patients continued to collect timed samples after their treatment ended. 15–20 ml urine was collected in universal containers containing 1 ml phosphate buffer, pH 10, which was added to raise the sample's pH above 6 to stabilise β_2M [23]. The samples were first stored at 4°C and then transferred to −20°C until analysed.

Analysis of urine

All urine samples were tested for blood and protein traces by dipstick (Boehringer).

NAG was measured by a spectrophotometric assay with an *ortho*-nitrostyryl substrate [24].The intra-assay coefficient of variation (CV) over the range of the standard curve (0–50 μmol/l) was 2.4–14.7% and the interassay CV over the measured range was 2.5–17.5%. Samples were assayed in duplicate, with a quality control standard run in each assay. The CV of the quality control sample was 6.2%. Duplicate samples differing by more than 10% were re-assayed. Urinary NAG activity was corrected for variations in urine concentration and output (and the dilution caused by addition of phosphate buffer, which does not interfere with this spectrophotometric method) by expressing the results as per mg of urine creatinine.

β_2M concentration was assayed with a commercially available radioimmunoassay Phadebas kit (Pharmacia). The limit of sensitivity of the assay is 0.1 mg/l and the intra-assay CV over the standard curve range of 0.1–16.0 mg/l was 1.2–7.6% (n = 12). Duplicate sample measurements differing by more than 10% were re-assayed. Corrections were done as for NAG.

Creatinine concentration was measured by the standard alkaline picrate reaction in an LKB 600 reaction rate analyser.

Measurement of plasma ifosfamide concentration and plasma nitrobenzylpyridine (NBP) alkylating activity

Plasma ifosfamide was measured with a specific gas liquid chromatography method [21, 22] and plasma NBP alkylating activity was measured as described [21, 22].

Statistical analysis

We investigated differences pre- and postchemotherapy treatment in the same regimen by the Wilcoxon's matched-pairs signed-rank test. Although this was not designed as a direct comparative study, differences between treatment groups were analysed by a one-way analysis of variance, followed by a Bonferroni correction for multiple comparisons. Correlations were investigated with linear regression. A two-tailed $P < 0.05$ was taken as the level of statistical significance.

RESULTS

We studied 10 patients who received 15 treatment courses of regimen A; 4 patients who received five treatment courses of regimen B, and 6 patients who received six treatment courses of regimen C. The clinical characteristics and ifosfamide/mesna dosages for the patients are illustrated in Tables 1 and 2. Figures 1 and 2 illustrate the maximum increase (peak) from baseline (pretreatment) in urine NAG activity/mg creatinine and urine β_2M concentration/mg creatinine, respectively. A one-way analysis of variance of the per cent increase above baseline in both maximum urinary NAG activity and maximum β_2M concentration between all treatment groups, revealed no significant differences.

Significant correlations were found between the percentage increase above baseline of the maximum urine NAG activity/mg creatinine and the percentage increase above baseline of the maximum urine β_2M concentration/mg creatinine in regimen A (r^2 = 0.432, P = 0.009), in regimen B (r^2 = 0.772, P = 0.049),

*Table 1. Clinical characteristics, pretreatment chemotherapy and ifosfamide/mesna doses for the patients who received regimen A**

Patient and course no.	Sex	Age (years)	Disease	Total IF dose (g)	Total mesna dose (g)	Previous CT/RT	Urinalysis (maximum HAEM/PR)
1A	M	23	OS	12.0	12.8	DOX/IF	ND/ND
1B				12.0	12.8		ND/ND
1C				12.0	12.8		ND/ND
2	M	34	AS	11.0	11.8	RT	2+/2+
3	M	26	Thymoma	12.5	13.4	IF	ND/1+
4A	F	51	CC	10.0	10.7	RT	2+/1+
4B				10.0	10.7	IF	ND/ND
5A	F	31	CC	10.5	11.2	Nil	ND/1+
5B				10.5	11.2	IF	ND/ND
6	M	55	CL	14.5	15.5	Nil	1+/2+
7	F	54	CO	12.5	13.4	RT	ND/1+
8	F	40	CC	12.5	13.4	RT	3+/3+
9	F	45	CC	11.2	12.0	RT	2+/1+
10A	F	52	CC	12.0	12.8	RT	2+/1+
10B				12.0	12.8	IF	3+/1+
Median		40		12.0	12.8		

*Fractionated ifosfamide 1.5 g/m² and mesna 1.6 g/m², daily for 5 days.
IF = Ifosfamide, CT = chemotherapy, RT = radiotherapy, HAEM = haematuria, PR = proteinuria, M = male, F = female, OS = osteosarcoma, DOX = doxorubicin, ND = none detected, AS = adenocarcinoma of sinus, CC = carcinoma of cervix, CL = carcinoma of lung, CO = carcinoma of ovary.

Table 2. Clinical characteristics, pretreatment chemotherapy and ifosfamide/mesna doses for patients who received regimens B and C

Patient and course no.	Sex	Age (years)	Disease	Total IF dose (g)	Total mesna dose (g)	Previous CT/RT	Urinalysis (maximum HAEM/PR)
Regimen B							
1	M	23	OS	8.0	13.4	DOX/IF	ND/ND
2	M	57	CP	8.7	14.7	RT	3+/1+
3A	M	72	CL	9.0	15.1	Nil	ND/ND
3B				9.0	15.1	IF	ND/ND
4	M	48	CP_a	9.1	15.3	Nil	ND/1+
Median		52		9.0	15.1		
Regimen C							
1	M	54	CL	10.0	16.0	Nil	2+/3+
2	M	56	CL	9.5	15.2	IF	1+/2+
3	F	40	CL	7.5	12.0	RT	1+/ND
4	M	63	CL	9.0	14.4	Nil	ND/1+
5	M	41	CL	8.5	13.7	Nil	ND/ND
6	M	71	CL	8.9	14.2	Nil	ND/1+
Median		55		8.9	14.3		

For abbreviations see Table 1; also, CP = carcinoma of pharynx, CP_a = carcinoma of pancreas.

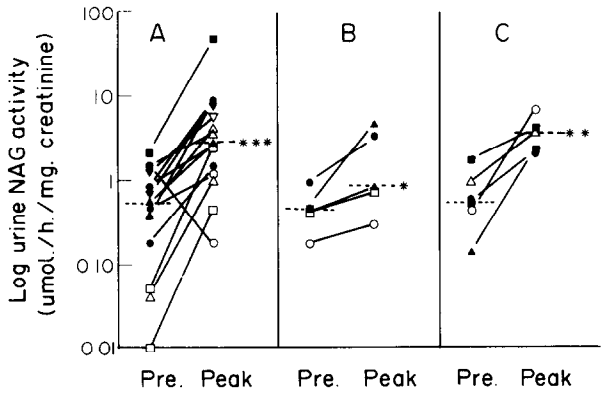

Fig. 1. The maximum rise (peak) from baseline (pretreatment) in the urine NAG activity per mg urine creatinine. Data are presented for each individual patient's course of ifosfamide/mesna in regimens A, B and C. ---- represents the median value of the group. Using Wilcoxon's matched-pairs signed-rank test with a two-tailed *P* value; * not significant, ** $P < 0.05$, *** $P < 0.005$.

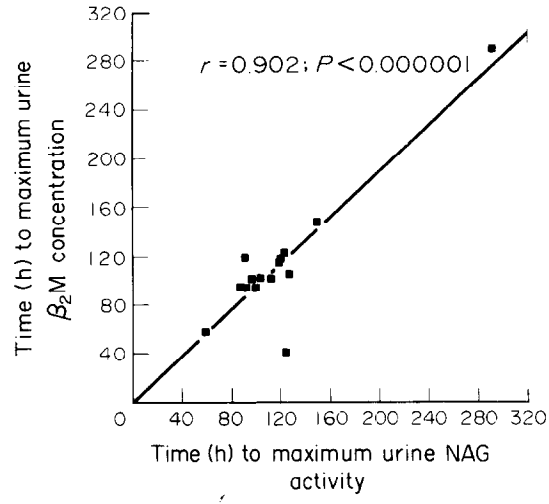

Fig. 3. The correlation between the time (h) to maximum (peak) urinary NAG activity, and the time (h) to maximum (peak) urinary β_2M concentration for the patients in regimen A.

and in regimen C ($r^2 = 0.987$, $P = 0.0006$). There were no significant correlations between either maximum (peak) urine NAG activity/mg creatinine and maximal grade of haematuria (regimen A, $M = 0.19$, $P > 0.05$; regimen B, $M = 0.23$, $P > 0.05$; regimen C, $M = 0.18$; $P > 0.05$) or maximum (peak) urine β_2M concentration/mg creatinine and maximal grade of haematuria (regimen A, $M = 0.25$, $P > 0.05$; regimen B, $M = 0.14$, $P > 0.05$: regimen C, $M = 0.09$, $P > 0.05$) using Jaspen's multiserial coefficient of correlation, in any treatment regimen studied.

The median (range) duration of collection of urine specimens from the time of initiation of ifosfamide/mesna therapy for regimen A was 364 h (116–477.5), for regimen B was 43.5 h (32.5–248), for regimen C was 43.75 h (21–269.5). The median (range) time to the maximum value of urinary NAG activity/mg creatinine and the maximum value of urinary β_2M concentration/mg creatinine in each treatment regimen was: regimen A:NAG_{Tmax} = 118 h (87–291), β_2M_{Tmax} = 102.5 h (80.5–291); regimen B:NAG_{Tmax} = 31.5 h (20.5–49.5), β_2M_{Tmax} = 33.5 h (22.5–62.0); regimen C:NAG_{Tmax} = 37.0 h (21–53.h), β_2M_{Tmax} = 37.5 h (21–53.5). In regimen A there was a significant correlation between the time to the maximum value of urine NAG activity/mg creatinine and the time to the maximum value of urine β_2M concentration/mg creatinine; $r^2 = 0.813$, $P < 0.000001$ (see Fig. 3).

Correlations between plasma NBP alkylating activity area under the concentration–time curve, ifosfamide pharmacokinetic parameters and urine NAG activity/mg creatinine and urine β_2M concentration/mg creatinine were examined. Plasma NBP alkylating activity area under the concentration–time curve on day 1 of regimen A correlated significantly with the percentage increase above baseline of both the maximum urine NAG activity/mg creatinine ($r^2 = 0.513$, $P = 0.0022$) and maximum urine β_2M concentration ($r^2 = 0.413$, $P = 0.0097$) (see Figs. 4 and 5, respectively). In regimen B, a weaker, but still statistically significant correlation was found between the plasma NBP alkylating activity area under the concentration–time curve and the percentage increase above baseline of both maximum urine NAG activity ($r^2 = 0.843$, $P = 0.03$) and maximum urine β_2M concentration ($r^2 = 0.78$; $P = 0.046$) (data not shown). In regimen C, no such statistically significant correlations were

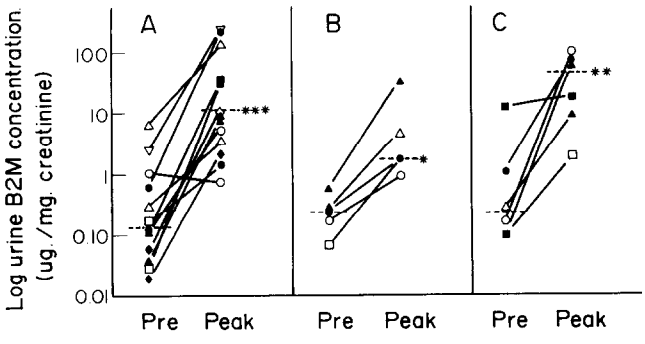

Fig. 2. The maximum rise (peak) from baseline (pretreatment) in the urine β_2M concentration per mg urine creatinine. Data are presented for each individual patient's course of ifosfamide/mesna in regimens A, B and C. ---- represents the median value of the group. Using Wilcoxon's matched-pairs signed-rank test with a two-tailed *P* value; * not significant, ** $P < 0.05$, *** $P < 0.005$.

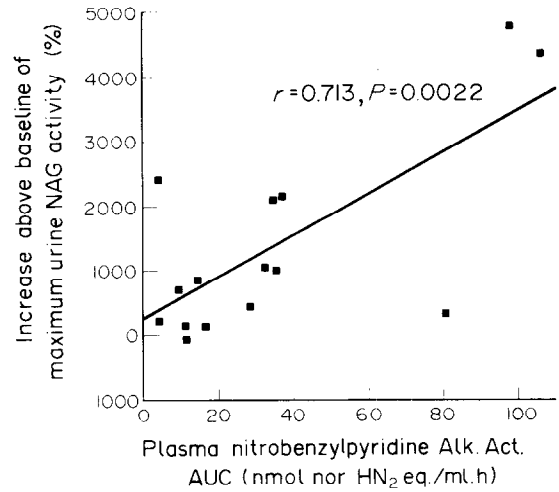

Fig. 4. The correlation between the plasma NBP alkylating activity area under the concentration–time curve, AUC_{0-24} (nmol equivalents of nor nitrogen mustard (HN_2)/ml.h) on day 1 of treatment and the percentage increase above baseline of the maximum urine NAG activity in regimen A.

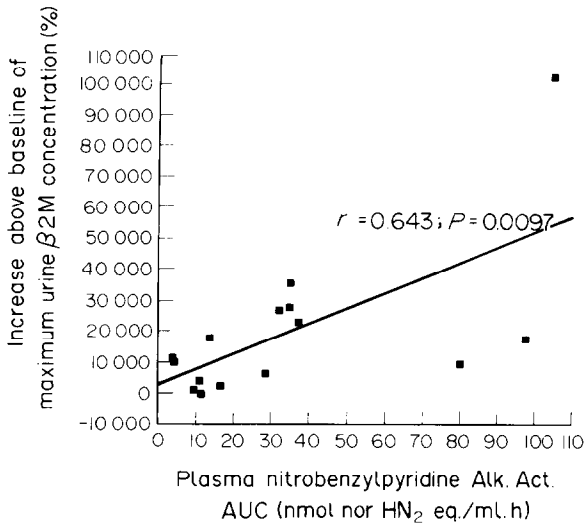

Fig. 5. The correlation between the plasma NBP alkylating activity area under the concentration–time curve, AUC_{0-24} (nmol equivalents of nor nitrogen mustard (HN_2)/ml.h) on day 1 of treatment and the percentage increase above baseline of the maximum urine β_2M concentration in regimen A.

found. Total body ifosfamide clearance did not correlate significantly with the percentage increases above baseline of either of these urine proteins, in any of the study regimens.

None of the patients in any of the treatment regimens studied had increases in serum creatinine concentrations above the normal range (60–125 μmol/l) post-treatment.

DISCUSSION

The simplest measure of ifosfamide/mesna nephrotoxicity is the assessment of the presence of haematuria and proteinuria by dipstick. In the three regimens we studied there was no correlation between either maximum (peak) urine NAG activity/mg creatinine or maximum (peak) urine β_2M concentration/mg creatinine and maximal grade of haematuria (Jaspen's multiserial coefficient of correlation). The measurement of these urinary proteins is undoubtedly a more sensitive measure of nephrotoxicity than simple dipstick testing [25]. Previous work has demonstrated that these proteins appear in increased amounts in the urine following tubular damage [19, 20, 26]. We therefore propose that increases in urinary NAG and β_2M content may indicate a different disease process to haematuria (which may originate from glomerular, ureteric or bladder epithelial damage), and could explain the absence of any correlation between them. These findings must be viewed as preliminary and interpreted with caution in view of the small patient numbers.

Measurement of the urine proteins NAG and β_2M has been used to assess the tubular toxicity of a number of nephrotoxic antineoplastic agents, e.g. cyclophosphamide and methotrexate [27] and cisplatin and iproplatin [25, 28, 29]. In patients who received cisplatin chemotherapy, abnormal elevations in urine NAG activity persisted well after treatment has been completed suggesting chronic renal tubular damage [25, 28]. In one study, the measurement of urine β_2M concentrations during and after cisplatin therapy was not found to be a good predictor of long-term nephrotoxicity [29]. These data, however, were not corrected for variation in urine concentration or output.

Our data show that in all three ifosfamide/mesna regimens studied there was evidence of significant transient renal tubular toxicity, which confirms previous studies [17, 18], and which was often subclinical. The data do not reveal any significant differences in the quantitative percentage increases in these urinary proteins between the different ifosfamide/mesna regimens. This study was not designed as a direct comparative study, and because of the small patient numbers, particularly in regimens B and C, the type II (β) error of this study is high.

In regimen A, there was a significant correlation between the time to maximum increase (peak) in urine NAG activity and the time to maximum increase in urine β_2M (see Fig. 3). In regimens A and B significant correlations were noted between the percentage increase above baseline in maximum urine NAG activity and the percentage increase above baseline of maximum urine β_2M concentration. These data suggest that urine NAG activity and β_2M concentration are measuring the same process in regimens A and B. The absence of such a correlation in regimen C may be caused by the small patient numbers.

The derangement of tubular function can extend well beyond the period of ifosfamide/mesna administration. In the few cases where data are available the markers of tubular damage return to baseline prior to initiation of the next course of chemotherapy. This provides preliminary evidence that the tubular damage caused by ifosfamide/mesna treatment does not necessarily lead to permanent renal damage. This tentative conclusion is supported in part by the absence of persistent, clinically significant elevations of serum creatinine, postifosfamide/mesna therapy. Our findings confirm previous studies of the effect of 5-day fractionated ifosfamide/mesna therapy on urine NAG activity in children by Goren *et al.* [17, 18]. These workers found that the mean percentage rise above baseline of maximum urine NAG activity was 733% (range 50–9600). This is lower than found in our patients, and their time-course of the increase in urine NAG activity and its subsequent decline back to normal within 1–2 days after chemotherapy was similar but less protracted than seen in our 5-day study. Goren *et al.* did not find any permanent effect of the temporary ifosfamide-induced NAG enzymuria on renal function [18, 30].

Several UK paediatric oncology groups, however, have reported permanent renal impairment with repeated courses of ifosfamide combination therapy [14, 15]. These studies revealed evidence of chronic glomerular, proximal and distal tubular dysfunction when ifosfamide combination therapy was used to treat solid extrarenal tumours in children. These data must be viewed with some caution, not only because they were obtained in children and should not be directly extrapolated to adults, but also because of the concomitant use of other antineoplastic agents (but not including cisplatin) and various anti-infectious drugs including aminoglycosides and amphotericin B, which can cause nephrotoxicity [14].

The plasma NBP alkylating activity area under the curve (AUC) in ifosfamide treatment regimens A and B correlated significantly with the percentage increase above baseline of both the maximum urine NAG activity and maximum urine β_2M concentration. Plasma NBP alkylating activity is known to be a non-specific measure of the metabolites produced by hepatic metabolism of ifosfamide [21]. In regimen A and B of these studies, plasma NBP alkylating activity AUC appears to be a more accurate reflection of the total exposure of the renal tubules to the nephrotoxic ifosfamide-related moieities than total-body ifosfamide clearance. These results in regimens A and B support the hypothesis that ifosfamide metabolites may be involved in the production of the renal tubular damage [6 ,7]. The absence

of such a correlation in regimen C may be due to the different administration regimen of ifosfamide/mesna, or the small patient numbers. Furthermore, plasma concentrations of parent drug or its metabolites are not necessarily the most accurate parameters to reflect the actual damage produced in the nephron. Interestingly, earlier studies [31] also suggested that the ifosfamide urotoxicity was related to plasma NBP alkylating activity AUC.

We found no evidence to suggest that the observed transient elevations of these urine proteins led to permanent renal impairment in the short term. This study was not, however, designed for long-term follow-up, nor did it utilise the most sensitive measures of renal function. Further studies of the relation between the magnitude and pattern of short-term increases in these urine proteins, and their relation to longer term effects on renal function in adults receiving different schedules of ifosfamide/mesna chemotherapy are needed. The use of more sensitive measures of renal function such as [^{51}Cr] ethylenediamine tetra-acetate (EDTA) glomerular filtration rate measurements in conjunction with sensitive markers of proximal and distal tubular function should be incorporated into future studies of ifosfamide/mesna chemotherapy schedules and renal toxicity.

1. Brade WP, Herdrich K, Varini M. Ifosfamide—pharmacology, safety and therapeutic potential. *Cancer Treat Rev* 1985, **12**, 1–47.
2. Zalupski M, Baker LH. Ifosfamide. *J Natl Cancer Inst* 1988, **15**, 556–566.
3. Van Dyk JJ, Falkson HC, Van Der Merwe AM, Falkson G. Unexpected toxicity in patients treated with isophosphamide. *Cancer Res* 1972, **32**, 921–924.
4. Czownicka Z, Utraeka-Hutka B. Clinical studies with Uromitexan—an antidote against urotoxicity of holoxan. Preliminary results. *Nowotwory*, 1980, **30**, 377–380.
5. Brock N, Stekar J, Pohl J, Niemeyer U, Scheffler G. Acrolein, the causative factor of urotoxic side effects of cyclophosphamide, ifosfamide, trofosfamide and sufosfamide. *Arzneim Forsch (Drug Res)* 1979, **29**, 651–661.
6. Cox PJ. Cyclophosphamide cystitis—identification of acrolein as the causative agent. *Biochem Pharmacol* 1979, **28**, 2045–2049.
7. Scheef W, Klein HO, Brock N, *et al.* Controlled clinical studies with an antidote against the urotoxicity of oxazaphosphorines: preliminary results. *Cancer Treat Rep* 1979, **63**, 501–505.
8. Bryant B, Jarman M, Ford HT, Smith IE. Prevention of isophosphamide induced urothelial toxicity with 2-mercaptoethane sulphonate sodium (mesna) in patients with advanced cancer. *Lancet*, 1980, **ii**, 657–659.
9. Klein HO, Wickramnayake PD, Coerper C, Christian E, Pohl J, Brock N. High dose ifosfamide and mesna as continuous infusion over five days—a phase I/II trial. *Cancer Treat Rev*, 1983, **10** (suppl. A), 167–173.
10. Scheulen ME, Niederle W, Bremer K, Schutte J, Seebers S. Efficacy of ifosfamide in refractory malignant diseases and uroprotection of mesna, results of a clinical phase II study with 157 patients. *Cancer Treat Rev* 1983, **10** (suppl. A), 93–101.
11. Stuart-Harris RC, Harper PG, Parsons CA, *et al.* High dose alkylating therapy using ifosfamide infusion with mesna in the treatment of adult advanced soft tissue sarcoma. *Cancer Chemother Pharmacol* 1983, **11**, 69–72.
12. Fossa SD, Talle K. Treatment of metastatic renal cancer with ifosfamide and mesnum without irradiation. *Cancer Treat Rep* 1980, **64**, 1103–1108.
13. Sangster G, Kaye SB, Calman KC, Dalton JF. Failure of 2-mercaptoethane sulfonate sodium (mesna) to protect against ifosfamide nephrotoxicity. *Eur J Cancer Clin Oncol*, 1984, **20**, 435–436.
14. Skinner R, Pearson ADJ, Price L, Coulthard MG, Craft AW. Nephrotoxicity after Ifosfamide. *Arch Dis Child*, 1990, **65**, 732–738.
15. Jenney M, Morris-Jones P, Gattamaneni HR, Lewis MA. Ifosfamide for children with solid tumours. *Lancet*, 1990, **335**, 1398.
16. Pattertson WP, Khojasten A. Ifosfamide induced renal tubular defects. *Cancer* 1989, **63**, 649–651.
17. Goren MP, Wright RK, Horowitz ME, Pratt CB. Ifosfamide induced subclinical tubular nephrotoxicity despite mesna. *Cancer Treat Rep*, 1987, **71**, 127–130.
18. Goren MP, Pratt CB, Viar MJ. Tubular nephrotoxicity during long term ifosfamide and mesna therapy. *Cancer Chemother Pharmacol* 1989, **25**, 70–72.
19. Price RG. Urinary enzymes, nephrotoxicity and renal disease. *Toxicology*, 1982, **23**, 99–134.
20. Peterson PA, Evrin PE, Bergaard I. Differentiation of glomerular, tubular and normal proteinuria: determinations of urinary excretion of beta-2-microglobulin, albumin and total protein. *J Clin Invest* 1969, **48**, 1189–1198.
21. Lewis LD, Fitzgerald D, Harper PG, Rogers HJ. Fractionated ifosfamide therapy produces a time dependent increase in ifosfamide metabolism. *Br J Clin Pharmacol* 1990, **30**, 725–732.
22. Lewis LD, Fitzgerald DL, Mohan P, Thatcher N, Harper PG, & Rogers HJ. The pharmacokinetics of ifosfamide when given as short and long infusions in cancer patients. *Br J Clin Pharmacol* 1991, **31**, 77–82.
23. Sanderson AR. Beta-2-Microglobulin: an immunological overview. Extracts from a symposium on beta-2-microglobulin and disease. Birmingham, U.K., April 1980, Pharmacia G.B. Ltd. pp. 6–9.
24. Yuen CT, Kind PRN, Price RG, Praill PFG, Richardson AC. Colorimetric assay for *N*-acetyl-β-D-glucosaminidase (NAG) in pathological urine using the omega-nirostyryl substrate: the development of a kit and the comparison of a manual procedure with the automated method. *Ann Clin Biochem* 1984, **21**, 295–300.
25. Jones BR, Bhalla RB, Miadek J, *et al.* Comparison of methods of evaluating nephrotoxicity of cisplatinum. *Clin Pharmacol Ther* 1980, **27**, 557–562.
26. Wright PJ, Plummer DT. The use of urinary enzyme measurements to detect renal damage caused by nephrotoxic compounds. *Biochem Pharmacol*, 1974, **23**, 65–71.
27. Fleming JJ, Parapia L, Morgan DB, Child JA. Increased urinary beta-2-microglobulin after cancer chemotherapy. *Cancer Treat Rep* 1980, **64**, 581–588.
28. Goren MP, Wright RK, Horowitz ME. Cumulative renal tubular damage associated with cisplatin nephrotoxicity. *Cancer Chemother Pharmacol* 1986, **18**, 69–73.
29. Sorenson PG, Nissen MH, Groth S, Rorth M. Beta-2-microglobulin excretion: an indicator of long term nephrotoxicity during cisplatinum treatment. *Cancer Chemother Pharmacol*, 1985, **14**, 247–249.
30. Goren MP, Pratt CB, Meyer WH, *et al.* Mesna excretion and ifosfamide nephrotoxicity in children. *Cancer Res* 1989, **49**, 7153–7157.
31. Creaven PJ, Allen LM, Cohen MH, Nelson RL. Studies on the clinical pharmacology and toxicology of isophosphamide (NSC 109724). *Cancer Treat Rep* 1976, **60**, 445–449.

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