Cancer Chemother Pharmacol (1994) 33: 391–398

Cancer Chemotherapy and Pharmacology

Preclinical pharmacokinetics and stability of isophosphoramide mustard

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Received 19 April 1993/Accepted 17 September 1993

Abstract. Stability and preclinical pharmacokinetics of isophosphoramide mustard (IPM), an active metabolite of ifosphamide, were investigated using analytical methods developed in this laboratory. For stability evaluation of IPM we used a rapid, high-pressure liquid chromatographic (HPLC) method by which IPM is analyzed directly from aqueous solutions without derivatization on a 10-μm C-18 reversed-phase column with theophylline as the internal standard. IPM in sodium phosphate buffers was found to undergo pH-dependent first-order degradations. At pH 7.4 and 38° C, the IPM solution showed a half-life of 45 min. A gas chromatographic–mass spectrometry (GC/MS) method for the analysis of IPM in plasma was also developed. This method utilized solid-phase extraction with deuterium-labeled IPM as the internal standard. The routine detection limit for the assay was 50 ng/ml with within-run and between-run coefficients of variation of 6% and 11%, respectively. By this method, stability of IPM in plasma and in RPMI 1640 tissue culture medium was evaluated, and its pharmacokinetics in the Sprague-Dawley rat following i.v. administration at 40 mg/kg were investigated. IPM was found to be more stable in these media, with half-lives in the range of 100 min. IPM plasma pharmacokinetics were found to decline monoexponentially with terminal half-lives ranging from 6.8 to 18.7 min and total clearance between 6.0 and 18.3 ml/min. Plasma protein binding of IPM was found to be 55%, and the partition ratio between plasma and red blood cells of 4.9 to 1, respectively. Cytotoxicity of IPM to L1210 cells was evaluated, and the results indicated that the IC_{50} with 1-h and 4-h exposure was 33 and 15 μM, respectively. Based on these data, IPM plasma levels in the rat declined below the IC_{50} in about 1 h at this dose. More frequent dosing or infusion may be necessary to maintain adequate drug levels for antitumor activity when IPM is administered directly.

Introduction

Ifosfamide (IP) is a structural isomer of cyclophosphamide (CP) with one of the chloroethyl side chains residing on the ring. IP was synthesized several years after CP, and both remain clinically the most useful alkylating agents to date. There are substantial differences in their spectrum of antitumor activity and toxicity [1–3], and these differences may be in part due to their difference in metabolism as well as the stability of several of their metabolites. Metabolic pathways of CP and IP have been largely elucidated. Both agents require the activation by microsomal enzymes to form the C-4 hydroxylated metabolites [4–10] followed by formation of the respective ultimate alkylating, cytotoxic metabolites, isophosphoramide mustard (IPM) for IP and phosphoramide mustard (PM) for CP [11–14]. While pharmacokinetics of CP and metabolites are largely understood [15–25], the relationship between the parent drug and metabolites has only recently been defined [25]. However, the pharmacokinetics of IP metabolites remain largely undescribed. Struck et al., following the identification of IPM as the metabolite of IP [26, 27], reported synthesis, the in vitro cytotoxicity, and the in vivo cytotoxicity evaluation of IPM in mice [28]. Bryant et al. [29] used a gas-chromatographic assay to quantify IPM in plasma from patients receiving IP. However, no definitive stability data for IPM have been reported. Since IPM is the ultimate, active metabolite of IP, as PM is to CP, its pharmacokinetics, stability, and cytotoxic activity are important and constitute the focus of this report.

The work reported was supported by USC Cancer Center Core grant CA 14089 from the National Cancer Institute

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Materials and methods

Chemicals. Isophosphoramide mustard (IPM) and [β-$^2H_4$] isophosphoramide mustard-d_4 (IPM-d_4) were synthesized in this laboratory (K. K. Chan and J. H. Wang, unpublished data). PM, as its cyclohexyl amine salt, was obtained from Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. All organic solvents purchased from E. M. Science (Gibbstown, N. J.) were of HPLC grade. *N,O*-bis(Trimethylsilyl)trifluoroacetamide (BSTFA) and *N*-trismethylsilylimidazole (TMSI) were obtained from Pierce (Rockford, Ill.). C-18 reversed-phase resin was obtained from Analytichem International (Harbor city, Calif.). The stainless steel HPLC column, 4.6 mm × 250 mm, packed with 10 μm, C-18 reversed-phase resin (Econosphere), was purchased from Alltech (Deerfield, Ill.).

HPLC analysis of IPM in aqueous buffers. IPM in aqueous buffer was analyzed by an HPLC method similar to that for PM [30]. Essentially, IPM and the internal standard theophylline were separated on the HPLC column described above, fitted to a Shimadzu (model LC-6A) HPLC (Cole Scientific, Calif.). The components were eluted by a mobile phase consisting of 0.005 M perchloric acid at a flow rate of 1 ml/min and detected via an ultraviolet detector set at 207 nm.

Extraction and derivatization. IPM along with the added internal standard, IPM-d_4, in plasma was extracted by a solid-phase method similar to that previously reported for PM [25]. Essentially, the plasma sample, spiked with the internal standard, was eluted through a 10-ml Poly-prep minicolumn (Bio-Rad, Richmond, Calif.) containing C18 reversed-phase resin by normal saline followed by methanol. The saline fraction was discarded while the methanol fraction collected and evaporated by N_2 gas. The residue was derivatized by treatment with 40 μl of a mixture of BSTFA and TMSI (5 : 1, v/v) at 120° C for 60 min.

Gas chromatographic–mass spectrometry analysis. GC-MS analysis of IPM was carried out on a Hewlett-Packard 5985A mass spectrometer coupled to a Hewlett-Packard 5840A gas chromatograph (Hewlett-Packard, Palo Alto, Calif.) via an all-glass jet separator. The mass spectrometer was operated with the following parameters: temperature for transfer line and jet separator at 200° C, ion source at 250° C. Chemical ionization mode was used, and ammonia as the reagent gas was introduced directly into the source by means of an isolation valve situated after the jet separator. A DB-1, 15 m × 0.5 mm ID capillary column with 1.5 μm thickness (J&W Scientific, Folsom, Calif.) was used. The oven temperature was programmed from 170° C to 230° C at 30° C min, and the injection temperature was set at 210° C. Helium was used as the carrier gas at a flow rate of 30 ml/min. Quantitation was performed by selected ion monitor mode and ions at m/z 329 and 333 corresponding to trimethylsilylated and dehydrochlorinated IPM and IPM-d_4, respectively, were selected.

Protein binding of IPM in rat plasma. IPM in fresh rat plasma at 20 μg/ml was incubated at 37° C for 30 min. A 0.1-ml aliquot was removed for the analysis of total IPM. The remaining plasma was placed into an ultrafiltration unit (Amicon Centrifree, W. R. Grace, Danvers, Mass.) and centrifuged at 3500 *g* for 10 min to obtain the ultrafiltrate. Then 50 μl of the filtrate was removed for unbound IPM analysis. IPM analysis was performed by the GC-MS method as described previously. The experiment was performed in triplicate.

Stability study of IPM in aqueous buffer. IPM solutions in 0.067 M sodium phosphate buffer (PBS) at 8 mM each were adjusted to pH values of 1.7, 3.0, 5.5, 6.0, 7.4, 9.0, 10.0 and 11.0 with small volumes of 0.1–1.0 *N* HCl or 0.1–1.0 *N* NaOH. Each of these solutions was incubated separately at 38° C in a water bath. According to an appropriate time schedule, typically at 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240 and 270 min, 20 μl of each IPM solution was removed and an appropriate amount of theophylline, was immediately added. The resultant solutions were frozen at –20° C until analysis, generally within 24 h. IPM concentrations of these solutions were measured by the HPLC method previously described for PM [30]. A duplicate experiment was performed for each pH value.

Stability of IPM in human plasma. IPM in human plasma at 20 μg/ml was incubated at 37° C in a water bath. According to a predetermined time schedule of 0, 5, 15, 30, 45, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 420 min, 100-μl aliquots were removed and an appropriate amount of IPM-d_4 was added to each as the internal standard. The samples were immediately frozen at –20° C until analysis, generally within 24 h. A duplicate experiment was performed for each time point. The analysis of IPM concentrations was performed using the previously described GC/MS method.

Stability of IPM in tissue culture medium. IPM in RPMI 1640 containing 10% of fetal bovine serum at 20 μg/ml was incubated at 37° C. With the time schedule as that for plasma, 100-μl aliquots were removed and an appropriate amount of IPM-d_4 was immediately added to each as the internal standard. The samples were frozen at –20° C until analysis, generally within 24 h. IPM concentrations in these samples were analyzed by the GC/MS method previously described.

Pharmacokinetic study. Animal experiments were carried out according to a protocol approved by the institutional animal use review committee. Six male Sprague-Dawley rats (Simonsen, Gilroy, Calif.) weighing between 250–320 g were used. Rat chow (Purina and Wayne, Rialto, Calif). and water were given ad libitum. The jugular vein of each rat was cannulated under ether anesthesia [31]. Essentially, the fur around the ventral right side of the neck was shaved. The skin was sterilized with iodine solution. A 2- to 3-cm incision was made, and the neck muscle gently separated to expose the right jugular vein. A small nick was made, and a 10-cm-long beveled cut piece of PE 50 tubing was inserted and pushed into the right atrium. The muscle and skin were then closed with stitches and the cannula was exteriorized under the skin at the back of the neck. The blood flow was reexamined and the tubing filled with heparinized saline solution (200 IU/ml). After the rat was completely conscious, 1 ml of IPM in normal saline (approximately 100 mg/ml) was injected into the jugular vein cannula. The dose used was 40 mg/kg. Blood samples, about 0.3 ml each, were collected in heparinized tubes according to a predetermined time schedule of 0, 2, 5, 10, 20, 30, 40, 50, 60, 75, 90, 105, 120 min. After collection of each sample, 0.3 ml normal saline was injected into the rat to replace the fluid lost. Plasma was obtained from each sample by centrifugation at 2000 *g* and at 4° C for 4 min and kept frozen at –20° C until analysis, generally within 24 h. Analysis for each sample was performed in duplicates. The pharmacokinetic parameters were calculated using PLOT4U [32] and MINSQ (MicroMath, Salt Lake City, Utah) computer programs.

Partition of IPM between plasma and red blood cells. Fresh heparinized rat whole blood was removed from a rat and the hematocrit measured by a Readacrit centrifuge (Clay Adams, Parsippany, N. J.). An appropriate amount of IPM was added to result in the concentration of 21.4 μg/ml and the resulting sample incubated at 37° C for 30 min. Then the blood sample was centrifuged at 1500 *g* for 10 min and plasma separated. A 0.1-ml aliquot of plasma was removed, and an appropriate amount of the internal standard was added. The sample was then processed for GC/MS analysis for IPM. A parallel plasma sample containing the same initial concentration of IPM was incubated for the same period of time and the resulting concentration determined to account for IPM degradation during the incubation.

Antitumor activity of IPM. L1210 and human CCRF-CEM leukemic cells were used in these experiments. To 22.5 ml of RPMI 1640 containing 10% fetal bovine serum and about 5×10^4 cells per ml in the tissue culture flask (Costar, Mass.) was added an appropriate volume of IPM stock solution in PBS buffer, to give separate final drug concentrations of 5, 10, 25, 50, and 100 μM using aseptic techniques. In the control, the drug solution was replaced by the same volume of PBS. After 1 and 4 h, for each concentration a triplicate of 3×2 ml was transferred to a 15-ml centrifuge cell culture tube and the tubes were

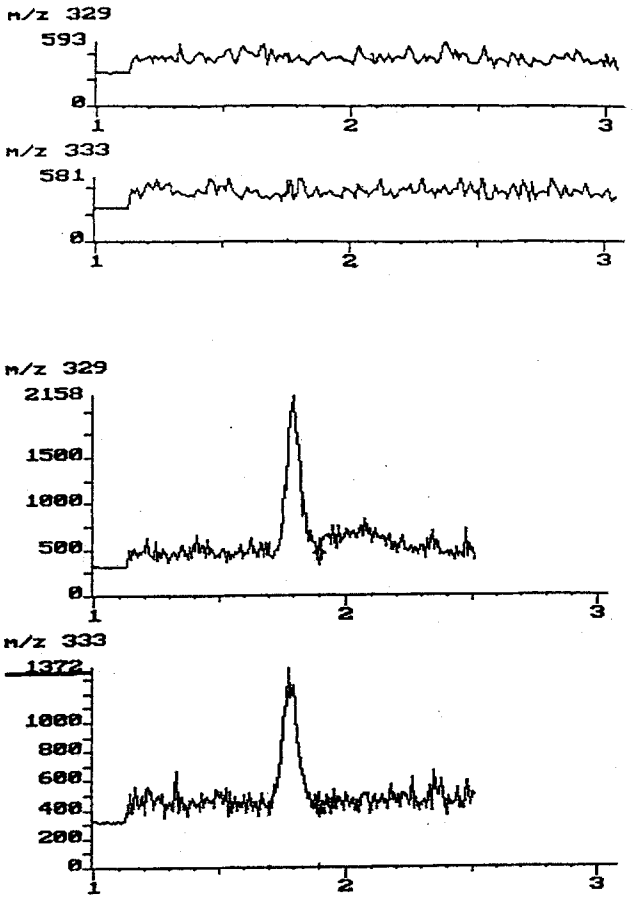

Fig. 1. A representative set of ion chromatograms of IPM (m/z 329) and the internal standard (β-$^2H_4$]IPM (m/z 333) in plasma. *Vertical axis* represents ion counts and *horizontal axis*, retention time in minutes. *Top panels* are blank plasma extracts and *bottom panels*, spiked plasma extracts

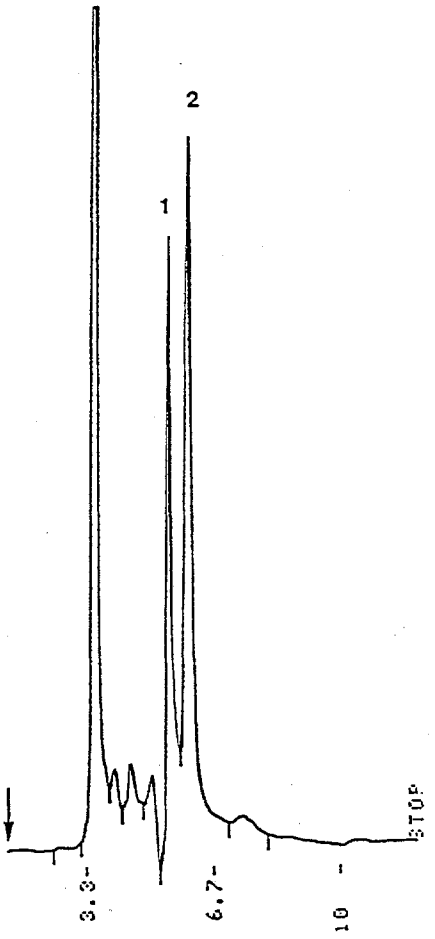

Fig. 2. A representative HPLC chromatogram of IPM (*1*) and its internal standard (*2*). *Arrow* indicates the injection point in time and the *horizontal marks* indicate the retention times in minutes

centrifuged at 1000 *g* for 10 min. The drug solution was removed and discarded. The incubation time of 1 h is commonly used for alkylating agents, but the 4-h period was selected on the basis of the optimal schedule for PM [61]. The pellet from each sample was washed once with 5 ml of PBS and the cells were resuspended in 2 ml of fresh medium. The cell suspension was transferred to each of a 24-well plate (Costar, Mass.) and the plate incubated in an incubator (Model 3331-2, National, Portland, Ore) maintained at 5% CO_2 atmosphere. After 72 h, the cell solutions were removed and cells counted by means of a Coulter counter (Coulter Electronics, Hialiah, Pa.). The entire experiment was repeated once.

Results

GC/MS assay of IPM in plasma

The GC/MS chromatograms of plasma blank and plasma sample containing IPM and its internal standard, IPM-d_4 are shown in Fig. 1. As shown, no interference in the ion regions at m/z 329 and 333 were found in the plasma blank. IPM and the internal standard was eluted at 1.8 min as symmetrical ion peaks. Sensitivity limit of the assay was 50 ng/ml, and a linear relationship between peak height ratios of IPM-d_0 to IPM-d_4 and the amounts of IPM was found from 50 to 2000 ng/ml monitored with a correlation coefficient (r^2) of 0.9985. The within-run and the between-run coefficients of variation (CV) of the assay were 6% (n = 7) and 11% (n = 7), respectively, at 500 ng/ml level. The recovery from the solid-phase extraction was essentially 100% at the concentration of 500 ng/ml.

Stability of IPM in aqueous buffer

A typical HPLC chromatogram as obtained from a freshly prepared IPM buffer solution containing the internal standard theophylline is shown in Fig. 2. As shown, IPM and theophylline were well-resolved as symmetrical peaks. IPM concentrations in PBS at all pH values were found to decline monoexponentially with time, and the data were therefore analyzed by regression to a logarithmic function using a standard computer program (PLOT4U) [32]. The first-order decay rate constants and half-lives were computed and are shown in Table 1. As shown, at pH 7.4 and 38° C IPM solution showed a $t^1/_2$ of 45 min, more stable than PM which was reported to have a half-life of 15 min under the same condition [30]. IPM was found to be most unstable at about pH 9.0. At acidic pH values or basic pH values higher than 9.0, IPM solution was found to be more

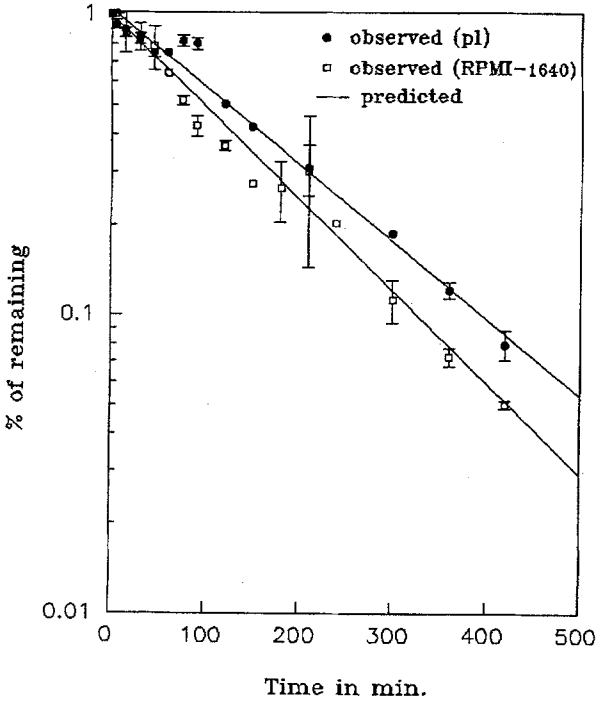

Fig. 3. IPM degradation profiles in human plasma (●) and RPMI 1640 cell culture medium with 10% fetal calf serum (□) at 37° C. The initial IPM concentrations in both media was 20 μg/ml, and *vertical bars* represent ± SD values

Table 1. First-order decay rate constants and half-lives of isophosphoramide mustard (IPM) in sodium phosphate buffers at different pH values

pH	Rate constants (min^{-1})	$T^1/_2$ (min)
1.7	0.0054	128
3.0	0.0093	75
5.5	0.0134	52
6.0	0.0117	59
7.4	0.0150	46
9.0	0.0169	41
10.0	0.0135	51
11.0	0.0048	143

stable. These results differed from those recorded with PM, which was found to be more stable at acidic pH values but not at high pH values. The reason for this difference is not apparent at this time.

Stability of IPM in human plasma and tissue culture medium

The degradation profile of IPM in fresh human plasma at 37° C is shown in Fig. 3. The concentration of the IPM was found to decline with an apparent first-order process with the half-life of 118.5 min. Similar to the degradation in PBS, IPM appears more stable than PM, which showed a degradation half-life of 55 min in human plasma [29]. The stability of IPM in the tissue culture medium RPMI 1640 containing 10% FBS was also evaluated, and the degradation profile was found to follow an apparent first-order process with the half-life of 98.28 min, similar to that in plasma (Fig. 3).

Pharmacokinetics of IPM in the rat

A typical pharmacokinetic profile of IPM in rat plasma at the dose of 40 mg/kg is shown in Fig. 4. As shown, the elimination of IPM in rat plasma following i. v. administration exhibits a monoexponential decline with an average half-life of 12.7 min and can therefore be described by one-compartment kinetics. The relevant pharmacokinetic parameters estimated from plasma disposition are summarized in Table 2. As shown, the mean total clearance value was 11.0 ± 4.4 ml/min (range 6.0–18.3) and the mean volume of distribution, 220 ± 156 ml. Protein binding of IPM in fresh rat plasma, as estimated by the ultrafiltration method, was found to be 55.1 ± 0.1%. The partition of IPM between plasma and red blood cells was also evaluated in fresh rat blood, and the result indicated that IPM partition into rbc less readily in the ratio of 4.9 : 1 in favor of plasma. Thus, these data indicated potential difference between plasma and blood pharmacokinetics for this agent.

Table 2. Relevant pharmacokinetic parameters of IPM in Sprague-Dawley rats following i. v. drug administration at 40 mg/kg. Parameters were derived from a one-compartment model; $T^1/_2$, half-life; AUC, area under the curve; Vd, volume of distribution; MRT, median residence time; k, elimination constant; Cl total clearance

Rat no.	Co (μg/ml)	T½ (min)	AUC (μg/min/ml)	Vd (ml)	MRT (min)	k (min^{-1})	Cl (ml/min)
1	72.23	17.46	1997	165.1	22.78	0.040	5.97
2	23.86	18.73	687	528.0	24.77	0.037	18.33
3	108.11	7.13	1586	136.9	11.35	0.097	9.33
4	86.96	7.61	1020	136.9	10.51	0.097	9.80
5	78.95	6.81	822	144.7	8.92	0.102	13.90
6	50.05	17.87	1344	231.5	26.07	0.039	8.62
Average	70.03	12.60	1243	220.2	17.40	0.068	10.99
SD or range	29.50	6.81–18.73	496	155.9	8.92–24.77	0.036	5.97–18.33

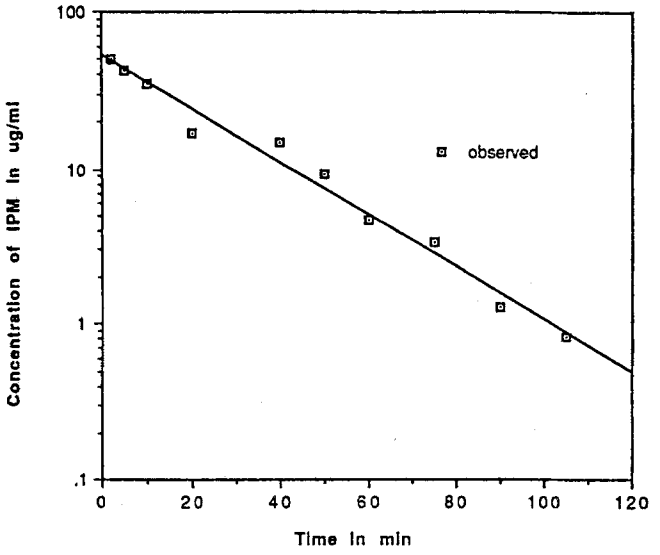

Fig. 4. A representative plasma concentration-time profile of IPM in the Sprague-Dawley rat following an i.v. dose of 40 mg/kg. The *line* presents the fitted values to a monoexponential equation

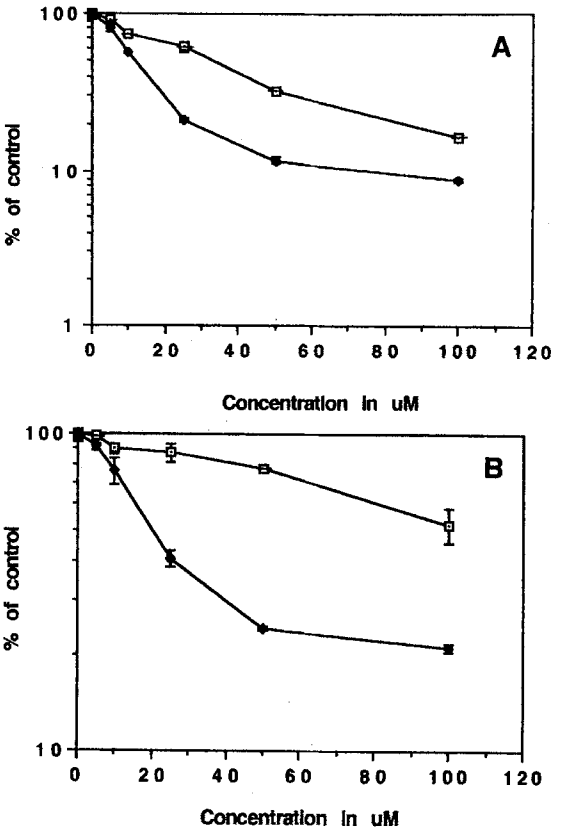

Fig. 5. Cytotoxicity of IPM in **A** L1210 and **B** human CCRF-CEM leukemic cells following 1-h and 4-h exposures as represented by an decrease of % of control (no drug) as a function of an increase in drug concentration; ⊡, L1210 1 h; ◆, L1210 4 h; ⊡, CCRF-CEM 1 h; ◆, CCRF-CEM 4 h

Cytotoxicity evaluation of IPM in L1210 and CCRF-CEM cells

IPM cytotoxicity to L1210 and CCRF-CEM cell lines at 1 h and 4 h exposures was evaluated, and the results are shown in Fig. 5 A and B, respectively. As shown, IPM was only slightly active against both L1210 and CCRF-CEM cells at concentrations above 25 μM after 1 h of exposure and quite active at 100 μM. However, the activity increased significantly at longer exposure time of 4 h. At 4 h, IPM inhibited L1210 cell growth to about 80% at 25 μM compared with only 35% at the same concentration at 1 h of exposure. Similar results were obtained for CCRF-CEM cells.

Discussion

For stability study of IPM, it would be advantageous to utilize a rapid and simple method such as the HPLC method developed and employed here. However, this method could not be applied for IPM in biologic fluids because of the presence of interfering substances. In addition, the higher sensitivity requirement and the need for simultaneous assay of metabolites necessitated the development of an alternate method. Several assay methods for cyclophosphamide and metabolites have been published, including gas chromatography [33], high-pressure liquid chromatography [34, 35], thin-layer chromatography with radioactivity detection [35, 36] or with densitometric determination [37], and gas chromatographic-mass spectrometry [38, 39] with stable isotope dilution techniques [25, 30, 40]. Similar methodologies for ifosphamide and metabolites have been relatively scarce. Because of the instability of some of the active metabolites of oxazaphosphorines (e.g. 4-OH IP, 4-OH CP, PM), analytical procedures without the use of isotopically labeled internal standard, especially for those involving labor-intensive manipulations, could suffer from inherent loss and produce less reliable and more scattered data. The present analytical method for IPM combines stable isotopically labeled IPM as the internal standard, which compensates procedural losses, with highly specific mass spectrometry, and offers a distinct advantage. The mass-spectrometric method has been generally viewed as an expensive and overly sophisticated instrument; however, the availability of low-cost mass detectors (MSD) with PC-based computer interface has rendered this type of analytical tool amenable to widespread laboratory use. Thus, the present method can be used to support clinical pharmacokinetics studies of IP.

Using the GC/MS methodology, pharmacokinetics of IPM were assessed in the rat following its direct administration. The results show a rapid disposition with an average $t_{1/2}$ less than 15 min, similar to that previously reported for PM [40]. On the other hand, following administration of IP, it has been previously reported that IPM plasma levels sustained [29] in human, though no data recorded in rats were available. In the case of PM, following its direct administration PM plasma levels declined significantly more rapidly than those generated from CP. This has been explained as partly due the well-known phenomenon of a flip-flop model [41] for drug metabolite and the existence of a diffusional barrier for polar metabolites [42]. Thus, it is

likely that in the case of IP, a similar phenomenon exists, though no data are available at present.

Increasing attention has been focused on the role of the active metabolites in contributing to the antitumor activity of the parent oxazaphosphorines [43–50]. It is commonly accepted that PM and IPM are the ultimate intracellular cytotoxic metabolites for their respective parent drugs, CP and IP. However, there is still disagreement on the relative significance of these mustards and their respective immediate 4-hydroxy precursors in the overall cytotoxic and pharmacokinetic roles upon dosing of the parent compounds [13, 39, 51–53]. Because of the polar nature of PM and IPM, it is commonly agreed and demonstrated that there is a penetration barrier for their cellular entrance [25, 48, 54]. The low partition behavior to rbc found for IPM is consistent with this contention. On the other hand, the precursors, 4-OH CP and 4-OH IP, would readily diffuse into cells and are thus believed to be *the* transport forms for PM and IPM [25, 48, 54].

When PM was evaluated clinically, prior to its identity as a CP metabolite being discovered, the results were generally unimpressive [55]. Preclinical pharmacokinetic data indicated that the agent has a short plasma t½ [40, 56, 57]. Thus, considerable efforts have been made to develop the pre-activated form of oxazaphosphorines for clinical use. In the case of cyclophosphamide, 4-hydroperoxycyclophosphamide has been developed as a bone marrow purging agent [46, 47] and ASTA Z 7557, 4-(2-sulfonatoethylthio)-cyclophosphamide, has already been subjected to the clinical phase of evaluation [49]. However, these approaches have one serious problem in common, in that these pre-activated oxazaphosphorines still carry a toxicophore, acrolein. Convincing evidence has been published showing that acrolein is primarily responsible for the hemorrhagic cystitis in patients [58, 59]. Furthermore, 4-OH CP was also found to possess even shorter plasma t½ than PM following its direct administration [25, 48, 54] and has been shown to be extremely unstable in vitro [60]. Similar property probably exists for 4-OH IP, though there are no corresponding data available. On the other hand, though PM has been shown to be rather unstable in buffer (30), it is more stable in plasma (30). Similar properties were found for IPM in the present study, although IPM appeared to be considerably more stable than PM. Thus, PM and IPM possess better properties for development for clinical use.

In the present study, IPM was found to be more stable than PM at basic pH values. This observation is somewhat surprising; however, it is possible that it may be due to a methodologic problem. At high pH values, IPM is first deprotonated followed by formation of presumably the aziridine anion and then the diazirdine anion. The latter two species may not be separable from the parent IPM under the current HPLC condition. Consequently, the stability was artificially increased. It may be that there is no similar situation exist for PM.

Following an i.v. bolus dose of IPM at 40 mg/kg to the rat, IPM concentrations achieved ranged from 50 μg/ml (227 μM) to 1 μg/ml (4.5 μM) in a time-span of about 90 min (Fig. 4). It would be of interest to determine whether significant cytotoxic activity could be achieved at these concentration ranges. Thus, cytotoxicity of IPM on L1210 murine and CCRF-CEM human leukemic cell lines was separately evaluated. We have found that the degree of cytotoxicity of IPM was dependent on the exposure time. IPM required longer than the standard 1-h exposure time to exhibit significant cytotoxic activity similar to that of PM [61]. In fact, following a 4-h exposure time, the cytotoxicity of IPM is rather high. This finding is similar to the data reported by Struck et al. [28]. Thus, following an i.v. administration of IPM at 40 mg/kg, significant cytotoxic drug concentrations can be achieved, albeit with somewhat short duration. However, it was found that sustained circulating levels of PM [62] and probably IPM [29] could be produced following administrations of the corresponding prodrugs CP and IP, respectively. These prodrugs, however, require metabolic activation and carry a urotoxic acrolein moiety. The significant cytotoxicity of IPM coupled to these pharmacokinetics data suggests that IPM should be developed directly as an important antitumor agent. The short plasma t½ could probably be overcome by appropriate formulation, manipulation of dose schedules, or other prodrug development.

In conclusion, a sensitive and specific GC/MS assay for IPM in plasma has been developed, which can be used to support routine pharmacokinetic studies of IPM directly and when it is generated from IP. This method was used to study pharmacokinetics of IPM in the rat, and results indicated rapid disposition of IPM. Another HPLC method has also been developed for the analysis of IPM in buffers. This method is rapid and does not require derivatization, but it cannot be used for IPM in complex matrix. With this method, IPM has been found to be unstable in buffer, though more stable than PM. The stability was influenced by the pH values in the medium and more stable at acidic and basic pH values. IPM has been found to be apparently more stable in plasma and tissue culture medium than in buffer. Cytotoxic activity evaluation in L1210 indicates significant activity of IPM when an adequate exposure time was used, a phenomenon that can be achieved in vivo.

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