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Original Paper

Ifosfamide Nephrotoxicity: Limited Influence of Metabolism and Mode of Administration During Repeated Therapy in Paediatrics

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This study investigated the relationship between both acute and chronic nephrotoxic effects of ifosfamide (IFO) and its metabolism. 15 paediatric patients (4 girls) were investigated. Each received 6–9 g/m² IFO over 15 days, repeated every 3 weeks for up to 16 courses. The pharmacokinetics and metabolism of IFO were measured during its administration, either as a continuous 72 h infusion or as three bolus doses of 3 g/m² on consecutive days. In 8 patients, the metabolism of IFO was investigated during one early course and one late course to determine the magnitude of any changes following repeated administration. Acute measures of renal toxicity were not correlated with any of the IFO pharmacokinetic or metabolic parameters in the same course, whether the drug was administered as a bolus or by continuous infusion. Chronic renal toxicity, determined 1 month ($n = 13$) or 6 months ($n = 8$) after treatment, did not correlate with any of the IFO pharmacokinetic or metabolic parameters in any individual course of treatment. The overall degree of nephrotoxicity, however, was correlated with the changes in metabolism between late and early courses ($n = 8$). There was a negative correlation between the change in area under the curve of the dechloroethylated metabolites of IFO and the overall nephrotoxicity at 1 month or 6 months after treatment (both $r^2 = 0.66$, $P = 0.014$). The results imply that patients in whom metabolism via dechloroethylation decreases are at a greater risk of chronic nephrotoxicity. This is contrary to the hypothesis that the systemic production of chloroacetaldehyde is the mechanism by which IFO causes nephrotoxicity. The importance of acute and chronic changes in renal function for long-term outcome remains to be determined. Copyright © 1996 Elsevier Science Ltd

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INTRODUCTION

IFOSFAMIDE (IFO) is used in the treatment of many different tumours, including a number of paediatric malignancies [1]. The drug itself is inactive and requires metabolic activation via a P450-mediated 4-hydroxylation reaction [2, 3]. The 4-hydroxyifosfamide intermediate ultimately undergoes β-elimination to release isophosphoramide mustard (IPM), which is thought to be the ultimate alkylating species [4]. Inactivation can occur by oxidation of an intermediate to form the carboxy (CX) metabolite, a reaction mediated by a cytosolic aldehyde dehydrogenase enzyme [5]. As well as the activation pathway, IFO can also undergo oxidative dealkylation to yield 2- and 3-dechloroethyl metabolites (2DC and 3DC) [4]. This reaction is mediated by the same P450 enzyme as that involved in 4-hydroxylation [3], and results in the formation of an equimolar quantity of chloroacetaldehyde. As dechloroethylation can account for up to 30% of a dose of IFO [6], substantial amounts of chloroacetaldehyde can be formed. Chloroacetaldehyde has been shown to be toxic to renal tubular cells [7] and to hepatocytes [8], and has been shown to form DNA cross-links [9]. Another aspect of IFO metabolism is that clearance of the drug appears to increase after continuous or repeated administration over several days [10, 11]. This is thought to be due to auto-induction of drug-metabolising enzymes, and results in the increased production of dechloroethylated metabolites [11, 12].

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In contrast to cyclophosphamide, non-haematological toxicities may limit the use of IFO. The acute haemorrhagic cystitis seen in the early use of this drug has now been overcome by concomitant administration of sodium mercaptoethanesulphonate (Mesna) [13]. An acute, reversible toxicity in the central nervous system has been reported in some patients [14] and the incidence of this toxicity is higher following oral administration as compared to intravenous administration [15]. The toxicity is less common in paediatric patients [16], but renal toxicity can be a major problem in younger patients [17].

We have been conducting studies of the pharmacokinetics and metabolism of IFO in paediatric patients. These studies have included investigations of intersubject variability [6], intrasubject variability in different courses of treatment [18] and a comparison of continuous infusion with intermittent bolus administration [12]. Simultaneously with these drug metabolism studies, the renal function of the patients has been intensively studied. Measures of acute toxicity have been determined during these courses when metabolism was investigated and, where possible, post-treatment follow-up measures of chronic renal toxicity have been performed. The data presented in this report comprise a comparison of renal toxicity with the pharmacokinetics and metabolism of IFO determined during individual courses or with the variation in metabolism during repeated administration.

PATIENTS AND METHODS

The 15 patients (4 female) studied were treated with various protocols for a variety of cancers, mostly soft-tissue sarcomas. The details of each patient, diagnosis and IFO treatment are given in Table 1. No patient had received prior platinum chemotherapy or irradiation involving the kidneys, nor had any patient undergone nephrectomy prior to treatment. No other nephrotoxic drugs were administered to these patients [6]. IFO was administered intravenously at a dose of 3 g/m^2/day for 2–3 days, together with an equivalent dose of Mesna. Treatment was repeated every 3 weeks for up to 16 courses. The pharmacokinetics and metabolism of IFO were determined in each patient in up to three courses when the drug was administered as a continuous, 72 h infusion. Measurements of the pharmacokinetics and metabolism were also performed in one course when IFO was administered as a short (1 h) infusion on 3 consecutive days (bolus administration). In 8 of the patients, measurements were performed during one early course (1–3) and one late course (later than course 8) to determine the magnitude of any changes following repeated administration.

Plasma and urine samples were collected during and for 24 hours after drug administration. IFO and its metabolites were determined in plasma and urine by a quantitative, high-performance thin-layer chromatography-photographic densitometry technique (HPTLC-PD) [19]. Exposure to IFO and each of its metabolites was quantified as the area under the plasma concentration against time curve (*AUC*, μM/h) as calculated by the trapezoidal rule. In each course, and for each dose of the 1 h infusion courses, pharmacokinetic parameters were determined using model-independent analysis. Clearance (*Cl*, l/h^1/m^2) was calculated as the *AUC* of IFO divided by the dose administered. Half-life ($t_{1/2}$, h) was calculated by fitting a mono-exponential equation to the terminal phase of the postinfusion data. The exponential coefficient of this equation, *K*, is equal to $0.693/t_{1/2}$. Volume of distribution ($Vd\beta$, l/m^2) was calculated as *Cl*/*K*. For each course, the magnitude of auto-induction was quantified as the percentage change in IFO concentration from its peak to the end of the infusion. For bolus administration, the *AUC* of parent drug on day 3 was compared to that on day 1. The degree of increased dechloroethylation was quantified as the corresponding increase in plasma concentration of the predominant 3DC metabolite. Where available, parent drug and metabolite concentrations in urine were used to calculate the percentage

Table 1. *Details of patients studied*

Patient	Sex	Age (years)	Body surface area (m^2)	Diagnosis	Courses studied*	Total dose (g/m^2)†	Follow-up (months)
1	M	16.0	1.5	Ewing's	2, 4, 5(b)	27	1 only
2	M	7.0	0.9	Emb Rhab	2, 3(b)	54	1 only
3	M	3.8	0.6	Pm Emb Rhab	2, 6(b), 13	144	1 and 6
4	M	1.9	0.5	Pm Emb Rhab	2, 8(b)	153	1 and 6
5	M	0.8	0.3	Schwannoma	3, 6(b)	90	1 and 6
6	F	5.1	0.7	Orb Emb Rhab	3(b), 4	81	None
7	M	1.0	0.4	PNET	1, 2(b), 9	81	1 and 6
8	M	5.0	0.8	Pelvic Emb Rhab	2(b), 3, 15	141	1 and 6
9	F	4.3	0.7	Ewing's	4(b), 8	84	1 and 6
10	M	6.8	0.9	Emb Rhab	5, 15(b), 16	153	1 only
11	F	1.6	0.4	Triton tumour	3, 4(b)	36	None
12	M	16.5	1.8	Ewing's	6, 10(b)	84	1 only
13	F	6.4	0.8	Orb Emb Rhab	2(b), 3	81	1 and 6
14	M	10.9	1.0	Ewing's	3, 4(b), 8	123	1 and 6
15	M	12.5	1.1	Epith sarc	3, 8(b), 15	153	1 and 6

Ewing's, Ewing's sarcoma; Emb Rhab, embryonal rhabdomyosarcoma; Pm, parameningeal; Schwannoma, of left femoral nerve; Orb, orbital; PNET, primitive neuroectodermal tumour; Epith sarc, epitheloid sarcoma of thumb.

*b, bolus; †doses were administered as 6 or 9 g/m^2 cycles every 3 weeks. Concomitant therapy included etoposide, actinomycin D and vincristine. Dexamethasone, ondansetron and metaclopramide were administered as anti-emetics. Doses of ifosfamide were administered as a continuous infusion, except one cycle that was administered as three 1 h infusions of 3 g/m^2 on consecutive days ('bolus' administration).

of the dose excreted unchanged and as each metabolite. Renal clearance of IFO was calculated as the product of the fraction of the dose excreted unchanged and *Cl*. Changes in the pharmacokinetics and metabolism of IFO during repeated courses ($n = 8$) were measured by the magnitude of changes (expressed as a percentage) in the pharmacokinetic parameters or *AUC* values, comparing an early course to a late course.

In each course, renal function was investigated in each patient on day 1 before treatment with IFO commenced and again within 36 h of completion of the IFO administration. Blood and urine were collected and the serum creatinine, serum CO_2 and early morning urine osmolality (*EMUO*) were measured. Glomerular filtration rate (*GFR*) was measured by plasma clearance of ^{51}Cr-EDTA. The renal tubular threshold for phosphate (*Tmp/GFR*) and fractional excretion of glucose (*FEGluc*) were calculated. At 1 and 6 months after treatment, these investigations were repeated. Details of these methods have been published elsewhere [20]. For the assessment of overall chronic nephrotoxicity, a composite nephrotoxicity score (*NTX*) was determined according to the aggregate degree of abnormality from four of the measures of renal function (*GFR*, *Tmp/GFR*, serum CO_2, *EMUO*) [17]. This score reflects the severity of clinically relevant nephrotoxicity.

Changes in renal function were correlated with the pharmacokinetics and metabolism of IFO during individual courses (acute toxicity), and also by comparing chronic renal toxicity with the pharmacokinetic parameters and *AUCs* from individual courses. Analysis was performed using multiple regression. To correct for prior exposure to IFO and for variation in the age of the patients, course and patient age were included as independent covariates when considering individual courses. To determine whether changes in the pharmacokinetic parameters of IFO and its metabolites during repeated therapy affected long-term nephrotoxicity, these changes were compared with the *NTX* score for 8 patients where this information was available. These latter relationships were determined by Spearman's rank correlation. Analysis of variance was used to check for treatment and period effects between different modes of administration.

RESULTS

Renal toxicity

All the patients showed evidence of subclinical renal damage, both acutely when renal function was measured before and after a course of therapy, and when measured 1 or 6 months after the end of treatment. The acute reduction in renal function varied amongst the patients and, for an individual patient, with the course and mode of drug administration. Median changes, together with ranges, for acute renal toxicity are given in Table 2. *GFR*, measured prior to the administration of IFO, was not used as a measure of acute toxicity, but did not seem to decline with repeated dosing. The most sensitive measures of acute renal toxicity appeared to be serum CO_2 and *Tmp/GFR*, which both showed consistent decreases 5 days after the start of each course of treatment (Figures 1 and 2).

Chronic toxicity was found in most of the patients studied, with abnormalities in serum CO_2, *Tmp/GFR*, *EMUO*, *FEGluc* and *GFR*. Abnormalities in *Tmp/GFR* were most common (Figure 3). Median values and ranges for the different measures of nephrotoxicity are given in Table 3. Of the 12 patients studied 1 month after treatment, 2 showed no nephrotoxicity, 4 mild nephrotoxicity, 5 moderate nephrotoxicity and 1 severe nephrotoxicity, according to the classifications described previously [17] (Figure 4). At 6 months after treatment, of the 8 patients available for assessment, 1 had no nephrotoxicity (patient 14), 4 had mild and 3 moderate nephrotoxicity.

Pharmacokinetics and metabolism

The pharmacokinetics and metabolism data for these patients have been reported previously [6, 12, 18]. The major finding relevant to the present paper is a lower *AUC* for the dechloroethylated metabolites following bolus administration compared to continuous 72 h infusion of the same dose [12]. A large degree of intersubject variation in the pharmacokinetics and metabolism was observed, together with an unexpectedly large degree of intrasubject variation [18]. Neither of these types of variation appeared to be due to patient characteristics, such as age, sex, renal function or order of treatment. Intrasubject comparison showed that, in most patients, *AUC* of dechloroethylated metabolites declined with repeated courses of therapy and that *AUC* of IFO was inversely correlated with age [18].

Comparison of nephrotoxicity and IFO parameters

Acute nephrotoxic effects were compared to the pharmacokinetics and metabolism of IFO in individual courses. No correlation was seen between any measure of renal function and any of the parameters of IFO pharmacokinetics and metabolism, including the observed degree of auto-induction. This was true for both infusion and bolus courses. There was no difference between infusion and bolus courses with regard to nephrotoxicity.

Similarly, it was not possible to demonstrate a relationship between chronic nephrotoxicity and the pharmacokinetics and metabolism of IFO determined on any one course. In part, this may be due to the large intrasubject variability described above, so that no one course is representative of an individual's exposure to IFO and its metabolites throughout a treatment schedule involving up to 16 cycles of therapy. However, in the 8 patients who were studied during an early course (1–3) and a late course (>8), a correlation was observed between changes in the degree of dechloroethylation between courses studied and overall *NTX* at both 1 and 6 months (both $r^2 = 0.66$, $P = 0.014$). That is to say, those patients whose *AUC* of 3DC decreased following repeated administration of IFO showed a greater degree of nephrotoxicity at 1 and 6 months (Figure 5). Similarly, an increase in *AUC* of the 3DC metabolite was associated with an absence of or mild nephrotoxicity.

DISCUSSION

IFO nephrotoxicity was first reported as a Fanconi-like syndrome [21] with hypophosphataemic rickets [22] and renal tubular acidosis [22, 23]. Although more common in children, Fanconi's syndrome after treatment with IFO has also been reported in adults [24, 25]. Because many of the patients treated with IFO are very young, growth retardation may be seen due to hypophosphataemic rickets and renal tubular acidosis [22, 26]. Recent studies have shown that pre- or concomitant treatment with platinum cytotoxic drugs [27, 28], nephrectomy [28], pelvic disease [22], age [29] and the cumulative dose of IFO [30] are important factors in determining the probability of IFO nephrotoxicity. Simple measures of renal function, such as serum creatinine, are inadequate to assess IFO nephrotoxicity because the primary

Table 2. Measures of acute renal toxicity in paediatric patients receiving ifosfamide (IFO)

Treatment	*GFR*	Serum creatinine	Serum CO_2	*Tmp/GFR*	Urine osmolality	*FEGluc*
Infusion	126	1.5	−2	−0.3	−68	0
	80–189	−22–34	−8–6	−0.6–0.5	−681–477	−1.3–1.0
Bolus	126	−4	−2	−0.5	−65.5	0.1
	92–209	−20–7	−7–−1	−1.2–−0.1	−787–339	−0.1–64.1

Values are given as a median and range. Glomerular filtration rate (*GFR*) is the pretreatment value and is provided as an indictor of overall renal function in the patients studied. Measures of acute toxicity are expressed as the absolute changes comparing the parameter before and after treatment with IFO. Details of normal ranges for these parameters and of the nephrotoxicity score are given in the article by Skinner and associates [30]. Revised normal range of the fractional excretion of glucose (*FEGluc*) ⩾ 0.1. *Tmp/GFR*, tubular threshold for phosphate.

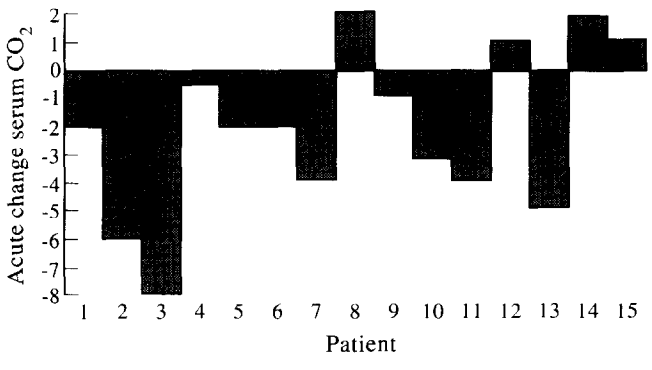

Figure 1. Acute changes in serum CO_2 following a single course of ifosfamide, 9 g/m², administered as a continuous infusion. A decrease is indicative of acute toxicity.

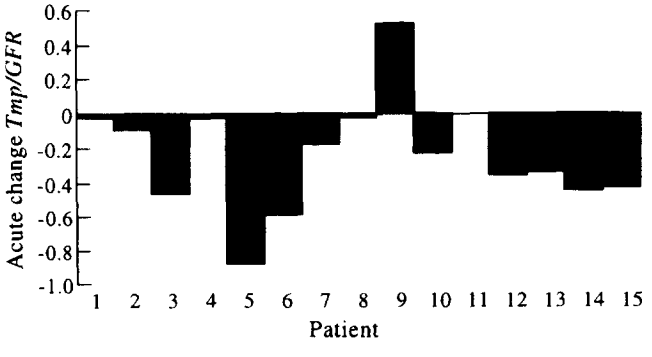

Figure 2. Acute changes in the tubular threshold for phosphate *Tmp/GFR* following a single course of ifosfamide, 9 g/m², administered as a continuous infusion. A decrease is indicative of acute toxicity.

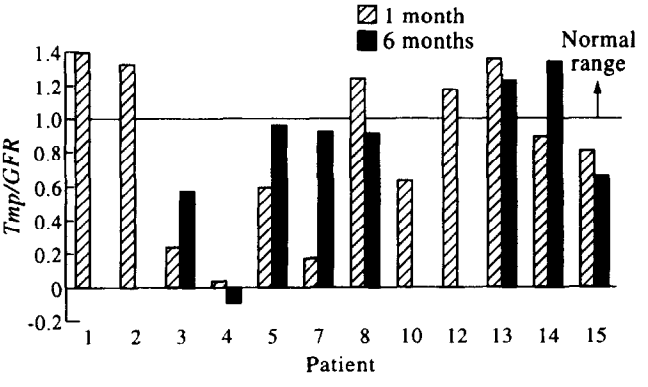

Figure 3. Tubular threshold for phosphate (*Tmp/GFR*) in patients studied at 1 or 6 months follow-up after the end of treatment with ifosfamide.

site of renal damage is in the proximal tubule, with glomerular impairment as a secondary process. Assessment of IFO nephrotoxicity should take account of both glomerular and renal tubular function [20, 27]. A grading system based on different aspects of clinically relevant renal tubular function and *GFR* has been proposed for assessing the overall degree of nephrotoxicity [17].

The encephalopathy and nephrotoxicity associated with IFO therapy have not been seen with the structural isomer cyclophosphamide [31]. Since cyclophosphamide does not undergo extensive dechloroethylation [32, 33], this has led to the suggestion that chloroacetaldehyde is responsible for these toxicities [17]. Chloroacetaldehyde is undoubtedly toxic to many physiological systems. It may be further oxidised to chloroacetic acid that also possesses toxic effects. There has been one report of increased levels of chloroacetaldehyde in the urine or plasma of patients with CNS toxicity [34], and we have observed elevated levels of dechloroethylated metabolites in the urine and plasma of such patients (data not shown). However, other metabolites, such as the 4-hydroxy intermediates, IPM and CX, interfere with ion transport in cultured renal tubular cells [35]. As yet, no unambiguous evidence for the mechanisms of IFO nephrotoxicity has been produced.

Following initial reports of IFO nephrotoxicity, several investigators have sought to elucidate the mechanism of this toxicity or to define predisposing patient or treatment factors [22, 29]. No attempt has yet been made to relate the metabolism of IFO to any measure of renal damage. Several papers have been published that attempt to define the most sensitive or meaningful measurements for diagnosing IFO nephrotoxicity. These include measurements of specific amino acids [28], tubular enzymes [36] and proteins in urine [36]. Those suggested by Skinner and associates [20], and employed in this study, are designed to indicate differential toxic effects at the glomerulus, proximal tubule and distal tubule.

The observations of apparent relationships between chronic IFO nephrotoxicity and the pharmacokinetics and metabolism of the drug described in this paper may offer some insight into the mechanisms underlying this toxicity. In the present study, neither mode of administration nor intra- or intersubject

Table 3. Measures of chronic renal toxicity in paediatric patients 1 and 6 months after treatment with ifosfamide

Follow-up time	*GFR*	Serum creatinine	Serum CO_2	*Tmp/GFR*	Urine osmolality	*FEGluc*	*NTX* score
1 month	102	53	20	0.79	564	0.1	3
	77–244	32–87	16–24	0.1–1.4	132–833	0–11.9	0–9
6 months	65	65	17	0.90	688		3
	44–114	38–92	14–21	−0.1–1.3	391–1051	ND	0–7

Measures of chronic toxicity are given as absolute values. Details of normal ranges for these parameters and of the nephrotoxicity score (*NTX*) are given in the article by Skinner and associates [30]. Revised normal range of the fractional excretion of glucose (*FEGluc*) ≥ 0.1. *GFR*, glomerular filtration rate; *Tmp/GFR*, tubular threshold for phosphate; ND, not determined.

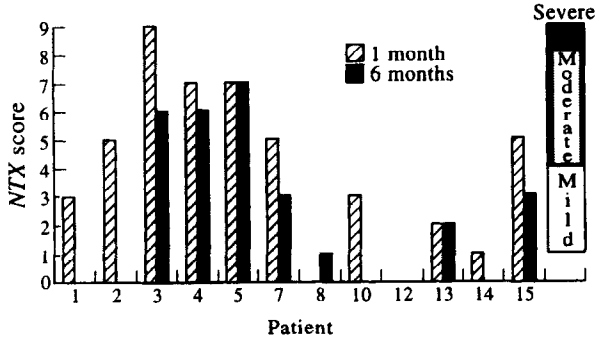

Figure 4. Total nephrotoxicity score (*NTX*) in patients studied at 1 or 6 months follow-up after the end of treatment with ifosfamide.

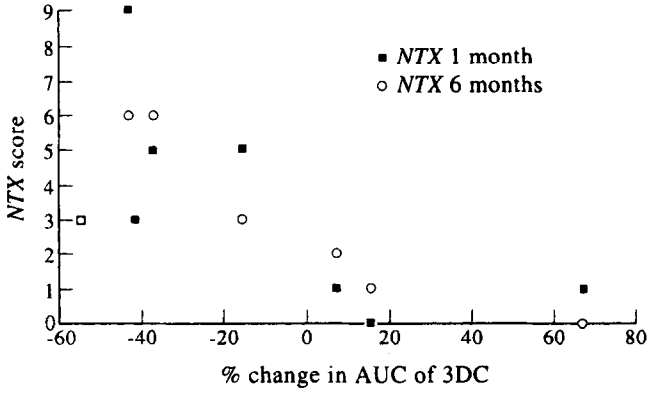

Figure 5. Correlation of overall nephrotoxicity (*NTX*) 1 and 6 months post-treatment with % change in the area under the curve of the metabolite 3-dechloroethyl during repeated courses of ifosfamide. $r^2 = 0.66$, $P = 0.014$.

variations correlated directly with measures of nephrotoxicity, but there was some indication that dechloroethylation and the production of chloroacetaldehyde are not associated with this toxicity. The degree of dechloroethylation, as determined by *AUCs* for the 2DC and 3DC metabolites, was lower when the drug was administered as repeated boluses than as a continuous infusion. There was no difference in acute nephrotoxicity between the administration methods, however, and no direct correlation, positive or negative, could be shown between acute nephrotoxicity and dechloroethylation. The total nephrotoxicity score was greater in those patients who showed a decrease in dechloroethylation during repeated courses. This relationship is the opposite to that which has been suggested by a review of previous studies [17]. The hypothesis that greater dechloroethylation would be expected to result in greater nephrotoxicity is based on the formation of an equimolar quantity of chloroacetaldehyde in the dechloroethylation reaction. However, no direct relationship between chloroacetaldehyde production and nephrotoxicity has been demonstrated *in vivo*. Our data, based on observations of acute and chronic nephrotoxicity, appear to contradict this hypothesis.

The results indicate that systemic metabolism has some influence on IFO nephrotoxicity, but other mechanisms are also possible. Cytochrome P450 enzymes have been detected in renal tubular cells, in particular CYP3A enzymes [37], the major contributors to IFO metabolism [3], have been reported to be expressed in kidney cells. CYP3A4, the major enzyme in the liver, is polymorphically expressed in the kidney, i.e. some individuals do not express the enzyme in the latter tissue [38]. In contrast, CYP3A5, which is polymorphically expressed in the liver, is expressed consistently in the kidney [38]. A role for CYP3A5 in IFO metabolism has been reported [39]. It may be that this differential expression of the two human iso-forms of CYP3A in different individuals contributes to nephrotoxicity, independent of variability in systemic metabolism. In keeping with the theory that metabolism in the kidney is significant for both IFO and cyclophosphamide is the observation that the carboxy metabolite of both drugs is often undetectable in plasma, but represents up to 25% of the administered dose found in urine [6, 40].

In conclusion, we have observed that no simple relationship exists between either acute or chronic nephrotoxicity and the pharmacokinetics and metabolism of IFO in a single course of therapy. By comparing different courses of therapy, it is possible to conclude that a greater degree of nephrotoxicity is associated with a lesser degree of dechloroethylation. Further studies will be required to fully elucidate the exact mechanism underlying IFO nephrotoxicity.

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