Anti-Cancer Drugs, 3, pp. 19–23

Clinical pharmacokinetics of ifosfamide in combination with *N*-acetylcysteine

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The pharmacokinetics of ifosfamide were studied in 20 patients with soft tissue and bone sarcomas. Drug was administered as a 30–60 min i.v. infusion at 1.2 or 2.0 mg/m²/day for five consecutive days. Some patients also received 1.5 g/m² of *N*-acetylcysteine (NAC) administered 3 times per day during the course of therapy. NAC had no effect on ifosfamide pharmacokinetics. There were significant differences in plasma half-life, area under the concentration–time curve and plasma clearance on day 1 versus day 5 of ifosfamide administration. Myelosuppression and granulocytopenia correlated better with day 1 versus day 5 ifosfamide pharmacokinetics suggesting that the alteration of ifosfamide pharmacology with multiple dosing has a significant effect on drug activity.

Key words: Ifosfamide, *N*-acetylcysteine, pharmacokinetics.

Introduction

Ifosfamide is a structural isomer of cyclophosphamide (CTX) that differs from it by the transposition of a β-chloroethyl group from the exocyclic nitrogen to the N-6 endocyclic nitrogen. Like CTX, ifosfamide is metabolized by the microsomal mixed function oxidase system of the liver. The metabolism of ifosfamide produces the alkylating metabolite acrolein and the cytotoxic nitrogen mustard derivative isophosphoramide mustard.[1] Acrolein causes the hemorrhagic cystitis observed with both drugs[2] while isophosphoramide mustard accounts for the anti-tumor activity and myelosuppression of ifosfamide.[3] Unlike CTX, ifosfamide loses its chloroethyl groups, through action of the liver, to form dechloroifosfamide, dechlorocyclophosphomide and chloroacetaldehyde, which is likely responsible for the neurotoxicity of ifosfamide.[4,5]

The bladder toxicity of ifosfamide is dose related and can be reduced by either increasing urine flow with hydration and diuretics or using multiple dose schedules.[6] Multiple dosing, however, induces ifosfamide metabolism by the hepatic enzymes, increasing the drug's plasma clearance and decreasing its half-life ($t_{1/2}$) and area under the concentration–time curve (AUC).[7–11] The most important factor in the reduction of bladder toxicity is the use of nucleophillic thiol compounds, such as mesna and *N*-acetylcysteine (NAC), which are thought to react with acrolein.[12] Although bladder cystitis is the dose-limiting toxicity when ifosfamide is used alone, uroprotection with sulfhydryl compounds causes the limiting toxicity to become myelosuppression.[13] A recent report suggests a significant reduction of anti-tumor responses in patients with lung cancer when treated with ifosfamide and NAC as compared to ifosfamide alone.[14]

A clinical trial at our institution assessed the effects of i.v. NAC with hydration on ifosfamide bladder toxicity.[15] In conjunction with the clinical trial, a pharmacological study was performed to determine ifosfamide pharmacokinetics and whether NAC alters these parameters, thus contributing to an alteration of ifosfamide efficacy.

Supported in part by a grant from Bristol-Myers Squibb Inc.

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Materials and methods

Patients

Twenty patients with histologically proven metastatic soft tissue or bone sarcomas were treated with ifosfamide with or without NAC and hydration. All patients had normal renal and hepatic function and peripheral blood counts and had received prior standard therapy. The patients were stratified and randomized into three groups to receive one of the following regimens: Group 1 ($n = 7$), ifosfamide 1.2 g/m^2 i.v. daily for 5 days; Group 2 ($n = 5$), ifosfamide 1.2 g/m^2 i.v. daily for 5 days + NAC 1.5 g/m^2 i.v. 3 times daily for 5 days; or Group 3 ($n = 8$), ifosfamide 2.0 g/m^2 i.v. daily for 5 days + NAC 1.5 g/m^2 i.v. 3 times daily for 5 days. Ifosfamide was administered by 30 min infusion; NAC was given by 15 min infusion at 0.5, 3 and 8 h after the start of the ifosfamide infusion.

Sample collection and storage

Blood (10 ml) was collected in vacuum tubes containing EDTA at the following times: pretreatment; 15 min into and at the end of the infusion; and at 1, 3, 5, 7, 9, 11 and 24 h after the infusion on the first and fifth days of drug administration. Plasma was obtained by centrifugation of blood samples at 1000 × g in a refrigerated (5°C) centrifuge. The plasma was stored at −20°C until analyzed.

Urine was collected before drug treatment and then for intervals of 0–6, 6–12 and 12–24 h after infusion on days 1 and 5. After urine volumes were measured, 5 ml aliquots were stored at −20°C until analyzed.

Sample preparation and analysis

The procedure for extraction of ifosfamide from biological fluids was that of Margison *et al.*[16] To 1 ml of biological sample (plasma or urine) in borosilicate glass test tubes, 25 µl (12.5 µg) of a 0.5 mg/ml aqueous solution of CTX as internal standard was added. After addition of 1 ml of 0.1 M NaOH, the mixture was vortexed, chloroform (10 ml) was added, and the tubes were shaken at high speed for 5 min and then centrifuged at 1500 r.p.m. for 10 min at 15°C. The layers were separated and residual water was removed from the organic layer by passing it through 9 cm Whatman Phase Separator filter paper (silicon treated, Fisher Scientific) in glass funnels. The filter paper was discarded and the funnel washed with 2 ml of chloroform. The solvent was evaporated from the samples under nitrogen at 50–60°C. The residues were reconstituted with 250 µl of acetonitrile:H_2O (22:78). Then, 100 µl of this solution was analyzed by HPLC using the previously reported procedure.[16] The HPLC apparatus consisted of a Waters 6000A pump, U6K injector and a 990 photodiode array detector/integrator (Waters Associates). The separations were accomplished on a Sherisorb 5 C_8 column (250 × 4.6 mm, Phenomenex, Torrence, CA) at a flow rate of 1.5 ml/min and detector wavelength of 190 nm. The elution solvent was acetonitrile:H_2O (22:78). The retention time of ifosfamide was 5 min and that of CTX was 10 min. Ifosfamide concentrations in the biological fluids were calculated by the peak height ratio method with CTX as the internal standard. Standard curves were prepared by spiking blank plasma with known amounts of ifosfamide and a constant amount of CTX and processing the samples was as described above for the biological samples. The standard curves were linear for concentrations from 1 to 50 µg/ml.

Pharmacokinetic parameters and statistics

The ifosfamide concentrations for each patient were fitted to equations derived by non-linear regression analysis using the PCNONLIN Version 02 (Statistical Consultants Inc., Lexington, KY) and RSTRIP Version 4.03 (MicroMath, Inc., Salt Lake City, UT) microcomputer programs. Pharmacokinetic parameters were calculated from the coefficients and exponents of the regression equations using the formulas published by Wagner.[17] Paired t-tests of significance were used to determine the significance of differences between the pharmacokinetic values on days 1 and 5. A two sample (independent samples) test of significance was used to compare the parameters of the groups. These calculations were performed using the Minitab statistical program for DOS microcomputers, Version 7 (Minitab Inc., State College, PA). The 'best subsets of regression' routine of the Minitab program was used to identify those subsets of regression parameters [$t_{1/2}$, AUC, plasma clearance (Clt), volume of distribution at steady state (V_{dss}), maximum concentration (C_{max})] that best correlated with decreases in white blood cells (WBCs) and

granulocytes. Final selection of the best fit was accomplished using the statistical parameters provided by the program. Differences were considered significant at the 95% confidence level.

Results

The plasma clearance of ifosfamide decreased rapidly in a biphasic manner as shown in Figure 1(a and b). These data are from individual patients, but are representative of results observed with all patients. The curves in Figure 1(a) show drug clearances on days 1 and 5 from a patient after receiving 1.2 g/m^2 of ifosfamide by daily 30 min i.v. infusions. Figure 1(b) shows ifosfamide plasma clearance in another patient receiving the same dose of ifosfamide but also receiving NAC (1.5 g/m^2 by 15 min i.v. infusion 3 times daily for 5 days). The mean values of the pharmacokinetic parameters are

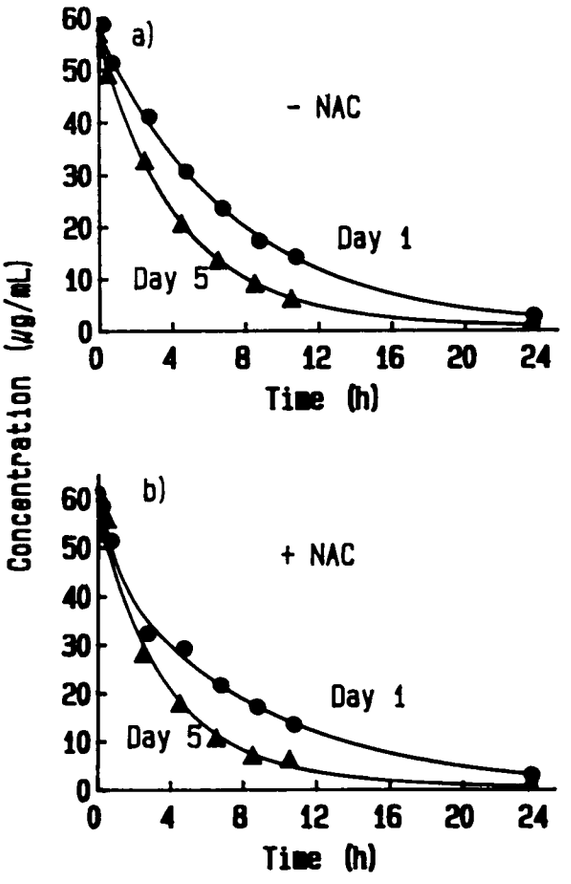

Figure 1. Ifosfamide (1.2 g/m^2) plasma clearance on day 1 (●) and day 5 (▲): (a) without and (b) with administration of NAC (1.5 g/m^2).

presented in Table 1. There were no significant differences (two-way *t*-test) between the parameters of groups 1 and 2 when compared on the same days. However, there were statistically significant differences (paired *t*-test) between the $t_{1/2}$ on days 1 and 5 within both groups and the AUC and Clt in Group 1 (Table 1). Similarly, for Group 3, $t_{1/2}$, AUC and Clt were different on days 1 and 5 (Table 2), while $t_{1/2}$, Clt and V_{dss} were not different from the corresponding values for Groups 1 and 2. The percentage of parent drug excreted in the urine by 24 h was the same for all three groups.

The extent of dependence of myelosuppression and granulocytopenia on specific pharmacokinetic parameters was assessed by multivariate regression analysis that identified the combinations of parameters that gave the best correlations with the decrease of cell counts. These calculations indicate that the decrease of WBCs correlated better with day 1 than with day 5 parameters. The day 1 correlation was: % decrease WBC $= -52.0 + 0.056\text{AUC} + 15.3\text{Clt} + 0.25C_{max} + 1.1V_{dss}$ ($r = 0.90$, $p < 0.001$). While the correlation with day 5 parameters was: % decrease WBC $= -65.6 + 4.0t_{1/2} + 0.19\text{AUC} + 17.7\text{Clt}$ ($r = 0.76$, $p = 0.004$).

Correlations between pharmacokinetic parameters and granulocyte count were not as good, but still significant. Again, the day 1 parameters were better predictors of cell decrease: % decrease granulocytes $= -71.1 + 0.07\text{AUC} + 17.5\text{Clt} + 1.7C_{max}$ ($r = 0.89, p < 0001$). The best correlation between granulocytes and day 5 parameters was: % decrease granulocytes $= -81.6 + 8.3t_{1/2} + 19.4\text{Clt} + \text{Clt} + 0.55C_{max}$ ($r = 0.76, p = 0.004$).

Discussion

Ifosfamide and CTX are useful for the treatment of a variety of solid tumors. Ifosfamide has been suggested to be superior to CTX in lymphomas, testicular cancer, ovarian cancer, lung cancer and sarcomas.[6,18] Because of a slower rate of metabolic activation a 5 times larger dose of ifosfamide compared to CTX must be administered for similar anti-tumor activity.[7] Ultimately, this leads to greater bladder toxicity. Since the anti-tumor activity and the bladder toxicity of ifosfamide are caused by different entities, eliminating the toxicity should not affect the anti-tumor activity and, thus, the therapeutic index would be increased.[2,12]

Hydration, diuresis, fractionated doses and administration of sulfhydryl compounds have been

Table 1. Pharmacokinetic parameters (mean ± SD) of ifosfamide (1.2 g/m^2) on days 1 and 5 with and without NAC (1.5 g/m^2)

	$t_{1/2}$ (h)	AUC (μgh/ml)	Clt (l/h/m^2)	V_{dss} (l/m^2)	C_{max} (μg/ml)	24 h excretion (%)
Day 1						
−NAC	6.8 ± 3.3	593 ± 325	2.4 ± 0.76	20.4 ± 2.0	55.9 ± 7.1	17.2 ± 5.8
+NAC	4.9 ± 2.8	504 ± 332	3.3 ± 2.0	20.7 ± 8.1	48.3 ± 10.7	19.1 ± 7.0
Day 5						
−NAC	3.9 ± 0.9[a]	289 ± 38[a]	4.2 ± 0.49[a]	21.8 ± 3.9	64.8 ± 15.8	17.2 ± 8.2
+NAC	2.3 ± 1.2[a]	233 ± 56	5.4 ± 1.4	21.7 ± 2.7	54.9 ± 12.4	14.2 ± 5.8

[a] Significantly different from day 1 ($p < 0.05$).

Table 2. Pharmacokinetic parameters of ifosfamide (2.0 g/m^2 day × 5) with NAC (1.5 g/m^2 3 × day × 5)

	$t_{1/2}$ (h)	AUC (μgh/ml)	Clt (l/h/m^2)	V_{dss} (l/m^2)	C_{max} (μg/ml)	24 h excretion (%)
Day 1	7.0 ± 2.2	84.2 ± 282	2.6 ± 0.64	22.9 ± 5.7	94.7 ± 16.4	25.5 ± 6.8
Day 5	4.0 ± 1.6	471.2 ± 93.7	4.4 ± 0.89	24.1 ± 6.2	103.0 ± 19.9	22.9 ± 5.7
p	0.0015	0.0013	<0.0001	0.64	0.36	0.56

used to alleviate the bladder toxicity of ifosfamide. To be effective, uroprotective agents must not reduce the anti-tumor efficacy of ifosfamide. Mesna almost completely eliminates bladder toxicity without changing the pharmacokinetics of ifosfamide or its active metabolite, isophosphoramide mustard.[12] Much less is known about NAC; its utility was limited initially because only an oral dosage form was available and its odor caused excessive nausea and vomiting. Recently, phase I studies of an i.v. preparation have been conducted, but these have shown NAC to be a less effective uroprotector than mesna.[15] As indicated by the data in Table 1, the pharmacokinetics of ifosfamide did not change with the co-administration of NAC. Increasing the dose of ifosfamide to 2.0 g/m^2/day for 5 days yielded comparable pharmacokinetic results (Table 2). The values of the pharmacokinetic parameters observed in this study are consistent with those reported previously.[9] Any suggestion that NAC reduces ifosfamide anti-tumor efficacy[14] cannot be attributed to an alteration of the pharmacokinetics of the parent drug.

Ifosfamide pharmacokinetics were altered by multiple dosing (Figure 1a and b; Table 1). A significant decrease of $t_{1/2}$ occurred on day 5 compared with day 1, indicating self induced alteration of ifosfamide pharmacology. This phenomenon has been previously observed for both ifosfamide[7,11] and CTX[19,20] and has been attributed to an induction of liver mixed function oxidase metabolism. Myelosuppression was observed in all patients. The percentage decrease of WBCs was found to correlate with the pharmacokinetic parameters. This result contrasts with that of Lind *et al.*,[9] who found no correlation between ifosfamide pharmacokinetic parameters, singly or in combination, and toxicity. Besides the obvious conclusion that ifosfamide causes myelosuppression and granulocytopenia, the results of the multiple regression analysis suggest that toxicity depends more on day 1 drug administration than on day 5 administration. This is reminiscent of the 'priming' effect observed with CTX in which pretreatment with this drug reduces gastrointestinal and bone marrow toxicity in chemotherapeutic regimens.[21] The alteration of ifosfamide pharmacokinetics with multiple dosing and the dependence of myelosuppression on the initial drug dose suggest that alternate schedules of ifosfamide administration, such as use of a priming dose, may need to be examined.

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(Received 11 November 1991; accepted 10 December 1991)