

The pharmacokinetics and metabolism of ifosfamide during bolus and infusional administration: a randomized cross-over study

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Summary In a randomized cross-over trial, 11 patients received ifosfamide (IFOS) in 21-day cycles, which alternated between 3 g m⁻² × (2 or 3) days given as a 1-h bolus doses, or the same total dose as a continuous infusion. Patients who received four or more cycles also alternated between two cycles on dexamethasone 4 mg 8 hourly for 3 days starting 8 h before IFOS, and two cycles off dexamethasone. A total of 34 patient cycles were studied and serum and urinary levels of IFOS, 2 dechloroethylifosfamide (2DC), 3 dechloroethylifosfamide (3DC), carboxyifosfamide (CX) and isophosphoramidate mustard (IPM) were measured by thin-layer chromatography. No significant differences could be detected in the areas under the curve (AUCs) of serum concentration, nor in the proportion of IFOS or its metabolites found in the urine. There was no significant effect of dexamethasone on IFOS metabolism. These results indicate that there is no identifiable pharmacokinetic basis for insistence on either bolus or infusional methods of IFOS administration.

Keywords: ifosfamide; pharmacokinetics; thin-layer chromatography; schedule; dexamethasone

The oxazaphosphorine, ifosfamide (IFOS) is used to treat a large number of malignancies (Brade et al, 1985). IFOS is a prodrug, requiring biotransformation for activity or toxicity (Allen and Creaven, 1972; Creaven et al, 1974). Metabolism appears to be mainly in the liver where cytochrome P450 enzymes produce the active intermediate 4 OH ifosfamide (Brock and Hohorst, 1963). This metabolite exists in equilibrium with its tautomer aldo-ifosfamide, which may be oxidized to carboxyifosfamide (CX) or decompose spontaneously, by β elimination to produce isophosphoramidate mustard (IPM) (Brock, 1983). In addition to ring oxidation, side-chain oxidation at the exo- or endo-cyclic nitrogen may occur, yielding the metabolites 2- and 3-dechloroethyl ifosfamide (2DC), (3DC) and chloroacetaldehyde (CAA) (Figure 1).

IPM is the ultimate alkylating metabolite of IFOS, causing DNA interstrand cross-links. The metabolites responsible for nephrotoxicity and neurotoxicity are unknown, although CAA has been implicated as this is a reactive metabolite and produced in large quantities (Wagner, 1994). Concentrations of 2DC and 3DC are an indirect measurement of the unstable metabolite CAA, as this is formed in equimolar amounts in the *N*-dechloroethylation process.

A number of clinical pharmacokinetic studies have now been published showing considerable inter- and intra-individual variability in serum and urine levels of parent drug and metabolites (Creaven et al, 1974; Hartley et al, 1994; Boddy et al, 1993; 1995a; 1996). Some of this variability may be due to the differences in detection method (Nelson et al, 1976; Wagner et al, 1994), the schedule of administration, schedules, age (Lind et al, 1989a), the

volume of distribution (Lind et al, 1989b), and the patient's age and hepatic and renal function. Metabolic factors may also be responsible such as genetic differences in the mixed function oxidases or drug interactions involving the cytochrome P450 systems (Lind et al, 1990; Kaijser et al, 1993; 1994; Kurowski & Wagner, 1993).

In standard IFOS doses (5–10 g m⁻² per cycle), many schedules of administration are used, such as a bolus dose in 30–60 min over 2–5 days and 24 or 72 h continuous infusions. It is unclear whether there is an optimal schedule, either for anti-tumour effect or for the reduction of toxicity, and whether the pharmacokinetics of IFOS and its principal metabolites are significantly influenced by schedule. Animal studies have shown that four fractionated doses of IFOS were superior to a single bolus in producing a higher tumour cell kill for less haematological and urothelial toxicity (Klein et al, 1984). Clinical studies are limited but also suggest that continuous infusion IFOS is haematologically less toxic than bolus doses for the same anti-tumour effect (Rodriguez et al, 1976; Morgan et al, 1982; Klein et al, 1984; Anderson et al, 1993). Thus, it is possible that increasing fractionation of IFOS may confer some sparing of normal tissue toxicity. In children, Boddy et al (1995b) have compared the pharmacokinetics of IFOS and metabolites under bolus and infusion; however, there have been no similar measurements in adults.

IFOS metabolism has been studied *in vitro* using rat and human liver microsomes (Ruzicka & Ruenitz, 1992; Murray et al, 1994; Walker et al, 1994). Weber and Waxman (1993) have shown that pretreatment of rats with dexamethasone (DEX) increased liver microsomal activation of ifosfamide sixfold. DEX is commonly used in combination with ifosfamide because of its antiemetic properties. Its use may therefore alter the metabolic pattern of ifosfamide with therapeutic or toxic consequences.

The aims of this study were, therefore, to compare the pharmacokinetics of 1-h bolus dose vs continuous infusion IFOS in a

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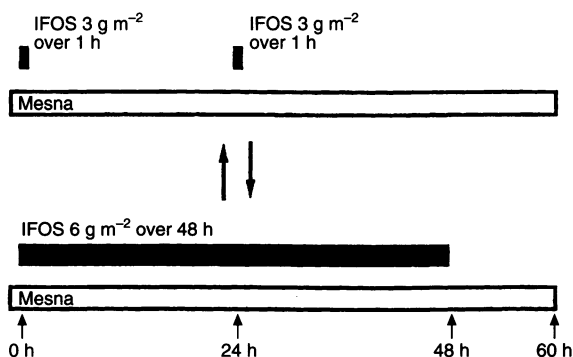


Figure 2 Treatment schedules for bolus (upper) and infusion (lower) administrations

Extraction of parent drug and metabolites from patient urine was as described previously (Boddy and Idle, 1992; Hartley et al, 1994). Samples were applied to prewashed XAD-2 columns and eluted with methanol, dried and reconstituted in a small volume of methanol. An aliquot (100 mg) of silicic acid and 3 ml of methanol were added to the residue, vortexed and then centrifuged at 4000 r.p.m. for 10 min. The supernatant was collected and evaporated to dryness. Serum samples were treated similarly, but the XAD-2 columns were washed with 0.05 M Tris buffer pH 5.5 before sample application.

Samples were then reconstituted in 70 μ l of methanol and 50 μ l was applied to high-performance thin-layer chromatography (HPTLC) plates using a 'Linomat IV' TLC sample applicator (Camag, Germany). The TLC plates were placed in glass tanks containing dichloromethane-dimethylformamide-glacial acetic acid (90:7:1) and allowed to run the full height of the plate twice to ensure good resolution of all metabolites. After drying, the plates were placed in a second mobile phase of chloroform-methanol-glacial acetic acid (9:6:1) and allowed to run to a height of 3.0 cm twice. Once dry, the plates were then sprayed with 5% 4-(4-nitrobenzyl)pyridine (NBP) in acetone/0.2 M acetate buffer pH 4.6 (8:2, v/v) twice, heated at 150°C for 10–15 min and then dipped in 3% potassium hydroxide in methanol to develop the plates. Immediately after development, the TLC plates were scanned using a reflecting laser densitometer. With the exception of serum IPM, clean and reproducible chromatograms were obtained.

Concentrations were calculated using known standards of each metabolite and urine and serum controls. Although IPM was usually visible on the chromatograms, the recovery was poor. As a consequence, the values of the serum IPM levels were regarded as too unreliable and were disregarded. The recovery of serum IFOS, 3DC, 2DC and CX, all at 80 μ M, from the internal controls was 95%, 80%, 60% and 40% respectively. The recoveries for IFOS, 3DC, 2DC, CX and IPM, all at 80 μ M, in urine were 93%, 80%, 85%, 45% and 25% respectively. The coefficient of variation of the urinary assays was previously reported to be 3.2%, 5%, 7%, 13% and 32% respectively (Hartley et al, 1984) and for the serum assays for IFOS, 3DC, 2DC and CX were 22%, 23%, 21% and 12% respectively.

Pharmacokinetic analyses of urinary and serum concentrations

Urinary collections were made over consecutive 6-h periods. No urinary drug or metabolite could be detected in the collections

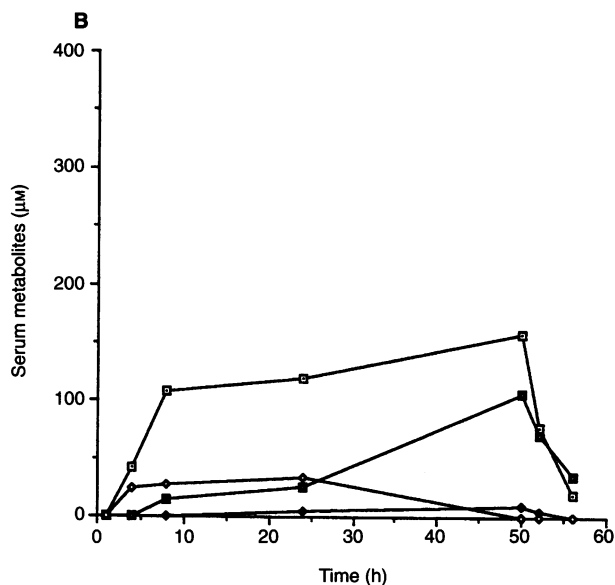
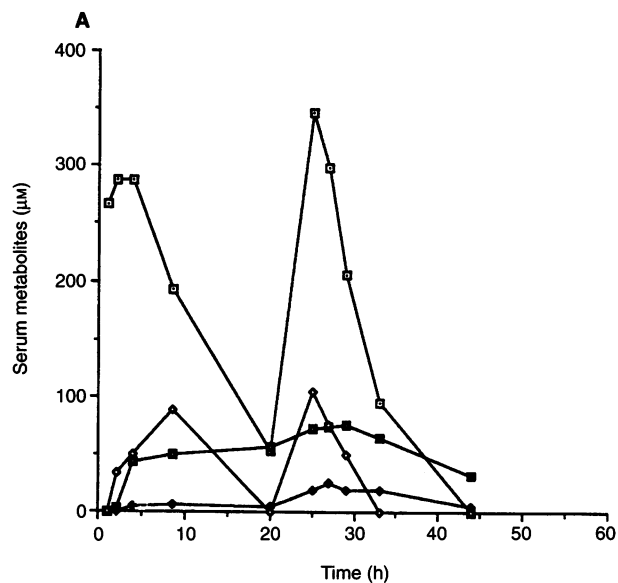


Figure 3 Serum concentration of IFOS and three metabolites in a patient under bolus (A) and continuous infusion (B) administration. \square , IFOS; \blacklozenge , ZDC; \blacksquare , 3DC; \diamond , CX

after 60 h from the start of administration, and so, for each cycle, the total urinary amounts of drug and metabolites over the first 60 h were calculated. These were then expressed as the molar fractions of the administered dose, and are referred to hereafter as a *urinary fraction* of dose.

For the serum determinations the area under the curve (AUC_∞) from time zero to infinity of the concentration vs time was assessed primarily from the experimental data points by the linear trapezoidal rule. In those cases in which the concentration of the last sample had not reached the baseline, it was necessary to estimate the area in the tail portion. This was based on extrapolation of the last few data points using an exponential decay curve. To adjust

Table 1 Mean and s.d. of peak serum concentrations (μM) of IFOS and its metabolites aggregated over all patients and treatment cycles (total of n cycles) and categorized according to mode of administration (A) or by absence/presence of co-administration of dexamethazone (B). The P values are those relating to within patient comparisons from an ANOVA (see text)

A							
	Bolus dose			Continuous infusion			P -value
	Mean	s.d.	n	Mean	s.d.	n	
IFOS	380.1	157.2	14	185.4	109.7	13	0.002
2DC	52.7	52.5	9	26.3	21.0	12	>0.05
3DC	104.9	39.9	10	92.5	97.3	12	>0.05
CX	69.1	34.2	10	56.4	55.6	10	>0.05

B							
	Dexamethazone			No dexamethazone			P -value
	Mean	s.d.	n	Mean	s.d.	n	
IFOS	268.9	136.4	15	308.2	202.4	12	>0.05
2DC	44.2	50.8	11	30.3	19.8	10	>0.05
3DC	111.0	97.9	12	82.7	32.6	10	>0.05
CX	71.3	63.9	9	55.8	23.2	11	>0.05

Table 2 Mean and s.d. of AUC (mm h) of IFOS and its metabolites aggregated over all patients and treatment cycles (total of n cycles) and categorized according to mode of administration (A) or by absence/presence of co-administration of dexamethazone (B)

A							
	Bolus dose			Continuous infusion			P -value
	Mean	s.d.	n	Mean	s.d.	n	
IFOS	6.89	2.03	14	6.96	4.40	13	
2DC	0.98	1.27	9	0.64	0.44	12	
3DC	3.14	2.01	10	2.92	2.73	12	
CX	0.91	0.55	10	1.52	2.36	10	

B							
	Dexamethazone			No dexamethazone			P -value
	Mean	s.d.	n	Mean	s.d.	n	
IFOS	7.20	4.10	15	6.57	2.09	12	
2DC	1.04	1.12	11	0.50	0.41	10	
3DC	3.33	3.00	12	2.65	1.38	10	
CX	1.57	2.51	9	0.92	0.48	11	

for minor differences in dose, all values of AUC and C_{max} , the peak concentration, were normalized to correspond to a total dose of 11.54 g, the mean value over all patients and cycles.

Statistical methods

Analyses of variance (ANOVA) were made using the SPSS statistical package (SPSS, Chicago, IL, USA). As observations were generally not available for all combinations of the factors of interest (for example patient number, bolus/infusion administration, cycle number, use/absence of dexamethazone), it was only

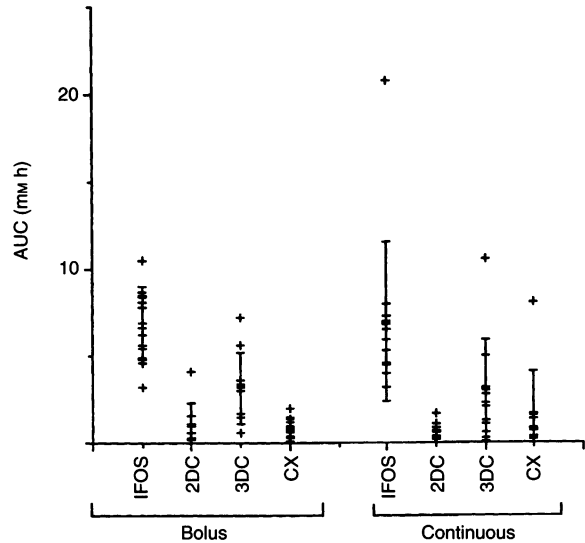


Figure 4 Scattergram with overall mean and s.e. of the area under the curve (AUC) of the serum concentration of ifosfamide and metabolites for all cycles of bolus and infusion administrations

possible to test for the main effects. In all analyses the first of these factors, patient number, was always included in the ANOVA model. With the cross-over design of the investigation, this allowed for the testing for any effects due to any of the remaining three factors on a within-patient basis. Effects were regarded as statistically significant for $P < 0.05$.

RESULTS

Eleven patients were studied over a total of 34 cycles of treatment. Of these, two patients declined venesection and one had insufficient urine collection. Seven patients were treated with IFOS over 48 h and four patients were treated with IFOS over 72 h.

Serum levels

Figure 3 shows typical measurements of serum concentration of IFOS and metabolites, measured in the infusion and the bolus treatments of a patient receiving IFOS over 48 h. The concentration profile of IFOS shows an immediate rise after the bolus administration and a gradual rise after infusional administration. There are minor differences in the profiles of the metabolites in the example in Figure 3, but these were not seen systematically in the rest of the dataset. In general, comparing the two types of administration, the serum metabolite profiles were quite similar in both shape and magnitude.

Summary values

Summary values of C_{max} for all patients and cycles under the two administration modes, and with and without DEX are listed in Table 1. The ANOVA showed no effect due to DEX but the effect of the administration mode was significant for IFOS ($F = 13.3$; d.f. 1,13; $P = 0.002$) but not for any of the three metabolites. In summary, therefore, for the peak serum concentrations of IFOS and its metabolites, only those of IFOS were significantly different (by a factor of 2.05) in the bolus dose against the infusion.

Table 3 Mean and s.d. of urinary fraction (%) of IFOS and its metabolites aggregated over all patients and treatment cycles (total of *n* cycles) and categorized according to mode of administration (A) or by absence/presence of co-administration of dexamethazone (B). The *P*-values are those relating to within patient comparisons from an ANOVA (see text)

A							
	Bolus dose			Continuous infusion			<i>P</i> -value
	Mean	s.d.	<i>n</i>	Mean	s.d.	<i>n</i>	
IFOS	21.0	9.0	16	25.2	14.5	15	>0.05
2DC	5.3	2.1	16	5.6	3.5	15	>0.05
3DC	12.4	4.9	16	12.2	6.1	15	>0.05
CX	16.1	6.8	16	13.5	8.7	15	>0.05
IPM	10.2	6.9	16	9.5	3.7	15	>0.05

B							
	Dexamethasone			No dexamethasone			<i>P</i> -value
	Mean	s.d.	<i>n</i>	Mean	s.d.	<i>n</i>	
IFOS	20.9	6.4	18	26.0	16.9	13	>0.05
2DC	5.1	2.5	18	5.9	3.2	13	>0.05
3DC	11.8	5.8	18	13.0	5.0	13	>0.05
CX	15.8	6.3	18	13.5	9.6	13	>0.05
IPM	10.5	3.8	18	9.1	7.4	13	>0.05

Table 4 Urinary fraction of dose as a percentage: grand mean and within-patient standard deviations

	Grand mean	Within-patient s.d.
IFOS	23.0	11.1
2DC	5.4	2.3
3DC	12.3	4.6
CX	14.8	7.2
IPM	9.9	4.8
Total	66.4	20.6

A preliminary analysis was made on the AUC of those treatment cycles consisting of a pair of bolus doses at a 24 h interval. Owing to the rapid decay of the concentration of IFOS after the first dose, it was possible to resolve the AUC into components contributed by each of the two doses. In ten treatment cycles in six patients, the AUC components contributed by the first and second dose had means of 4.96 mm h and 2.56 mm h respectively. A paired *t*-test showed that these were significantly different ($t = 3.33$, d.f. = 9, $P = 0.009$) and this seems to give clear evidence of induction of IFOS metabolism. It was not possible to apply this type of analysis to the AUCs of the metabolites as it was impossible to resolve the AUC into the components contributed by the two doses.

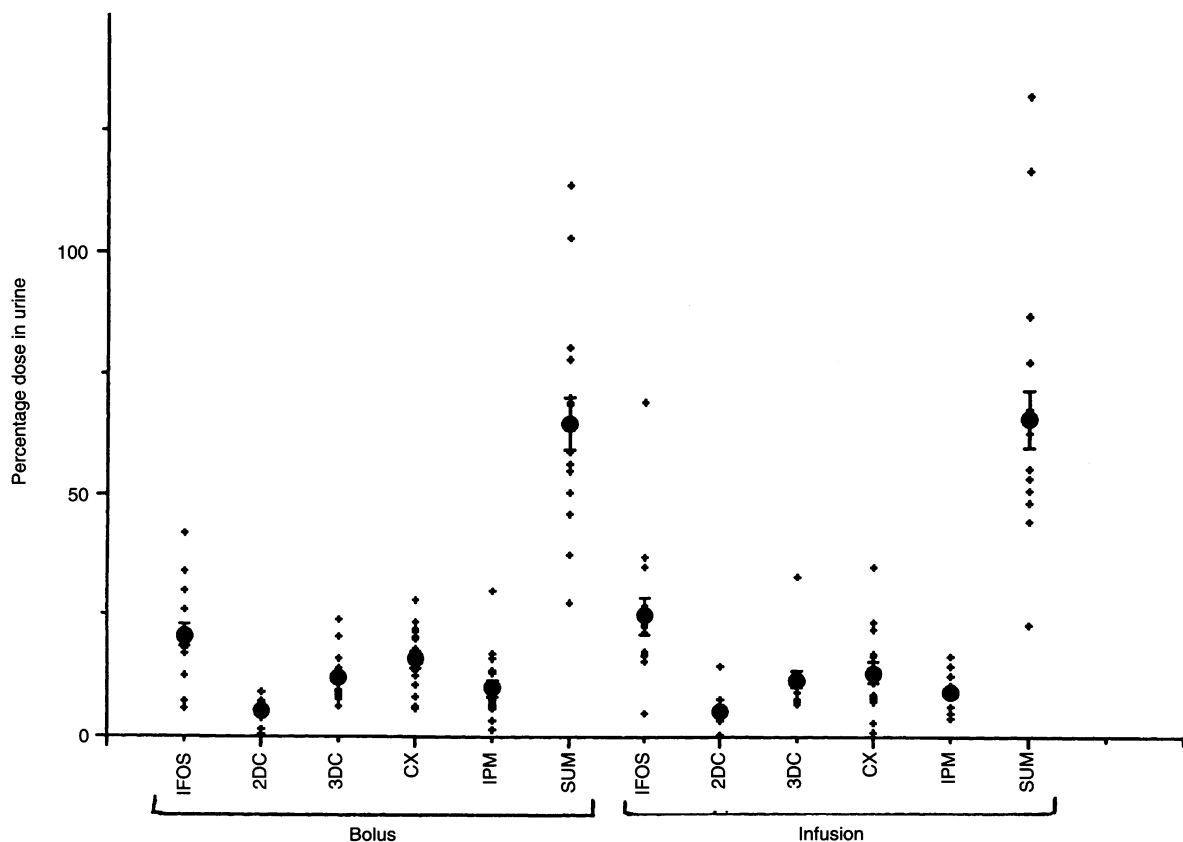


Figure 5 Scattergram with overall mean and s.e. of the urinary fraction of dose (as percentage) for ifosfamide and metabolites for all cycles of bolus and infusion administrations

Subsequent analyses were made in terms of the total AUC over the treatment cycle. Figure 4 gives a scatter plot of AUCs taken over all patients and cycles for bolus and infusion administrations, and Table 2 gives corresponding summary data. ANOVA of these with DEX, administration mode and cycle number as factors, either singly or in combination did not reveal any significant effects.

Urine analysis

For each cycle the total urinary amounts of IFOS and metabolites were expressed as molar fractions of the amount of IFOS given. Patients receiving 72 h treatments were also included in this part of the analysis, giving a total of 31 cycles in ten patients. Figure 5 gives a scatter plot of these values, for bolus and infusion administration, and Table 3 gives corresponding summary data. The ANOVA did not reveal any significant differences on the urinary fractions of parent drug or metabolite of bolus/infusion administration or of absence/presence of DEX co-administration. The effect of sequential cycles was also examined, but the analysis did not reveal significant alteration in the proportion of metabolite generated with successive cycles. Thus, it appeared unlikely that there was induction of IFOS metabolism between successive cycles.

As the patients underwent more than one cycle of treatment, it was possible to derive from the ANOVA an estimate of the within-patient variability (i.e. from cycle to cycle) of the urinary fractions. For this, all urinary results were pooled (as no significant differences due to administration of DEX had been detected) and the values of the within-patient s.d. given in Table 4. These results reveal a considerable degree of within-patient variability in urinary excretion from cycle to cycle.

DISCUSSION

Ifosfamide is commonly administered, either as a series of intravenous boluses over several days or as an infusion, yet there has been no direct pharmacokinetic comparison between the two schedules. Klein et al (1984) reported a study in which rats were treated with either a single or four bolus doses and demonstrated that the fractionated IFOS regimen resulted in a decreased LD_{50/30} and higher tumour cell kill. Phase I/II clinical studies also indicated that fractionating the dose resulted in less microscopic and macroscopic haematuria (Rodriguez et al, 1976; Klein et al, 1984). More recently, Anderson et al (1993) randomized 159 patients with small-cell lung cancer to receive 5 g m⁻² IFOS, either over 24 h or 7 days with an ambulatory pump infusion, and found the survival to be the same but the haematological toxicity to be less in the 7-day infusion arm.

Previous pharmacokinetic studies have been reviewed recently by Wagner (1994) and Kaijser et al (1994). A number of studies were performed on patients who received either infusional or bolus IFOS, but which measured only the parent drug (Creaven et al, 1974; Allen and Creaven, 1975; Nelson et al, 1976). Of the reports that have made measurements on both IFOS and its principal metabolites, Kurowski and Wagner (1993) studied 11 patients receiving five daily boluses of IFOS and measured IFOS, 2DC, 3DC, and CAA and the unstable 4 hydroxyifosfamide. Other groups (Boddy and Idle, 1992; Boddy et al, 1993; 1995a; Hartley et al, 1994) have measured both IFOS and its principal metabolites in patients receiving infusional IFOS. In general, these studies have

shown that there is induction of IFOS metabolism after approximately 3 days of treatment. With repeated daily bolus doses over 5 days, Lind et al (1989) reported a decrease in half-life that forms a mean of 6.2 h on the first day to 3.8 h on the fifth day and that this decrease was almost entirely explained by an increase in clearance.

In the example shown (Figure 3) of the profiles of each metabolite, there are some minor differences between that 1 h bolus and infusion administrations. However, over the whole dataset little systematic difference could be discerned, and cross-over comparison between 1 h bolus and infusional IFOS did not reveal a significant difference in the AUC. This is compatible with the cross-over study in 17 children reported by Boddy et al (1995b). Any alteration in the relative amounts of metabolites produced may be of therapeutic advantage. Less dechloroethylation would result in a reduction of 2DC, 3DC and CAA and, possibly, less toxicity. From our data, it appears that with bolus administration the metabolites are sufficiently slow to appear in the serum their concentration profiles are essentially no different from that with infusion administration. Thus, any differences in therapeutic or toxic effect of the two administration regimes do not appear to be reflected in differences in serum pharmacokinetics of these metabolites.

In those patients receiving cycles of two bolus doses, the AUC attributable to the second dose was less than that of the first. Although this was not the primary purpose of this investigation and the result was based on small numbers, it offers some evidence of induction and it parallels the findings of Lind et al (1989) referred to above.

In the second part of the study, we examined the effect of DEX on the metabolism of IFOS. Weber and Waxman (1993) showed that, in rats, phenobarbital induced cytochrome P450 subfamily 2B1 for the metabolism of both IFOS and cyclophosphamide. They also reported that DEX induced cytochrome P450 3A to increase the metabolism of IFOS sixfold (but not that of cyclophosphamide) and suggested the co-administration of DEX clinically may enhance response. Walker et al (1994) have suggested that the same enzyme is responsible for both IFOS activation and dechloroethylation in humans. In our study, patients were crossed over from no DEX to DEX 4 mg t.d.s. for 3 days, the latter being chosen as that in routine clinical use in conjunction with antiemetics. No difference in any of the pharmacokinetic parameters could be detected and so, at these dose levels of DEX, changes in the measured IFOS metabolites do not appear important. This is of clinical interest as DEX is widely used as an antiemetic for cytotoxic chemotherapy. For cyclophosphamide Yule et al (1995) reported an association of DEX pretreatment in children with the presence of serum ketocyclophosphamide. In our patients the levels of ketoifosfamide were barely detectable by the TLC technique and we were unable to confirm or deny any such similar association.

Several previous reports have drawn attention to the wide variability in IFOS handling between patients, and this is the case in our patients. However, it is apparent from our results (Table 4) that there is also a large degree of within-patient variability, i.e. from cycle to cycle. The total fraction of dose recovered in urine, for example, has a within-patient s.d. that corresponds to a coefficient of variation of 31%. Only a small part of this variability can be attributed to uncertainties in the experimental technique. The coefficient of variation of this assay has been reported previously by this group and for IFOS was found to be 4% (Hartley et al, 1994). Boddy et al (1996) recently reported a high degree of within-patient variability in ifosfamide pharmacokinetics in children. In

11 patients the within-patient variation from cycle to cycle in serum AUC of drug was twofold and that of metabolites was up to tenfold. The large variability from cycle to cycle may mean that differences due to saturation or induction, stemming from mode of administration, or of the co-administration of DEX, may not be revealed, even in cross-over studies such as that reported here. Indeed, it could be argued that any such differences would be relatively unimportant clinically compared with the inherent variability of drug handling within a patient.

We conclude that, against this pattern of variability in drug handling in patients from cycle to cycle, we can detect no consistent significant differences in the serum pharmacokinetics, nor the urinary excretion, of IFOS and its metabolites, when under bolus as against infusion administration. From the pattern of serum pharmacokinetics and urinary excretion in adults, we can find no reason to prefer one regimen over the other in the clinical use of the drug. This is broadly in line with the conclusions of Boddy et al (1995b) for children.

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