Cancer Chemother Pharmacol (1998) 41: 413–416

SHORT COMMUNICATION

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Acute changes in urine protein excretion may predict chronic ifosfamide nephrotoxicity: a preliminary observation

Received: 27 August 1996 / Accepted: 25 July 1997

Abstract *Purpose*: To evaluate proteinuria occurring early after ifosfamide therapy and to assess the use of changes in proteinuria in the prediction of severe chronic nephrotoxicity. *Methods*: One-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis was used to characterize urine protein excretion in 12 children with solid tumours before and after the first course of ifosfamide treatment, and in 24 healthy children. Chronic nephrotoxicity was evaluated at 6 months after ifosfamide treatment and graded as none, mild, moderate or severe. *Results*: Urine from healthy children and from 10 of 12 patients before ifosfamide therapy showed a protein band with a molecular weight (95.4 kDa) corresponding to that of Tamm-Horsfall protein but no lower molecular weight proteins. After the first course of ifosfamide this 95.4-kDa protein was lost in six of ten patients with a concomitant appearance of a low molecular weight proteinuria (< 70 kDa) in eight. Tamm-Horsfall protein was lost in two of five patients who subsequently developed no or mild nephrotoxicity and in four of five patients who subsequently developed moderate or severe nephrotoxicity. *Conclusions*: Early subclinical changes in urine protein excretion after ifosfamide, manifested by a loss of Tamm-Horsfall protein excretion, may be predictive of subsequent chronic nephrotoxicity.

Key words Ifosfamide · Proteinuria · Tamm-Horsfall protein · Nephrotoxicity · Children

Introduction

Ifosfamide is used in the treatment of several childhood cancers including Ewing's sarcoma, rhabdomyosarcoma and other mesenchymal tumours [12, 13]. However, both glomerular and renal tubular toxicity may occur after treatment with ifosfamide. Although often subclinical, this nephrotoxicity may be severe in some children [7, 16]. Higher cumulative doses of ifosfamide, younger patient age at treatment [2, 18], prior nephrectomy and preexisting renal impairment, including that caused by cisplatin, have all been associated with an increased risk of nephrotoxicity [14, 17, 18]. However, it is not possible to predict before therapy begins whether or not an individual child will develop nephrotoxicity. Current strategies to prevent or reduce renal damage in children receiving ifosfamide include limiting the total dose received, and monitoring renal function during treatment with the intention of modifying treatment when clinically significant damage becomes apparent.

Proteinuria is consistently reported after ifosfamide treatment [16] with an acute increase in the urinary excretion of immunoglobulin, transferrin, albumin and several low molecular weight proteins (<70 kDa) such as retinol binding protein (RBP), α1-microglobulin (α1-M) and β2-microglobulin (β2-M) [7]. With further therapy these abnormalities may become persistent [7]. Urinary β2-M and RBP excretion have been proposed as possible predictors of chronic nephrotoxicity [2, 7]. One-dimensional sodium dodecyl sulphate polyacrylamide gel electrophoresis (1D SDS-PAGE) has been used to characterize proteinuria after ifosfamide treatment [7]. The aim of this study was to characterize the changes in urine protein excretion occurring early after ifosfamide treatment and to evaluate the possible role of these changes in the prediction of severe chronic nephropathy.

Materials and methods

Patients

Three females and nine males, aged from 1 to 16 years, received ifosfamide as part of combination chemotherapy for sarcoma (11 patients), and primitive neuroectodermal tumour (1). At diagnosis all subjects had normal renal function (normal plasma

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concentrations of electrolytes, creatinine, phosphate and normal dipstick urine analysis, including blood and protein). None had evidence of urinary tract obstruction by tumour and none received potentially nephrotoxic chemotherapy (other than ifosfamide) or radiotherapy to the kidney. The study received ethical approval and all parents and patients, when of appropriate age, gave informed consent.

Controls

Early morning urine specimens were collected from 13 healthy children of staff, and from 11 healthy children (10 males, 14 females, aged 1 to 18 years, median 7 years).

Treatment

Ifosfamide was administered as a continuous intravenous infusion of 3 g/m^2 for 3 consecutive days, with intravenous hydration and mesna continuing until 12 h after ifosfamide. The two patients with Ewing's sarcoma received 2 g/m^2 ifosfamide administered over 1 h on 3 consecutive days. Mesna was given as 3 g/m^2 per day for 3.5 days in all patients apart from those with Ewing's sarcoma who received 2 g/m^2 per day for 3 days followed by 1.5 g/m^2 per day for 12 h.

Other potentially nephrotoxic supportive treatment included intravenous vancomycin (four patients), acyclovir (one), amikacin (one) and amphotericin (three) for between 0 and 24 days (median 0). In every case this was administered after the second course of chemotherapy.

Evaluation of proteinuria

Urine was collected from each patient immediately before and 5 days after the start of the first course of ifosfamide and stored at −20 °C. Protein levels were measured using the assay described by Bradford [4]. SDS-PAGE was performed using a MINI-PROTEAN-II system (BioRad Laboratories, Hemel Hempstead, UK), which required the use of 2 μg of protein per sample. Coomassie brilliant blue was used to stain the gels. The limit of protein detection was estimated at 0.1 μg. In order to determine molecular weight densitometry was performed using a Kodak Megaplus camera (model 1.4) fitted with a 0.3 neutral density filter and a 600-nm band-pass filter camera connected to a Sun Workstation computer (Sun Micro-Systems, Calif.) using the Bio-Image Whole Band Analyser software (Millipore, Watford, UK). The molecular weight of sample proteins was calculated from the migration distance relative to molecular weight standards.

Assessment of chronic nephrotoxicity

The ifosfamide nephrotoxicity score (potentially ranging from 0 to 16) was calculated at 6 months after completion of ifosfamide by grading the glomerular filtration rate (measured by ^{51}Cr-labelled ethylenediaminetetra-acetic acid plasma clearance), the renal tubular threshold for phosphate, plasma bicarbonate concentration and early morning urine osmolality. The full details of the scoring system are presented elsewhere [20]. Patients were divided into two groups according to their nephrotoxicity score at 6 months: group 1

Table 1 Grading system for ifosfamide nephrotoxicity. Total score (i.e. sum of GFR + Tm_p/GFR + HCO_3 + EMUO): 0, no nephrotoxicity; 1–3, mild nephrotoxicity; 4–7, moderate nephrotoxicity; ≥8, severe nephrotoxicity (*DDAVP* desmopressin test[a]; *EMUO* early morning urine osmolality, mOsm/kg; *GFR* glomerular filtration rate, ml/min/1.73 m^2; *HCMA* hyperchloraemic metabolic acidosis[b]; *HCO_3* plasma bicarbonate concentration, mmol/l; *HR* hypophosphataemic rickets[c]; *NDI* nephrogenic diabetes insipidus[d])

Nephrotoxicity grade	GFR	Tm_p/GFR[e]		HCO_3[f]		EMUO
		<12 months	≥1 year	<12 months	≥1 year	
0	≥90	≥1.10	≥1.00	≥18.0	≥20.0	≥600 or normal response to DDAVP (if tested)
1	60–89	0.90–1.09	0.80–0.99	15.9–17.9	17.0–19.9	500–599
2	40–59	0.70–0.89	0.60–0.79	12.0–14.9	14.0–16.9	400–499
3	20–39	No symptoms but 0.60–0.69	0.50–0.59	No symptoms but 10.0–11.9	12.0–13.9	No symptoms but 300–399 with no response to DDAVP (if tested)
4	≤19	HR or myopathy or <0.60	<0.50	HCMA or <10.0	<12.0	NDI or <300 with no response to DDAVP (if tested)

[a] A normal response is defined by a urine osmolality ≥800 mOsm/kg

[b] Defined by biochemistry (moderate with severe hyperchloraemia ≥112 mmol/l; moderate or severe metabolic acidosis HCO_3 <15 at age <12 months, <17.0 at ≥1 year), with or without clinical symptoms/signs (e.g. Kussmaul respiration)

[c] Defined by biochemistry (moderate or severe hypophosphataemia <0.90 mmol/l at age <12 months, <0.80 at ≥1 year), with either clinical signs (genu valgus, bow leg, ricket rosary, cranial tabes, swollen wrists and ankles, abnormal gait, painful limp) or radiological features (wide epiphyseal plate, expanded metaphysis, reduced bone density, secondary hyperparathryoidism with subperiosteal erosions), or with both

[d] Defined by clinical symptoms/signs (polyuria and polydipsia, dehydration) with or without biochemistry (moderate or severe hypernatraemia >150 mmol/l), with lack of response to desmopressin (DDAVP)

[e] Tm_p/GFR is the renal tubular threshold for phosphate (mmol/l) and is calculated from the plasma and urine phosphate and creatinine concentrations

$$Tm_p/GFR = Pp - Up \times Pcr/Ucr$$

where P and U are plasma and urine concentrations, and p and cr are phosphate and creatinine, respectively

[f] If a patient is receiving long-term supplementation with bicarbonate (or citrate) for acidosis, the HCO_3 grading should be based on the last plasma HCO_3 concentration measured before starting the supplements. It is recommended that the need for continued treatment should be reviewed at appropriate intervals – if the supplements are stopped (temporarily or permanently), the $HC0_3$ score can be regraded as appropriate

patients had no or mild nephrotoxicity (score 0–3); group 2 patients had moderate or severe nephrotoxicity (score ≥4). Details are given in the Table 1. The results from the two groups were compared using the Mann-Whitney *U*-test.

Results

The clinical features of groups 1 and 2 are shown in Table 2. There were no statistically significant differences between the two groups in age or ifosfamide dose.

Patterns of proteinuria

Control subjects

No low molecular weight proteinuria was detected, but a protein band of molecular weight of 95.4 kDa (denoted band A) was excreted by all individuals.

Patients receiving ifosfamide

The pretreatment profile of proteinuria in both groups of patients was the same as the control group, except that two patients in group 2 did not excrete band A at diagnosis. After the first course of ifosfamide, of the ten patients who excreted band A at diagnosis, this band was not present in two of five patients in group 1 and four of five patients in group 2. Low molecular weight protein (< 70 kDa) bands were detected after the first course more commonly in group 2 patients. For example band I (23.3 kDa) was present in two of five group 1 patients and five of seven group 2 patients after the first ifosfamide course.

Discussion

This small descriptive study showed that treatment with ifosfamide resulted in the loss of band A and the appearance of low molecular weight proteins in many patients. These changes were apparent after the first course in a higher proportion of children in group 2.

Protein band A appears to be a normal constituent of urine whose excretion is reduced after tubular damage. It has a molecular weight (95.4 kDa) very close to that of Tamm-Horsfall protein (THP) (94 kDa). THP excretion has not been previously studied after ifosfamide treatment The changes in band A excretion were initially reversible but with repeated therapy a progressive reduction in excretion was seen (data not shown). Since THP is normally produced in the thick ascending limb of the loop of Henle (TALH) [9], the extensive tubular damage known to occur after ifosfamide [19] might lead to reduced excretion. Therefore a reduction in THP excretion would provide further evidence of tubular damage, but of a different nature to that causing low molecular weight proteinuria. The relevance of a loss or reduction in THP secretion as a predictor of subsequent

Table 2 Clinical details of patients and changes in urine THP excretion. All patients were previously untreated. All schedules were three weekly

Diagnosis	Sex	Age (years)	Total dose (g/m^2)	Dose/Course (g/m^2)	Nephrotoxins also used (days)	Renal toxicity (group)[a]	THP present at diagnosis	THP present after 1st dose
Rhabdomyosarcoma	M	7	48	9	Vancomycin (3) Amphotericin (3)	None (1)	Yes	No
Ewing's sarcoma	M	10	123	6 or 9	Vancomycin (19) Amikacin (1) Acyclovir (3)	None (1)	Yes	No
Ewing's sarcoma	M	16	84	6 or 9	Nil	None (1)	Yes	Yes
Rhabdomyosarcoma	M	3	141	8 or 9	Nil	Mild (1)	Yes	Yes
Rhabdomyosarcoma	F	6	81	9	Nil	Mild (1)	Yes	Yes
Primitive neuroectodermal tumour	M	1	81	9	Nil	Moderate (2)	No	No
Rhabdomyosarcoma	M	3	144	9	Amphotericin (1)	Moderate (2)	No	Yes
Rhabdomyosarcoma	F	5	81	9	Nil	Moderate (2)	Yes	No
Rhabdomyosarcoma	M	6	153	9	Vancomycin (16)	Moderate (2)	Yes	Yes
Rhabdomyosarcoma	F	7	144	9	Nil	Moderate (2)	Yes	No
Epithelioid sarcoma	M	12	153	9	Amphotericin (5) Vancomycin (8)	Moderate (2)	Yes	No
Rhabdomyosarcoma	M	1	153	9	Nil	Severe (2)	Yes	No

[a] Group 1 no or mild renal damage; group 2 moderate or severe renal damage

chronic tubular damage is unknown. However, THP may have a role in sodium and chloride reabsorption in the TALH by assisting in the countercurrent mechanism of water reabsorption. Impaired reabsorption at this site could contribute to an ifosfamide-induced nephrogenic diabetes insipidus. Although a rise in THP excretion has been reported after cisplatin administration [8, 22], other groups have found a fall in excretion after cyclosporin treatment [6], with acute tubular necrosis [11] and in chronic renal disease [10, 22].

Since a constant amount of protein was loaded onto each gel, the results reflect the relative rather than the absolute urine excretion of each band. However it was noted that protein band A disappeared or could be absent even if there was not a concomitant increase in intensity or number of protein bands present. Therefore, although we cannot exclude the possibility that a relative increase in low molecular weight proteinuria obscured the excretion of an unchanged absolute amount of THP, we think this is unlikely.

Other protein bands detected in the urine in children after ifosfamide treatment in this study had molecular weights similar to transferrin, RBP (band I), lysozyme, and β2-M. An acute low molecular weight proteinuria is consistent with previous reports of increased excretion of β2-M, RBP and α1-M in most patients treated with ifosfamide [1, 5, 7, 15].

Individual differences in oxazaphosphorine metabolism, leading to variable tubular exposure to potentially nephrotoxic ifosfamide metabolites, might explain some of the inter-individual differences in acute and chronic ifosfamide nephrotoxicity. This would explain the range of cumulative dose at which ifosfamide-induced nephrotoxicity occurred in this and previous studies [19]. There is considerable interest surrounding individual differences in oxazaphosphorine metabolism [3] and how these may modulate the risk of chronic ifosfamide nephrotoxicity.

In conclusion, this preliminary descriptive study of proteinuria after ifosfamide treatment demonstrated a low molecular weight proteinuria and loss of a normally excreted protein with a molecular weight similar to that of THP. After the first course of ifosfamide there was a tendency for this pattern of protein excretion to be seen more commonly in individuals who later developed severe clinical renal damage. It is hypothesized that early subclinical changes in urine protein excretion may be predictive of the likelihood of subsequent chronic nephrotoxicity.

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