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COMPARATIVE PHARMACOKINETICS OF ORAL AND INTRAVENOUS IFOSFAMIDE/MESNA/METHYLENE BLUE THERAPY

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ABSTRACT:

Oral treatment with ifosfamide results in dose-limiting encephalopathy. Methylene blue is effective in reversal and prophylaxis of this side effect. In the present study, the pharmacokinetics of ifosfamide after iv and po therapy in combination with prophylactic administration of methylene blue were investigated. Nine patients with metastatic non–small cell lung cancer were treated by a combination of ifosfamide (3 days), sodium 2-mercaptoethane sulfonate (4 days), and etoposide (8 days). Cycles were repeated every 28 days. Ifosfamide was administered orally, with the exception of one of the first two cycles, when it was administered as a short infusion (randomly assigned). The patients received methylene blue in doses of 50 mg po 3 times daily; an initial dose of 50 mg was given the evening before chemotherapy. Urine samples were collected over the entire treatment period, and concentrations of ifosfamide and its major metabolite, 2-chloroethylamine, were measured by gas liquid chromatography. By the same technique, 2- and 3-dechloroethylifosfamide were determined in plasma and urine. Overall alkylating activity in urine was assayed by reaction of the alkylating metabolites with 4-(4′-nitrobenzyl)-pyridine. The chemotherapeutic regimen was well-tolerated by all of the patients studied. There was no evidence of a shift in the metabolic pattern dependent on the route of administration. From the data, we conclude that methylene blue has a neuroprotective effect and that the pharmacokinetics of ifosfamide are not influenced by its comedication.

Ifosfamide (ifos[1]), encephalopathy is occasionally seen after iv application but occurs regularly after po treatment, which makes it the dose-limiting toxicity for po regimens (Cerny *et al.*, 1986; Wagner and Drings, 1986; Lind *et al.*, 1989; Lind *et al.*, 1990, Manegold *et al.*, 1992; Vincent *et al.*, 1995). Although reversible in most cases, the encephalopathy represents a major disadvantage of ifos and has lead to extended discussions about its clinical value and cost-effectiveness (Kamen *et al.*, 1995). The oral application of ifos in an outpatient setting would therefore be interesting from an economical point of view but would also represent major progress in patient's quality of life. Despite various proposals for management (Scheef, 1983; Schlenzig, 1995; Cerny *et al.*, 1990) and risk assessment of ifos encephalopathy (Meanwell *et al.*, 1986) in the past, this untoward effect often remained not only unpredictive and dose-limiting for oral ifos but also a drawback of iv ifos (Fields *et al.*, 1995), necessitating interruption of chemotherapy (Keizer *et al.*, 1995). This type of encephalopathy might become equally important for new congeners of ifos, which are currently undergoing preclinical study (Pohl *et al.*, 1995; Kutscher *et al.*, 1995). At present, the causative agent of encephalopathy and the reason for the excessive toxicity of oral regimens remain speculative (Cerny and Küpfer, 1992). Based on a systematic investigation of an ifos overdose case, which revealed a glutaric aciduria type II–like biochemical defect, we have successfully used methylene blue (mb) for reversal and prophylaxis of ifos encephalopathy (Küpfer *et al.*, 1994). The effectiveness of mb in the context of ifos encephalopathy has been confirmed by other investigators (Zulian *et al.*, 1995; Ferreiro *et al.*, 1995; Alonso *et al.*, 1996; Demandt and Wandt, 1996).

In order to investigate the prophylactic potential of oral mb, we decided to conduct a clinical study with oral ifos at escalating doses. In order to allow comparison of po and iv pharmacokinetics, each patient was to receive the drug on one of the first two cycles as a short infusion, then orally on all other cycles. After both modes of application, ifos and its major metabolites were measured in urine and plasma. Overall activation of ifos was measured in urine.

Materials and Methods

Experimental Protocol in Man. Nine patients (six men and three women, aged 44 to 71 years; median age, 61 years) with non–small cell lung cancer were included in the study after having given written consent and after approval of the study by the local ethics committee. The oral chemotherapy consisted of an active regimen of ifos and etoposide (Cerny *et al.*, 1989). Ifos was administered orally as a fixed single dose in the morning for 3 days. One of the first two cycles, which was randomly assigned, consisted of short infusions (20 min). The ifos dose was increased from patient to patient during the study, according to clinical observations. Etoposide was administered at a daily dose of 100 mg po for 8 days. For uroprotection, sodium 2-mercaptoethane sulfonate (mesna) was administered intravenously in the range of 60%

[1] Abbreviations used are: ifos, ifosfamide; mb, methylene blue; mesna, sodium 2-mercaptoethane sulfonate; 2-DCE, 2-dechloroethylifosfamide; 3-DCE, 3-dechloroethylifosfamide; NBP, 4-(4′-nitrobenzyl)-pyridine; 2-ClEA, 2-chloroethylammonium chloride; HPLC, high-performance liquid chromatography; *AUC*, plasma concentration-time curve; AUC_{po}, *AUC* after po administration of ifos; AUC_{iv}, *AUC* after iv administration of ifos; *F*, mean bioavailability; *CL*, mean total body clearance.

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of the ifos dose and supplemented by two po mesna doses of 800 mg 4 hr and 8 hr after ifos administration. Methylene blue (50 mg po three times daily) was given for prophylaxis of ifos encephalopathy; an initial dose of 50 mg was given the evening before chemotherapy started. In order to provide supplementation of glucose, the patients were asked to drink at least 2 liters of fruit juice daily. Chemotherapy cycles were repeated every 28 days; urine and blood samples were collected for analysis during the first two cycles. Blood samples were drawn at 0, 0.05, 0.3, 0.6, 3, 8, and 24 hr after the first ifos administration and assayed for ifos and for its dechloroethylated metabolites (2-DCE and 3-DCE) by gas liquid chromatography. Four milliliters of blood was drawn into heparinized tubes and centrifuged, and the plasma was decanted and stored at −20°C until analysis. Urine was collected, starting with the first mb intake on the evening before chemotherapy, for 3 days and pooled to 24-hr samples. Urine samples were stored at −20°C until the time of analysis and assayed for overall alkylating activity (NBP Test), for 2-CIEA, ifos, 2-DCE, and 3-DCE. We also obtained blood and urine samples from one female patient being treated with ifos for recurrent leiomyosarcoma. The patient had a long history of disease and had undergone multiple previous ifos therapies in the past, some of which had to be postponed because of severe encephalopathy episodes. The patient received four additional cycles of ifos, combined with mb for prophylaxis of encephalopathy. During the first two cycles, ifos was administered as a continuous infusion at a dose of 3.2 g daily, combined with doxorubicin and oral mb as described above. The third and fourth cycles of this patient consisted of oral ifos monotherapy combined with mb. Dosage, mode of administration of ifos, scheduling, and accompanying cytostatics of the patients described in the present study are summarized in table 1. Central nervous system side effects of the chemotherapy were staged according to the MD Anderson score (Castallanos and Fields, 1986).

Drug and Metabolite Analysis. *Materials.* Analytical standards for ifos, 2-DCE, 3-DCE, trofosfamide, and hard gelatin capsules containing 400 mg of mesna were a gift from Asta Medica (Wangen, Switzerland). 2-CIEA for synthesis, sodium carbonate, disodium hydrogenphosphate, and acetone were purchased from Merck (Zürich, Switzerland). Methanol (HPLC grade), ethyl acetate *puriss*. and *N*,*N*-dimethylacetamide (internal standard) were obtained from Fluka (Buchs, Switzerland). 4-(4′-Nitrobenzyl)-pyridine (NBP) for synthesis, sodium acetate, and sodium hydroxide were from Merck (Zürich, Switzerland). Diethylether for HPLC and triethylamine *puriss*. were obtained from Fluka (Buchs, Switzerland). Methylene blue Ph H VI was purchased from Hänseler (Herisau, Switzerland). Gelatin capsules containing 50 mg mb were prepared at the pharmacy of the University Hospital of Berne, Switzerland.

Analytical techniques. 2-CIEA in urine was analyzed according to a modified gas liquid chromatographic method of the US Pharmacopoeia XXIII. The gas chromatograph was a Perkin Elmer (Norwalk, CT) model 3920 equipped with a nitrogen- and phosphorus-selective thermionic detector. The stationary phase was 10% Carbowax 20 M 2% KOH on 80/100 Chromosorb WAW (Supelco, Bellefonte, CA), packed in a glass column (o.d. = 1.8 mm, length = 1.8 m). Nitrogen, at a flow of 40 ml/min, served as the carrier gas. The detector gases were hydrogen and air, at a flow rate of 2 and 150 ml/min, respectively. The analysis was performed under isothermal conditions at an oven temperature of 100°C. Injector and detector temperatures were set at 200°C. An integrator model Spectraphysics SP 4920 (San Jose, CA) was used. The extraction procedure was as follows: 300 μl of urine was made alkaline with 300 μl of saturated sodium carbonate solution, 25 μl of 0.1% *N*,*N*-dimethylacetamide was added, and the mixture extracted into 300 μl of ethyl acetate. Calibration graphs in blank urine were linear in the range of 200 μM to 5 mM. The limit of detection was 0.1 mM. Overall alkylating activity in patient urine was measured with the NBP Test. An modified method of Friedman and Boger (Friedman and Boger, 1961; Preussmann *et al.*, 1969) was used. Five hundred microliters of urine was diluted with 1 ml of 2 M sodium acetate buffer (pH, 4.6), and 500 μl of 10% NBP in acetone was added. After mixing on the vortex mixer, the sample was placed in a heating block at 100°C for 40 min. After cooling, 4 ml of 5% triethylamine in diethylether and 1 ml of acetone were added. After mixing on the vortex mixer, 1 ml of sodium hydroxide 6N was added. After mixing, the absorption of the organic phase was measured at 540 nm. A Pye Unicam PU 8610 UV/VIS spectrophotometer (Cambridge, UK) was used. In order to obtain quantitative results, a calibration graph was constructed by adding known amounts of 2-CIEA to blank urine. The graph was linear in the range of 150 μM to 5 mM. Results were expressed as equivalents of 2-CIEA. Addition of mesna or mb did not interfere with the assay. Ifos, 2-DCE, and 3-DCE were determined in plasma and urine by means of capillary gas liquid chromatography with nitrogen- and phosphorus-selective thermionic detection, according to a modified method by Kurowski and Wagner (Kurowski and Wagner, 1993). The gas chromatograph was a Hewlett Packard HP-5890 series II (Palo Alto, CA). The column was a capillary column HP PAS-1701 (length = 10 m; o.d. = 0.32 mm; film thickness = 0.25 μm). Detector and injector temperatures were kept at 250°C. The carrier gas was nitrogen, at a flow rate of 1.7 ml/min; the initial column pressure was kept at 5 pounds per square inch. Data was acquired with a Hewlett Packard HP-3392 integrator. Calibration graphs were constructed daily by adding known amounts of ifos, 2-DCE, 3-DCE, and trofosfamide (for internal standardization) to bovine plasma and human blank urine, respectively. All standard solutions were kept at −20°C and were stable for 8 weeks. Calibration graphs in bovine plasma were linear in the range of 5 μM to 200 μM for the unchanged drug and of 3.5 μM to 125 μM for 2-DCE and 3-DCE, the detection limit being 4 μM for ifos and 2.5 μM for 2-DCE and 3-DCE. Calibration graphs in urine were linear in the range of 4 μM to 1.5 mM for the unchanged drug and of 25 μM to 1 mM for 2-DCE and 3-DCE, the detection limit in urine being 4 μM for ifos and 25 μM for 2-DCE and 3-DCE. As for the determination in patient plasma, 200 μl of plasma was transferred into a conical tube, and 50 μl of a solution of trofosfamide 200 μg/ml as internal standard was added. The sample was diluted with 100 μl of distilled water and 1 ml of ethyl acetate was added. The samples were subsequently shaken vigorously by means of a vortex rotation mixer, and phase separation was achieved by centrifugation. The organic phase was collected and evaporated in a water bath at a temperature of 40°C under a gentle stream of nitrogen. The residue was redissolved in 200 μl ethyl acetate and 1 to 2 μl of residue was injected into the gas chromatograph. The oven temperature was kept at 190°C for 2 min and was then raised to 200°C at a rate of 10°C/min; the final temperature of 200°C was kept for 13 min, and the split ratio of the carrier gas was 1:10. As for the analysis of patients' urine samples, 50 μl of urine was diluted with 100 μl distilled water, and 50 μl of a solution of trofosfamide (200 μg/ml) was added for internal standardization. Fifty microliters of 0.1 M disodium hydrogenphosphate buffer (pH 9.3) was added, and the mixture was subsequently extracted into 750 μl of ethyl acetate. After centrifugation, 1 to 2 μl of the organic phase was used for analysis. The initial oven temperature was 120°C and was raised to 210°C at a rate of 20°C/min; the final temperature of 210°C was kept for 11.5 min, and the split ratio of the carrier gas was 1:5. The extraction recovery rates from plasma were 92.9% for ifos, 32.7% for 2-DCE, 32.4% for 3-DCE, and 101.7% for trofosfamide. For urine, the recovery rates were 80.8% for ifos, 39.9% for 2-DCE, 33.7% for 3-DCE, and 88.2% for trofosfamide. The inter-day coefficient of variation was 7.14% for ifos, 19.4% for 2-DCE, and 37.45% for 3-DCE. The intra-day coefficient of variation was 2.34% for ifos, 5.03% for 2-DCE, and 5.0% for 3-DCE.

Pharmacokinetics and Calculations. A non-compartmental approach was used to estimate clearance (*CL*) and bioavailability (*F*) of ifos. The areas under the plasma concentration-time curves (AUC) of ifos, 2-DCE, and 3-DCE were calculated, applying the trapezoidal rule. Total body clearance of ifos was calculated by dividing the administered dose by the AUC of ifos. Bioavailability of ifos was calculated by dividing the AUC after po administration (AUC_{po}) by the AUC after iv administration (AUC_{iv}) for each subject on each day of treatment. Urinary recovery values were calculated for ifos, 2-DCE, 3-DCE, and 2-CIEA and expressed as percentages of the total ifos dose administered. The percentage of 2-CIEA excreted in urine was calculated under the assumption that 1 mole of ifos generates 2 moles of 2-CIEA. Total alkylating activity in urine was determined as equivalents of 2-CIEA and then expressed as percentages of total dose. To compare parameters between the iv and the oral group, the Mann-Whitney test was performed.

Results

The chemotherapeutic regimen presented in this study was well-tolerated in all patients, with no occurence of central nervous system toxicity after either mode of ifos application in mb-protected patients during the first two cycles. In the following po cycles, however, four cases of encephalopathy in a total of 36 po cycles were observed (table 1). This was due to compliance problems in ambulatory patients

TABLE 1

Patient population, dosage, scheduling, accompanying cytostatics and cases of encephalopathy in the present study

Patient	Diagnosis	Site	Sex	Age	Nr. cycle studied	Ifos Dosage	Mode of application	Mesna Dosage	Other Cytostatic	Total Number of PO Cycles	Encephalopathy Grade After PO	MB Taken	Total Number of IV Cycles	Encephalopathy Grade After IV	MB Taken
						g/d, d_1–d_3		*d_1–d_3*							
BC	SCLC	Bone Brain	M	71	1	2.5	po	60% of ifos dose iv 2 × 800 mg po	Etoposide	6	1×4*	Not fully	1	0	Yes
					2	2.5	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide						
GA	SCLC	Extensive	M	67	1	2.5	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide	5	0	Yes	1	0	Yes
					2	2.5	po	60% of ifos dose iv 2 × 800 mg po	Etoposide						
BW	SCLC	Brain	M	44	1	3	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide	2	1×1	NO	1	0	Yes
					2	3	po	60% of ifos dose iv 2 × 800 mg po	Etoposide						
WE	NSCLC	Lung	F	62	1	3	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide	4	0	Yes	1	0	Yes
					2	3	po	60% of ifos dose iv 2 × 800 mg po	Etoposide						
FS	Sarcoma	Uterus	F	59	1	3	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide	3	0	Yes	1	0	Yes
					2	3	po	60% of ifos dose iv 2 × 800 mg po	Etoposide						
GD	SCLC	Extensive	F	52	1	2	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide	3	0	Yes	1	0	Yes
					2	2	po	60% of ifos dose iv 2 × 800 mg po	Etoposide						
KP	NSCLC	Bone Brain	M	52	1	2	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide	3	0	Yes	1	0	Yes
					2	2	po	60% of ifos dose iv 2 × 800 mg po	Etoposide						
HM	NSCLC	Bone Lung	M	70	1	2.5	po	60% of ifos dose iv 2 × 800 mg po	Etoposide	5	1×4	No	1	0	Yes
					2	2.5	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide						
BG	SCLC	Bone	M	57	1	3	iv short	60% of ifos dose iv 2 × 800 mg po	Etoposide	2	1×1	Yes	1	0	Yes
					2	3	po	60% of ifos dose iv 2 × 800 mg po	Etoposide						
GR	Metastatic		F	61	1	3.2	cont. iv	2.8 g cont iv/d	doxorubicin	1					
	recurrent				2	3.2	cont. iv	2.8 g cont iv/d	doxorubicin	2					
	leiomyosarcoma				3	2, 2.5, 2 × 1.5	po	2.8 g cont. iv/d		3	0	Yes	2	0	Yes
					4	2 × 1.5	po			4					

NSCLC, non–small cell lung cancer; SCLC, small cell lung cancer; d, day; cont, continous; short, short infusion.
* Gastrointestinal-intolerant to mb, received mb iv, no further encephalopathy with oral ifos and mb iv in cycles 3–6.

TABLE 2

Pharmacokinetics and total akylating activity in urine on 3 successive days of ifosfamide administration

Parameter	*N*		IV			PO		
	iv	po	Day 1	Days 1–2	Days 1–3	Day 1	Days 1–2	Days 1–3
Recovery ifos (% dose)	10	10	5.3 ± 1.6	10.8 ± 2.5	15.6 ± 4	4.9 ± 2.7	9.4 ± 4.8	13.6 ± 6.3
Recovery 2-DCE (% dose)	10	10	0.9 ± 0.5	2 ± 1	3.7 ± 1.7	0.4 ± 0.5	1.4 ± 1	4.6 ± 4.9
Recovery 3-DCE (% dose)	10	10	1.4 ± 0.4	3.8 ± 0.8	6.9 ± 1.5	1.3 ± 0.4	4.2 ± 1.6	7.0 ± 2.2
Recovery 2-CIEA (% dose)	6	6	4.1 ± 4.3	8.9 ± 6.7	12.7 ± 6.7	3.2 ± 1.2	5 ± 3.1	6.5 ± 3.3
NPB in urine (% dose)	4	4	4.5 ± 1.2	10 ± 2.4	17.8 ± 6.2	3.9 ± 0.6	11.5 ± 1.8	21 ± 2.7

N, number of patients evaluated; iv, intravenous; po, peroral; recovery, cumulative urinary recovery; ifos, ifosfamide; 2-DCE, 2-dechloroethylifosfamide; 3-DCE, 3-dechloroethylifosfamide; 2-CIEA, 2-chloroethylamine; NBP, NBP test for total alkylating activity. All values are expressed as mean ± standard deviation.

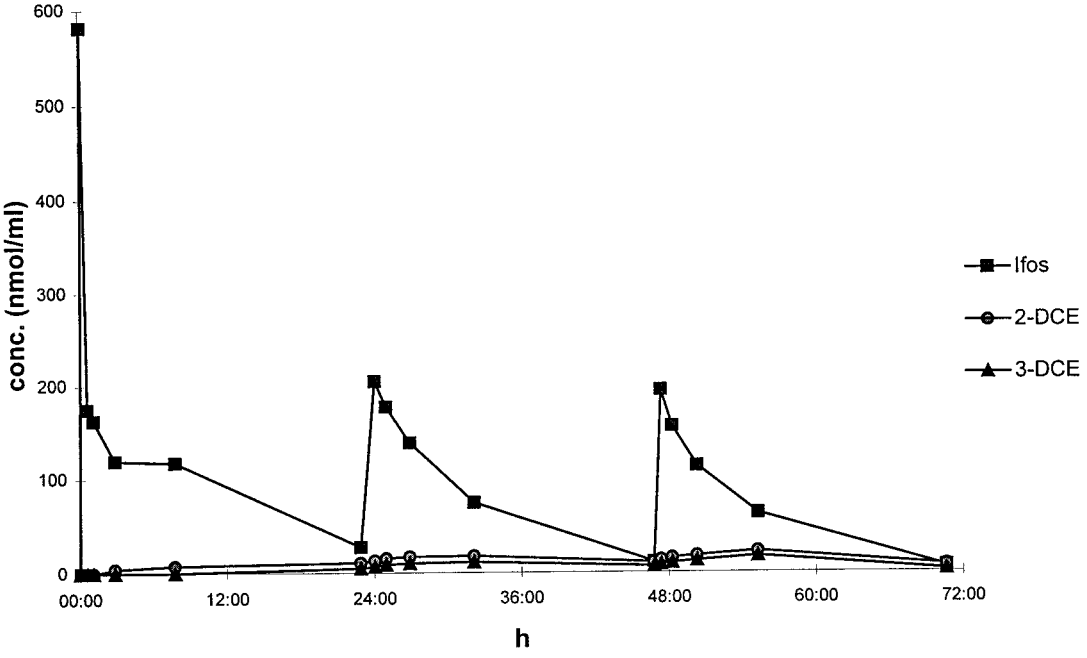

FIG. 1. *Plasma concentration vs. time curve in one representative patient receiving 3 g ifos iv once daily for 3 days.*

in three instances (no mb taken) and, in one further patient, to uncontrolled emesis with mb.

Ifos and its dechloroethylated metabolites could be detected in the plasma and urine of all patients (table 2). Figures 1 and 2 display the plasma concentration *vs.* time curve for ifos, 2-DCE, and 3-DCE after po and iv application in one representative patient, who had received a fixed dose of 3 g ifos daily. 2-CIEA was measured in the urine of six patients. This metabolite was not detectable in plasma when the methodology identical to that for urine was applied. For all pharmacokinetic parameters evaluated, the interindividual variability was pronounced. After oral administration, ifos was demonstrated to be completely bioavailable on each day of chemotherapy, expressed as the mean bioavailability (*F*), calculated from 10 patients and 11 courses (table 3). The AUC values displayed in table 3 are not corrected for the patients' body surface or mass, which explains why no linear relationship between dose and AUC is observed. The mean total body clearance of ifos (*CL*), calculated from 10 patients and 11 courses, was not statistically significant different between the two modes of application (table 3). Dechloroethylation of ifos was demonstrated to be independent of route of administration on the second and third days of chemotherapy, as reflected by the mean po/iv ratio of the AUCs for 2-DCE and 3-DCE being close to 1 (table 3). On the first day of chemotherapy, this ratio was markedly higher than 1, indicating a higher dechloroethylation after po administration, but here the excessively large standard deviation has to be taken into consideration. The interindividual comparison of the two routes of administration on the first day of chemotherapy is displayed in fig. 3. Ifos was completely bioavailable in all of the patients studied. Two patients (KP and BC) had markedly higher plasma levels of 2-DCE and 3-DCE, respectively, after po administration on the first day of treatment. As for the urinary pharmacokinetics, no statistically significant difference in the overall recovery of ifos, the dechloroethylated metabolites, or 2-CIEA and NBP activity could be demonstrated for the two routes of administration (fig. 4). Independently of route of administration, an increase in ifos metabolism within the chemotherapy cycle, reflected by an increase in average ifos clearance (table 3) matched by a decrease in average ifos AUC (table 4), was observed in all patients. In contrast, higher plasma concentrations of the dechloroethylated metabolites at the end, compared with those of the first day of chemotherapy, were measured in all but one patient (table 4). Excretion of the parent drug in urine decreased during chemotherapy, matched by an increasing excretion of both of the alkylating metabolites (NBP activity) and the dechloroethylated metabolites (fig. 5). A markedly stronger enhancement of ifos metabolism after po, compared with iv, administration was observed only in patient BC; in all other subjects, the degree of enhancement of ifos metabolism was similar in both routes of administration (table 4). In all of the patients studied, the degree of dechloroethylation during chemotherapy increased independently of the route of administration, with the excep-

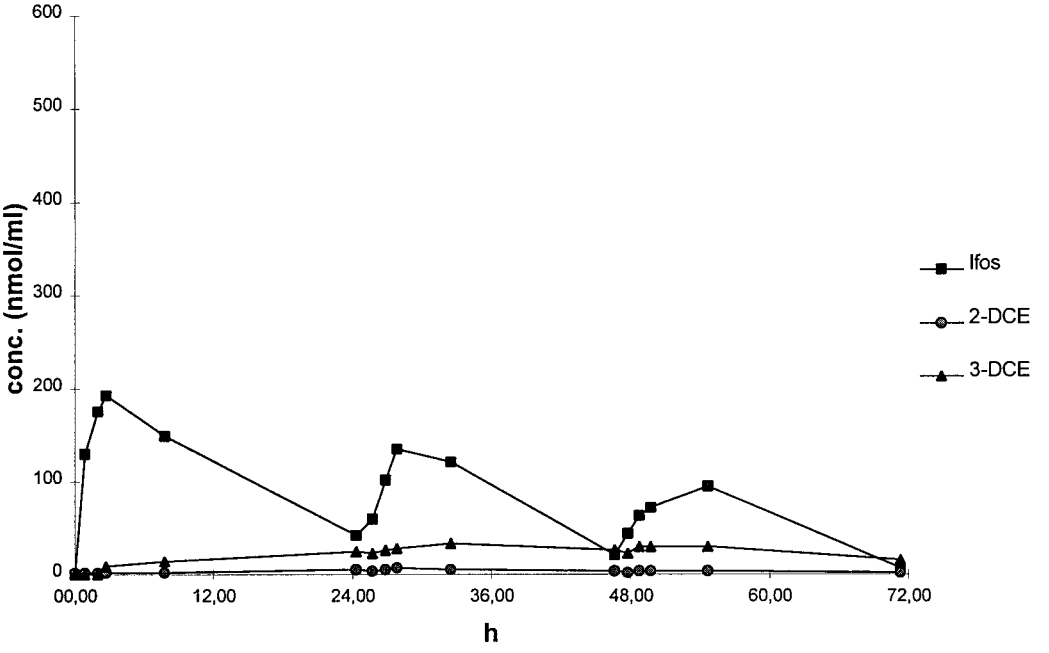

FIG. 2. *Plasma concentration vs. time curve in one representative patient receiving 3 g ifos po once daily.*

TABLE 3

Pharmacokinetics in plasma on 3 successive days of ifosfamide administration

Parameter	*N*		IV Bolus			PO		
	iv	po	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3
AUC_{ifos} (mg · hr/liter) 3 g ifos/day	3	3	603 ± 148	448 ± 58	379 ± 80	599 ± 166	415.7 ± 50	327 ± 67
AUC_{ifos} (mg · hr/liter) 2.5 g ifos/day	2	2	574 ± 40	409 ± 40	329 ± 36	860 ± 280	440 ± 39	365 ± 4
AUC_{ifos} (mg · hr/liter 2 g ifos/day	3	3	637 ± 88	456 ± 128	310 ± 72	646 ± 109	454 ± 68	373 ± 51
CL_{total} ifos (L/hr)	9	10	3.7 ± 1.6	5.2 ± 2.1	6.1 ± 2.6	3.1 ± 1.4	4.6 ± 2	5.6 ± 2.7
F		9				1.2 ± 0.3	1 ± 0.2	0.96 ± 0.3
AUC/AUC_{iv} 2-DCE		9				5.2 ± 10.2	0.9 ± 0.3	0.9 ± 0.1
AUC_{po}/AUC_{iv} 3-DCE		9				2.7 ± 4.6	1.1 ± 0.7	1.1 ± 0.5

CL_{total}, total body clearance, calculated as dose divided by area under the plasma time-concentration curve (*AUC*); *F*, bioavailability of ifosfamide, calculated as AUC_{po} divided by AUC_{iv}; AUC_{po}/AUC_{iv}, area under the plasma concentration time curve po divided by area under the plasma concentration time curve iv. All values are expressed as mean ± standard deviation.

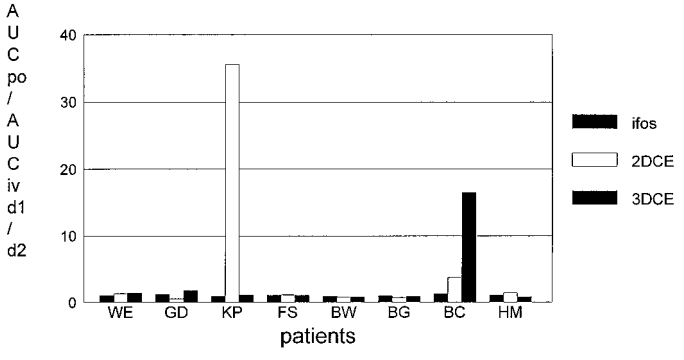

FIG. 3. *Interindividual comparison of bioavailability of ifos and the first-pass metabolism of 2-DCE and 3-DCE after 24 hr of chemotherapy.*

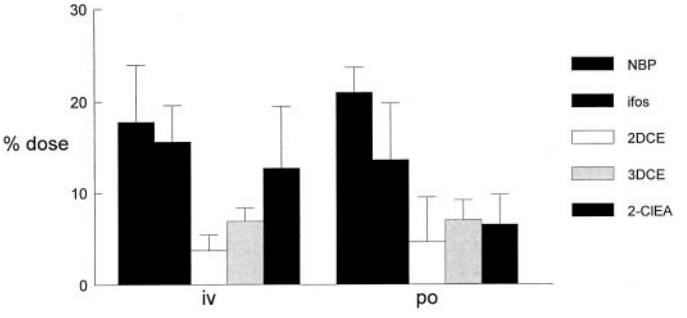

FIG. 4. *Comparison of overall urinary recovery of unchanged drug and major metabolites of ifos, in % of dose after 3 days of iv and po administration.*

tion of patient BC, who showed less dechloroethylation at the end of his po cycle, compared with the first day of po chemotherapy.

Discussion and Conclusions

According to the experience and the literature presented so far (summary in table 5), the maximal daily single dose of 3 g ifos used in the present study (corresponding approximately to 1.8 g/m^2) administered to four patients should be associated with a high risk of encephalopathy. In an almost identical protocol conducted with oral ifos and etoposide, but without mb, central nervous system toxicity was reported to occur in 30% of all cycles, necessitating even termination of treatment in one case (Cerny *et al.*, 1989). The data collected so far indicate a good clinical efficacy of mb in the prevention of ifos encephalopathy.

There is a wealth of data available concerning the pharmacokinetics of ifos. The data presented herein is in good accordance with previously published results. We found no significant shift in the metabolic pattern dependent on route of administration. This confirms the orig-

TABLE 4

Autoinduction: intraindividual comparison ratio $AUC_{day\ 1}$ divided by $AUC_{day\ 3}$ for ifosfamide and dechloroethylated metabolites

Patient	Ifos Dose	IV				PO			
		Ifos	2-DCE	3-DCE	Ifos	Ifos	2-DCE	3-DCE	Ifos
	g/day	AUC_{d1}/AUC_{d3}	AUC_{d1}/AUC_{d3}	$AUC_{d1}AUC_{d3}$	Cl_{d1}/Cl_{d3}	$AUC_{d1}AUC_{d3}$	$AUC_{d1}AUC_{d3}$	$AUC_{d1}AUC_{d3}$	Cl_{d1}/Cl_{d3}
WE	3	1.58	0.64	0.23	0.63	1.72	0.8	0.34	0.58
GD	2	1.84	0.61	0.57	0.29	1.83	0.38	0.64	0.55
KP	2	1.7	0.03	0.74	0.59	1.64	1.08	0.76	0.61
FS	3	1.7	0.54	0.58	0.59	2.15	0.92	0.78	0.46
BW	3	1.43	0.62	0.57	0.70	1.43	0.46	0.42	0.70
BG	3	1.62	0.51	0.56	0.62	1.93	0.38	0.59	0.52
BC	2.5	1.68	0.49	0.17	0.59	3.16	1.77	1.17	0.32
GR		Cont. infusion	Cont. infusion	Cont. infusion	No fixed dose	No fixed dose	No fixed dose	No fixed dose	0.15
HM	2.5	1.82	0.35	0.68	0.55	1.57	0.52	0.56	0.64
Mean		1.67 ± 0.123	0.47 ± 0.189	0.51 ± 0.191	0.57 ± 0.11	1.93 ± 0.48	0.8 ± 0.42	0.8 ± 0.46	0.50 ± 0.16

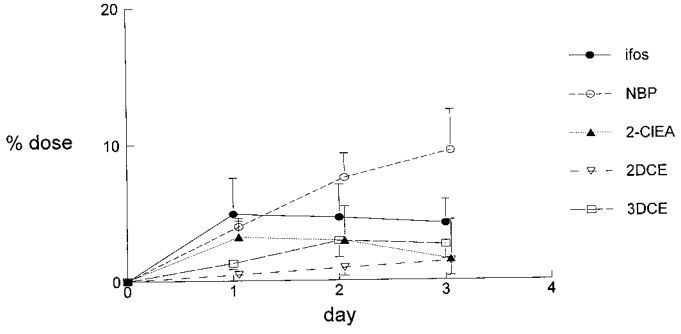

FIG. 5. *Urinary excretion of ifos and its major metabolites after po administration, expressed as % of dose.*

inal finding of an almost complete bioavailability of ifos (Cerny *et al.*, 1986; Wagner and Drings, 1986). In order to eliminate the effect of metabolism enhancement, we chose the data from day one for comparison with other studies. As for the iv bolus administration, Boos and coworkers (Boos *et al.*, 1991) investigated the urinary excretion of ifos, 2-DCE, and 3-DCE in children after various treatment schedules and found that a mean of 21% ± 6% of the applied ifos dose was excreted unmetabolized; the overall urinary recovery of 2-DCE was 4% ± 2%, and that of 3-DCE was 14% ± 5%. Kurowski and coworkers (Kurowski *et al.*, 1993) investigated the pharmacokinetics of ifos and its metabolites after a 5-day fractionated iv bolus therapy at a dose of 1.5 g/m^2. They reported a geometric mean for the AUC of ifos on day one of 1780 nmol · h/ml (corresponding to 464.6 mg · h/liter), 111 nmol · h/ml (22 mg · h/liter) for the AUC of 2-DCE, and 146 nmol · h/ml (29 mg · h/liter) for the AUC of 3-DCE. As for the iv application, we have determined a urinary recovery of ifos of 15.6% ± 4% and a recovery of 3.7% ± 1.7% and 6.9% ± 1.5% for 2-DCE and 3-DCE, respectively. Lind and coworkers reported a median AUC of 680.651 μg · h/ml and a median total body clearance of 51.334 ml/min (corresponding to 3 liters/hr) after application of oral ifos as single agent at a dose level of 1.5 g/m^2 on the first day of treatment. A mean urinary recovery of 6.49% (range, 0.61%–26.63%) is reported for the sum of both dechloroethylated metabolites on the first day of treatment, displaying a large interpatient variability for dechloroethylifosfamide excretion (Lind *et al.*, 1990). We calculated a mean AUC of 599 ± 166 mg · h/liter, a mean total body clearance of ifos of 3.1 ± 1.4 liters/hr, and a mean urinary recovery of 0.5% and 1.4% for 2-DCE and 3-DCE, respectively, for a fixed dose of 3 g ifos daily.

No statistically significant difference in cumulative urinary NBP activity after po ifos, compared with iv administration, was observed. This finding confirms the results of Lind and coworkers, who found no statistically significant difference between po and iv administration of ifos concerning overall excretion of alkylating metabolites (Lind *et al.*, 1990). These authors report a median value for total alkylating metabolite excretion of approximately 22% for the oral group as determined by thin-layer chromatography with NBP detection. Although we studied another protocol and used a different analytical

TABLE 5

Previously published studies conducted with po administration of ifosfamide

Authors and Year of Publication	Number of Patients/Cycles	Total dose of po Ifos per Cycle	Duration of Administration (days)	% Incidence of encephalopathy	Comparison With iv Application
McNeil and Morgan, 1981	5/9	5 g	10	0	Yes
	8/8	10 g	10	0	
	2/2	15 g	10	0	
Cerny *et al.*, 1986	7	1 g	1	0	Yes
	7	2 g	1	0	
	3	5 g	1	100	
Wagner and Drings, 1986	12/12	1 g/m^2	1	0	Yes
	6/6	2 g/m^2	1	80	
Cerny *et al.*, 1989	65/390	6 g	3	30	No
Lind *et al.*, 1989 and 1990	10/10	7.5 g/m^2	5	50	Yes
Kurowski *et al.*, 1991	7/7	1 g/m^2	1	0	Yes
	5/5	1.5 g/m^2	1		
Manegold *et al.*, 1992	15/?	3 g/m^2 = 5 g	5	13	No
	19/?	6 g/m^2 = 10.5 g	14	42	
	23/?	7.5 g/m^2 = 14 g	14	35	
	7/?	10 g/m^2 = 17.5 g	14	56	
Kurowski and Wagner, 1993	11/10	7.5 g/m^2	5	0	No
Vincent *et al.*, 1995	38/38	500 mg/day	Continuous	17	No

technique, our average recovery of alkylating metabolites in urine after oral administration, calculated from four patients, accounts for 21%, which is close to the finding of Lind *et al.*. This implies that mb does not interfere with hepatic activation of ifos. Measurements of the active metabolite 4-OH-ifosfamide, as described by Kurowski and coworkers (Kurowski *et al.*, 1991) was not carried out in this study. Kurowski *et al*. reported higher levels of 4-OH-ifosfamide after po administration of ifos, compared with iv administration. Recent studies of ours, performed in recirculating rat liver perfusions, indicate that bioactivation of ifos is not impaired by mb (Aeschlimann *et al.*, 1997).

As for the pathway of oxidative *N*-dealkylation, which leads to formation of 2-DCE and 3-DCE and the potentially toxic by product 2-chloroacetaldehyde, no statistical difference between the two routes of administration was observed. However, the large interindividual variability concerning *N*-dealkylation after po administration on day one is noteworthy. Two patients of ten studied displayed a marked *N*-dechloroethylation of ifos after po administration on day one; it is interesting to note that these two patients were free of central nervous system toxicity. Lind and coworkers made similar observations, as cited from their publication of 1990: "Disappointingly, urinary dechloroethyl-ifosfamide levels failed to correlate with the development and severity of neurotoxicity. . ."; however, in this study, no plasma levels of the dechloroethylated metabolites nor 2-chloroacetaldehyde was measured in plasma or urine. It was already reported in 1989 (Cerny T, Küpfer A, Stabilization and quantitive determination of the neurotoxic metabolite chloroacetaldehyde in the plasma of ifosfamide patients, ECCO5, abstract 147, 1989) that ifos patients can exhibit higher plasma levels of 2-chloroacetaldehyde than the toxic threshold suggested by Goren and coworkers (Goren *et al.*, 1986), which casts some doubt on the hypothesis that 2-chloroacetaldehyde is the sole causative agent of central nervous system toxicity. Secondary metabolites of 2-DCE and 3-DCE have been identified in the rat (Wang and Chan, 1995). Generation of these secondary metabolites is partly coupled to the release of 2-chloroacetaldehyde. At least theoretically, 2-CIEA is also a potential source of 2-chloroacetaldehyde in ifos patients, being a substrate of plasma amine oxidase (Neumann *et al.*, 1975). When all of this is considered together, we conclude that measurements of 2-DCE and 3-DCE might not adequately reflect generation of 2-chloroacetaldehyde. The relative toxicity of hepatic and extrahepatic formation of 2-chloroacetaldehyde (Aeschlimann *et al.*, 1997) and the positive influence of mb on the intrahepatic redox status, which controls the systemic availability of 2-chloroacetaldehyde, has recently been discussed (Küpfer *et al.*, 1996).

The phenomenon of increased metabolism during ifos chemotherapy has been well documented in the past by several authors and has been investigated systematically by Lewis (Lewis *et al.*, 1990). This observation has generally been explained by autoinduction. Large interpatient variability of urinary excretion of 2-CIEA was also found. It has been shown that 2-CIEA can combine with bicarbonate, yielding oxazolidin-2-one (Highley *et al.*, 1995). We assume that the large interpatient variability is due to variable pH and bicarbonate content in the urine. The role of 2-CIEA and oxazolidin-2-one in the context of ifos encephalopathy is still unclear. The bicarbonate loss occurring under ifos chemotherapy (Fields *et al.*, 1995) might not only decrease the formation of oxazolidin-2-one from 2-CIEA but might also necessitate supplementation of acetate or glucose. The potential of acetate as an antidote for encephalopathy remains hypothetical as long as the role of oxazolidin-2-one in ifos encephalopathy is unclear. The assay described in this article was not sensitive enough for measurement of 2-CIEA in plasma; nevertheless, Highley and coworkers documented the presence of 2-CIEA in the plasma of ifos patients by using another method (Montmerency and Van Cauwenberghe, 1994).

From the present study, we conclude that mb is effective in prevention of ifos encephalopathy and that the pharmacokinetics of ifos are not influenced by mb comedication. Further investigations concerning the dosing, pharmacokinetics, and mechanism of action of mb have to be conducted.

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