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Original article

Saturable metabolism of continuous high-dose ifosfamide with Mesna and GM-CSF: A pharmacokinetic study in advanced sarcoma patients

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Summary

Background: The aim of this study was to assess the pharmacology, toxicity and activity of high-dose ifosfamide/mesna ± GM-CSF administered by a five-day continuous infusion at a total ifosfamide dose of 12–18 g/m^2 in adult patients with advanced sarcomas.

Patients and methods: Between January 1991 and October 1992 32 patients with advanced or metastatic sarcoma were entered the study. Twenty-seven patients were pretreated including twenty-three with prior ifosfamide at less than 8 g/m^2 total dose/cycle. In 25 patients (27 cycles) extensive pharmacokinetic analyses were performed.

Results: The area under the plasma concentration-time curve (AUC) for ifosfamide increased linearly with dose while the AUC's of the metabolites measured in plasma by thin-layer chromatography did not increase with dose, particularly that of the active metabolite isophosphoramide mustard. Furthermore the AUC of the inactive carboxymetabolite did not increase with dose. Interpatient variability of pharmacokinetic parameters was high. Dose-limiting toxicity was myelosuppression at 18 g/m^2 total dose with grade 4 neutropenia in five of six patients and grade 4 thrombocytopenia in four of six patients. Therefore the maximum tolerated dose was considered to be 18 g/m^2 total dose. There was one CR and eleven PR in twenty-nine evaluable patients (overall response rate 41%).

Conclusion: Both the activation and inactivation pathways of ifosfamide are non-linear and saturable at high-doses although the pharmacokinetics of the parent drug itself are dose linear. Ifosfamide doses greater than 14–16 g/m^2 per cycle appear to result in a relative decrease of the active metabolite isophosphoramide mustard. These data suggest a dose-dependent saturation or even inhibition of ifosfamide metabolism by increasing high dose ifosfamide and suggest the need for further metabolic studies.

Key words: high-dose ifosfamide, metabolism, pharmacology, sarcomas

Introduction

The alkylating agent ifosfamide (IFO) in conjunction with the uroprotective agent mesna (2-mercaptoethanesulfonate sodium) has demonstrated clinical activity against a wide range of tumor types [1]. For treatment of soft tissue sarcoma only the anthracyclines, DTIC and the oxazaphosphorine IFO have consistently shown response rates of $\geq$20% in non-pretreated sarcoma patients [2–4]. Combination chemotherapy seems to improve the response rate, but it is not certain that this translates into an overall survival benefit [5–7]. Serious side effects of IFO include myelosuppression, nausea and vomiting, alopecia, uro- and CNS-toxicity [8].

Preliminary results of high dose IFO/mesna (IFO-dose $\geq$ 10 g/m^2 per cycle) in sarcoma patients with different subtypes indicate therapeutic efficacy even in pretreated patients [9–14]. Continuous IFO/mesna application has been shown to be less toxic as compared to fractionated i.v. infusions and preclinical and clinical data are consistent with a higher therapeutic index for the continuous infusion schedule over several days [15–18]. For this reason we chose a schedule with continuous IFO when this trial commenced. Subsequently, a report suggested that repetitive 2-hour infusions every 12 hours to a total dose of 14 g/m^2 may be more effective at inducing clinical response than the same total dose delivered by continuous infusion over three days [14]. In contrast, using a conventional dose of IFO (6 to 9 g/m^2), there was no demonstrable difference between a bolus IFO-administration and a continuous infusion schedule of the same total dose [19].

Ifosfamide is a prodrug which is metabolised *in vivo* to produce a variety of active and potentially toxic metabolites (Figure 1). The bioactivation is mediated by a cytochrome *p*450 enzyme (CYP3A4) and results in hydroxylation at the carbon-4 position of the oxazaphosphorine ring. This 4-hydroxy-ifosfamide (4-OH-IFO) is unstable and exists in equilibrium with its tautomeric form aldoifosfamide (aldo-IFO). Aldo-IFO decomposes

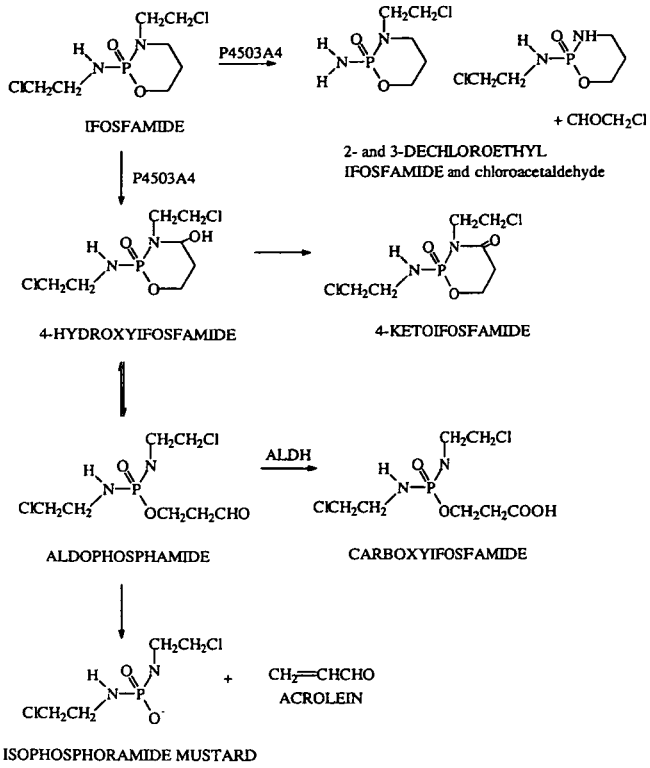

Figure 1. Pathways of metabolism of ifosfamide.

spontaneously to form the alkylating agent isophosphoramide mustard (IPM) and acrolein. By its alkylating ability IPM is assumed to be the active metabolite of IFO. The by-product acrolein is believed to be responsible for the urotoxic effects of IFO [20]. Alternatively aldo-IFO can be oxidised by an aldehyde dehydrogenase enzyme (ALDH1) to carboxy-IFO (CX) which is an inactive metabolite. Approximately half of the dose of IFO undergoes oxidative N-dealkylation to produce either 2- or 3-dechloroethyl-IFO (2-DCI, 3-DCI respectively). An equimolar quantity of chloroacetaldehyde (CA) is formed in each of these reactions and this toxic metabolite has been implicated in the neurotoxicity which may accompany IFO-therapy [21] and may furthermore be associated with nephrotoxicity. Oxidation of 4-hydroxyifosfamide results in another inactive metabolite which is 4-keto-IFO (KETO).

Pharmacokinetic and metabolism data are scarce for patients treated with high-dose IFO [22, 23]. Since IFO is a prodrug and needs to be activated *in vivo*, there may be saturation of metabolism which would preclude very high dose treatment with this agent. A similar saturation phenomenon has been noted with cyclophosphamide [24, 25]. We therefore examined the pharmacokinetics of IFO and its metabolites in patients with high-dose IFO/mesna, in combination with hematopoietic growth factors, where indicated, in this dose escalation study. Pharmacokinetic data for high-dose mesna and endogenous sulphydrils have been published elsewhere [26].

Patients and methods

Patients

Patients with a histologically confirmed diagnosis of inoperable soft tissue, osteo- or chondrosarcoma with measurable disease were eligible for study. Pretreatment with chemotherapy was allowed. Prior treatment with IFO was limited to less than 8 g/m^2 per cycle. Furthermore a WHO performance status ≤ 2 and age ≤ 70 years was mandatory. A creatinine-clearance ≥ 60 ml/min, liver enzymes $\leq 2\times$ normal values, leukocytes $\geq 3.5 \times 10^9$/l, thrombocytes $> 100 \times 10^9$/l and hemoglobin ≥ 10 g/dl were other inclusion criteria. The patients had to give informed consent and the study was accepted by the local ethical committees

Treatment plan

In order to define the maximum tolerated dose (MTD) of ifosfamide as a continuous infusion over five days the treatment design was to escalate the daily dose from level 1 to level 3 as described below At each level the inclusion of four patients was planned. If a granulocyte-nadir of $< 0.1 \times 10^9$/l with an infectious episode (requiring i.v antibiotics) and/or a thrombocyte-nadir of $< 50 \times 10^9$/l or any other important non-hematological toxicities (excluding alopecia or nausea and vomiting) such as CNS toxicity grade 3 or higher occurred in the first course of two patients of a particular dose level, this level was considered as the maximal tolerated dose (MTD). As we did not reach the MTD in any patient in level 3 and observed encouraging responses we decided after patient 20 (the 12th patient on level 3) to escalate the dose to a further level 4 for the following patients. The first patient at level 1 had a partial remission after seven cycles and received a second-line treatment at level 4 later on when his tumor became progressive again. MTD was achieved at dose level 4. Therefore, the recommended dose was considered to be 16 g/m^2 of IFO per cycle (level 3) and seven additional patients were treated at this level (Table 1).

IFO was given in 2 liters of normal saline or dextrose 5% daily as a continuous i.v. infusion. Mesna was added to IFO in the same infusion bag. On day 6 mesna was administered at the same daily dose for an additional 24 hours. An antiemetic agent was given and bicarbonate was replaced at the discretion of the treating physician. Because grade 4 granulocytopenia was repeatedly encountered at level 1, all subsequent patients starting at level 2 received GM-CSF (leucomax) at a dose of 5 µg/kg sc. once daily starting on day 7 until day 16 (total of 10 doses). If granulocytes had not reached $\geq 0.5 \times 10^9$ on day 16, GM-CSF application was continued until day 20. The next chemotherapy cycle was started on day 22 if the following criteria were met: leukocytes $\geq 3.5 \times 10^9$/l, thrombocytes $\geq 100 \times 10^9$/l, Cr-clearance ≥ 60 ml/min. Otherwise the cycle was postponed by one week or if the criteria could still not be met, the patient was taken off treatment.

Toxicity and response assessment

Pretreatment evaluation included standard history, physical examination, a complete blood cell count, electrolytes and biochemical assessment of liver and kidney function as well as urinalysis. History, physical examination and the laboratory tests were repeated before each cycle. A chest X-ray and tumor measurement of all evaluable lesions by conventional X-ray or computed tomographic scan at baseline were mandatory. Tumor imaging and chest X-ray were repeated every second cycle. Complete blood cell count with differential count was obtained every week. A complete laboratory and radiological work up was performed after the last chemotherapy cycle. Toxicity was evaluated according to WHO criteria, except for neurotoxicity which was measured by M.D. Anderson score [27].

Complete response (CR) was defined as the disappearance of all clinical evidence of tumor Pathologic complete response (pCR) was defined as no viable tumor in the resected specimen that represented the residual radiological abnormality. Partial response (PR) was defined

Table 1. Patient characteristics and treatment results.

Dose level	No.	Sex	Age	Primary tumor type	Grading (NCI)	Stage (AJCC)	Initial WHO performance	No. of pre-treatments	Pretreatments incl. ifos	No. of cyles	Response	Resp. duration (weeks)	Survival (weeks)
1	1	m	48	STS*	3	4B	1	1	1	9	PR	44	129
	2	f	40	CHS	3	4B	1	1	0	6	NC	–	40
	3	m	33	CHS	2	4B	2	1	0	7	PR	26	57
	4	m	65	OS	–	4B	2	3	2	2	NC	–	30
2	5	m	37	STS	3	4B	0	1	1	9	PR	84	161
	6	m	51	STS	3	4B	1	1	1	6	PR	52	73
	7	m	46	CHS	3	4B	0	1	1	5	NC	–	76
	8	m	62	STS	3	3B	0	1	1	1	ne	–	2
3	9	m	52	STS	3	4B	0	1	1	2	PR	13	13
	10	f	22	STS	3	4B	0	1	0	4	PR	48	67
	11	f	39	STS*	3	4A	0	0	0	3	CR	31	40
	12	f	51	STS*	3	4B	0	0	0	2	PD	–	37
	13	m	27	STS*	3	4B	0	3	2	4	NC	–	61
	14	f	71	STS	3	4A	1	1	1	4	PR	40	47
	15	m	40	OS	–	4B	1	2	1	1	PD	–	6
	16	m	22	NB	3	3B	1	0	0	3	NC	–	77
	17	m	46	STS	3	3B	2	1	1	5	PR	31	39
	18	f	42	STS*	2	4B	0	1	1	2	PD	–	37
	19	f	44	STS*	2	4A	0	0	0	2	NC	–	83
	20	f	54	STS	2	4B	2	1	1	1	PD	–	35
4	21	m	36	CHS	2	4B	2	1	1	2	PD	–	46
	22	m	61	STS	3	4A	2	2	2	1	PD	–	35
	23	f	65	STS*	3	4B	0	1	1	1	uk	–	59
	24	f	53	STS*	2	4B	0	1	1	2	PR	24	41
	25	m	52	STS*	3	4B	1	1	1	1	PD	–	17
3	26	m	47	OS	–	4B	0	3	1	2	NC	–	144**
	27	f	24	OS	–	4B	1	2	0	4	PR	77	134**
	28	m	23	STS	3	4B	1	0	0	4	NC	–	55
	29	f	64	STS	2	4A	1	1	1	2	NC[1]	–	29
	30	f	48	STS	2	4B	1	3	1	4	PR	25	73
	31	m	63	STS*	3	4B	1	1	1	2	PD	–	36
	32	f	46	STS	3	4B	1	1	1	1	uk	–	11

Abbreviations: STS – soft tissue sarcoma; STS* – leiomyosarcoma, OS – osteosarcoma; CHS – chondrosarcoma; NB – neuroblastoma; ne – not evaluable; NC[1] – PR but no confirmatory X-ray; uk – unknown; ** – lost for follow up, week patient last seen.

as a $\geq 50\%$ reduction in the sum of the products of the biperpendicular diameters of measurable lesions, without the appearance of new lesions for at least three weeks. Minor response (MR) was defined as a decrease in tumor size between 25% and 49%. Stable disease (SD) was defined as a less than 25% change in the dimensions of the tumor, and progressive disease (PD) was defined as a $\geq 25\%$ increase in the sum of the perpendicular diameters and/or appearance of new lesions. Survival was measured in weeks from the first day of treatment until the date of death. Response duration in patients with complete or partial response was calculated as the time from the first day of treatment until the date of diagnosis of relapse.

Pharmacokinetics and metabolite analyses

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

During the first cycle blood samples were collected immediately before and at 12, 24, 36, 48, 72, 96, 120, 132 and 144 hours after the start of treatment. Blood was heparinised and plasma was separated and frozen immediately at −20 °C prior to analysis. Urine was collected at 24-hour intervals throughout the infusion and for 24 hours after. The volume of each urine sample was measured and an aliquot was frozen at −20 °C for subsequent analysis

Concentrations of ifosfamide (IFO), isophosphoramide mustard (IPM), carboxyifosfamide (CX), 2- and 3-dechloroethylifosfamide (2- and 3-DCI) and 4-ketoifosfamide (KETO) were determined in urine and plasma, using gas chromatography (GC) for IFO [28] and a quantitative thin-layer chromatography-photography densitometry technique (TLC) for the metabolites [29].

Exposure of each patient to ifosfamide and each of its metabolites was expressed as the area under the plasma concentration-time curve (AUC) for that species. This was calculated by the trapezoidal method. When comparing AUCs for different metabolites at different doses, AUC divided by dose was used as a measure of comparison. The AUC for IFO was used to calculate clearance (Cl). Due to the sparcity of ifosfamide data beyond the end of the infusion and the induction of its own metabolism, it was not possible to calculate other pharmacokinetic parameters such as half-life and volume of distribution. Recoveries of parent drug and metabolites in urine were expressed as a percentage of the administered dose. Both AUC and percents of dose were corrected for molecular weight.

Statistics

Due to small sample size, patient characteristics, response and toxicity were presented by descriptive statistic only. Survival probabilities were estimated by Kaplan–Meier method. Spearman rank correlation was used to determine the relationships between drug and metabolite pharmacokinetics and dose. The Mann–Whitney U-test was used to compare data from different dose levels.

Results

Patient characteristics

Thirty-one patients with advanced or metastatic sarcoma and one patient with a neuroblastoma were entered into this Swiss national study between January 1991 and October 1992. The latter was included even though this tumor does not fulfil the histological criteria of a sarcoma because this type of tumor also responds well to ifosfamide and because the main aim of the study was to examine the pharmacokinetics of this drug. Table 1 shows the patients characteristics. Median age of the patients was 45 years (range 22–71 years).

WHO performance status on starting the study was 0 or 1 in 26 patients each and 2 in 6 further patients. Fourteen patients were female, eighteen patients were male. The histological diagnosis was osteosarcoma in 4, chondrosarcoma in 4, neuroblastoma in 1 and soft tissue sarcoma in 23 patients. Of the soft tissue sarcoma patients there were 10 with the diagnosis of leiomyosarcoma. Stage according to AJCC was IIIB in 3 patients, IVA in 5 patients and IVB in 24 patients. Overall 27 patients were pretreated, of whom 7 had more than 2 pretreatments.

Nineteen patients had extensive plasma and urinary pharmacokinetics performed during the first cycle, seven further patients during the second cycle and one patient during the third cycle. The total number of cycles given was 104. Our first patient received seven cycles in level 1 and later on two cycles in level 4. Another patient received nine cycles in level 2. Only 5 of 32 patients had no pretreatment and 23 patients had been previously treated with the standard dose of IFO ($\leqslant 8$ g/m^2/cycle).

Response data

Table 1 shows the response of each individual patient. There was 1 CR and 11 PR in 29 evaluable patients with confirmed response, resulting in an overall response rate of 41%. Of the 11 patients with a PR, 8 had been pretreated with IFO. One further patient (patient 29) had a PR after two cycles, but refused further treatment and PR could not be confirmed due to refusal of X-rays. He was therefore counted as no change. Median duration of response was eight months (range 3.0–19.4 months). Median survival was 11 months (2 weeks – 3.1 years). These results are listed in Table 1.

Toxicity

Dose limiting toxicity was neutropenia and thrombocytopenia with consequent infections (Table 2). According to WHO-criteria fever and infection grade 1 and 2 were found in 30%, grade 3 in 13% and grade 4 in 2% of 104 cycles. There was one toxic death of a 62-year-old patient at dose level 2, who died during the first cycle (day 14) due to septicemia with *E. coli* in therapy induced agranulocytosis. At level 4 (18 g/m^2 total dose) grade 4 neutropenia was found in five of six patients during cycle 1 and led to severe infections in two of six patients. Furthermore at this dose level, thrombocytopenia grade 4 was found in four of six patients during the first cycle. Therefore level 4 was found to be too toxic for further continuation in this group of mainly pretreated patients and the recommended dose was considered to be 16 g/m^2 (level 3). Independent of the dose level, anemia grade 3 and 4 was found in 25% of the patients. The overall rate of infections per patient was 17%. CNS-toxicity was usually mild (grade $\leqslant 2$ M.D. Anderson score [27]) and was documented in 22% of the

Table 2. Toxicity WHO grades 3 + 4.

	Levels								
	1. cycle				2. cycle				All cycles
	1	2	3	4	1	2	3	4	$n = 104$
No. of patients treated	4	4	19	6	4	3	16	3	32
Leukocytes	3/4	3/3[b]	18/19	5/6	4/4	2/2[b]	13/15[b]	3/3	90/99[b]
Thrombocytes	0/4	1/3[b]	5/19	4/6	1/4	0/2[b]	5/15[b]	1/3	23/99[b]
Hemoglobin	1/4	0/3[b]	4/19	2/6	2/4	0/3	5/15[b]	1/3	25/100[b]
Infections	0/4	2/4	4/19	2/6	1/4	0/3	2/16	1/3	16/104
Neurotoxicity[a]	0/4	0/4	0/19	0/6	0/4	0/3	0/16	0/3	1/104
Urotoxicity	0/4	0/4	0/19	0/6	0/4	0/3	0/16	0/3	0/104
Nausea/vomiting	0/4	0/3[b]	0/19	0/6	0/4	0/3	0/15[b]	0/3	0/100[b]

Level 1: 12 g/m^2/5d IFO; level 2: 14 g/m^2/5d IFO; level 3: 16 g/m^2/5d IFO; level 4: 18 g/m^2/5d IFO.

[a] M.D. Anderson neurotoxicity scale [27].

[b] Some cycles with missing hematological values and incomplete toxicity reports.

patients. By using the M.D. Anderson neurotoxicity score there was only one patient with grade 3 neurotoxicity which presented as progressing peripheral neuropathy. In all instances neurotoxicity was fully reversible within three days. CNS-toxicity was more frequent during the first two cycles than thereafter. Nausea and vomiting were frequently encountered (58% of all cycles), but were of only moderate degree (21% WHO grade 1, 37% WHO grade 2) because prophylactic 5-HT_3-blockers and steroids were used. Mild urotoxicity (grade 1) was seen in 36% of courses, but there was a grade 2 urotoxicity in only one cycle. No grade 3 or 4 urotoxicity has been observed. Alopecia occurred in all of the patients, many of whom had alopecia at pretreatment due to prior chemotherapy. There was one patient with an unexplained short episode of apnoea on day 4 of the first cycle which was possibly due to chlorpromazine. This patient recovered promptly.

Pharmacokinetics

Ifosfamide and its metabolites were detectable in plasma and urine from all investigated patients during IFO administration using the quantitative thin-layer chromatography–photography densitometry technique (TLC). Parent drug could also be measured by gas chromatography (GC), which gave more reliable results at the high concentrations seen during high-dose treatment. For consistency, the values quoted for parent drug were derived from GC analysis. During the infusion period, the concentration of IFO in plasma rose to a peak after the first 24 hours (Figure 2). Thereafter, plasma concentration declined towards the end of the infusion so that the day 5 value was on average 50% (range 0%–80%) less than that on day one, despite a continuous, constant rate of administration. This was accompanied by an average 50% increase in plasma concentration of dechloroethylated metabolites (range 0%–300%). The time-course of this effect varied. Some patients showed a rapid decline in ifosfamide concentration to reach a lower steady-state concentration on day two with no further change. Others showed a steady decline in drug concentration from day one to day five with no sign of attaining a steady-state level. After the end of the infusion, concentrations of IFO rapidly fell below the limit of detection, with concentrations of metabolites persisting slightly longer in some patients.

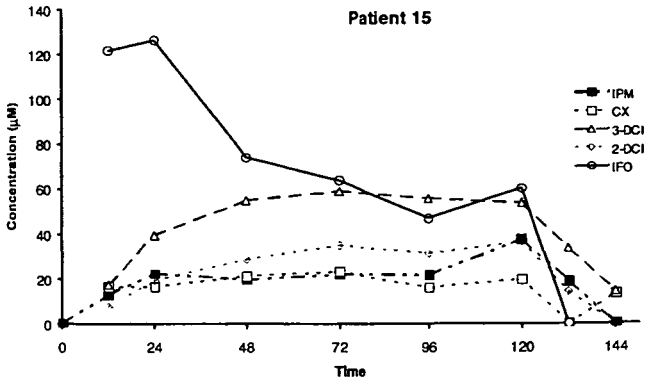

Figure 2. Plasma concentration-time profile of ifosfamide and its metabolites in patient 15.

Plasma and urinary pharmacokinetics for IFO and its metabolites at each dose level are given in Tables 3 and 4. Only the AUC for parent drug increased linearly with dose (Figure 3). However, the AUCs of the metabolites measured in plasma by TLC did not

Table 3 Plasma pharmacokinetics of total dose IFO over five days.

IFO dose ($g \cdot m^{-2}$)	No. of cycles	IFO	IPM	CX	3-DCI	2-DCI	IFO Cl ($ml/min/m^2$)
12	7	9.7 (8.3–14.2)	4.8 (0.9–5.6)	1.3 (0.7–2.6)	5.5 (2.5–8 7)	2.7 (0.4–5.4)	4.77 (3 33–5.56)
14	8	13.0 (9.3–15.2)	2.8 (2.1–5.1)	1.3 (0.2–3.6)	4.2 (2.8–8.3)	2.4 (1.6–3.5)	4.13 (3.54–5.81)
16	7	16.1 (7.4–18.7)	2.0 (0–5.3)	1.5 (0.7–3.1)	5.9 (2.6–6.6)	2.2 (1.2–3.5)	3.82 (3.29–8.27)
18	5	16.3 (12.8–20.2)	3.8 (2.1–5.6)	2.4 (0.8–3.9)	6.4 (4.6–9.8)	4.2 (2.5–4.5)	4.24 (3.43–5.42)

Mean values and range (between parentheses) of AUC (in mM·hr) of IFO and metabolites; data for each metabolite given as AUC (mM·hr); total clearance of IFO in $ml/min/m^2$.

Table 4. Urinary pharmacokinetics of total dose IFO over five days.

IFO dose g/m^2	No. of cycles	Recovery (% of dose)					
		IFO	IPM	CX	3-DCI	2-DCI	KETO
12	7	11.5 (9.6–28.2)	6.3 (1.1–14.0)	6.0 (0.9–12.7)	8.6 (1.7–10.7)	4.9 (1.1–6.9)	0.1 (0–0.6)
14	8	13.5 (10.0–18.9)	6.9 (3.8–11.3)	10.7 (6.5–16.5)	8.8 (5.0–22.1)	8.4 (3.7–16.0)	0.2 (0–0.6)
16	7	12.6 (9.5–17.4)	4.2 (1.6–4.4	6.9 (4.3–14.9)	6.1 (3.1–8.5)	5.4 (2.2–7.2)	0.0 (0–0.3)
18	5	17.3 (14.3–22.3)	5.0 (3.2–7.0)	7.3 (4.8–12.3)	6.2 (3.1–12.9)	3.1 (2.7–8.3)	0.3 (0.2–0.6)

Recovery of IFO and metabolites. Mean values and range (between parentheses) in mM·hr.

increase with dose, particularly that of IPM. This supposedly active metabolite did not increase with increasing doses of IFO (Figure 3). Likewise, the AUC of the inactive metabolite CX did not increase with dose. Thus, the lack of increase in IPM-AUC cannot be attributed to an increase in inactivation by aldehyde dehydrogenase. Similarly, the AUCs of the inactive dechloroethylated metabolites did not increase in proportion to the dose. The 4-keto-ifosfamide (KETO) was a minor metabolite in all cases (recovery < 0.7% of the dose or undetectable).

The excretion of IFO unchanged in urine tended to increase with dose, but this trend was only just significant ($P < 0.05$). One subject (patient 26) excreted a large percentage (28%) of a 12 g/m^2 dose as unchanged drug. If this subject is excluded, the correlation of excretion of unchanged drug with dose is highly significant ($P < 0.01$). The recovery of the active metabolite IPM as a percentage of the dose administered decreased on increasing the dose. This inverse relation was most significant between the excretion of IPM at the dose level of 14 and 16 g/m^2 IFO ($P = 0.008$). In contrast the recovery of CX increased up to 14 g/m^2 and declined thereafter similar to the 2-dechloroethyl metabolites (Table 4).

The weak point of a dose-escalation study is that individual patients are not studied at successive dose levels. In the present study, two patients were analyzed at different doses. Patient 1 had a partial remission after seven cycles with 12 g/m^2 and received two further cycles in level 4 18 g/m^2 when his tumor became progressive again. The pharmacokinetic results are summarized in Figure 4. Values of AUC for metabolites are unchanged or decreased at the higher dose, while the AUC of ifosfamide increases proportionately to dose and the percent of dose unchanged in urine is doubled. This comparison of different dose levels in the same patient again indicates saturation of metabolism.

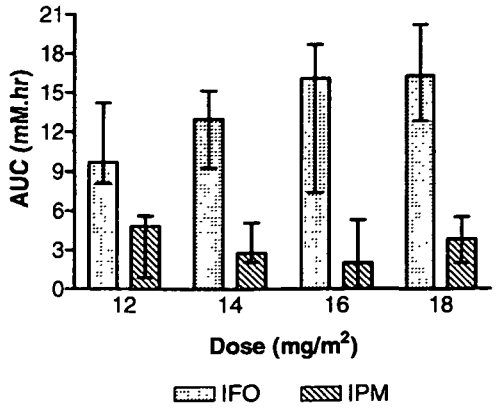

Figure 3 AUC of ifosfamide and isophosphoramide mustard at different dose levels.

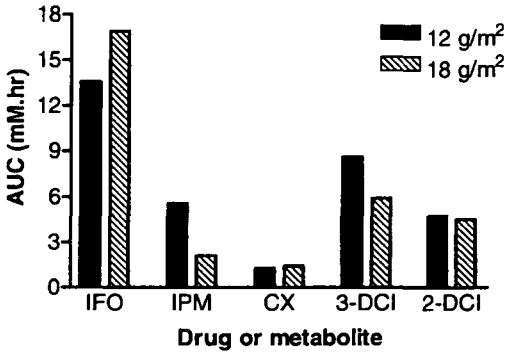

Figure 4. Plasma AUCs (a) and urine recoveries (b) of ifosfamide and metabolites at doses of 12 g/m^2 and 18 g/m^2 in patient 1.

Discussion

High-dose ifosfamide, defined as ≥ 10 g/m^2 per cycle, is a very active single agent therapy even in patients previously treated with standard dose ifosfamide. As a prodrug ifosfamide must be activated through the liver cytochrome *p*450 enzyme system and this metabolic step may be saturated in high dose regimens. Our results show that in the population studied here, there are important dose-related alterations in IFO metabolism. Furthermore there is a high interindividual variability. The well documented phenomenon of enzyme induction during treatment with IFO [22, 29–31] was confirmed and the time course of the enzyme inductive effect also varied. Some patients showed a rapid decline in IFO concentrations over five days to reach a lower steady state concentration on day 2, whereas other patients showed no sign of a steady state level even after five days. This indicates a substantial individual variability in induction of IFO metabolism. These findings are in agreement with results from pharmacokinetic studies of children [32].

As regards the four dose levels studied, only the AUC for the parent drug increased linearly with dose indicating that the pharmacokinetics of IFO itself are not dose dependent over the dose range studied here. This has already been shown in previous lower dose studies [32, 33]. This is reflected in a total clearance and renal clearance similar to those reported previously [33–35]. However the AUCs of the metabolites measured in plasma by TLC did not increase with the IFO dose, particularly that of IPM which is thought to be the main alkylating metabolite of IFO. The AUC of IPM did not increase as much as that of IFO, implying that the activation pathway of IFO was saturated already at the dose levels studied here. In patients treated with standard dose IFO, IPM metabolism was unchanged and showed no tendency to decrease with increasing dose [36]. Interestingly the AUC of the inactive metabolite CX also does not increase with dose. This phenomenon cannot be attributed to a decrease in inactivation by aldehyde dehydrogenase enzyme (ALDH1) because the AUCs of the inactive dechloroethylated metabolites do not increase in proportion to the dose either. This implies that the inactivation pathways of IFO metabolism may also be saturated at the dose levels studied. Therefore the excretion of unchanged IFO in urine tended to increase with dose.

The profile of excretion of IFO and metabolites in urine provides further evidence of saturable metabolism. The decrease in recovery of the active metabolite IPM, of the inactive metabolite CX and the dechloroethyl metabolites as the dose of IFO was increased is consistent with saturable metabolism and may reflect product-inhibition of the activating enzymes by IFO metabolites. This inverse relation was most significant between the IFO dose level of 14 and 16 g/m^2. A similar phenomenon has been reported for enzyme inhibition by products of cyclophosphamide metabolism [37]

In patient 1 a dose dependency of IFO metabolism could be investigated in an intraindividual comparison of dose level 1 and 4. This young patient responded at the 12 g/m^2 level and was later retreated at 18 g/m^2. The comparison of the pharmacokinetic results confirm a clear saturation of IFO metabolism with regard to both the active and inactive metabolites. By further increasing IFO from 12 to 18 g/m^2 no augmentation in active IFO metabolites could be achieved. Excretion of unchanged IFO at the higher dose level was double that at the lower. Overall these results suggest that for high-dose continuous IFO therapy 12–14 g/m^2 might be the optimal dose level. In a recent paper [14] it has been questioned if short infusions compared to continuous infusions of the same dose of IFO might be more active. The hypothesis that continuous IFO could inhibit the activation of IFO to a greater extent than after a short infusion would have to be tested in a further study.

Toxicity was predominantly hematological (Table 2) in this mostly intensively pretreated group of patients, with WHO grade 3 and 4 leucopenia in >90% of the cycles. Since we encountered severe leucopenia at level 1 (12 g/m^2/cycle) all patients received GM-CSF thereafter. This allowed further dose escalation up to 18 g/m^2/cycle. At this level, leucopenia with infection was clearly dose limiting and this dose cannot be recommended in pretreated patients. Therefore we consider 16 g/m^2 to be the recommended dose in such patients. Infections, WHO grade 3 and 4, were seen in 15% of all cycles with one toxic death. The unexpected low rate of serious CNS toxicity might be due to administration as a continuous infusion [16]. Nausea occurred frequently and was usually of moderate degree with prophylactic application of 5 HT_3 blockers and steroids. No grade 3 or 4 nephro- or urotoxicity was observed. We also analyzed mesna, cysteine and glutathione pharmacokinetics in these patients [26]. Mesna was highly effective in preventing bladder toxicity and mesna urinary excretion did not change significantly with time. Interestingly however, there was a depletion of circulating total cysteine, glutathione and homocysteine. This marked derangement of sulphydril and disulphide homeostasis could modulate the efficacy and toxicity of ifosfamide/mesna therapy.

The response rate of 41% in this mostly intensively pretreated group of patients was remarkable with 1 CR and 11 PRs in 29 evaluable patients. More significantly, 8 of the 11 patients with a PR had been pretreated with standard-dose IFO, suggesting a dose response relationship for IFO in the treatment of sarcoma patients. Furthermore, a response was seen in three of ten patients with leiomyosarcoma (1 CR and 2 PR) which usually is considered to be resistant to conventional doses of IFO. In this small group of patients we could not study the relationship between IFO metabolism and response to chemotherapy. In view of the heterogeneity of the disease as well as the status of pretreatment, a far higher number of patients would be necessary to study this.

Based on pharmacological considerations, we conclude that in previously treated patients high-dose IFO is optimal at doses of 12–14 g/m^2, with an MTD of 18 g/m^2. For non-pretreated patients the optimal dose for high-dose IFO treatment has yet to be defined. The results of our study indicate that higher doses may produce only limited benefit due to the saturable pharmacokinetics of IFO and its metabolites. With the support of peripheral stem cells, very high-dose IFO-combination regimens with up to 24 g/m^2/cycle are now used with significant toxicities [38]. It might be that such high doses of IFO are not contributing additional therapeutic benefit but result in increased toxicity. Pharmacological studies would provide useful information concerning the likely therapeutic benefit of such high doses. In sarcoma, prior therapy with standard-dose IFO should not exclude patients from further treatment with IFO at higher dose levels. The response rate of high-dose IFO monochemotherapy is promising and should be accompanied by pharmacological studies in order to elucidate individual metabolism on a broader range of patients.

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