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Alkylating activity in serum, urine, and CSF following high-dose ifosfamide in children

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Summary. The pharmacokinetics of alkylating activity were studied in 17 children treated i.v. with ifosfamide (IF) at 3 g/m^2 as a 1-h infusion for 2 consecutive days every 3 weeks, with mesna as a uroprotector. Two patients were treated for a newly diagnosed rhabdomyosarcoma according to the current SIOP (International Society of Pediatric Oncology) protocol. The other 15 patients were treated in a phase II study and presented with one of the following malignancies in relapse: neuroblastoma (7), osteosarcoma (3), soft tissue sarcoma (2), Wilms' tumor (1), non-Hodgkin's lymphoma (1), and acute lymphoblastic leukemia (1). Plasma alkylating activity levels determined by using 4(4′-nitro-benzyl)-pyridine showed considerable inter-individual and intercyclic variations and decreased biphasically, with mean α and β half-lives of 60 min and 6–7 h, respectively. Probably as a result of liver mixed-function oxidase induction, on the 2nd day of treatment the terminal half-lives were shorter, the plasma exposures were lower, and the mean plasma clearances were higher. Renal excretion was almost complete after 24 h, accounting for a mean of 19% of the injected dose. The CSF alkylating activity levels, obtained in four children, were always lower than the plasma levels and ranged from 8 to 51 μg/ml, with a mean CSF/plasma ratio of 0.53 ± 0.23 during the first 12 h. We conclude that IF alkylating activity was biphasically cleared from the plasma, with significant interindividual and intercyclic variability, that the renal contribution to the clearance was low, and that high levels of CSF alkylating activity could possibly contribute to the CNS toxic side effects observed in pediatric patients treated with high-dose IF/mesna.

Introduction

The oxazaphosphorine ifosfamide (isophosphamide or Holoxan, IF) is an analog of cyclophosphamide (Endoxan) whose chemical structure differs from Endoxan in that the two alkylating chloroethyl groups are not on the same nitrogen residue [3]. The main advantage it has over its congener is lower myelotoxicity, enabling higher doses to be given [1, 9]. However, early clinical trials in adults [9] and children [11] have established hemorrhagic cystitis as the major dose-limiting toxicity.

A 1-h infusion of IF given on 2 consecutive days in combination with vincristine has been shown to be effective in some pediatric tumors, with mesna (sodium 2-mercaptoethane sulfonate) as a bladder protector [10]. The paucity of pharmacokinetic data recorded in children following IF administration [17] stimulated us to determine the levels of alkylating activity in biological fluids after the i.v. administration of IF to children. Among others, the objectives of this study were to confirm the recently reported evidence that after repeated administration, the half-lives and the area under the plasma concentration vs time curves decreased and the plasma clearance increased as a result of induction of hepatic enzymes [12]. We were also interested in verifying alkylating activity in the CSF after i.v. administration of IF, since CNS toxicity occurs following the treatment of adult [9] and pediatric [18] subjects with IF and mesna.

Patients and methods

Patients. A total of 17 children aged from 2 to 16 years (median, 6 years) were given 3 g/m^2 IF as a 1-h infusion for 2 consecutive days every 3 weeks. Mesna was used as a uroprotector. Two patients were treated for a newly diagnosed rhabdomyosarcoma according to the current SIOP (International Society of Pediatric Oncology) protocol for malignant mesenchymal tumors. The remaining 15 patients were treated with IF in a phase II study and presented with one of the following malignancies in relapse: neuroblastoma (7), osteosarcoma (3), soft-tissue sarcoma (2), Wilm's tumor (1), non-Hodgkin's lymphoma (1), and acute lymphoblastic leukemia (1).

Sampling. The pharmacokinetics of IF were studied on at least one and usually several occasions in plasma (15 patients), urine (8 patients), CSF (4 patients), and ascites (1 patient). Blood samples (2–3 ml) were taken before and at the end of the 1-h infusion and 1, 2, 3, 4, 7, 11, 15, 19, and 23 h after IF administration for the 2 consecutive days. Urine samples were collected every 4 h up to 48 h after therapy and kept at 4° C prior to analysis. CSF was collected by lumbar puncture or through an Omaya reservoir.

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Determination of IF alkylating activity

A colorimetric assay for cyclophosphamide has been developed by Friedman and Boger [6], based on the reaction of alkylating metabolites with 4-(4′-nitrobenzyl)-pyridine (NBP). The generated quaternary pyridinium compound is transformed at basic pH into a highly colored pigment that absorbs at 575 μm. This tedious and difficult method has been modified by Christian et al. [4] to enable higher stability of the coloration, this modified technique was used in the present study for IF.

To 500 μl biological fluid (plasma, urine, CSF, or ascitic fluid), 1.5 ml perchloric acid (2.67%) was added. After mixing and centrifugation for 3 min in a bench centrifuge at full speed (2,000 *g*), 0.5 ml of the clear supernatant was heated for 20 min at 100° C and then cooled, after which 250 μl 0.2 *M* acetate buffer (pH 4.6), 250 μl 0.6 *N* NaOH, and 375 μl 3.3% NBP in acetone were added. After this mixture was heated at 100° C for 20 min and cooled, 1.25 ml triethylamine-acetone (1:1 v/v) was added. The mixture was vortexed and centrifuged for 3 min at 2,000 *g*, and the absorbance of the clear supernatant was read at 575 nm.

Pharmacokinetic analysis

Plasma alkylating activity levels (C) vs time (t) data obtained for each day, treatment, and patient were fitted to a biexponential equation. The pharmacokinetic parameters were calculated, assuming an open two-compartment model for IF disposition, as follows [15]:

$\ln C = \ln (A \cdot e^{-\alpha \cdot T} + B \cdot e^{-\beta \cdot t})$
AUC = area under the C vs t curve = $(A/\alpha) + (B/\beta)$
Cl = systemic clearance = dose/AUC
k_{el} = elimination constant = $\alpha \cdot \beta \cdot (A + B)/(A \cdot \beta + B \cdot \alpha)$
V_d = apparent volume of distribution = Cl/K_{el}
$t_{1/2}\alpha$ = first-phase half-life = $\ln 2/\alpha$
$t_{1/2}\beta$ = second-phase half-life = $\ln 2/\beta$

The individual pharmacokinetic parameters obtained for each treatment and patient were averaged and are reported in Table 1. The mean plasma levels of alkylating activity were averaged for each patient and from the pharmacokinetic parameters deduced from them, the C vs t curves for 12 patients were plotted (Fig. 1).

Table 1. Mean pharmacokinetic parameters of alkylating activity after the i.v. infusion of IF in children on 2 consecutive days

Number	Patient	From plasma data:								From urinary data:	
		n[a]	Co (μg/ml)	Vd (l/m^2)	AUC ($kg \cdot h \cdot l^{-1}$)	Kel (h^{-1})	Cl ($l \cdot h^{-1} \cdot m^{-2}$)	$t_{1/2}\alpha$ (min)	$t_{1/2}\beta$ (h)	% Dose elim	$t_{1/2}\beta$ (h)
Day 1:											
1	J.F.D.	1	448	19.2	1.33	0.29	2.26	41.9	7.7		
2	F.G.	3	189	37.0	1.57	0.13	2.03	44.5	8.1		
3	O.T.	3	366	25.4	1.02	0.31	3.04	57.8	6.4		
4	J.H.	2	99	58.7	0.79	0.15	6.55	6.3	5.7	22.6	4.2
5	P.K.	10	120	50.8	0.93	0.17	6.47	87.6	6.3	13.7	8.6
6	C.M.	8	582	28.8	2.93	0.28	2.05	35.9	5.9	19.3	8.1
7	N.D.	9	286	25.3	2.47	0.23	2.70	30.9	7.5	14.2	7.0
8	J.D.	1	97	36.0	0.76	0.13	3.95	–	3.2	–	–
9	G.M.	10	262	38.5	3.41	0.41	4.98	34.0	5.5	17.2	6.4
10	F.M.	3	149	26.4	3.18	0.17	3.57	152.5	4.9	19.2	4.2
11	J.S.	1	127	43.6	1.08	0.12	2.65	42.4	12.0	26.1	12.2
12	S.S.	1	141	66.2	0.70	0.20	4.72	112.9	13.5		
13	M.D.	1						–	8.7		
14	D.F.	1								17.4	5.4
15	L.K.	1						–	5.8		
16	M.M.	1						–	2.4		
Mean			239	38.0	1.68	0.22	3.74	58.8	6.9	18.7	7.0
±SD			156	14.5	1.02	0.09	1.61	42.4	2.9	4.1	2.6
Day 2:											
1	J.F.D.	1	270	17.2	0.97	0.26	3.16	106.8	6.1		
2	F.G.	3	485	22.7	1.01	0.48	3.01	26.7	6.5		
3	O.T.	3	699	38.1	0.66	0.22	5.01	66.5	6.0		
4	J.H.	2	614	40.3	1.24	0.54	2.45	18.0	3.8	32.1	4.8
5	P.K.	8	119	86.9	0.68	0.22	9.30	51.5	4.5	12.0	6.2
6	C.M.	7	394	24.6	2.24	0.33	2.73	51.6	5.3	23.5	6.0
7	N.D.	8	287	29.9	1.95	0.25	2.97	23.6	5.5	15.1	8.0
8	J.D.	1	145	42.8	0.93	0.16	3.22	116.5	11.4		
9	G.M.	8	241	31.4	2.77	0.36	6.00	30.7	4.6	19.1	4.5
10	F.M.	2	270	23.8	2.37	0.41	5.51	64.4	6.0	16.1	–
11	J.S.	1	102	36.8	0.54	0.19	5.54	108.4	5.5	20.6	6.0
12	S.S.	1	103	51.2	0.44	0.23	6.83	91.5	7.8		
13	M.D.	1							2.5		
Mean			303	37.1	1.32	0.30	5.39	63.0	5.8	19.7	5.9
±SD			209	18.4	0.80	0.12	2.73	35.5	2.1	6.6	1.2

[a] Number of treatment courses

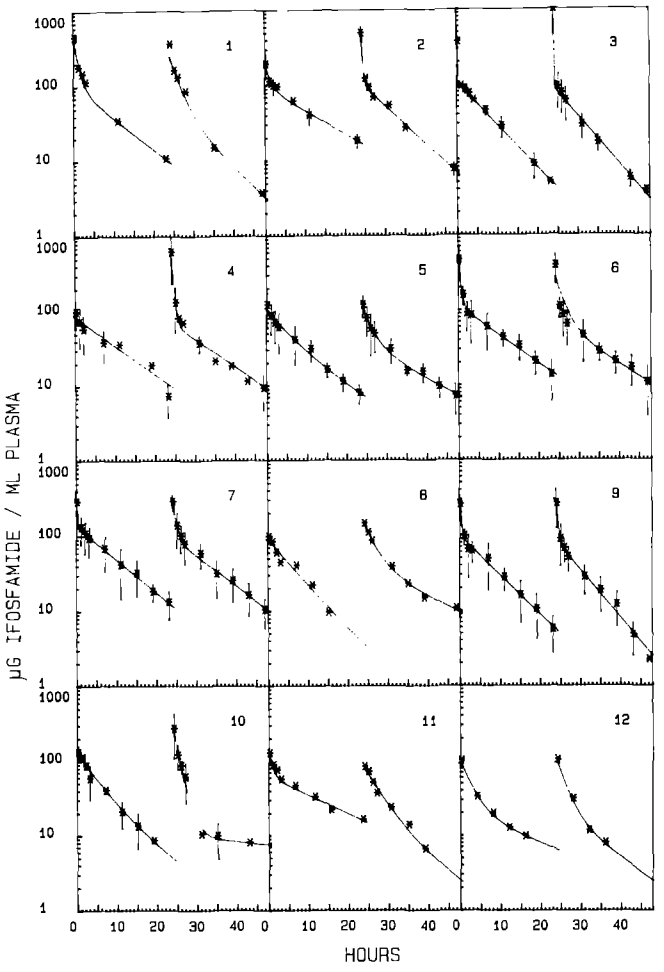

Fig. 1. Mean plasma disappearance of alkylating activity in 12 children after a 1-h i.v. infusion of 3 g/m^2 IF for 2 consecutive days every 3 weeks

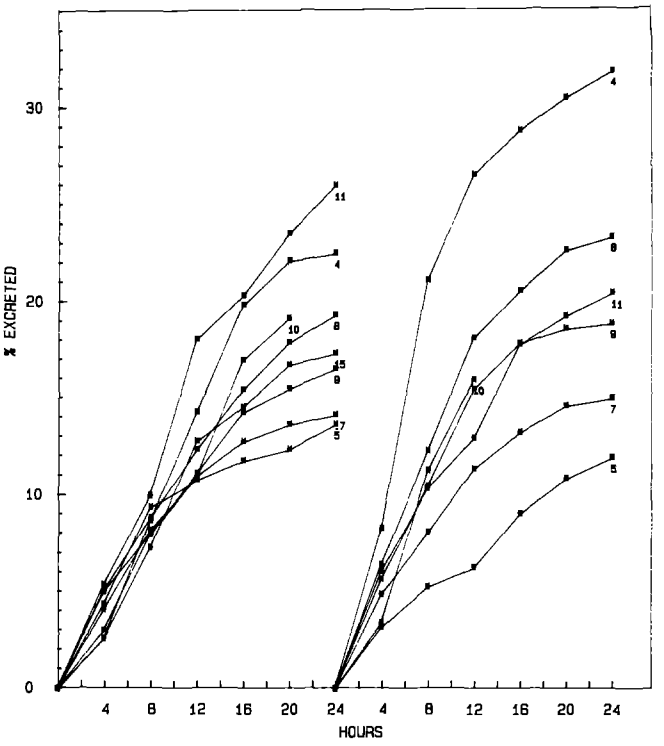

Fig. 2. Mean cumulative urinary excretion of alkylating activity after the administration of IF every 3 weeks at 3 g/m^2 (excretion curve labelled for each patient)

Results

The levels of plasma alkylating activity measured after the i.v. administration of IF at 3 g/m^2 on 2 consecutive days to children with various tumors showed considerable individual variation. Variations were also noted between different treatment cycles and between day 1 and day 2 treatments in the same patients. The initial plasma levels (C_o) were usually >100 µg IF equivalent/ml (range, 97–699 µg/ml) and decreased at least biphasically (Fig. 1). The mean half-lives for the first phase were respectively, 59 min (range, 6–153 min) and 63 min (range, 18–117 min) for day 1 and day 2 treatment courses (Table 1). For the 1st day of treatment, the mean elimination phase half-life was 6.9 h and 7.0 h from the plasma and urinary data, respectively. On the 2nd day of treatment the values were lower, 5.8 and 5.9 h, respectively. For 11 of 13 patients the $t_{½}$ β values were shorter on the 2nd day of treatment by a mean of 2.3 h.

The mean distribution volumes (V_d) ranged from 17 to 87 l/m^2, with an average of 38 l/m^2 and no difference between values on days 1 and 2. For each patient, the mean plasma exposure to the alkylating activity estimated by the AUC was higher on the 1st day of treatment in 10 of 12 cases, ranging between 0.44 and 3.41 kg·h/l. The values of the elimination constant (K_{el}) were less variable than the other pharmacokinetic parameters and were generally slightly higher on the 2nd day of treatment. The plasma clearance (Cl) of the alkylating activity in the individual treatment courses was higher in 38 of 44 cases on the 2nd day of therapy. The average of the mean Cl values on days 1 and 2 were 3.7 and 5.4 l·h^{-1}·m^{-2}, respectively, but this difference was not significant.

The percentage of the dose alkylating activity that was eliminated in the urine during the first 24 h showed clear patient-to-patient variation (Fig. 2) as well as intraindividual variation during different treatment courses (unpublished results). After the first 24 h, the renal excretion was almost complete. For eight patients on day 1 of treatment and six on day 2 there were enough data points to determine the terminal urinary half-life. The results, reported in Table 1, show that the values were no different from those obtained from the plasma data. In one patient (patient 17) presenting with a neuroblastoma in relapse, no detectable alkylating activity was found in the ascitic fluid 28 h after the i.v. administration of 3 g/m^2 IF.

The levels of alkylating activity in CSF and the corresponding plasma levels were obtained for four children (Fig. 3). The CSF concentration of alkylating activity was always lower than the plasma concentration, the higher concentration being observed 1 h after the end of the i.v. infusion. The CSF levels were between 8 and 51 µg IF equivalent/ml, and the mean CSF-to-plasma concentration ratio during the 1st 12 h was 0.53.

Discussion

Although IF has extensively been used in adults in various combination regimens and has proved to be one of the most active antitumor drugs introduced into clinical trials

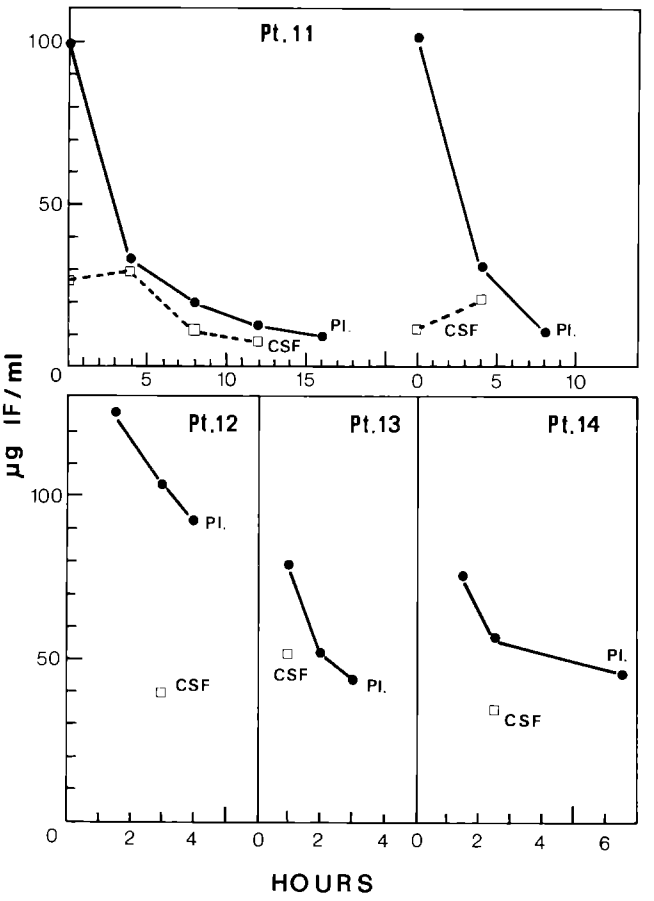

Fig. 3. Plasma and CSF levels of alkylating activity after the i.v. infusion of IF to four children

by the NCI [15], its use in children has only recently been considered [11, 14, 19]. In this age group, it may be superior to cyclophosphamide for highly resistant tumors [17]. IF is an inactive prodrug, analogous to cyclophosphamide in that it must be conversted by liver microsomal mixed-function oxidases to alkylating metabolites, producing DNA-DNA and DNA-protein cross-links [7]. The NBP method we used did not determine IF itself or its nonalkylating metabolites but only the active alkylating species, which react with NBP to form a highly colored quaternary pyridinium compound.

In our study we observed a strong variability in the disposition of IF alkylating activity between patients and between treatment courses in the same patient. This variation, typical of the oxazaphosphorines, probably results from the variability of the activation of IF by mixed-function oxidase, which is also known to be more important in children [7]. The plasma alkylating activity of IF decreases at least biphasically during the 1st 24 h after its i.v. administration, and the elimination half-lives determined from plasma and urinary data were not significantly different. The mean value of 7 h falls in the range of values previously reported in adults: 5.3 h [13]–9.2 h [12].

The volume of distribution did not vary between the 2 days of treatment. However, in children treated on 2 consecutive days every 3 weeks, we observed a decrease in the elimination half-life and in the plasma exposure to alkylating species concomitantly with an increase in the plasma clearance. These results suggest that the repeated administration of IF may induce hepatic enzymes. The same observations have recently been made in adults treated for 3 and 5 days, respectively, with IF [12, 13].

The renal contribution to IF clearance was low, since only a mean of 19% of the dose given was eliminated in the urine during the 1st 24 h, with no additional urinary IF excretion on the 2nd day of treatment. During the 1st 12 h, a mean alkylating activity of 1%/h was eliminated in the urine, and at 24 h the renal excretion was almost complete.

The information concerning the CSF concentration of IF is anecdotal. In three adult patients 2–3 h after infusion, the CSF levels of IF were 5%–30% of those measured in plasma [5]. In our study on four children treated by 1-h infusion of IF at 3 g/m^2 for 2 consecutive days, the peak drug concentration in CSF was reached within 4 h, and up to 12 h after the end of infusion the mean CSF/plasma concentration ratio was 0.53.

The finding of high alkylating levels in CSF following high-dose IF administration by a 1-h infusion encourage the use of this protocol for the treatment of some brain tumors or tumors that have spread to the CNS. Finally, the renal excretion of alkylating activity observed in this study suggests that a dose of IF be given every 12 h instead of 24 h to maintain high plasma levels of alkylating activity over 24 h. Such a study is in progress in our clinical center.

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