Nonpredictable Pharmacokinetic Behavior of High-Dose Cyclophosphamide in Combination with Cisplatin and 1,3-Bis(2-chloroethyl)-1-nitrosourea

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ABSTRACT

Our objective was to assess whether the total area under the curve (AUC) of high-dose cyclophosphamide (CPA), combined with cisplatin and 1,3-bis(2-chloroethyl)-1-nitrosourea, could be predicted from its AUC on the first day of treatment. We reviewed the AUC of CPA in 470 patients who underwent pharmacokinetic monitoring of the drug. All patients received the same high-dose regimen of CPA, cisplatin, and 1,3-bis(2-chloroethyl)-1-nitrosourea (STAMP-I) with identical antiemetic support. Subsequently, patients who experienced a toxic death, relapsed after high-dose chemotherapy, or remained relapse-free at a minimum follow-up of 1 year after high-dose chemotherapy were analyzed for a correlation between the total AUC of CPA and both relapse-free survival and toxic death. The AUC of CPA decreased from day 1 to day 2 in most patients. However, its changes from day 2 to day 3 varied significantly. Neither the value of AUC on day 1 nor its decreasing trend from day 2 to day 3 could predict the AUC on day 3 and the total AUC. Our pharmacodynamic analysis in 335 patients failed to show a correlation between the total AUC of CPA and either toxic death or relapse-free survival. The significant intersubject variability in the AUC of CPA makes the final AUC of the drug unpredictable from an initial measurement on day 1. Thus, in this combination, measurement of levels of parent CPA, with the objective of real-time therapeutic monitoring of this drug, is not informative.

INTRODUCTION

HDC with stem cell support attempts to overcome cell resistance to standard-dose chemotherapy by exploiting the dose-response effects of combinations of antineoplastic drugs. Considering the high potential for toxicity with HDC, ongoing research is pursuing the application of TDM to HDC. TDM prospectively adjusts for interindividual variability of concentrations achieved after the first dose of a drug to a final target PK parameter. This strategy is based on two requirements: (a) the knowledge that, for a particular drug, a certain PK parameter is associated with either a toxic or a therapeutic pharmacodynamic effect; and (b) the demonstration that the drug’s PK behavior is consistent and predictable using an initial measurement.

Some successful applications of TDM to HDC include the prospective dose adjustment of busulfan to reduce the incidence of hepatic venoocclusive disease (1). PK-guided Phase I trials of busulfan have been proven to be feasible (2). A recent prospective randomized trial in childhood acute lymphoblastic leukemia compared conventional dosing of methotrexate, teniposide, and cytarabine, based on the body surface area, with individualized dosing of the three drugs based on a target AUC range (3). The outcome of patients with B-cell acute lymphoblastic leukemia was significantly improved with TDM compared to conventional dosing.

CPA is widely used in HDC for hematological and solid malignancies. Its toxic profile at high doses includes cardiac necrosis, venoocclusive disease of the liver, and hemorrhagic cystitis. Ayash et al. (4) retrospectively reported an inverse correlation between the AUC of the parent compound and both cardiotoxicity and tumor response. Their results suggest the potential need for TDM of CPA.

Four hundred seventy patients underwent PK monitoring of high-dose CPA at the University of Colorado Bone Marrow Transplant Program. CPA was delivered in combination with CDDP and BCNU. We reviewed the PKs of CPA to determine whether day 1 AUC could be predictive of AUCs on subsequent days and of the total AUC.

PATIENTS AND METHODS

Patient Population. As of March 1998, PK monitoring of CPA had been performed on 470 patients treated with the same CPA-containing combination. Diagnoses were breast cancer (n = 406), NHL (n = 50), and HD (n = 14). All patients had a Southwest Oncology Group performance status of ≤2 and acceptable pretransplant organ function (creatinine clearance,
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The treatment schema is depicted in Fig. 1. The doses of CPA, CDDP, and BCNU were those previously described by Peters et al. (5) On days −6, −5, and −4, CPA was given as a daily 1-h infusion starting between 9 and 11 a.m., at 1875 mg/m²/day (total dose, 5625 mg/m²), and CDDP was administered as a 72-h CI at 165 mg/m². On day −3, 600 mg/m² BCNU was infused over 2 h. All doses of chemotherapy were calculated on the actual body weight, unless that weight was ≥20% over the ideal body weight, in which case the average between the actual and ideal body weight was used to calculate the body surface area. Unselected marrow or PBPCs were infused on days −1, 0, and +1, whereas CD34-selected PBPCs were infused on day −1. All patients received granulocyte-colony stimulating factor-mobilized PBPCs were performed prior to admission.

Chemotherapy. The treatment schema is depicted in Fig. 1. The doses of CPA, CDDP, and BCNU were those previously described by Peters et al. (5) On days −6, −5, and −4, CPA was given as a daily 1-h infusion starting between 9 and 11 a.m., at 1875 mg/m²/day (total dose, 5625 mg/m²), and CDDP was administered as a 72-h CI at 165 mg/m². On day −3, 600 mg/m² BCNU was infused over 2 h. All doses of chemotherapy were calculated on the actual body weight, unless that weight was ≥20% over the ideal body weight, in which case the average between the actual and ideal body weight was used to calculate the body surface area. Unselected marrow or PBPCs were infused on days −1, 0, and +1, whereas CD34-selected PBPCs were infused on day −1. All patients received granulocyte-colony stimulating factor-mobilized PBPCs were performed prior to admission.

Supportive Care. On day −7, i.v. hydration at 250 ml/m²/h, to ensure a urinary output of >200 ml/h, and continuous bladder irrigation were initiated and continued through day −3, after the delivery of BCNU. Prior to chemotherapy, patients were transfused irradiated packed RBCs to a target hematocrit of ≥42. No transfusions were given during chemotherapy. All patients received the same antimetic regimen, consisting of prochlorperazine (loading dose of 10 mg i.v. followed by 1 mg/m²/h as a CI), diphenhydramine (25 mg every 6 h i.v.), and lorazepam (1 mg/m² every 4 h i.v.). A standard prophylactic antimicrobial regimen was initiated on day −2.

Analytical Methods. Blood samples for CPA were obtained in 10-ml heparinized collection tubes, stored at 4°C, and assayed within 24 h using a modification of the method of El-Yazigi and Martin (6). Blood samples were obtained remote from the site of CPA infusion at 30, 60, 90, 120, 180, 300, 420, 540, 780, 1020, and 1260 min after the start of each daily infusion. After the samples were centrifuged at 2000 rpm for 10 min, 1 ml of the supernatant plasma and 1 ml of the internal standard solution, 50 µg/ml diphenhydramine, were dispensed into plastic disposable tubes. Solid-phase extraction columns (LC-SI 3-ml Extraction Columns; Supelco Inc., Bellefonte, PA) were preconditioned with 3 ml of the elution solution (85% high-pressure liquid chromatography-grade ethyl acetate and 15% high-pressure liquid chromatography-grade methanol) followed by 3 ml of water. The plasma containing internal standard was then added to the column. The columns were eluted with 1.5 ml of the elution solution. The eluant was centrifuged at 2000 rpm for 5 min, and 0.8 ml of the supernatant was transferred to 1-ml autosampler vials. One µl of each vial was injected, with a split ratio of 20:1. A capillary column-equipped gas chromatograph with nitrogen-phosphorus detection was used. The time/concentration data were analyzed using WIN-NONLIN Version 1.1 nonlinear regression software (SCI Software, Cary, NC). The detection limit of this assay was 0.1 µg/ml. Its intra- and interrun precision coefficient variation was <10%. This analysis can be performed overnight with results available the next morning prior to the subsequent CPA dose.

Pharmacodynamic Analysis. A pharmacodynamic analysis of CPA was performed in patients who experienced a toxic death, relapsed after HDC, or remained free of evidence of recurrence at a minimum follow-up of 1 year. A total of 335 patients were evaluated for a potential correlation between the total AUC of CPA and the occurrence of toxic death. Median follow-up for this sample is 28 months (range, 1–89 months).

This patient sample was broken down into the following subgroups to ascertain the correlation of CPA AUC with a therapeutic end point (relapse-free survival) within each of the subgroups: stage II–III breast cancer (n = 115); stage IV breast cancer (n = 163), including both patients with no evidence of disease (n = 38) and those with macroscopic tumor at the time of transplant (n = 125); NHL (n = 44); and HD (n = 13).

Statistical Methods. Using patients as the random variable and time as the fixed factor, we fitted a mixed model to test the relationship between the values of the AUC of CPA and time (days 1, 2, and 3). The relationships between AUCd1 and AUCd2 and between AUCd2 and AUCd3 were studied by fitting simple linear models. A χ² test was used to test the distribution of proportions of four patterns of AUC changes. These patterns were “decrease” (the AUC consistently decreasing from day 1 to day 3), “up-down” (the AUC increasing from day 1 to day 2 but then decreasing to day 3), “down-up” (the AUC decreasing from day 1 to 2 but then increasing from day 2 to 3), and “increase” (the AUC consistently increasing throughout the 3 treatment days). ANOVA and the t test were used to detect differences in the magnitude of AUCd1 among the four groups as well as differences in the decreasing scale from AUCd1 to AUCd2 between decrease and down-up groups.

The total AUC of CPA was compared in patients with and without a toxic death using the Kruskal-Wallis test. A two-tailed t test was used to evaluate the association of CPA AUC with relapse. All statistical calculations were performed using the SAS software package Version 6.12 (SAS Institute Inc., Cary, NC).
RESULTS
Overall, the values of CPA AUC tended to decrease over the three days of treatment, as shown in Table 1. The mixed model demonstrates that the mean value of AUC decrease is 6,300 μg·min·ml⁻¹ per day, when considering the patient population as a whole. When the group of patients was studied at the subject level, four different patterns of changes in the AUC values became apparent (Table 2). A consistently decreased AUC value over time (decrease pattern) was seen in 66.5% of the subjects. Of these, 30.6% demonstrated a decrease in the AUC value over time (decrease pattern) was seen in 66.5% of the patients had an increase from day 2 to day 3 but an increase from day 2 to day 3 (down-up); 0.2% and 2.6% of the patients had increase and decrease patterns, respectively. The χ² test showed that the proportions of the four patterns were not uniform (P = 0.001).

The data were analyzed in an attempt to identify a pharmacological reason for the four different patterns. The value of the AUCd1 was compared among the four groups (Table 3). The ANOVA test showed no significant difference of AUCd1 among the four patterns (P = 0.4). The decreasing trend in the AUC from day 1 to day 2 but an increase from day 2 to day 3 (down-up); 0.2% and 2.6% of the patients had increase and up-down patterns, respectively. The χ² test showed that the proportions of the four patterns were not uniform (P = 0.001).

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Pharmacodynamic Analysis. The association between the occurrence of toxic death and the total CPA AUC did not reach statistical significance (P = 0.18) in the sample of 335 patients analyzed.

In addition, the AUC of CPA did not correlate with relapse in any of the groups evaluated: stage II–III breast cancer (P = 0.36), all stage IV breast cancer patients (P = 0.45), stage IV breast cancer patients with no evidence of disease (P = 0.74), stage IV patients breast cancer with macroscopic lesions at transplant (P = 0.18), NHL (P = 0.15), and HD (P = 0.84).

DISCUSSION
Our retrospective analysis of the AUC of CPA shows that, although AUC generally decreased from day 1 to day 2 and exhibited an overall reduction throughout the 3 treatment days, there were significant variations in the changes of the AUC from day 2 to day 3. For each individual patient, neither the value of AUCd1 nor the decreasing trend from AUCd1 to AUCd2 could predict the direction of the subsequent change in the last treatment day.

In addition, we did not find a correlation in our series between the total AUC of CPA with either a toxic (treatment-related death) or a therapeutic end point (relapse-free survival). In contrast, Ayash et al. (4), showed a significant inverse association between the AUC of CPA and both end points using a different high-dose regimen of CPA, thiopeta, and carboplatin (STAMP-V).

CPA is an inactive prodrug that undergoes a complex metabolic process, which is summarized in Fig. 2 (7). It is hydroxylated by the cytochrome P450 system to its active metabolite 4-OHCPA, which equilibrates with its acyclic tautomer AP. 4-OHCPA and AP enter the cell, where AP undergoes β-elimination of acrolein to form phosphoramide mustard, the ultimate bialkylating moiety. Hepatic or erythrocyte aldehyde dehydrogenase isoenzymes, particularly aldehyde dehydrogenase-1, can oxidize AP to inactive metabolites that are excreted in the urine. In addition, CPA can also be excreted unchanged in the urine or undergo P450-mediated side-chain oxidation to inactive compounds.

Measurement of the parent compound for pharmacodynamic analysis has important limitations. The P450-mediated hydroxylation of CPA is subject to interactions with other drugs, such as busulfan (8), phenytoin (8), CDDP (9), ondansetron (10), or dexamethasone (11). High-dose CPA demonstrates nonlinear elimination kinetics, in both a concentration- (12) and time-dependent (13) fashion. A 7-fold intersubject difference in exposure to 4-OHCPA was observed after CPA/total body irradiation, in which CPA is given first (8). Chen et al. (13) described nonlinear clearance and altered hepatic metabolism of CPA combined with thiopeta. These authors noticed a large range in Kₐ in the P450-mediated oxidation of CPA for patients receiving single-agent CPA (14).

Because the population in this study was homogeneous in terms of concomitant antineoplastic drugs, hydration, concurrent antiemetics, and pretreatment hematocrit and liver function...
tests and because these variables remained homogeneous throughout the 3 treatment days, the intrapatient variability of CPA AUC seems attributable to less correctable factors. The disposition of high-dose CPA by the P450 enzymes is a saturable process (12). On the other hand, CPA autoinduces its own P-450 metabolism (15–18). Chen et al. (13) observed that autoinduction is time dependent, occurring at an average of 29 h after the start of the CPA infusion. Thus, nonpredictable variations in the balance between enzyme saturation and autoinduction, which did not become apparent until after the second day of treatment, may explain the intrapatient differences in the AUC of CPA throughout the days of treatment we observed.

Studies on the correlation between the AUC of CPA and 4-OHCPA/AP show contradictory data. Slattery et al. (8) have shown an inverse correlation between the AUC of CPA and that of 4-OHCPA, which is consistent with the clinical observations from Ayash et al. (4) In contrast, Chen et al. (14) found a positive correlation between both AUCs. In addition, these authors found no correlation between these two AUCs when CPA was given concurrently with thiotepa. Therefore, it has been argued that it should be the active metabolites that are monitored in first place. Study of the actual exposure to these reactive and extremely unstable intermediates has been limited in the past by analytic methodology. Real-time TDM requires rapid sample analysis with results available the next morning prior to the subsequent CPA dose, and accurate and reliable assays for the determination of 4-OHCPA or AP have only recently been developed. Anderson et al. (19) described a method that includes immediate derivatization of the metabolites to form a stable oxime. This assay involves gas chromatography/mass spectrometry technology and requires a specific deuterium-labeled internal standard (20). Slattery et al. (8) developed an assay to quantify 4-OHCPA based on the bedside formation of a stable derivative.

An important limitation to the monitoring of the active metabolites may be the variability in the relative contribution of activating and inactivating reactions to their disposition. Busse et al. (21) showed a reduction in the activation of CPA and an increase in the inactivation of 4-OHCPA/AP and CPA at high doses of the drug, compared to conventional doses given to the same patients. These intrapatient changes showed, in turn, wide interindividual variability. Thus, the varying shift in the metabolic pathways of 4-OHCPA toward inactivating reactions suggests that the measurement of plasma levels of 4-OHCPA, like the quantitation of the parent drug, may not reflect the intracellular levels of phosphoramide mustard, ultimate responsible for the alkylating effects of CPA. In addition, Dockham et al. (22) showed a 3-fold interindividual variation in the erythrocyte

![Metabolism of CPA](Fig. 2)
fraction of aldehyde dehydrogenase-1, an enzyme that seems to play an important role in the detoxification of 4-OHCPA. Thus, variations in the hematocrit may also change drug disposition.

In conclusion, our study shows that, with the CPA, CDDP, and BCNU regimen, unpredictable intrapatient variations in the AUC of CPA occur. This strongly suggests that prospective TDM of CPA based on the measurement of the parent compound is not feasible. Observations cited above suggest a similar variability in the reactive metabolites, although further analyses are needed. Investigation of other alkylating agents which may be amenable to real-time TDM in the transplant setting seems warranted.

REFERENCES


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