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ORIGINAL ARTICLE

Alan V. Boddy · S. Murray Yule · Ruth Wyllie
Lisa Price · Andrew D.J. Pearson · Jeffrey R. Idle

Intrasubject variation in children of ifosfamide pharmacokinetics and metabolism during repeated administration

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Abstract The aim of this study was to investigate intrasubject variability in ifosfamide (IFO) pharmacokinetics and metabolism which may influence clinical effect, since the pharmacology of this drug is dependent on metabolism. A group of 11 patients (ages 1–16 years) were studied on at least two occasions. IFO, 9 gm^{-2}, was administered as a continuous infusion over 72 h. Plasma and urine samples were collected and concentrations of IFO and its metabolites were determined. Comparisons were made between courses in the same subject, allowing for differences in age and prior IFO treatment. There was a wide variation in drug (twofold) and metabolite (up to tenfold) AUCs between courses in the same patient. Although some patients did show an increase in clearance between courses (up to threefold), there was no significant consistent change in overall pharmacokinetics among the different courses studied in the same patient. There was a significant decrease (up to 63%) in the AUC of the inactive metabolite 3-dechloroethylifosfamide (3-DCI) in later courses compared with the first course studied ($P = 0.032$, paired t-test). This was matched by an increase in the AUC of the total dechloroethylated metabolites with course ($P = 0.015$, paired t-test). None of the other metabolites measured showed any consistent change in plasma or urine levels between courses. Overall, the AUC of parent drug correlated with age ($r^2 = 0.86$, $P = 0.011$), and postinfusion half-life correlated with plasma bilirubin ($r^2 = 0.89$, $P = 0.007$). This study demonstrated large and seemingly unpredictable intrasubject variability in IFO pharmacokinetics and metabolism during repeated administrations. Investigations relating the clinical effects of IFO to pharmacokinetics and metabolism must take this variation into account.

Key words Ifosfamide · Pharmacokinetics · Metabolism · Variability · Pediatrics

Introduction

Ifosfamide (IFO) acts as an alkylating antitumor agent and is used in the treatment of a number of malignancies. Different protocols for the administration of this drug use a large range of doses, modes of administration, duration of treatment and combination with other drugs [20, 23]. Many therapeutic regimens for the treatment of childhood cancers require IFO to be administered as multiple courses, regularly spaced over a period of several months [23]. Oxazaphosphorines such as IFO are prodrugs which require metabolic activation to exert their antitumor effect. Specifically, activation of IFO has been shown to be dependent on an initial 4-hydroxylation reaction followed by a sequence of spontaneous reactions resulting in the liberation of the alkylating species isophosphoramide mustard (IPM) [24]. Oxidation of an intermediate in this reaction by an aldehyde dehydrogenase enzyme produces the inactive carboxy metabolite (CX). The initial 4-hydroxylation reaction has recently been shown to be mediated by the cytochrome P450 enzyme CYP3A4 [6, 27], with a possible contribution from CYP2B6 [6]. Another P450-mediated route of metabolism is oxidative dechloroethylation of IFO, which results in the formation of the inactive metabolites 3-dechloroethylifosmide (3-DCI) and 2-DCI and

A.V. Boddy (✉)[1] · J.R. Idle
Department of Pharmacological Sciences, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

S.M. Yule · R. Wyllie · L. Price · A.D.J. Pearson
Department of Child Health, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, UK

Present address:
[1]Cancer Research Unit, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, UK

Table 1 Patient and study details. Concomitant chemotherapy: *E* etoposide 200 mg/m^2 as a 2–4 h infusion on days 1, 2 and 3 of IFO treatment, *Dox* doxorubicin 20 mg/m^2 as a 4–6-h infusion on days 1, 2 and 3 of IFO treatment, *V* vincristine 1.5 mg/m^2 as a bolus on day 1 of IFO treatment, *A* actinomycin D 1.5 mg/m^2 as a bolus on day 1 of IFO treatment, *O* ondansetron, *Cot* cotrimoxazole, *D* dexamethasone, *F* frusemide, *Mic* miconazole oral gel *P* paracetamol, *Im* imipenem. *Tc* Teicoplanin, *N* nystatin oral, *L* lactulose, *M* metoclopramide *Cod* codeine, *Nor* norethisterone, 1-*a* 1-alphatocopherol, *SK* SandoK, *Bis* bisacodyl, *Flu* flucloxacillin, *Car* carbamazepine

Patient	Sex	BSA (m^2)	Weight (kg)	GFR[a] (ml min^{-1})	Age (years)	Course	Changes in medication
1	M	0.6	14.8		3.8	2	E, A, Cot, O
		0.6	13.8	121	4.6	13	E, A, Cot, Mic, O, P, Im, Tc
2	M	1.9	74.4	Not done	16.0	2	O, D, N, V, A
		1.9	67.5		16.1	4	O, D, N, V, A, L, M
3	F	1.6	56.5	Not done	12.0	1	O, D, V, A, Cot, Nor, N, Cod, F. P
		1.6	57.4		12.1	3	O, D, V, A, Cot, Nor
4	M	0.42	9.1		1.0	1	E, Cot, A
		0.45	9.9	209	1.5	9	E, Cot, N, O, P, 1-a
5	M	0.9	24.2	184	6.8	5	E, Cot, O, vancomycin
		0.9	23.7	122	7.4	15	E, Cot, O
6	F	0.8	21.3	117	6.4	2	E, D, O, Cot, P
		0.9	24.2	92	6.8	8	E, D, O, Cot
7	M	1.0	27.2	104	10.9	3	V, Cot, O, Dox, P
		1.0	29.7	96	11.7	16	V, Cot, O, A
8	M	0.5	11.4	115	1.9	2	E, Cot, O, L
		0.5	11.7	158	2.3	9	E, Cot, O, L
		0.5	11.8	101	2.7	15	E, Cot, O, M, acyclovir, SK
9	M	0.8	20.4	176	5.0	2	E, Cot, O
		0.8	23.0	132	5.4	9	E, Cot, O, D, Bis, F
		0.85	24.7	143	5.8	15	E, Cot, O, D, Bis
10	M	1.1	30.7	128	12.5	3	E, Cot, O, Flu, P
		1.1	30.7		12.8	9	E, Cot, O, D, P, F, morphine
		1.1	35.7	67	13.1	15	E, Cot, O, D, P
11	M	0.28	8.3	126	0.75	3	E, Cot, P, Cod, F, O, Car
		0.3	9.6	100	1.0	9	E, Cot, P, Cod, F, O, Car

[a]Corrected to 1.73 m^2

the toxic species chloroacetaldehyde. This reaction is also mediated by CYP3A4 [27].

The clinical effects of IFO are thought to be dependent on the relative contributions of these metabolic pathways in vivo [24]. Thus, variation in metabolism amongst different individuals and intrasubject variation in metabolism following repeated administration could give rise to variation in both therapeutic and toxic effects [24]. The oxazaphosphorines are known to induce their own metabolism [3, 13, 21] and to interact with a number of drug-metabolizing enzymes [16] so that long-term changes in drug metabolism are likely. Another potential source of intrasubject variability is age-dependent change in drug-metabolizing enzymes in pediatric patients.

The metabolism of cyclophosphamide and IFO has been intensively studied in adults [9, 13, 17], but relatively few studies have been performed in children [2, 4, 5]. In a previous study we determined the interindividual variability in IFO metabolism in a group of pediatric patients [2]. In the present study we investigated intrasubject variation in IFO metabolism by determining parent drug and metabolites in plasma and urine on at least two occasions during repeated administration. The aim was to determine whether patients could be characterised with regard to IFO metabolism by studying a single course, or if intrasubject variation was so large and unpredictable as to make such an approach inappropriate.

Materials and methods

IFO and its metabolites were obtained from Asta Medica, Frankfurt, Germany. Cyclophosphamide and 4-nitrobenzylpyridine (NBP) were purchased from Sigma, Poole, UK. All other reagents were of appropriate analytical grade.

A group of 11 patients two females were being treated for Ewings tumor (three patients), rhabdomyosarcoma (five patients), primitive neuroectodermal tumor, epithelial sarcoma or malignant schwannoma (one patient each) with up to 16 courses of IFO at 3-week intervals. Ages ranged from 1 year to 16 years. Patients received IFO as a continuous infusion (Gemini PC-2 volumetric infusion pump, Imed, San Diego, Calif.) at a dose of 3 gm^{-2} each day for 3 days. This was accompanied by 3 l m^{-2} of hydration each day and Mesna (3 g m^{-2} per day), infused during and for 12 h after IFO administration. Other chemotherapy and concurrent drug administration are listed in Table 1. No attempt was made to limit other drug treatment during the study, as this was dictated by clinical criteria. Other chemotherapy (etoposide, actinomycin-D, vincristine and doxorubicin) was the same in all courses, except for patients 4 and 7 who received actinomycin-D or doxorubicin on only one course. Most other treatment was consistent between courses and

comprised analgesia (paracetamol or codeine), antibiotics (mostly prophylactic co-trimoxazole), antiemetics (dexamethasone, metaclopramide and ondansetron) topical antifungal agents (nystatin, miconazole), frusemide or minor gastrointestinal or dietary treatments. One patient was on long-term carbamazepine treatment (20 mg twice daily) which did not alter during the study. The influence of other drug treatment on the pharmacokinetics and metabolism of IFO will be discussed below. Patients' clinical status, renal function (indicated by plasma creatinine or glomerular filtration rate (GFR) by ^{51}Cr-EDTA), liver function (alanine transaminase (ALT), bilirubin and albumin) were measured throughout the treatment period. Hematological and other toxicities were recorded throughout treatment, but these data are not considered here due to the different treatments received and variable clinical status of the patients. Renal toxicity will be considered in a separate report. The study was approved by the Joint Ethical Committee of the University of Newcastle upon Tyne and Newcastle Health Authority and that of the Royal Marsden Hospital, London. Written, informed consent was obtained from parents or guardians as appropriate. The initial course studied for nine of the patients has been included in our previous report of inter-subject variation of IFO metabolism [2].

Eight patients were studied during two courses, up to 9 months apart, with three patients studied on three occasions. Blood samples (3–5 ml depending on the size of the child) were collected immediately before, at 3, 6, 12, 18, 24, 36, 48 and 60 h after the start of the infusion, at the end of the infusion and at 1, 2, 4, 6, 12, 18 and 24 h after the end of the infusion. Blood was anticoagulated with EDTA, and plasma separated and frozen immediately at -20°C prior to analysis. Urine was collected from the older children at 6-h intervals throughout the infusion and for 24 h after. Each passage of urine was stored at 5°C until the end of the collection period. The volume of each urine collection was measured and an aliquot frozen at -20°C for subsequent analysis.

Concentrations of IFO, IPM, CX, 2-DCI, 3-DCI and 4-ketoifosfamide (KETO) were determined in urine and plasma using a quantitative thin-layer chromatography–photography densitometry technique [1]. Briefly, urine (1 ml) and plasma (0.75 ml) samples, with 50 µl internal standard (cyclophosphamide 500 µg ml^{-1} in methanol), were extracted and applied to silica gel TLC plates (E. Merck, Darmstadt, Germany). After chromatography and visualization of the alkylating species, the plates were photographed. The negative was enlarged to the exact size of the original plate and the photographs of the plates were scanned. The peak areas for IFO and metabolites were divided by the area under the internal standard (cyclophosphamide) peak and the peak area ratio used for calibration. Each plate contained samples and at least six tracks derived from spiked urine or plasma containing known concentrations of authentic standards (2–50 µg ml^{-1}). Calibration curves were obtained for IFO and each of the metabolites and used to determine the concentrations in patient urine and plasma samples. This assay had a within-plate coefficient of variation ranging from 5.9 to 10.2% for the different species measured, with interplate coefficients of variation of 7.4 to 15.7%.

A noncompartmental approach was used to estimate clearance (Cl) from concentrations of IFO in plasma. A monoexponential equation was fitted to the post-infusion data to estimate half-life ($t_{1/2}$) and volume of distribution (V_β) for each subject. Exposure of each patient to IFO and each of its metabolites was expressed as the area under the plasma concentration–time curve (AUC) for that species. Recoveries of IFO and metabolites in urine were expressed as a percentage of the administered dose. Both AUC and percentage of dose were corrected for molecular weight. Renal clearance (Cl_R) of IFO was determined from the product of Cl and the fraction of the dose recovered unchanged in the urine. The ability of IFO to induce its own metabolism was measured as the percentage change in parent drug or metabolite concentration between 24 h and the end of the infusion. Intrasubject variability was determined by comparing pharmacokinetic and metabolite variables in the different courses. Because different courses with different intervals between study were used for each patient, direct comparison within a patient may have been confounded by variation in prior treatment. Regression analysis, allowing for the prior exposure to IFO, was used to determine changes in parameters between courses studied Correlations of pharmacokinetic, metabolite and patient variables were analysed using linear regression with correction for course and study effects. Changes of greater than 20% in any pharmacokinetic or metabolite parameter were considered to be of potential clinical relevance.

Results

IFO therapy was well tolerated by all the patients studied except patient 4 whose treatment was stopped due to severe renal toxicity. Of those completing therapy, three obtained a complete remission and three a partial response. The other patients were not evaluable. Ten patients were disease free at 17–42 (median 25) months following diagnosis. One patient relapsed at 21 months and died of progressive disease. While accompanying hematological and gastrointestinal toxicity was monitored throughout treatment, no direct comparisons between different courses could be made because of differing concomitant chemotherapy and possible cumulative myelosuppression. No attempt was made to control concomitant therapy during study courses, and this resulted in some variation in the drugs received. However, in most patients there was only minor variation in therapy between courses (single dose analgesia, antiemetic or diuretic) and in others the only variation in therapy was the addition of laxatives or dietary supplements, often a single dose of paracetamol. All therapy is reported here for the sake of completeness. The other drugs administered which could potentially interact with IFO metabolism are dexamethasone and miconazole. However, the doses of dexamethasone were very low (2–4 mg three times daily for 4 to 5 days) compared with those used to achieve enzyme induction in rats (100 mg/kg on 4 consecutive days). In studies on cyclophosphamide metabolism, these antiemetic doses of dexamethasone have been shown to have no effect on pharmacokinetics [29], although more prolonged and high-dose treatment does increase cyclophosphamide Cl. Although miconazole was given as an oral gel (5 ml of 25 mg/ml four times daily), there has been at least one report of a drug interaction with this formulation of miconazole [7]. This patient did show a slight decrease in IFO Cl during the second course when miconazole was administered. Omission of this patient did not invalidate the subsequent analysis.

Plasma concentrations of IFO itself ranged from 20 to 120 µ*M* during the infusion, with concentrations of metabolites ranging from 10 (lower limit of detection) to 40 µ*M*. Intrasubject variability in the pharmacokinetic and metabolite profiles determined on different courses was observed. Clearance of parent drug was relatively unchanged (mean 26% increase compared with first course), although in patient 10 it

Table 2 Pharmacokinetic parameters of IFO following repeated administration (*Cl* plasma clearance = dose (mg)/AUC ($mg\,l^{-1}\,h$) of IFO, V_β volume of distribution, $t_{1/2}$ postinfusion hlf-life)

Patient	Cl (1) ($l\,h^{-1}\,m^{-2}$)	Cl (2) ($l\,h^{-1}\,m^{-2}$)	Cl (3) ($l\,h^{-1}\,m^{-2}$)	V_β (1) ($l\,kg^{-1}$)	V_β (2) ($l\,kg^{-1}$)	V_β (3) ($l\,kg^{-1}$)	$t_{1/2}$ (1) (h)	$t_{1/2}$ (2) (h)	$t_{1/2}$ (3) (h)
1	7.26	5.66		1.10	2.70		2.51	6.48	
2	3.97	5.19		0.59	1.31		4.05	6.24	
3	3.96	3.53		0.27	0.42		1.67	3.04	
4	7.20	7.30		0.75	1.22		1.57	2.55	
5	5.80	5.07		0.62	0.27		1.99	0.98	
6	6.43	5.23		0.55	0.61		1.59	2.17	
7	5.33	6.10		0.44	1.42		1.56	4.78	
8	4.91	7.04	9.30	0.40	0.81	0.70	1.29	1.86	1.24
9	8.17	6.87	5.79	0.74	0.89	0.40	1.60	2.59	1.39
10	3.19	6.78	9.08	0.41	0.90	0.55	2.48	2.54	1.37
11	14.0	14.0		1.61	0.62		2.36	0.92	

increased by over 100% from course 3 to course 9 and by as much again at course 15 (Table 2). Changes in $t_{1/2}$ ranged from a 60% decrease to a 200% increase compared with the first course studied. There were also changes in the AUCs of the metabolites (Fig. 1A–K) and in recovery in urine (Fig. 2), however, few of these changes were observed consistently within the study group. Also, since different courses were studied for different patients, the effect of time-dependent changes in parameters had to be considered. Thus, although $t_{1/2}$ appeared to increase in later courses, this was not significant for the group as a whole when prior exposure to IFO was considered. Concurrent drug treatment was considered as a source of intrasubject variation, but no consistent effect was observed. One patient treated with carbamazepine (patient 11) had the highest Cl (Table 2), which would be expected, as this anticonvulsant is a known inducer of CYP3A4 [18]. Levels of dechloroethylated metabolites in this patient were higher than those in the rest of the group (Fig. 1K).

With correction for differences in course studied (Table 1), there was no significant consistent change in Cl, V_β or $t_{1/2}$ between courses in the same patient (Table 2). A subgroup of four patients did show an increase in Cl following repeated therapy of between 20 and 190%, six patients varied (increase or decrease) by less than 20% and one had a decrease in Cl of 29% (patient 1, treated with miconazole oral gel). There was a significant decrease in the AUC of the 3-DCI metabolite in later courses ($P = 0.032$, paired t-test). This trend was confirmed if the AUCs of the total dechloroethylated products were considered together ($P = 0.015$, paired t-test) and there was a negative correlation of this combined AUC with the number of prior courses ($r^2 = 0.88$, $P = 0.033$). The decrease in AUC of dechloroethylated metabolites varied from 20 to 60% in six patients, four patients varied by less than 20% between courses and one patient had a 40% increase. These changes correlated inversely with changes in Cl ($r^2 = 0.95$, $P = 0.004$), indicating that some other route of elimination governs intrasubject variability in the overall Cl of IFO. There were no other significant consistent changes in the AUCs (Figure 1A–K) or urinary recoveries (Fig. 2) of IFO or its metabolites. The autoinductive effect, as determined by changes in steady-state concentrations of IFO or its dechloroethylated metabolites did not vary significantly between courses.

Taking into consideration the different courses that were studied for different individuals it was possible to consider the effect of other patient variables on the pharmacokinetics and metabolism of IFO. The AUC of IFO correlated positively with patient age ($r^2 = 0.86$, $P = 0.011$), but there was no correlation of other pharmacokinetic parameters with this patient characteristic. Half-life was positively correlated with liver function as reflected in plasma bilirubin ($r^2 = 0.89$, $P = 0.007$), but not with any other measure of liver function (ALT, albumin).

Discussion

Although the oxazaphosphorines IFO and cyclophosphamide have been used for many years, the design of dosage regimens is largely empirical and based on arbitrary dose fractionation. Little is known about how such prolonged treatment affects the metabolism of these drugs, or if changes in metabolism may be indicators of acute or chronic toxicity or of therapeutic efficacy. Previous studies have characterized the metabolism of IFO in a particular individual during a single course and so obtained some measure of the degree of interindividual variability [2, 5, 8, 17]. In the present study we attempted to characterize the relative contribution of intrasubject variation to total variability in IFO metabolism and pharmacokinetics. A recent study has indicated a high degree of intrasubject variability in cyclophosphamide pharmacokinetics [19].

IFO induces its own metabolism, resulting in complicated pharmacokinetics. The estimate of Cl obtained by noncompartmental analysis was an average over the time-course of administration and the $t_{1/2}$ determined

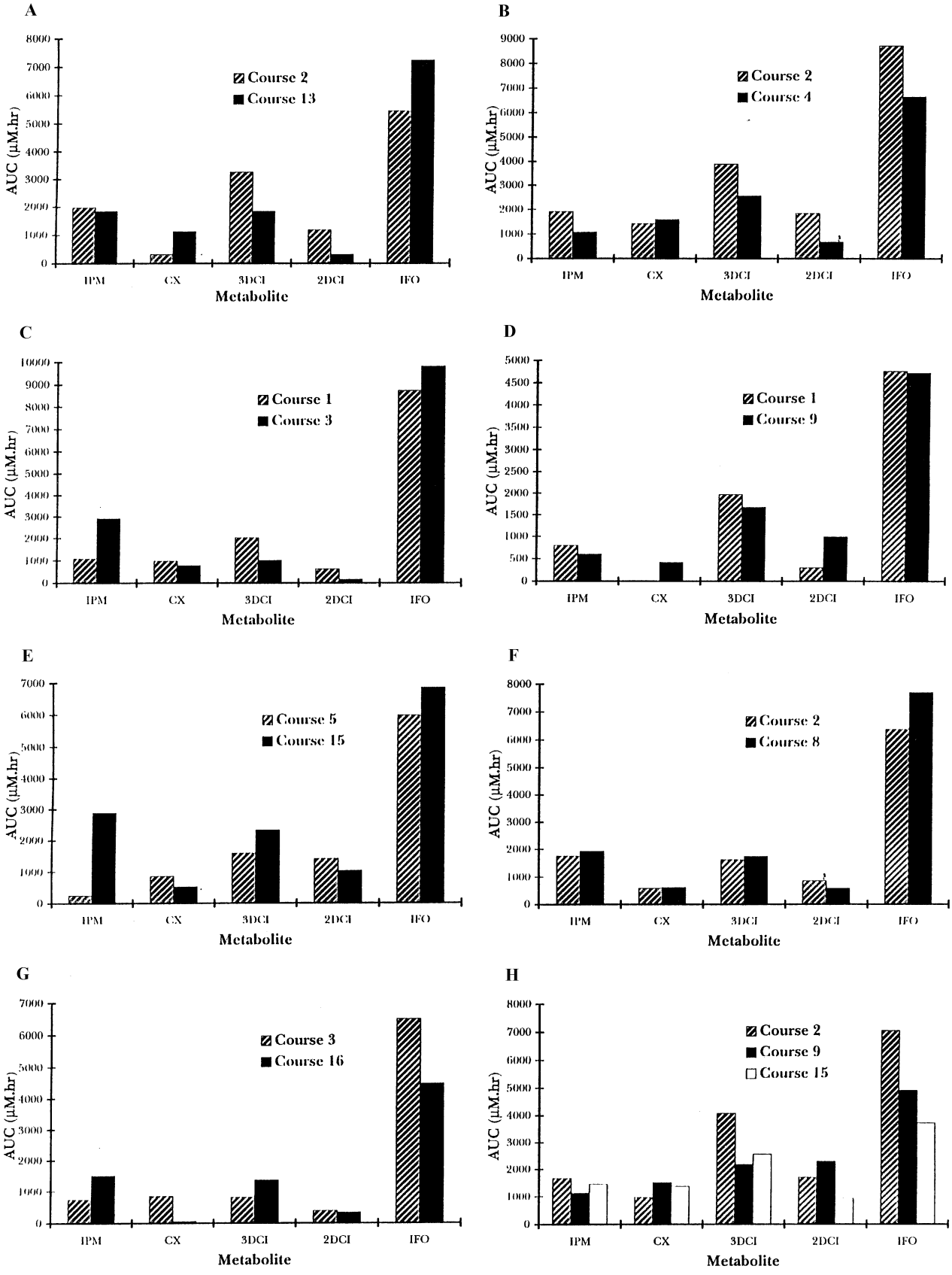

Fig. 1A–1K Comparison of IFO and metabolite AUC values determined on different courses in the same patient for 11 patients (**A** patient 1, **B** patient 2, **C** patient 3, **D** patient 4, **E**, patient 5, **F** patient 6, **G** patient 7, **H** patient 8, **I**, patient 9, **J**, patient 10, **K**, patient 11)

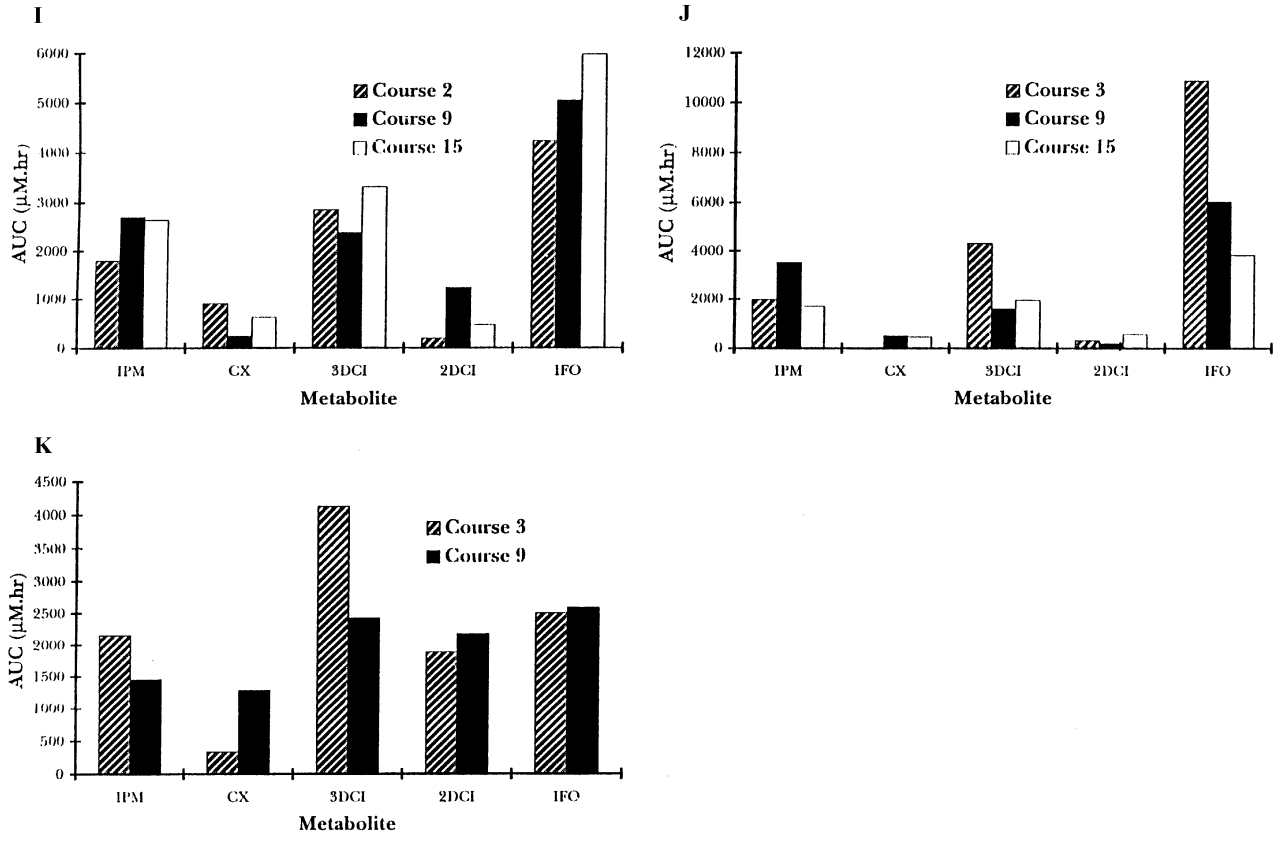

Fig. 1 (Continued).

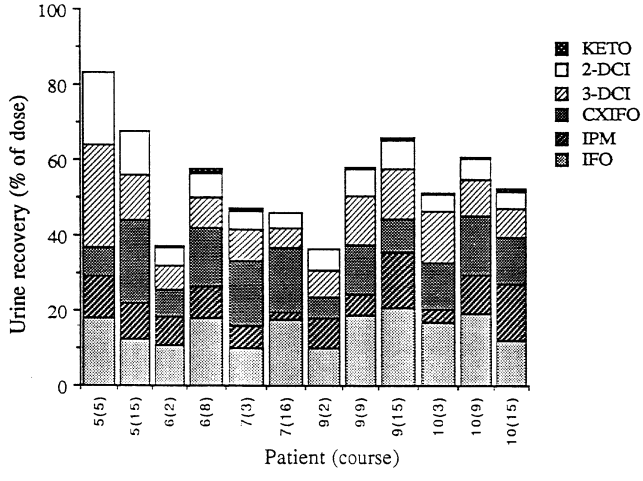

Fig. 2 Changes in urine recovery in five patients studied on different courses

at the end of the infusion reflected the maximally induced rate of elimination. Similarly, the estimate of V_β was strongly dependent on the slope of log plasma concentration at the end of infusion and so on the degree of autoinduction. This should be remembered in making comparisons between different courses in the same patient. Also in making any comparison between courses, some account must be taken of the prior exposure to IFO. Although every effort was made to study patients in a uniform fashion, i.e. on the same courses, this was not possible due to clinical and logistical constraints. Therefore, our comparison of IFO metabolism and pharmacokinetics was based on regression analysis with course as a covariable.

With these caveats, we would conclude that there was no significant, consistent intrasubject variation in the pharmacokinetics of IFO in this patient group. However, 4 of 11 patients did show an increase in Cl on repeated therapy. Although several patients did show an increase in $t_{1/2}$ (up to 200%), this did not reach statistical significance when the effect of course was included in the analysis.

Studies of IFO metabolism should ultimately yield information about the relationship between such metabolism and clinical effects. While metabolism in a single course may be related to acute toxicity, the usefulness of such studies for relating metabolism to chronic toxicity or therapeutic outcome depends on the degree of intrasubject variation. If such variation is large, a single course among many repeated courses may not be representative of the patient's overall exposure to parent drug or metabolites. From the present study it

would appear that only the total Cl of drug is at all reproducible between different courses, but even this parameter increased twofold in some patients. AUCs of metabolites varied greatly between courses, with intrasubject variations of up to tenfold and threefold for IPM and CX, respectively. Variation in AUC of dechloroethylated metabolites was less marked (− 60 to 40%), but reduction in this route of metabolism was the only consistent change in IFO metabolism with repeated doses. A study of IFO metabolism in adults also showed minor changes in urine recovery of dechloroethylated metabolites with repeated cycles of therapy [11]. The correlation of decreasing AUC of dechloroethylated metabolites with increasing Cl indicates that this increase is not due to augmented dechloroethylation. Rather it suggests that another route of elimination is increased, reducing the availability of IFO substrate for the dechloroethylation reaction. It is also possible that there is a parallel increase in the elimination of IFO and of its dechloroethylated metabolites by a common elimination pathway. 4-Hydroxylation of dechloroethyl metabolites of IFO has been reported in rats [28]. Removal of both dechloroethyl groups would result in a metabolite which would not be detected by the TLC method employed here. However, using ^{31}P-NMR, this metabolite has not been detected in the urine of patients receiving IFO [9]. In our previous study of interpatient variability in IFO metabolism, we found an increase in dechloroethylation with prior treatment [2], but this did not take into account the degree of intrapatient variability.

The correlation of $t_{1/2}$ of IFO with liver function impairment, as determined by plasma bilirubin, is an interesting observation, especially since postinfusion $t_{1/2}$ is determined mostly by the degree of autoinduction. Previous studies of drug metabolism have reported similar findings of reduced drug Cl in hyperbilirubinemic patients. Reduced Cl or prolonged $t_{1/2}$ have been associated with elevated bilirubin concentrations in pharmacokinetic studies with adriamycin [12], fluvoxamine [10], amonafide [22], and unbound etoposide [25]. Significantly, the pharmacokinetics of isradipine, a dihydropyridine substrate for CYP3A, are dependent on plasma bilirubin [14]. None of the patients in the present study was suffering from clinically relevant hyperbilirubinemia and an interaction with protein binding is unlikely for IFO which has low protein binding, but it is possible that the observed correlation of $t_{1/2}$ with bilirubin levels is merely due to their codependence on liver function. Bilirubin is cleared by glucuronidation and it has been reported that pretreatment of rats with cyclophosphamide can increase glucuronidation rates in hepatic microsomes [15]. Also, inducers of P450 enzymes have been shown to increase the expression of bilirubin glucuronyltransferases in human liver [26]. As oxazaphosphorines induce their own metabolism, this may indicate coregulation of CYP3A enzymes and glucuronyltransferase enzymes.

We would conclude that, due to the high degree of intrasubject variability, it is not possible to characterize an individual with regard to IFO metabolism by studying a single course. The source of this variability is not known and although there was some effect of pretreatment and patient age, these effects were not consistent in all patients studied. The relevance of this intrasubject variability on antitumour effect or on toxicity is yet to be determined. Studies seeking to characterize individuals with regard to IFO pharmacokinetics and metabolism, and to relate these to clinical effect, need to account for intrasubject variability.

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