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Pharmacokinetics, Metabolism and Clinical Effect of Ifosfamide in Breast Cancer Patients

A. V. Boddy, M. Proctor, D. Simmonds, M. J. Lind and J. R. Idle

Ifosfamide (IFO) at a dose of 5 g/m², was administered as a 24-h infusion to 15 patients with metastatic (12) or locally advanced (3) breast cancer (age range 33–59 years, median 46). Concurrent chemotherapy was doxorubicin (40 mg/m²) or epirubicin (60 mg/m²). Ifosfamide and its metabolites were measured in plasma and urine during and for 24 h after the infusion using a high performance thin layer chromatography (HPTLC) technique. Patients' haematological toxicity and biochemistry were monitored during treatment and patients were followed for up to 2 years after therapy. At the time of evaluation, 5 of the patients were alive, 2 of whom had not relapsed. A marked variation was observed in the pharmacokinetics and metabolism of ifosfamide in the evaluable patients. Clearance, volume of distribution and half-life of the drug were 3.48 ± 0.88 l/h/m², 0.56 ± 0.22 l/kg and 4.68 ± 2.01 h, respectively. There was no apparent correlation between these pharmacokinetic variables and patient age, weight or renal function. AUCs of the ultimate alkylating species isophosphoramide mustard (IPM) varied over 6-fold, as did those of the inactivated metabolite carboxyifosfamide (CX). AUCs of dechloroethylated metabolites varied 4-fold (3-dechloroethylifosfamide, 3-DCI) or 8-fold (2-DCI), while that of the parent compound varied only 2.5-fold. Variation in recovery of the metabolites in urine varied over an even wider range, total recovery varying from 17.5 to 81.8% of the dose administered. There was little apparent correlation between pharmacokinetic and metabolite parameters of IFO and haematological toxicity. However, there was a marked negative correlation between both progression-free interval and survival and the AUCs of the products of IFO activation (IPM and CX). In addition, the recovery of IPM in urine was higher in patients experiencing a partial response compared to those with progressive or stable disease. Recovery of dechloroethylated metabolites correlated positively with survival, if 1 poor prognosis patient was excluded. Although far from conclusive, these results give some insight into a possible mechanism of action of ifosfamide and indicate that some species other than IPM, as measured systemically, is responsible for the pharmacological effects of this drug.

Key words: ifosfamide, pharmacokinetics, metabolism, variability, clinical response
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INTRODUCTION

THE ALKYLATING agent ifosfamide (IFO) was introduced into clinical trials in 1970, but its early use was limited by severe haemorrhagic cystitis. Further research led to the development of Mesna as a safe and effective means of regional uroprotection [1]. Following this discovery, studies demonstrated activity against a wide range of tumour types, and IFO is presently included in combination chemotherapy for several tumours including small-cell lung cancer [2, 3], soft tissue sarcomas [4,5] and carcinoma of the breast [6, 7]. Adverse effects of IFO include myelosuppression, nausea and vomiting, alopecia and urotoxicity [8, 9]. Urotoxicity is minimal when Mesna is administered concurrently with IFO [9]. Encephalopathy is a serious consequence of therapy, but is relatively rare following intravenous administration [10, 11]. These side-effects are unpredictable and are often severe enough to restrict treatment. Moreover, just as host toxicity varies among individuals, some tumours are chemosensitive and curable whilst others remain resistant or recur following an initial response.

Ifosfamide itself possesses little cytotoxic effect. It is a prodrug which is metabolised *in vivo* to produce a variety of therapeutically active and potentially toxic metabolites [12, 13]. Thus, for a given individual, variation in metabolism between host and tumour tissue may result in variability in toxicity and in differences in chemosensitivity of the tumour. The initial activation reaction in the metabolism of IFO is mediated by the cytochrome P450 enzyme CYP3A4 (Figure 1) [14, 15]. Hydroxylation at the carbon-4 position of the oxazaphosphorine ring produces 4-hydroxyifosfamide, which exists in equilibrium with its tautomeric form, aldo-ifosfamide. The latter form may then either be oxidised by an aldehyde dehydrogenase enzyme (ALDH1) [16, 17] to carboxyifosfamide (CX), an inactive metabolite, or spontaneously decompose to form isophosphoramide mustard (IPM). The mustard is thought to be the primary alkylating agent [18]. Acrolein is formed as a by-product of the latter reaction and is believed to be responsible for the urotoxic effects of IFO. Up to 50% of a dose of IFO undergoes a separate oxidative *N*-dealkylation reaction, resulting in the loss of one or other of the chloroethyl side chains to produce either 2- or 3-dechloroethylifosfamide (2-DCI and 3-DCI) [19–21]. An equimolar quantity of chloroacetaldehyde is formed in both of these reactions, and this toxic metabolite has been implicated in the neurotoxicity which may accompany IFO therapy [22], and may also be associated with nephrotoxicity [22]. Other inactive metabolites include 4-ketoifosfamide (KETO) which is thought to result from oxidation of 4-hydroxyifosfamide [12].

Large interpatient differences in IFO metabolism have been reported in adults [13] and children [24] including wide variation in CX excretion. Studies on the urinary metabolites of cyclophosphamide, an isomer of IFO, indicate that certain individuals may be totally deficient in the excretion of this metabolite [25, 26]. It has been suggested that this may be the result of phenotypic variation in ALDH activity, which would be expected to apply equally to IFO metabolism, assuming both oxazaphosphorines are metabolised by the same ALDH enzyme.

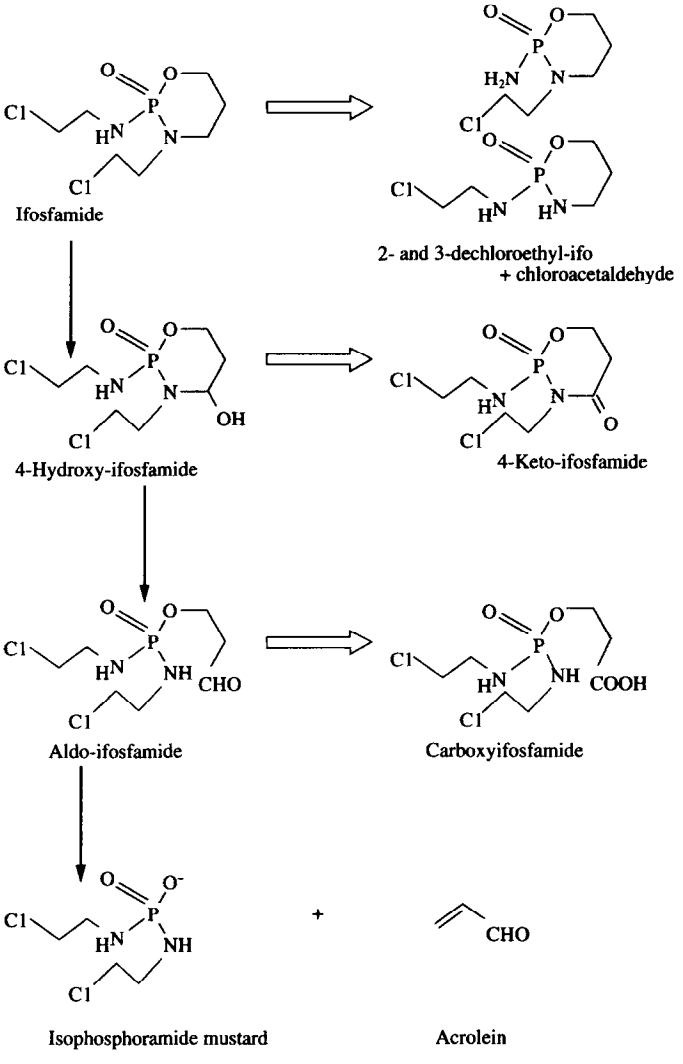

Figure 1. Activating (filled arrows) and inactivating (outline arrows) pathways of ifosfamide metabolism.

Patients with low levels of ALDH activity may deactivate IFO less efficiently and may be at increased risk of toxicity, possibly accompanied by apparently greater tumour sensitivity. Conversely, tumour inactivation of IFO by ALDH may result in chemoresistance. Similarly, the activation of cyclophosphamide and ifosfamide may be subject to great inter-individual variability [26, 27]. The human cytochrome P450 enzymes responsible for the initial hydroxylation reaction and inactivating dechloroethylation reactions have recently been identified [15]. Environmental and genetic factors may lead to large inter-individual differences in activities of these enzymes, which would in turn have an influence on the balance of activation and inactivation of a dose of IFO in an individual. To investigate the variation in metabolism of IFO and its relation to clinical outcome, we have studied the pharmacokinetics and metabolism following administration of a continuous infusion over 24 h in the treatment of breast cancer.

PATIENTS AND METHODS

Chemicals

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

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Patients and drug administration

15 patients with either metastatic or locally advanced carcinoma of the breast were being treated with IFO combined with either doxorubicin (50 mg/m^2) or epirubicin (60 mg/m^2). Patient characteristics were median 46 years (range 33–59); median body surface area 1.6 m^2 (range 1.5–1.8 m^2); median weight 62 kg (55–73); median creatinine clearance 95 ml/min (range 33–125). Patients received up to four doses of 5 g/m^2 of ifosfamide every 3 weeks as a continuous infusion over 24 h. This was accompanied by hydration (3 l) and a total of 5 g/m^2 of Mesna. Other concurrent therapy, including anti-emetics and haematological growth factors, is listed in Table 1. Patients' clinical status, renal function (creatinine clearance estimated from plasma creatinine), liver function (alanine transaminase (ALT), bilirubin and albumin) and haematological toxicity were monitored throughout the treatment period. The study was approved by the Ethical Committees of the Medical School of the University and of the General Hospital, Newcastle upon Tyne.

Pharmacokinetic sampling and analysis

During one course of ifosfamide, blood samples were collected immediately before, at 3, 6, 12, 18 and 24 h after the start of the infusion and at 1, 2, 4, 6, 12, 18 and 24 h after the end of the infusion. Blood was anti-coagulated with EDTA, and plasma separated and frozen immediately at −20°C prior to analysis. Urine was collected at 6-h intervals throughout the infusion and for 24 h after. The volume of each urine sample was measured and an aliquot frozen at −20°C for subsequent analysis.

Concentrations of IFO, IPM, CX, 2- and 3-DCI and KETO were determined in urine and plasma using a quantitative thin-layer chromatography-photography densitometry technique [28]. Briefly, 1 ml of each urine sample and 50 μl of internal standard (cyclophosphamide 500 μg/ml in methanol) were applied to an XAD-2 Spe-Ed solid phase extraction cartridge (500 mg/3 ml, Laboratory Impex Ltd., Teddington, U.K.). The cartridge was washed with 3 ml of water and dried. Drug and metabolites were eluted with methanol which was evaporated to dryness.

Plasma (750 μl) was added to 750 μl of cold acetonitrile and 50 μl of internal standard. After vortex mixing and centrifugation, the clear supernatant was evaporated to dryness.

Dry residues from the above procedures were reconstituted in methanol (70 μl) and 30 μl were applied to silica gel TLC plates (E. Merck, Darmstadt, Germany), which had been pre-eluted with methanol and dried at 150°C for 10 min. An automated Linomat IV TLC sample applicator was used (CAMAG, Berne, Switzerland). Chromatography was performed in glass TLC tanks, saturated with solvent. The mobile phase was dichloromethane-dimethylformamide-glacial acetic acid (90:8:1 v/v/v), which was allowed to rise to a height of at least 9.5 cm. After drying, the plates were run again in a second mobile phase of chloroform-methanol-glacial acetic acid (90:60:1) to a height of 2 cm. The plates were dried again and sprayed for at least 10 s with 5% NBP in acetone-0.2M acetate buffer, pH 4.6 (8:2 v/v), dried and then resprayed for 10 s. Plates were heated in an oven at 150°C for 10 min and left to cool.

Plates were dipped in 3% methanolic potassium hydroxide to reveal the blue spots formed from alkylated NBP. The plates were photographed within 10 s of dipping because of the unstable nature of the chromophore. To ensure uniform exposure and printing, a Kodak standard grey scale was photographed with each plate. The negative was enlarged to the exact size of the original plate. Uniform exposure was ensured by comparison of the Kodak grey scale with the original. The photographs of the plates were scanned with a CAMAG Scanner II densitometer using the program CATS3 (CAMAG) to integrate the areas under the chromatogram peaks. 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

Table 1. Patient and study details

Patient	Diagnosis	Concomitant medication	Course studied
1	M	Dox, Frus, Cpz, Ond.	3
2	M	Epi, GCSF, Dom, Dex, Lor.	1
3	LA	Epi, GCSF, Ond, Dom, Dex, Dicl.	2
4	M	Epi, GCSF, Desm, Dom, Dex, Met, Tem, CoP	1
5	M	Epi, GCSF, Dom, Dex.	1
6	M	Dox, MST, Ran, Pir, Dex	1
7	M	Epi, GCSF, Dom, Tem, Ond, DHC, Ibu, Lac, CoD	2
8	LA	Epi, GCSF, Dex, Dom, Ran, Met, CoC, Lac, Ond	3
9	M	Epi, GCSF, Nap, Tfp, Dom, Dex, HC.	1
10	LA	Epi, GCSF, Dom, Dex, Ond, Par.	2
11	M	Epi, GCSF, CoC, Tem, Par, Mor, Dom, Lor, Dex, Ran.	1
12	M	MST, Dom, Dex, Tem, CoP.	1
13	M	Epi, GCSF, CoP, Par, Dia, Pcz, Ond.	1
14	M	Epi, GCSF, Dom, Dex, Ond, Cpz.	1
15	M	Dox, Frus.	1

M, Metastatic disease; LA locally-advanced disease; Dox, doxorubicin; Epi, epirubicin; Frus, frusemide; Pir, piroxicam; CoC, cocodamol; CoD, codanthrusate; CoP, coproxamol; Cpz, chlorpromazine; Desm, desmopressin; Dex, dexamethasone; DHC, dihydrocodeine; Dicl, diclofenac; Dom, domperidone; GCSF, granulocyte colony-stimulating factor; HC, hydrocortisone; Ibu, ibuprofen; Lac, lactulose; Lor, lorazepam; Met, metoclopramide; Mor, morphine sulphate; MST, morphine sulphate (slow release); Nap, naproxen; Ond, ondansetron; Par, paracetamol; Pcz, prochlorperazine; Ran, ranitidine; Tem, temazepam; Tfp, trifluoperazine.

least six tracks derived from spiked urine or plasma containing known concentrations of authentic standards (2–50 μg/ml). Calibration curves were obtained for IFO and each of the metabolites and used to determine the concentrations in patient urine and plasma samples.

Non-compartmental methods were used to calculate clearance (Cl), volume of distribution (V_{dss}) and half-life ($t_{1/2}$) 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 % 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.

Statistics

Comparisons among patient groups were made using the Mann–Whitney U test. Correlations of pharmacokinetic, metabolite and patient variables were analysed using normal linear regression or Spearman's rank correlation where appropriate. Where appropriate, logarithmic transformations of the data were performed prior to analysis.

RESULTS

The treatment with a combination of IFO and an anthracycline was generally well-tolerated, nausea and vomiting being countered by anti-emetics. Administration of granulocyte colony-stimulating factor (G-CSF) was necessary in 11 patients to ameliorate haematological toxicity. A partial response was seen in 10 patients, 3 had progressive disease and 2 stable disease. At the time of evaluation, 13 patients had relapsed and only 5 were still surviving (as MS).

Pharmacokinetic parameters were determined in 14 patients (plasma samples were not available for patient 15). Clearance was 3.48 ± 0.88 l/h/m², V_{dss} 0.56 ± 0.22 l/kg, half-life 4.68 ± 2.01 h and Cl_R 0.41 ± 0.18 l/h/m². Clearance is corrected for surface area and V_{dss} has been corrected for weight to allow for comparison with other studies. A representative plasma profile of parent drug and metabolites is shown in Figure 2a, with the corresponding time course of recovery in urine in Figure 2b. Apparent steady-state concentrations of IFO in plasma were achieved after 8–12 h of infusion, but in some patients this was followed by a decline in plasma concentration towards the end of the infusion. Plasma concentrations of dechloroethylated metabolites continued to increase during and after the infusion, despite constant or declining concentrations of parent drug. At the end of the 24-h post-infusion collection period, the concentration of IFO was below the limits of detection of the assay, but measurable quantities of dechloroethylated metabolites and IPM were still present. Similarly in urine, parent drug excretion was almost complete after 48 h from the start of infusion, but excretion of other metabolites did not decline in parallel.

With the caveat that only the 48-h collection period is included, plasma AUCs and urine recoveries of parent drug and metabolites are given in Table 2. For the same dose, based on patient surface area, IFO AUC varied over a 2-fold range. Inter-individual variation in the AUCs of metabolites was greater (4- to 9-fold), with IPM and 3-DCI being the major metabolites observed in plasma. Similar degrees of variation were observed in urine. The recovery of parent drug, varying from 5.3 to 19.4% of the administered dose, indicated that excretion made only a

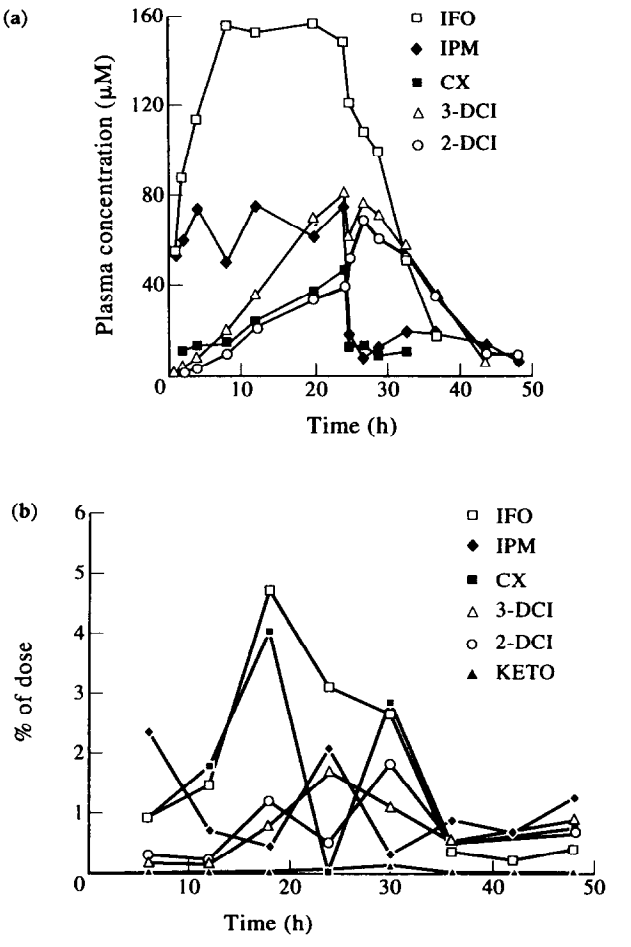

Figure 2. (a) Representative plasma profiles of parent drug and metabolites during and after a 24-h infusion of 5 g/m² ifosfamide (patient 1). (b) Time course of urinary excretion of parent drug and metabolites during and after a 24-h infusion of 5 g/m² ifosfamide (patient 1).

minor contribution to total clearance. In contrast to the situation in plasma, the carboxy metabolite was a major component of recovery in urine, accounting for up to 22.9% of the dose. Isophosphoramide mustard and 3-DCI were the other major metabolites in urine with lesser quantities of 2-DCI and only trace amounts of the KETO metabolite. Total recovery was very variable (17.5–81.8% of dose).

There was no apparent correlation between the pharmacokinetic parameters and the physical characteristics of the patients, other than a negative correlation of volume of distribution at steady-state (V_{dss}) with weight ($P = 0.048$). There was no apparent correlation between patient variables and AUCs or urine recoveries of drug or metabolites, other than that recovery of the carboxy metabolite (CX) was directly related to creatinine clearance ($P = 0.001$). There were strong positive correlations among the AUCs and recoveries of parent drug and the individual metabolites. The AUCs of the two dechloroethylated metabolites were strongly correlated ($P = 0.001$) and these two were correlated with AUCs of the CX metabolite ($P = 0.018$) and the parent drug ($P = 0.013$). The recoveries of the dechloroethylated metabolites were strongly correlated ($P < 0.001$) and the combination of these two was strongly correlated with the recoveries of all other metabolites ($P = 0.001$ (CX), 0.006 (IFO) and 0.034 (KETO)) except IPM alone, but including the recoveries of IPM and CX combined ($P = 0.002$). There were

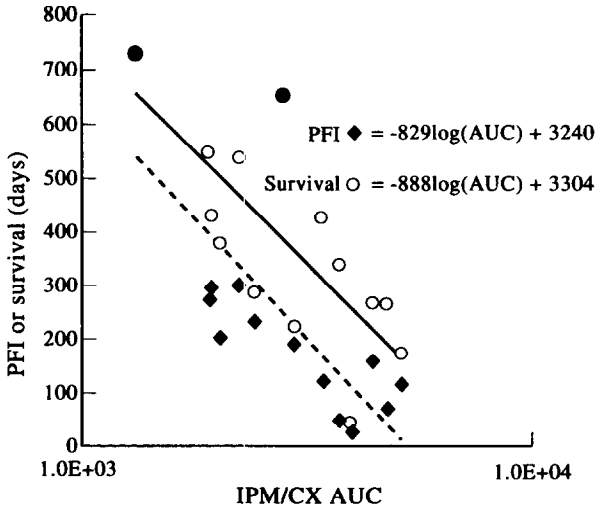

Figure 3. Inverse correlation of progression-free interval (PFI) and survival from time of diagnosis with log of the combined AUCs of IPM and CX. Two patients were alive and progression-free at the time of assessment (filled circles).

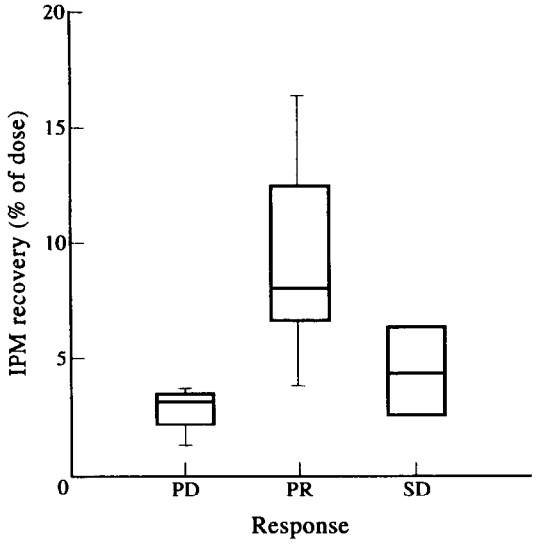

Figure 4. Recovery of IPM in urine from patients showing partial response (PR), stable disease (SD) or progressive disease (PD). Box plot shows median, interquartile range and absolute range.

no correlations between plasma AUC and urine recovery for individual metabolites. However, if patient 7 was excluded, there was a strong negative correlation of recovery of IPM with both IPM AUC (P = 0.021) and combined AUC of IPM and CX (P = 0.003).

With regard to the clinical outcome, there was little association of markers of haematological toxicity to pharmacokinetic parameters, although there was a positive correlation of Cl, corrected for body surface area, with nadirs of both granulocytes and total white cells (P = 0.006 and 0.005, respectively) and a strong negative correlation of decrease in platelets with V_{dss}, whether corrected for weight or not (P = 0.004). There was no correlation of pharmacokinetic parameters with progression-free interval (PFI) or survival (from time of diagnosis). There were negative correlations of both PFI (P = 0.007) and survival (P = 0.003) and AUC of IPM. Similar negative correlations were seen with the combined AUCs of IPM and CX (P = 0.018 and 0.004). These correlations were performed on log transformed data (Figure 3). In contrast, recovery of IPM in urine was significantly higher in those experiencing a partial response, compared to those with progressive or stable disease (P = 0.028) (Figure 4). The AUC of dechloroethylated metabolites was higher in survivors than in patients who were dead at the time of evaluation (P = 0.014) and there was a positive correlation of the recovery of these metabolites with survival greater than 100 days (P = 0.004).

DISCUSSION

The dependence of oxazaphosphorine antitumour effect and toxicity on metabolism has led to intense interest in how such metabolism influences the efficacy and toxicity of these drugs and the phenomenon of tumour resistance [12, 29–31]. Host metabolism is probably the most important determinant of tumour response, but metabolism by the tumour itself may be important in some instances. Variation in the initial activation of ifosfamide and cyclophosphamide may underlie inter-individual variation in tumour response [12]. The neurotoxicity which sometimes accompanies administration of IFO has been associated with the generation of choroacetaldehyde, a by-product of dechloroethylation [22]. This route of metabolism is relatively minor for cyclophosphamide which does not exhibit the same toxicity. Increased ALDH activity is associated with resistance to oxazaphosphorines in tumour cell lines [29, 31] and inhibition of this enzyme restores sensitivity [32–34]. Similarly, the selective tissue toxicity of oxazaphosphorines has been associated with distribution of ALDH isoenzymes [30]. The clinical implications of inter-individual variation in the metabolism of these drugs are far from clear.

Table 2. Areas under concentration–time curves (AUC mM.h) of ifosfamide and metabolites in plasma following 24-h infusion and recoveries of drug and metabolites in urine (% of dose) based on 48-h urine collection

Patient	IFO	IPM	CX	3-DCI	2-DCI	KETO	TOTAL
Plasma AUC							
Median	5.44	2.08	0.59	2.26	0.80		
Range	3.51–8.67	0.75–4.69	0.20–1.53	1.05–4.03	1.05–4.03		
Urine recovery							
Median	14.4	6.7	7.5	7.0	4.0	0.1	43.9
Range	5.3–19.4	1.2–16.4	2.1–22.9	2.7–21.3	0.6–10.7	0.0–0.9	17.5–81.8

For abbreviations, see text.

The pharmacokinetics and metabolism of IFO in the present study indicates a wide degree of inter-individual variability. As well as complications due to multiple drug therapy, the study of the pharmacokinetics of oxazaphosphorines may be confounded by induction of their own metabolism [35–41]. However, in the present study, only 3 patients (subjects 11, 12 and 14) showed an increase in clearance during the infusion, indicated by a decrease in the apparent steady-state concentration. The pharmacokinetic parameters have been derived assuming constant clearance, since the clearance values for those patients showing a decline in steady-state concentration were not significantly higher than the rest of the group. There was some indication that metabolism to dechloroethylated metabolites was greater during the latter stages of the infusion, as previously reported in paediatric patients [24], but this could not be determined unequivocally.

Comparing the pharmacokinetic and metabolite parameters calculated in the present paper with those in the literature, it is necessary to consider the time-course of drug administration. Lind and colleagues [40] found that the half-life of IFO in adults ranged from 2.4 to 5.0 h after 3 consecutive days of 1.5 g/m^2/day administered as a short infusion. These authors saw an increase in Cl from a median of 69.2 to 94.0 ml/min during the first 3 days of a 5-day regimen, with a relatively constant median volume of distribution of 40 l. This compares to mean ± S.D. values in the present study of 4.68 ± 2.01 h, 58.0 ± 14.7 ml/min/m^2 and 0.559 ± 0.216 l/kg for half-life, clearance and volume of distribution, respectively. Converting to an average adult body size (1.73 m^2 and 70 kg), IFO clearance is slightly higher in the group of patients studied here, while volume of distribution is similar and half-life distributed over a wider range. The renal clearance of ifosfamide in the former study was 15.1 ml/min compared with 11.6 ± 5.1 ml/min.

Few other studies have examined the plasma concentrations of IFO metabolites, although some investigations have examined their excretion in urine. Boos and colleagues [20] determined the excretion of the enantiomers of IFO and its dechloroethylated metabolites in urine in a group of children. Their results are consistent with ours, 3-CDI representing the major dechloroethylated metabolite and the total recovery of unchanged drug, 2- and 3-DCI being around 30–40%. Allen and colleagues [35] reported recoveries of unchanged drug of 20–50%, rather higher than the recoveries reported here. Lind and associates [13] reported much lower recoveries of the individual metabolites, using a quantitative TLC method similar to that employed in the present study. However, modifications to the chromatographic technique have allowed us to separate and more reliably quantify both the dechloroethylated metabolites and IPM. Recently, the technique of quantitative phosphorus-31 NMR has been applied to the measurement of IFO and its metabolites in urine [42]. This technique is very selective for the metabolites, and obviously provides an authentication of the species being measured. It is, however, rather time-consuming and lacking in sensitivity. The total recovery, recovery of unchanged drug and of dechloroethylated metabolites and carboxyifosfamide are very similar to ours, but they did not detect significant quantities of the mustard metabolite in their urine samples. This may be due to instability of the mustard under the NMR conditions employed, although the authors state that stability was verified during the prolonged analysis time period (up to 24 h). It is also possible that the TLC technique employed here is insufficiently selective to distinguish IPM from other alkylating material, although it has been refined from the technique employed in a previous study of ifosfamide metabolites in urine [13], which also failed to detect significant quantities of IPM. A recent report of a GC-MS assay for IPM may allow for more sensitive and selective determination of IPM in plasma and urine [43].

The 2.5-fold variation in plasma AUC of the parent drug is not great, given the variation of age and renal function within the patient group. However, the variation in the AUCs of the metabolites was greater (up to 9-fold) and could not be directly related to the AUC of the parent drug. Since the pharmacological action of IFO depends on its metabolic activation, this variability in metabolism would be expected to make a contribution to variation in clinical outcome, including toxicity. Similar variability was seen in urine recoveries of drug and metabolites and, despite carefully monitored urine collection, up to 80% of the dose was unaccounted for. Metabolism to some species not detected by the current techniques remains a possibility, but studies using radiolabelled material or ^{31}P-NMR have failed to account for a significantly greater fraction of the administered dose [12, 21]. Other alternatives are conjugation of parent drug or reactive metabolites to Mesna or endogenous species such as glutathione, possibly followed by biliary excretion and removal in the faeces. In a study with radiolabelled cyclophosphamide, only 6.5% of the dose was recovered as total radioactivity in bile [44].

An apparent deficiency in the excretion of the carboxy metabolite of cyclophosphamide has been reported in some individuals [25, 26]. However, comparable studies with IFO have not demonstrated this unequivocally [13, 24]. In the present study, carboxyifosfamide could be detected in plasma and urine of all patients and the variability in AUC of CX (0.2–1.53 mM.h) and in urine recovery (2.1–22.9% of dose) was similar to that for the other metabolites. This again suggests that there is no evidence for a polymorphism or deficiency of carboxy metabolite formation for IFO. The discrepancy between the apparent deficiency in carboxycyclophosphamide excretion and the lack of such a phenomenon with IFO can only be explained if a different isozyme of ALDH is involved in the oxidation of each of the aldehyde intermediates. The ALDH enzyme involved in oxazaphosphorine metabolism has been identified with cyclophosphamide metabolism [17], and there have been few comparisons with the corresponding metabolism of IFO. Cross-resistance to cyclophosphamide and IFO intermediates in tumour cell-lines overexpressing cytosolic ALDH has been reported [45], but this situation is far removed from that seen *in vivo*. Other possible reasons for this difference between cyclophosphamide and IFO lie in the relative instability of the carboxy metabolite of the former drug [46] and in the limits of sensitivity of the assay. The greater contribution of the CX metabolite to total recovery in urine compared to the relatively low concentrations of this metabolite in plasma are consistent with previous observations in a paediatric study of IFO where CX could not be detected in the plasma of some patients, despite the presence of high concentrations in the urine [24].

The lack of correlation between pharmacokinetic parameters and patient weight is in contrast to previous findings of an increase in volume of distribution with total body weight [47]. Indeed, there was an inverse correlation of volume of distribution at steady-state with body weight in the present study. This may be due to the comparatively narrow range of weights covered compared to the normal and obese patient groups in the former study. The lack of correlation between urinary recoveries and renal function, except for the CX metabolite, is also surprising. In the case of the parent drug, this indicates how much its

elimination is dominated by the processes of metabolism. The correlations among the products of the different metabolic pathways indicate that they may be mediated by a common enzyme system. Recent work with human liver microsomes indicates that the same isoenzyme is involved in both 4-hydroxylation [14, 15] and *N*-dechloroethylation reactions [15]. Thus, although these reactions are competing for the same substrate, the amount of the separate products formed are directly related, presumably determined by the amount of enzyme present in each individual. The fact that the recovery of IPM in the urine correlates inversely with the AUC of this metabolite in plasma is not easy to account for, but may be due to the dependence of IPM elimination on renal clearance. Thus, the higher the renal clearance of this metabolite, the smaller is its AUC and more metabolite appears in the urine. If renal clearance is lower, however, plasma AUC will be greater, less will be excreted in urine and more may be eliminated by non-renal routes.

The correlation of Cl of parent drug with nadirs of granulocytes and total white cells indicates that a higher rate of metabolism is associated with a lesser degree of toxicity. This is the opposite of the relationship between cyclophosphamide clearance and cardiotoxicity reported by Ayash and associates [48] but is probably a function of the greater contribution of non-activating metabolism to IFO clearance compared to that of cyclophosphamide. Correlation of the decrease in platelets with volume of distribution is probably an artefact.

The data on clinical response and pharmacokinetics and metabolism of IFO should be treated with caution due to the small numbers of patients in the study and the mixing of patients with metastatic and locally active disease. Nevertheless, the observed relationships between progression-free interval, survival and AUCs and recoveries of metabolites may provide some clues as to the exact mode of action of IFO in the treatment of breast cancer. The inverse correlations of PFI and survival with the AUCs of the products of the activation reaction (IPM and CX) appear to indicate that the therapeutic effect of IFO is mediated by some species other than the mustard as measured in plasma. This provides evidence that it is the 4-hydroxy metabolite, formed either in the liver or in tumour cells by metabolic oxidation, which releases a cytotoxic species intracellularly [27, 49]. That which decomposes in the extracellular fluid (including plasma) to release IPM or is metabolised to CX is ineffective and the measurement of these end-products in plasma reflects the extent of an inactivation process. Alternatively, the higher recovery of IPM in the urine of patients who had shown a partial response compared to those with stable or progressive disease may indicate that urine recovery of IPM is a better measure of the total degree of IFO activation in an individual. Similarly, positive correlations of dechloroethylated metabolites with measures of survival may indicate the degree of activity of a drug metabolising enzyme common to both pathways.

In conclusion, we have determined the pharmacokinetics and metabolism of ifosfamide in a small group of breast cancer patients. The pharmacokinetics of the parent drug were fairly uniform in this group, with greater variability in the levels of metabolites in plasma and urine. There was an apparent link between activating and inactivating pathways of metabolism, which may indicate that they are mediated by identical or related enzyme systems. The clinical data from this small study group provide some indication of the mechanism of action of this drug, principally that the mustard metabolite, as measured in plasma, does not contribute to the therapeutic effect.

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