Fractionated ifosfamide therapy produces a time-dependent increase in ifosfamide metabolism

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1 Fifteen patients received 1.5 g m^{-2} of ifosfamide intravenously over 0.5 h every day for 5 days. Twenty-one courses of treatment were studied. Plasma was assayed for ifosfamide by gas liquid chromatography and plasma alkylating activity was measured using the nitrobenzylpyridine (NBP) reaction.

2 A pharmacokinetic analysis revealed a significant decrease in the median (range) elimination half-life of ifosfamide from 7.2 (2.8–14.2) h on day 1 to 4.6 (2.3–7.7) h on day 5 ($P < 0.001$, Wilcoxon's test) with a concomitant significant increase in the median (range) clearance from 66 (31–148) ml min^{-1} on day 1 to 115 (52–381) ml min^{-1} on day 5 ($P < 0.001$). There was no significant change in the volume of distribution on day 5 compared with day 1.

3 There was a highly significant 223% increase in the median (range) plasma nitrobenzylpyridine alkylating activity area under the curve on day 1 from 16 (0.6–105) nmol nor nitrogen mustard equivalents $\text{ml}^{-1} \text{ h}$ to 52 (13–238) nmol nor nitrogen mustard equivalents $\text{ml}^{-1} \text{ h}$ on day 5.

4 During five courses of treatment (in five patients in the group) 24 h urine samples were collected on days 1 and 5. The median (range) renal clearance of ifosfamide on day 1 was 6.8 (1.3–16.2) ml min^{-1} compared with 5.7 (1.3–15.3) ml min^{-1} on day 5. This difference was not significant. The median (range) metabolic clearance of ifosfamide in these five patients on day 1 was 78.6 (39.9–141.2) ml min^{-1} and 132.6 (54.6–149.5) ml min^{-1} on day 5. This difference in metabolic clearance did not reach statistical significance.

5 These data indicate that after 5 days fractionated intravenous ifosfamide at 1.5 g m^{-2} daily produces a time-dependent increase in ifosfamide metabolism.

Keywords ifosfamide pharmacokinetics

Introduction

Ifosfamide (an analogue of cyclophosphamide) is widely used to treat solid tumours (Brade *et al.*, 1985). The use of fractionated dosage regimes was based initially on experimental work in Yoshida ascites sarcoma of the rat (Brock, 1977) which suggested that the curative

effects of ifosfamide were manifested more than its toxic effects. Early clinical trials (Drings *et al.*, 1970) confirmed that fractionated ifosfamide was effective and further studies (Morgan *et al.*, 1982; Rodriguez *et al.*, 1976) have suggested reduced toxicity with 5 day regimes. Since

1971 fractionated ifosfamide chemotherapy has become one of the most commonly used modes of administration.

Ifosfamide does not possess inherent cytostatic activity *in vitro* and requires activation *in vivo* to achieve its therapeutic effect. This activation occurs primarily as a result of the activity of the hepatic mixed function oxidase system (Alarcon *et al.*, 1972; Allen & Creaven, 1972). The lung can also metabolise ifosfamide, but to a much smaller extent than the liver (Hill *et al.*, 1973). The metabolism of ifosfamide produces a number of reactive products which may be detected by their non-specific reaction with 4-gamma-nitro-benzylpyridine (NBP), as described by Friedman & Boger (1961). It is now apparent that in man the metabolic pathways of ifosfamide and cyclophosphamide are qualitatively similar (Colvin, 1982) but that they exhibit quantitative differences.

With the increasing use of ifosfamide in cancer chemotherapy, the relative paucity of pharmacokinetic data on the drug is surprising. Early work on fractionated dose ifosfamide (Nelson *et al.*, 1976) using 1.6–2.4 g m⁻² intravenously daily for 3 days and using radioassay for labelled ifosfamide in three patients noted a terminal drug elimination half-life of 6.8 h with a 20% reduction in this value over the 3 days of administration. Piazza and coworkers (1984) studied ifosfamide pharmacokinetics in 10 patients with lung cancer receiving 1.8 g m⁻² ifosfamide daily for 5 days and observed a 50% reduction in its elimination half-life on day 1 compared with day 5. Recent studies (Lind *et al.*, 1986a), using oral ifosfamide 2 g daily for 3 days in six patients with lung cancer, revealed an increase in ifosfamide clearance from 54 ml min⁻¹ on day 1 to 86 ml min⁻¹ on day 3.

Therefore, the available evidence suggests an increase in ifosfamide clearance with time. The aim of the study was to confirm these preliminary findings and to assess whether the change in clearance was due to a change in ifosfamide metabolism.

Methods

Patient selection and protocol

Fifteen patients with neoplastic disease who were undergoing cancer chemotherapy with ifosfamide as single agent gave their verbal consent to take part in the study. Five patients were studied on more than one occasion such that their courses of treatment were given at least 21 days apart. Prior to each study every

patient had normal bone marrow function (Hb > 11.0 g dl⁻¹, WBC > 3.5 × 10⁹ l⁻¹; platelet count > 150,000 mm⁻³) and normal renal and hepatic function as measured by standard biochemical investigations. Haematological toxicity was monitored by a nadir blood count and uroepithelial toxicity was monitored by ward dip-stick testing and WHO grading. Pregnant women and patients known to be hypersensitive to alkylating agents were excluded from the study. The physical and clinical details of each patient are shown in Table 1a. For ethical reasons it was thought unreasonable to discontinue other drug therapy. Antiemetic therapy with either prochlorperazine 12.5 mg and/or domperidone 10–20 mg 8 hourly was used, supplemented occasionally with intravenous metoclopramide (10 mg) as required. Patients on phenytoin, barbiturates, allopurinol or glucocorticosteroids were excluded. Temazepam 10–20 mg (if required) was used as night time sedation. Concomitant drug therapy for each patient during the study is shown in Table 1b.

Each patient received ifosfamide 1.5 g m⁻² by i.v. infusion in 250 ml of 0.9% saline over 0.5 h, daily for 5 days. Intravenous mesna (400 mg m⁻²), used as a uroprotector, was given as an i.v. bolus at time zero (pre-dose), and 3, 6 and 9 h post-ifosfamide each day. Venous blood samples (8 ml) were usually taken at time zero and at 1, 2, 4, 6, 8, 10 and 24 h after the dose of ifosfamide on days 1 and 5 of treatment for measurement of plasma ifosfamide concentration and nitrobenzylpyridine alkylating activity. Ifosfamide remains stable in plasma when stored at –20° C for at least 6 months.

A sub-group of five patients were subject on one occasion to more intensive venous blood sampling (8 ml) *viz.* at time zero (pre-dose) and 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10 and 24 h post-ifosfamide dose on days 1 and 5 in conjunction with a complete 24 h urine collection on these days for the measurement of unchanged ifosfamide and nitrobenzylpyridine alkylating activity.

The study protocol was approved by the local ethics committee.

Measurement of ifosfamide concentrations

Plasma and urine drug concentrations were measured by the method of Juma *et al.* (1978) which determines ifosfamide as its trifluoroacetyl derivative by gas chromatography with an alkali flame ionization detector. The intra-assay coefficient of variation for the plasma assay was 9.5% at 5 µg ml⁻¹ and 4.3% at 50 µg ml⁻¹. The

Table 1 a) Clinical and treatment details of patients receiving ifosfamide 1.5 g m⁻² daily for 5 days as fractionated chemotherapy

Patient + course number	Sex	Age (years)	Disease	Weight (kg)	B.S.A. (m ²)	Ifosfamide dose (g)	Previous chemotherapy or radiotherapy
1	M	60	Squamous Ca. larynx	54.0	1.55	2.25	DXT/CTX
2A	M	23	Osteosarcoma	57.0	1.60	2.4	CTX
2B						2.4	CTX
2C						2.4	CTX
3A	M	60	Squamous Ca. tongue	69.0	1.86	2.8	NIL
3B						2.8	CTX
4	M	55	Small cell Ca. lung	72.2	1.88	2.5	DXT
5	M	34	Adeno Ca. maxillary sinus	70.0	1.60	2.2	DXT
6	M	70	Squamous Ca. pharynx	59.0	1.70	2.5	NIL
7	M	26	Malignant thymoma	65.0	1.70	2.5	CTX
8A	F	51	Squamous Ca. cervix	42.3	1.40	2.0	DXT
8C						2.0	DXT/CTX
9A	F	52	Squamous Ca. cervix	62.3	1.60	2.4	NIL
9B						2.4	CTX
10A	F	31	Squamous Ca. Cervix	47.2	1.40	2.1	DXT
10C						2.1	DXT/CTX
11	M	55	Large cell Ca. lung	78.9	1.94	2.9	NIL
12	F	54	Adeno Ca. uterus	61.0	1.76	2.5	DXT
13	M	55	Adeno Ca. lung	81.5	1.98	3.0	NIL
14	F	40	Squamous Ca. cervix	76.5	1.70	2.5	DXT
15	F	45	Squamous Ca. cervix	51.7	1.50	2.25	DXT
Median		52		63.2	1.70	2.5	

DXT = Radiotherapy; CTX = Chemotherapy; B.S.A. = Body Surface Area; Ca = Carcinoma.

Table 1 b) Concurrent drug therapy during fractionated 5 day ifosfamide/mesna therapy

Patient + course number	Concurrent drug therapy
1	Diamorphine; diazepam; dapson; prochlorperazine; lorazepam; lactulose; heparin
2A	Distalgesic; diclofenac; temazepam; domperidone; heparin
2B	Diclofenac; temazepam; domperidone; sodium chromoglycate; heparin
2C	Paracetamol; temazepam; metoclopramide; heparin
3A	Diamorphine; domperidone; isosorbide dinitrate; lactulose; heparin
3B	Diamorphine; metoclopramide; morphine S.R.; dothiepin; heparin
4	Frusemide; dexamethasone; heparin; lactulose; haloperidol; amitriptyline; diamorphine
5	Domperidone; indomethacin; metoclopramide; diamorphine; cimetidine; flucloxacillin; penicillin V; heparin
6	Diazepam; chlorpropamide; metoclopramide; amoxycillin; heparin
7	Domperidone; prochlorperazine; temazepam; heparin
8A	Morphine; metoclopramide; prochlorperazine; temazepam; lactulose; heparin
8C	Morphine; domperidone; prochlorperazine; lorazepam; paracetamol; heparin
9A	Metoclopramide; domperidone; prochlorperazine; paracetamol; heparin
9B	Domperidone; prochlorperazine; coproxamol; heparin
10A	Morphine; domperidone; prochlorperazine; dexamethasone; naproxen; heparin
10C	Diamorphine; metoclopramide; prochlorperazine; naproxen; temazepam; heparin
11	Propranolol; dihydrocodeine; domperidone; prochlorperazine; naproxen; temazepam; heparin
12	Dexamethasone; buprenorphine; heparin
13	Morphine; prednisolone; domperidone; indomethacin; paracetamol; heparin
14	Warfarin; domperidone; prochlorperazine; coproxamol; heparin
15	Morphine; domperidone; prochlorperazine; lorazepam; naproxen; temazepam; metronidazole; heparin

inter-assay coefficient of variation was 5.5–11.3% over the concentration range 5–50 $\mu\text{g ml}^{-1}$. The intra-assay coefficient of variation for the assay of ifosfamide in urine was 7.4% at 5.0 $\mu\text{g ml}^{-1}$ and 10.3% at 50 $\mu\text{g ml}^{-1}$.

Measurement of plasma alkylating activity

This was performed by the method of Juma *et al.* (1979) which is a modification of the technique of Friedman & Boger (1961) using nitrobenzylpyridine. The intra-assay coefficient of variation was 9.6% at 2 nmol of nor nitrogen mustard ml^{-1} and 5.8% at 30 nmol of nor nitrogen mustard ml^{-1} . The limit of the assay was 0.6 nmol nor nitrogen mustard ml^{-1} .

Ifosfamide is itself a 'weak alkylator' in this system. However, there was a threshold plasma ifosfamide concentration of 141 $\mu\text{g ml}^{-1}$, above which there was a linear increase in alkylating activity with ifosfamide concentration. In the measurement of alkylating activity for all samples where plasma ifosfamide concentration exceeded 141 $\mu\text{g ml}^{-1}$, the quantity of alkylating activity produced by the ifosfamide alone was subtracted from that observed to yield the measured alkylating activity for that sample.

Pharmacokinetic analysis

The decline in plasma ifosfamide concentration with time following intravenous administration could be described by a monoexponential function in all of the patients studied. The terminal elimination rate constant was estimated by least squares regression analysis of the plot of the logarithm of the plasma ifosfamide concentration or plasma alkylating activity vs time.

The areas under the curve (AUC) for plasma ifosfamide and alkylating activity were estimated by the linear trapezoidal rule. The area beyond the latest datum point ($C(\text{last})$) was calculated from $C(\text{last})/\lambda_z$. In calculating areas under the curve of plasma ifosfamide and alkylating activity on day 5, the pre-treatment value was used with the terminal elimination rate constant to subtract the proportion of the area due to the dose of ifosfamide given on day 4.

The following equations were used to calculate other parameters:

$$\text{Elimination } t_{1/2,z} = \frac{0.693}{\lambda_z}$$

$$V = \frac{D}{\text{AUC} \cdot \lambda_z}$$

where D is the intravenous dose, V is the apparent volume of distribution during the terminal log-linear elimination phase.

$$\text{Total clearance } CL = \frac{D}{\text{AUC}}$$

$$\text{Renal clearance } CL_R = \frac{\text{Total urinary excretion of ifosfamide over 24 h}}{\text{AUC}}$$

$$\text{Metabolic clearance } CL_M = CL - CL_R$$

Statistical analysis

The pharmacokinetic data are summarised as median (range) values. Non-parametric analysis for differences was performed using Wilcoxon's matched pairs signed rank test. Parametric analysis was done using Student's t -test. Correlations were investigated using Spearman's rank correlation test.

Results

Figure 1 shows plasma ifosfamide concentration-time data for days 1 and 5 in a representative patient. Figure 2 illustrates the plasma alkylating activity vs time data for the same patient on days 1 and 5. Pharmacokinetic parameters derived from data from all patients are shown in Table 2.

The percent increase in ifosfamide clearance from day 1 to day 5 correlated significantly with

Figure 1 Representative plot of the logarithm of the plasma ifosfamide concentration ($\mu\text{g ml}^{-1}$) vs time (h) on treatment day 1 (○) and treatment day 5 (●) for one patient who received 5 day fractionated intravenous ifosfamide (1.5 g m^{-2} daily).

Figure 2 Representative plot of the logarithm of the plasma alkylating activity (nmol equivalents of nor nitrogen mustard (HN₂) ml⁻¹ as measured by the nitrobenzylpyridine assay) vs time (h) on treatment day 1 (○) and on treatment day 5 (●) for one patient (the same patient as in Figure 1) who received 5 day fractionated intravenous ifosfamide (1.5 g m⁻² daily).

the percent increase in plasma alkylating activity area under the curve from day 1 to day 5 ($r_s = 0.45$; $P < 0.05$). There was no significant correlation between the absolute change in plasma ifosfamide clearance from day 1 to day 5 and the absolute change in plasma alkylating activity.

In the subgroup of five patients who had 24 h urinary ifosfamide concentrations and alkylating activity measured the median percentage of the dose of ifosfamide excreted unchanged on day 1 was 4.6% (2.8–14.1%). On day 5 the median percentage dose of ifosfamide excreted as unchanged drug was 4.8% (2.3–9.1%) (no significant difference from the data for day 1). Table 4 shows values of the renal and metabolic clearances of ifosfamide in these patients.

The data for the excretion of alkylating activity over the 24 h periods on days 1 and 5 showed great variation (Table 3) some of which was due to the difficulties involved in obtaining complete 24 h collections. However, a greater problem in interpreting urinary alkylating activity measured by the nitrobenzylpyridine assay is the variable presence of other non-specific moieties which can produce the reaction.

There were no significant correlations between any of the pharmacokinetic parameters of ifosfamide or total alkylating activity and haematological or uroepithelial toxicity as measured by the World Health Organisation grading.

Discussion

Ifosfamide when first given in a dose of 1.5 g m⁻² to patients as part of a fractionated treatment regime has a median terminal elimination half-

Table 2 Pharmacokinetic parameters for plasma ifosfamide and plasma alkylating activity for patients receiving ifosfamide 1.5 g m⁻² daily for 5 days

<i>Ifosfamide</i>	<i>Day 1</i> Median (range) n = 21	<i>Day 5</i> Median (range) n = 21
Terminal elimination half-life $t_{1/2}$	7.2 (2.8–14.2)	4.6 (2.3–7.7)**
Area under the plasma concentration-time curve AUC (μg ml ⁻¹ h)	612 (225–1354)	360 (90–715)**
Clearance CL (ml min ⁻¹)	66 (31–148)	115 (52–381)**
Apparent volume of distribution V (ml kg ⁻¹)	635 (406–1047)	721 (336–1871)†
<i>Plasma alkylating activity</i>		
Terminal elimination constant λ_z (h ⁻¹)	0.463 (0.045–0.896)	0.246 (0.073–0.618)*
Area under the plasma concentration-time curve AUC(0,24 h) nmol nor nitrogen mustard eq ml ⁻¹ h	16 (0.6–105.4)	52 (13–238)***

*** = two tailed $P < 0.0001$, ** = two tailed $P < 0.001$, * = two tailed $P < 0.05$, † = not significant (Wilcoxon matched pairs test).

Table 3 24 h urinary excretion of ifosfamide and alkylating activity on days 1 and 5 in patients receiving fractionated five day ifosfamide dosage

Patient + course number	Day 1 24 h total urinary ifosfamide excretion (mg)	Day 5 24 h total urinary ifosfamide excretion (mg)	Day 1 24 h total urinary alkylating activity (nmol nor nitrogen mustard eq)	Day 5 24 h total urinary alkylating activity (nmol nor nitrogen mustard eq)
8C	92	63	73	94
9B	68	55	18	1
10C	296	191	136	48
14	263	179	32	56
15	85	108	132	43
Median	92	108	73	48

Table 4 Ifosfamide renal and metabolic clearance on days 1 and 5 of fractionated chemotherapy

Patient + course number	Renal clearance (CL_R) on day 1 (ml min^{-1})	Renal clearance (CL_R) on day 5 (ml min^{-1})	Metabolic clearance (CL_M) on day 1 (ml min^{-1})	Metabolic clearance (CL_M) on day 5 (ml min^{-1})
8C	6.8	4.3	141.2	132.6
9B	1.3	1.3	39.9	54.6
10C	16.1	15.3	86.0	149.5
14	11.1	11.5	78.6	133.4
15	2.4	5.7	58.9	113.7
Median	6.8 ⁺	5.7 ⁺	78.6 ⁰	132.6 ⁰

(⁺ and ⁰ = No significant difference—Student's *t* test or Wilcoxon's test).

life of 7.2 h (range 2.8–14.2 h). This value is similar to that reported by others (2.2–7.8 h (median 5.3 h)) (Bryant *et al.*, 1980a; Cerny *et al.*, 1986; McNeil & Morgan, 1981; Nelson *et al.*, 1976; Piazza *et al.*, 1984). The terminal elimination half-life of ifosfamide at this dose was similar to that for low dose cyclophosphamide (5.1 h) (Graham *et al.*, 1983). The plasma decay of unchanged ifosfamide at this dose was best fitted by a monoexponential function, as shown by others (Creaven *et al.*, 1976; Nelson *et al.*, 1976).

Values of the volume of distribution of ifosfamide (634.9–721.1 ml kg^{-1} (median values for days 1 and 5)) were similar to those reported by McNeil & Morgan (1981) (37–48.8 l) and Lind *et al.* (1986b, 1989) (32.8–38.2 l and 34.8–41.5 l). The absolute values for the volume of distribution of approximately 60–70% body weight would be anticipated for a drug which distributes largely in body water and is poorly bound to plasma and tissue proteins.

The value of the total clearance of ifosfamide on day 1 of administration (66 (range 31–141) ml min^{-1}) was similar to those reported by Lind *et*

al. (1989) (69.2 ml min^{-1}), and Cerny *et al.* (1986) (56.7–69.7 ml min^{-1}) using similar ifosfamide doses. However, Nelson & coworkers (1986) reported a much lower ifosfamide clearance when given in a dose of 1.6–2.4 g m^{-2} daily for 3 days (14.9–24.2 ml min^{-1}). However, this last study used radiolabelled ifosfamide and the assay method may have been non-specific. In a small group of patients given ifosfamide 500 mg orally up to 2000 mg intravenously McNeil & Morgan (1981) reported a clearance of 108.9–148.3 ml min^{-1} , although they do not state whether the treatments were part of a fractionated regimen.

The most interesting finding in the present study was the significant 76% increase in ifosfamide clearance from day 1, to day 5 ($P < 0.001$; Wilcoxon's test), without any change in the apparent volume of distribution. Concomitant with the increase in ifosfamide clearance there was a highly significant 223% increase in the plasma alkylating activity area under the curve on day 1 from 16 nmol nor nitrogen mustard eq ml^{-1} h to 52 nmol nor nitrogen mustard eq ml^{-1} h ($P < 0.0001$; Wilcoxon's test) on day 5.

Furthermore, there was a significant positive correlation between the percent increase in ifosfamide clearance and the percent increase in plasma alkylating activity area under the curve (r_s 0.46; $P < 0.05$). This suggests a time-dependent increase in ifosfamide metabolism over the 5 days. The fact that in a subgroup of patients there was no change in the renal clearance of ifosfamide further supports this conclusion. Data from patients who received fractionated ifosfamide on more than one occasion do not suggest that the increase in ifosfamide metabolism is a permanent phenomenon because the first treatment day 1 ifosfamide clearance was similar to the day 1 clearance on any subsequent treatment courses.

Our findings are similar to that of Lind *et al.* (1989) using 1.5 g m^{-2} ifosfamide intravenously daily for 5 days who found an increase in ifosfamide clearance from a median of $69.19 \text{ ml min}^{-1}$ on day 1 to a median of $123.19 \text{ ml min}^{-1}$ on day 5 without a change in ifosfamide distribution. Thus, the initial suggestions from small studies (Nelson *et al.*, 1976; Piazza *et al.*, 1984) of time-dependent metabolism for ifosfamide are confirmed. A similar phenomenon has been observed with cyclophosphamide (D'Incalci *et al.*, 1979; Graham *et al.*, 1983).

Most of the metabolism of ifosfamide occurs in the liver and is mediated by the cytochrome P-450 system (Alarcon *et al.*, 1972; Allen & Creaven, 1972). The fact that ifosfamide clearance is low suggests that the time-dependent change in metabolism is not due to a change in liver blood flow, but to an increase in hepatic enzyme activity.

Studies that have investigated the effect of different chemotherapeutic regimens on hepatic cytochrome P-450 activity in man are limited and

have yielded variable results. Lipton *et al.* (1978) used the [^{14}C]-labelled antipyrine breath test to measure hepatic mixed function oxidase activity in seven patients receiving intravenous *Corynebacterium parvum* and cyclophosphamide (40 mg kg^{-1}) and showed a significant decrease in six of them. However, when the agents were given alone, each to two patients, no significant change in hepatic enzyme activity was noted. Teunissen *et al.* (1984) reported a 25–30% increase in hepatic cytochrome P-450 activity (as measured by antipyrine clearance) in eight patients with testicular cancer treated with two to three courses of cisplatin, vinblastine and bleomycin. There is, therefore, a need for further research into time-dependent changes in the kinetics of chemotherapeutic agents used to treat cancer as such changes might have a significant bearing on treatment regimes.

From the data presented it is not possible to determine whether the observed increase in ifosfamide metabolism is due to enzyme induction, reduced enzyme degradation or to an increased activity of existing levels of hepatic cytochrome P-450 resulting from a change in the spin state of the haemoprotein of the P-450 complex (Gibson & Tamburini, 1984). It seems unlikely that an alkylating agent (which reduces DNA/RNA and protein synthesis) could increase enzyme protein synthesis. Certainly the time course for cytochrome P-450 enzyme induction, with a maximum at 7–8 days (Ohnhaus & Brundert, 1982), is longer than the time course of the change in ifosfamide metabolism (Lind *et al.*, 1986a, 1989).

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