ABSTRACT

The pharmacokinetics of cyclophosphamide (CP) and several important metabolites was studied in detail in six patients receiving CP alone and with a radio- and chemosensitizing agent, SR-2508. CP at 1000 mg/m² was either infused in 20 min alone or given 2 h before an infusion of SR-2508 at 5 g/m² over 20 min, both separated by 3 weeks, to the same patients in a randomized fashion. Plasma and 24-h urinary levels of CP and four metabolites: [4-hydroxycyclophosphamide (4-OH CP), phosphoramid mustard (PM), chloroethyl oxazolidin-2-one, and alcohophamide] were monitored by a gas chromatographic-mass spectrometric-stable isotope dilution assay. CP plasma levels were found to decline monoexponentially with the appearance of transient saturation kinetics in some and a mean t½ of 5.2 h for patients treated with CP alone. Plasma 4-OH CP levels showed a mean peak concentration of 2.4 μM and declined approximately in parallel to those of CP. The major circulating metabolite was found to be PM with a mean peak concentration of 40 μM and a terminal t½ of 15 h. The mean area under the plasma concentration curve (AUC) ratios between metabolites and CP were: 4-OH CP, 0.0158; PM, 0.4518; and chloroethyl oxazolidin-2-one, 0.179 with alcohophamide at low levels. No appreciable amount of nitrogen mustard was detected. Mean urinary excretion was: CP, 10.8; 4-OH CP, 0.5; PM, 39.0; alcohophamide, 0.4; and chloroethyl oxazolidin-2-one, 3.0, all expressed as a percentage of CP dose. No statistically significant difference was detected in all standard pharmacokinetic parameters determined for both CP and metabolites between patients with CP alone and with SR-2508. Plasma 4-(p-nitrobenzyl)pyridine activity was found to correlate the dosest with metabolites between patients with CP alone and with SR-2508. Plasma 4-(p-nitrobenzyl)pyridine activity was found to correlate the closest with PM profiles, with respect to both standard pharmacokinetic parameters and AUC values. When plasma PM AUC values were plotted against AUC values of circulating 4-(p-nitrobenzyl)pyridine activity, a correlation coefficient of 0.859 (P < 0.001) was obtained. Together with the significant cytotoxicity of PM these data support a significant contribution of circulating PM in the antitumor effect of PM.

INTRODUCTION

Extensive pharmacokinetic data on the most widely used anticancer alkylating agent CP have been published since its introduction for the treatment of neoplastic diseases and as a suppressant of immune responses following organ transplants (1–3). The majority of the studies focused on the disposition of the parent drug as measured by a variety of assay procedures (4–10) following i.v. (1, 11–14) and/or p.o. administration(s) (15–18) either singly (8, 11, 12) or in combination with other drugs (3, 9, 10, 13, 19, 20). Early knowledge of CP pharmacology confined the role of CP to that of a prodrug of some alkylating entities, particularly NNM. Consequently, many of the early studies attempted to probe the circulating alkylating activity by a rather simple colorimetric method, the NBP assay, which is a measurement of the ability of alkylating species to react with 4-(p-nitrobenzyl)pyridine (11, 19–21). This assay appears to fulfill the practical purpose of providing a gross alkyl index following drug administration, although it lacks information as to the specific metabolites generated. As information on the metabolic activation of CP was unraveled (21–30) and metabolites were identified (Fig. 1), efforts were made to quantitate specific important metabolites in urine and in circulation (1, 8, 9, 17, 31–37) with the aim of understanding the relationship between the antitumor activity and metabolite levels. However, because of the instability of many of these reactive metabolites (31–33, 38–42), only certain of the circulating metabolites have been adequately quantitated in a given study (1, 8, 9, 17, 31–37). Still, questions on the validity of the analytical methodology exist among some studies because of a lack of adequate internal controls (32–35, 43) and much information relative to the disposition of the active metabolites is lacking. Using stable isotope-labeled methodology we have established a comprehensive gas chromatographic-mass spectrometry system for the quantitative measurement of most of the active CP metabolites in circulation and in urine, and we have begun to reevaluate the clinical pharmacokinetics of CP with reference to the specific metabolite composition and metabolite pharmacokinetics (44, 45). In the present study, we report the clinical pharmacokinetics of CP alone and in combination with a chemosensitization agent SR-2508. The choice of SR-2508, a misonidazole analogue, for this investigation was due to the reported effects of some misonidazoles on pharmacokinetics and metabolism of CP and other alkylating agents (46) and to potentiation of their antitumor effects in experimental systems (47–49). Additionally, the correlation of plasma NBP-alkylating activity with circulating metabolite levels is presented.

MATERIALS AND METHODS

Clinical Protocol. As part of a Phase I clinical trial of SR-2508 and CP for the assessment of the impact of the former agent on CP-induced myelosuppression, patients were randomly assigned to receive either CP alone and then CP + SR-2508, or vice versa, over the first 2 cycles. The CP dose was 1.0 g/m² and the SR-2508 dose was 5.0 g/m². All remaining cycles were given the combination. The pharmacokinetics of CP and SR-2508 was studied in selected patients during these first two cycles and that of SR-2508 has been reported previously (13). Six patients were entered on the pharmacokinetic study protocol at the Wisconsin Clinical Cancer Center, and this protocol was approved by its Human Research Committee. CP alone was given to patients by a 20-min infusion, and in the combination, CP was given as a 20-min infusion followed in 2 h by SR-2508 infusion over 10–20 min. Blood samples were collected into heparinized syringes typically at 0 (before drug), 0' (postinfusion), 15, 30, 45, 60, 90, 120, 150, 180, 240, 360, 480, 540, 660, 720, 840, 900, 1020, 1440, and 1560 min and placed immediately in an ice bath. Following separation of the plasma, usually within 1 h, a 2.0-ml aliquot was frozen immediately in a dry ice-acetone bath for shipment to Los Angeles while the remaining (about 2.0 ml) was frozen until it was analyzed at the
The chemical ionization mode was used, and ammonia was used as the reagent gas with the electron energy set at 200 eV. The injection port temperature was 200°C. The temperatures of the transfer line, ion source, and jet separator were all maintained at 200°C. Graph via an all-glass jet separator were used for the analysis. The temperatures purchased from Curtin-Matheson Scientific (Brea, CA). All solvents and reagents were of analytical or HPLC grade and were used without further purification.

Chemicals and Reagents. CP and PM used as the cyclohexylamine salt were supplied by Drug Synthesis and Chemistry Branch, Division of Cancer Treatment, NCI (Bethesda, MD). 4-(Nitrobenzyl)pyridine and NNM were purchased from Aldrich (Milwaukee, WI). 4-OOHCPC, [β-2H8]-4-OOHCPC, [α,β-2H4]CP, [α,β-2H4]PM, [α,β-2H4]NNM were synthesized according to the literature procedures (30, 45, 50) with appropriate modification. 4-OOHCPC and [β-2H8]-4-OOHCPC were prepared by reduction of 4-OOHCPC and [β-2H8]-4-OOHCPC, respectively, with sodium thiosulfate immediately before use (51). CNM and perdeuterio CNM were prepared by reaction of NNM and [α,β-2H4]NNM in saline solution with an excess amount of NaHCO3 followed by centrifugation at 2000 × g at 4°C for 6 min. The residue was evaporated to dryness under a stream of nitrogen. The residue was then derivatized with 40 µl BSTFA at 120°C for 40 min. An aliquot of the derivatized sample, usually 1–2 µl in BSTFA, was analyzed by GC/MS. The procedure is depicted in Fig. 2.

For the analysis of total PM (45), an appropriate amount of [α,β-2H4]PM was added to 0.3 ml of the thawed plasma sample followed by brief mixing. The sample was passed through a PolyPrep mini column (Bio-Rad, Richmond, CA) packed with 400 mg of C18 reversed-phase resin. After centrifugation at 2000 × g at 4°C for 3 min the resin was washed with 0.5 ml of cold saline. The water was removed from the resin by centrifugation at 2000 × g at 4°C for 6 min. The resin was eluted with 2 ml of methanol into a Reactiv vial. Methanol was evaporated to dryness under a stream of nitrogen. The residue was then derivatized with BSTFA as described above. TMS derivatives of CP, dehydrochlorinated AlcP, and dehydrochlorinated allopaphosphanide-cyanohydrin adduct were separated on the DB-1 capillary column at the programmed temperature with retention times of 1.6, 1.8, and 2.1 min, respectively. Trimethylsilyl dehydrochlorinated PM was analyzed on the DB-1 capillary column and gave a retention time of 1.4 min at 157°C isothermally. Under this assay procedure the metabolite 4-OH CP, present in plasma, which was not stabilized totally decomposed to form PM. Thus, this step measured the composite of PM intrinsically present and that generated by 4-OH CP decomposition (total PM). True PM concentrations were obtained by subtracting molar 4-OH CP specific activity measured in the same sample as described above from total PM. The error due to subtraction was negligible since 4-OH CP concentrations were usually one to two orders of magnitude below those of PM. CNM was separated on the OV-275 column at 195°C with a retention time of 1.5 min. Appropriate ions for these compounds and their respective deuterium-labeled internal standards, except in the case of AlcP which used CP-d8 as the internal standard, were selected for monitoring as shown in Fig. 3.

All compounds except CNM were analyzed as trimethylsilyl derivatives which yielded the base peaks under ammonia chemical ionization-mass spectrometry. CNM was detected as an ammonia adduct ion. Levels of CP and metabolites were calibrated individually using appropriate calibration curves constructed for each species during analysis of each batch of samples and synthetic labeled and nonlabeled metabolites were used for this purpose. Assay characteristics for CP and metabolites have been partially described previously (44, 45, 50). Essentially, for CP the within-run CV in human plasma at 0.39 µM was 6.5% (n = 8) and the between-run CV was 10.5% (n = 8) with a sensitivity limit better than 0.1 µM. For 4-OOHCPC, the within-run CV at 0.36 µM was 6.2% (n = 8) and the between-run CV was 11.9% (n = 8) with a detection limit better than 0.1 µM. For PM, the within-run CV at 0.45 µM was 11.3% (n = 8) and the between-run CV was 10.3% (n = 8) with a sensitivity limit better than 0.2 µM. For AlcP, the within-run CV at 0.36 µM was 4% (n = 6) and the between-run CV was 4% (n = 6) with a detection limit of 0.07 µM.

The NBP assay was performed by the method of Friedman and Boger (21) with slight modification (52). SR-2508 did not interfere with the assay (13).

Pharmacokinetic Analysis. Pharmacokinetic fitting was performed either by PLOT4U (53) or by RSTRIP (MicroMath) with model selection criteria similar to the Akaike Information Criterion (54). Basically, the parent drug conforms to a one-compartment model and the metabolite to a 2-exponential

![Fig. 1. Metabolic pathways of cyclophosphamide.](image-url)
RESULTS

Plasma Pharmacokinetics of CP. Plasma CP profiles of all but three patients essentially declined monoexponentially and were thus fitted to a one-compartment model. The other three patients showed a transient distribution phase and were fitted to a two-compartment model. In several of these profiles slight positive deviation (convex) from linearity was evident, possibly suggesting some degree of saturation. A set of typical plasma concentration-time profiles of CP, 4-OH CP, PM, AlcP, and CNM of a patient receiving 1.0 g/m² of CP alone is shown in Fig. 4A and another set of profiles of this patient receiving the same dose of CP in combination with 5.0 g/m² SR-2508 is shown in Fig. 4B. Pharmacokinetic parameters of CP, such as the apparent zero-time intercept (C₀), terminal elimination rate constant (β), terminal half-life (t₁/₂β), median residence time, AUC, total body clearance (CLT), steady-state volume of distribution (Vdss), and urinary excretion of unchanged CP in 24-h urine were computed and shown in Table 1. As shown, t₁/₂βs of CP when given alone were within the range reported by most investigators (1, 4, 8–11, 13, 19) but somewhat shorter than those reported by some authors (9, 11). Similarly, the clearance values were nearly identical to those of Bailey et al. (13) but lower than those reported by several other investigators (1, 4, 8–11, 19). The urinary elimination of unchanged CP was about 11% in 24 h, consistent with the values reported by most investigators (1, 4, 12, 13) but different from that of 3.3% reported by Fuks et al. (19).
Metabolite Profiles and Metabolite Kinetic Parameters. Using stable isotope-GC/MS methodology, 4-OH CP, AlcP, PM, and CNM levels were detected in the plasma of all patients. Plasma levels of 4-OH CP, PM, and CNM were quantitated simultaneously in all patients using individual calibration curves as described in "Materials and Methods." AlcP levels were measured as five profiles in three patients due to inadequate samples. The typical profiles are shown in Figs. 4, A and B. Also shown in the same figures are alkylating profiles of plasma from this patient as measured by the NBP assay. As shown, peak times for these metabolites occurred at about 2 h for 4-OH CP, 3–4 h for AlcP, 6–7 h for PM, and 12–16 h for CNM, consistent with the order of formation in the metabolic scheme (Fig. 1). The earlier appearance of AlcP than of PM may suggest that the reduction of aldophosphamide to AlcP was rather rapid. The magnitudes of peak level or AUC values were in the descending order of PM, CNM, 4-OH CP, and AlcP, although in some patients, plasma levels of AlcP at some time points were slightly higher than those of 4-OH CP. A similar trend was observed in the percentage AUC ratios between the metabolites and the parent drug, reflecting their apparent relative magnitude of levels in circulation. Plasma 4-OH CP levels were quite variable among patients.

Plasma 4-OH CP levels on the average peaked at about 2 h and declined with variable $t_{1/2}$, in most instances, at levels parallel to or slightly longer than those of CP. The average $t_{1/2}$ (Table 2) appeared longer than that of CP but the difference is not statistically significant. The mean peak value of 2.4 μM was slightly higher than that of 1.7 μM as reported by Schuler et al. (1) as activated CP by the fluorescence assay, even at a lower dose of CP in the present study. PM levels appear to be the highest of all metabolites and 30–40 μM peak plasma concentrations were achieved. These values were higher than the 19 μM reported by Moore et al. (8), following a 2-h infusion of CP at 60 mg/kg, a dose higher than in the present study. The mean AUC value was also significantly higher than that of 260 μM h following i.v. infusion of cyclophosphamide at 50–60 mg/kg (56). PM levels were in general significantly higher than those of Jardin et al. (34), Juma et al. (35), and Struck et al. (18). Following the peak, PM concentrations declined monoexponentially. The average half-life was about 15 h with a range between 7 and over 30 h, significantly longer than that of CP. This observation was consistent with those of Wilkinson et al. (11) following i.v. administration of a comparable dose of CP. How-
Fig. 5. Urinary excretion profiles of CP and metabolites in patient 2 with and without SR-2508.

ever, the $t_{1/2}$ is somewhat longer than the 6.1 h reported by Honjo et al. (9) and that reported by Moore et al. (8). Thus, PM is the major circulating metabolite following CP administration.

Plasma levels of CNM were also rather variable among patients. We were unable to detect any significant levels of NNM despite significant effort spent. Previously we were also unable to measure NNM directly following CP administration to the rat (45, 57).

All of these metabolites were also detected and quantitated in urine. As was the case in plasma, PM was detected as the highest concentration of all the metabolites, and as much as 40% of the dose was recovered in 30-h urine. This was followed by CNM, AlcP, and 4-OH CP. The total dose recovered as unchanged drug and metabolites during this period was 54%. None of the other metabolites accounted for more than 5%, and the 24-h excretion of 4-OH CP was only 0.5%. We did not attempt to quantitate urinary carboxyphosphamide although it was detected. A significant amount of CP was eliminated in urine during the first 8 h, but the major fraction of the metabolites was eliminated after 8 h. A urinary excretion profile of CP and metabolites in one patient is shown in Fig. 5. These excretion data were in general similar to those of Hadidi et al. (58), but the total dose recovered in our study was significantly higher than their reported value of 36% in 24-h urine. The lower total recovery in their study might be due to the lower amount of PM (18.5%) found relative to our data. The discrepancy might be due to the instability of PM and/or the difference in assay methodology. Our total urinary excretion data were similar to the 60%
The activity is expressed as NNM equivalents and is plotted against time. Two typical profiles are shown in Fig. 4. As shown, the NBP activity rose, peaked in approximately the same time frame as PM, and declined in parallel to the levels of PM. The levels and shape of NBP activity between the patient treated with C\textsuperscript{P} alone and with SR-2508 are listed in Table 2. As shown, t\textsubscript{1/2} of 15.5 h when C\textsuperscript{P} was given alone are also similar to those of PM. The peak values of NBP activity in several profiles were close to those of PM, although the mean value of peak PM concentration was larger than that of the NBP activity. No apparent difference in levels and shapes of NBP activity between the patient treated with C\textsuperscript{P} alone and the patient treated with SR-2508 were detected, similar to those data reported previously (13). Similar results were seen when the AUC values between these two groups of patients were compared. When the 24-h AUC values of the NBP profiles were plotted against the 24-h AUC values of the NB\textsuperscript{P} activity between the patient treated with C\textsuperscript{P} alone and that with SR-2508, a highly significant correlation (P < 0.001) of r\textsuperscript{2} of 0.859 was found between NB\textsuperscript{P} and PM (Fig. 6). The correlations with other metabolites were rather poor giving r\textsuperscript{2} values between these two groups of patients were compared. When the AUC values of C\textsuperscript{P} and the metabolites between the same time interval in an attempt to make correlations, a highly significant correlation (P < 0.001) of r\textsuperscript{2} of 0.738 but correlations with other metabolites were rather poor giving r\textsuperscript{2} values of significantly less than 0.5. Thus, it is evident that NBP profiles correlate closely with those of PM.

**DISCUSSION**

Major problems in the elucidation of metabolite kinetics of C\textsuperscript{P} include its complex metabolism and the lack of proper analytical methodology. The latter problem was compounded by the instability and reactivity of several C\textsuperscript{P} metabolites such as 4-OH C\textsuperscript{P}, PM, and NNM. To circumvent the stability problem we have used appropriate radiolabeled material recovered in urine over 24-48 h reported by Grochow and Colvin (14).

**Comparison of CP Pharmacokinetics Alone and with SR-2508.** As shown in Fig. 4, no visual difference of CP profiles between that of CP alone and that with SR-2508 was detected. When comparing the pharmacokinetic parameters of CP alone with those given with SR-2508 (Table 1) no difference was detected at the significance levels indicated. This finding is consistent with similar data reported recently using the GC method (13).

**Comparison of CP Metabolite Pharmacokinetics Alone and with SR-2508.** Relevant pharmacokinetic parameters of the key active CP metabolites when giving CP alone and with SR-2508 are listed in Table 2. As shown, t\textsubscript{1/2} of 15.5 h when C\textsuperscript{P} was given alone are also similar to those of PM. The peak values of NBP activity in several profiles were close to those of PM, although the mean value of peak PM concentration was larger than that of the NBP activity. No apparent difference in levels and shapes of NBP activity between the patient treated with C\textsuperscript{P} alone and the patient treated with SR-2508 were detected, similar to those data reported previously (13). Similar results were seen when the AUC values between these two groups of patients were compared. When the 24-h AUC values of the NBP profiles were plotted against the AUC values of CP and the metabolites between the same time interval in an attempt to make correlations, a highly significant correlation (P < 0.001) of r\textsuperscript{2} of 0.859 was found between NBP and PM (Fig. 6). The correlations with CP provided a slightly lower r\textsuperscript{2} of 0.738 but correlations with other metabolites were rather poor giving r\textsuperscript{2} values of significantly less than 0.5. Thus, it is evident that NBP profiles correlate closely with those of PM.

**Correlation of Circulating Metabolite Levels with NBP Activity.** Plasma NBP activity was determined by the colorimetric method. The activity is expressed as NNM equivalents and is plotted against time. Two typical profiles are shown in Fig. 4. As shown, the NBP activity rose, peaked in approximately the same time frame as PM, and declined in parallel to the levels of PM. The levels and shape of all of the NBP profiles as expressed in NNM equivalents closely resembled those of PM but not of any other metabolites. The plasma NBP activity-time courses were fitted to a one compartment metabolite model, and the relevant pharmacokinetic parameters were computed and are shown in Table 2. The mean peak time of 7 h and mean t\textsubscript{1/2} of 15.5 h when CP was given alone are also similar to those of PM. The peak values of NBP activity in several profiles were close to those of PM, although the mean value of peak PM concentration was larger than that of the NBP activity. No apparent difference in levels and shapes of NBP activity between the patient treated with CP alone and the patient treated with SR-2508 were detected, similar to those data reported previously (13). Similar results were seen when the AUC values between these two groups of patients were compared. When the 24-h AUC values of the NBP profiles were plotted against the AUC values of CP and the metabolites between the same time interval in an attempt to make correlations, a highly significant correlation (P < 0.001) of r\textsuperscript{2} of 0.859 was found between NBP and PM (Fig. 6). The correlations with CP provided a slightly lower r\textsuperscript{2} of 0.738 but correlations with other metabolites were rather poor giving r\textsuperscript{2} values of significantly less than 0.5. Thus, it is evident that NBP profiles correlate closely with those of PM.

**DISCUSSION**

Major problems in the elucidation of metabolite kinetics of CP include its complex metabolism and the lack of proper analytical methodology. The latter problem was compounded by the instability and reactivity of several CP metabolites such as 4-OH CP, PM, and NNM. To circumvent the stability problem we have used appropriate deuterium-labeled internal standards for all CP-related species except AlCP, thus compensating for procedural loss or decomposition during workup. Additionally, appropriate stabilization procedures, e.g., cyanohydrin formation for aldophosphamide/4-OH CP and oxazolidinone formation for NNM, were used to increase the stability of the derivatives. In the case of the stable metabolite AlCP, CP-\textsubscript{d} was used as the internal standard. This choice is an acceptable practice for a quadrupole mass spectrometer. Thus, using the developed comprehensive analytical procedure for the reactive metabolites of CP we were able to measure most of the active CP metabolites in circulation. While for the most part the results were similar to those reported some significant differences were noted.

A major difference between our study and those of Jardin et al. (34) and Juma et al. (35) was our failure to detect significant levels...
of NNM in circulation. Both groups converted NNM to the acylated derivatives before analysis by GC or GC/MS. Previous attempts to use their procedure in our laboratory only yielded highly variable and poorly reproducible results. Thus, we have developed an alternate procedure (45, 57, 59) for the quantitation of NNM and have used it in the present study. Based on reaction kinetics (38, 59) our method would have detected any significant amount of NNM as C-13 CNM if NNM had been present. Similarly, we were previously unable to detect significant circulating levels of NNM in rodents (45). Others have also detected low NNM levels in urine (58). This discrepancy could be due to an artifact resulting from the decomposition of carboxyphosphamide (34) or PM during the assay procedure in studies by those groups that detected high levels of NNM.

The other major difference was in the magnitude of circulating PM levels and the accompanying AUC. Our data showed substantially higher circulating levels of PM than those found by many investigators (18, 34, 35, 56) and also higher 24-h urinary excretion than that reported (58). This difference may be due to the lack of use of an appropriate internal standard and breakdown of PM during processing in some studies. We have also previously detected higher PM levels in plasma of rats receiving CP i.v. (45). These differences would result in significantly different interpretation of the pharmacology of CP metabolites. While it is generally agreed that PM is the ultimate intracellular cytotoxic metabolite, two opposing views concerning the relative significance of PM and its precursor 4-OH CP in circulation still exist. Thus, several investigators (27, 60–62) favor PM while some (36, 56, 63–67) favor the precursors. On the basis of three points: (a) the higher cytotoxicity of a number of normal and tumor mouse and human cell lines to 4-OH CP following a 30-min exposure; (b) ionizability of PM at physiological pH; and (c) AUC-cytotoxic activity ratio, Sladek (36, 67) recently argued for the significance of 4-OH CP.

The current study has shed some light on this controversy: (a) our data have shown significantly higher circulating levels of PM than of 4-OH CP levels, in the ratio of >30, nearly 10-fold of that of Sladek et al. (56); and (b) pharmacokinetic parameters of the NBP profiles as well as their AUC values correlate well with those of PM but not of other metabolites or the parent compound. Thus, if NBP is a valid measurement of the in vivo antitumor activity, then it is quite clear that PM is the important circulating cytotoxic metabolite. Although PM is highly ionized at physiologic pH its partition ratio between RBC and plasma was found to be about 0.5 (45). Although <1, and the value may be smaller for tissue or tumor cells, this partition ratio would still provide PM with significant ability to penetrate into cells in light of its long exposure time and high AUC values. On the basis of the cytotoxic data and AUC ratio values, Sladek (36, 67) proposed a relative activity index between 4-OH CP and PM which was estimated by a ratio between the AUC values of 4-OH CP and PM after being normalized to their respective cytotoxic potencies (AUCx/Px/AUCy/Py). If the value is >1, 4-OH CP would be considered to be the important circulating metabolite and likewise the reverse would hold for PM. Sladek (67) found that in all mouse normal and malignant cell lines and human tumor cell lines evaluated, the relative indices for 4-OH CP were all significantly >1. Using the same formula for the cytotoxic data of Sladek coupled to our AUC values we recalculated these indices for the same cell lines and found that in all cases but one the values were smaller than unity for 4-OH CP. Thus, this calculation indicated the reverse role between 4-OH CP and PM and that PM is the important circulating metabolite. It should be pointed out that the standard cytotoxicity evaluation for many alkylating agents of 1 h or shorter exposure was found to be inadequate in some cases, such as for PM (68). PM has been found to be only marginally cytotoxic to L1210, human CCRF-CEM cells, and HT-29 cells (68) following a 1-h exposure but significantly more cytotoxic following a 72-h continuous exposure. This is not unreasonable for polar ionizable molecules which require adequate time to achieve adequate penetration and thus a cytotoxic effect. On the other hand, this requirement may not be important for 4-OH CP. The 30-min exposure might have reflected only the transport and not a true measurement for the cytotoxicity. In vivo, following CP administration, the generated PM remains in circulation continuously as governed by the pharmacokinetics of CP, allowing circumvention of the diffusional barrier (69, 70). In the present case the majority of the PM profiles provided PM levels at or above 10 μM, which was reported to be the 50% effective concentration (71) for PM upon 4–28 h plus of exposure. Thus, our data and their interpretation strongly support the proposal that circulating PM plays an important role in the clinical antitumor effect of CP. Our previous, detailed metabolite kinetic study in the rat suggested that the 4-OH CP once formed in the liver is fully available to circulation (45) providing an important transport role for this metabolite, especially in the initial delivery of active metabolites to organ tissues.

REFERENCES


Fig. 6. Correlation plots between the AUC values of circulating PM and those of NBP profiles.

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REFERENCES


61. Friedman, O. M., Wodinsky, T., and Myles, A. Cyclophosphamide (NSC-26271)-
related phosphoramide mustards: recent advances and historical perspective. Cancer
62. Hipkins, J. H., Struck, R. F., and Gurtoo, H. I. Role of aldehyde dehydrogenase in the
metabolism dependent biological activity of cyclophosphamide. Cancer Res., 41:
3571–3583, 1981.
63. Connors, T. A., Cox, P. J., Farmer, P. B., Foster, A. B., and Jarman, M. Some studies
of the active intermediates formed in the microsomal metabolism of cyclophospha-
64. Brock, N., and Hohorst, H-J. The problem of specificity of alkylating cytostatics:
65. Domeyer, B. E., and Sladek, N. E. Kinetics of cyclophosphamide biotransforma-
66. Hilton, J. Role of aldehyde dehydrogenase in cyclophosphamide resistant L1210
68. Zheng, J. W., Chan, K. K., Ardalan, B., Hengst, J., and Muggia, F. M. One hour
exposure is not adequate for cytotoxic evaluation of some alkylating agents. Proc.
69. Sladek, N. E., Powers, J. F., and Grage, G. M. Half-life of oxazaphosphorines in
70. Pang, K. S., Cherry, W. F., Terrell, J. A., and Ulm, E. H. Disposition of enalapril and
its diacid metabolite, enalaprilat, in a perfused rat liver preparation. Presence of
a diffusional barrier for enalaprilat into hepatocytes. Drug Metab. Dispos., 12:
71. Friedman, O. M. Studies of some newer phosphoramide mustards. Cancer
Clinical Pharmacokinetics of Cyclophosphamide and Metabolites with and without SR-2508

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