Plasma Pharmacokinetics of Cyclophosphamide and Its Cytotoxic Metabolites after Intravenous versus Oral Administration in a Randomized, Crossover Trial

Robert F. Struck, David S. Alberts, Katherine Horne, J. Gregory Phillips, Yei-Mei Peng, and Denise J. Roe

ABSTRACT

Since cyclophosphamide is used by both oral and i.v. routes in the treatment of hematological and solid malignancies, we designed a randomized, crossover clinical trial to evaluate the pharmacokinetics of this anticancer agent after either administration route. Plasma levels of cyclophosphamide and its two cytotoxic metabolites, 4-hydroxy-cyclophosphamide and phosphoramidium mustard, were determined in seven cancer patients randomly assigned to treatment initially with either orally or i.v. administered cyclophosphamide with a 30-day interim between alternate therapy courses. Oral treatment was used initially in five patients and i.v. treatment in two patients, and the pharmacokinetic parameter, area under the plasma disappearance curve, was determined for each metabolite in each patient for both routes of drug administration. Statistical comparison of area under the plasma disappearance curve values for this set of patients indicated no significant differences for either metabolite for oral versus i.v. drug treatment, suggesting equal efficacy for these two routes of cyclophosphamide administration.

INTRODUCTION

Juma et al. (1) reported that three times the plasma alkylating activity was observed in lymphoma patients after p.o. treatment with CPA as compared to plasma alkylating activity in the same patients treated i.v. Heretofore, investigations on levels of CPA metabolites in blood or plasma of experimental animals or humans involved use of i.v. or i.p. drug administration (2-6). These investigators (1) also suggested that a different pattern of alkylating metabolites might be produced if different routes of CPA administration were used. In response to this report, we (7) investigated the pharmacology of CPA in mice and found that p.o. versus i.v. drug treatment resulted in identical qualitative metabolism of CPA but different quantitative metabolism, based on comparative blood levels of parent drug and metabolites. AUC data indicated a p.o./i.v. blood ratio of 0.17 for CPA itself and an average ratio of 0.40 for the six, relatively abundant metabolites (4-hydroxy-CPA, 4-keto-CPA, N-dechlo-roethyl-CPA, alcohophosphamide, carboxyphosphamide, and PM). The pathway for formation of these metabolites is shown in Fig. 1. For two of the oncolytic metabolites (4-hydroxy-CPA and PM), the observed ratio was 0.75 and 0.72, respectively, suggesting slight superiority for i.v. administration in the mouse. This report describes a similar study in cancer patients in which plasma levels of 4-hydroxy-CPA and PM were determined after p.o. or i.v. cross-over treatment with CPA as a means of assessing directly the implication of the earlier report (1), which suggested superiority for the p.o. route in patients. This report is the first study to compare plasma levels of either or both of the cytotoxic metabolites of CPA in patients after i.v. versus p.o. drug administration.

MATERIALS AND METHODS

Patient Selection. Eligibility criteria for this pharmacokinetic study included the following: (a) patients with histologically confirmed metastatic solid cancers requiring chemotherapy including CPA; (b) chemotherapy schedule allowing CPA as a single agent on day 1 of each of at least two courses; (c) dose of CPA 600 or 1000 mg/m² p.o. or i.v.; (d) no intake of routine type drugs known to interact with hepatic microsomal enzymes within 30 days of pharmacokinetic studies; (e) serum bilirubin less than 1.5 mg/dl and serum creatinine greater than or equal to 1.5 mg/dl; (f) and age less than 75 years. Patients were randomly assigned to receive CPA treatment initially by the p.o. or i.v. route with the alternate route being used 30 days later on the subsequent course.

Drug Administration. CPA (600 or 1000 mg/m²; Mead Johnson) in sterile water was mixed with [3H]CPA (0.5 mCi, specific activity, 2.0 Ci/mmol; Amersham) and administered by i.v. push in 5 min or p.o. in elixir syrup. Seven patients were treated with CPA at a dose of either 600 or 1000 mg/m² p.o. or i.v. followed 30 days later with the same dose by the route not used for the first treatment. Two patients received the lower dose (i.e., 600 mg/m²) of CPA, and five patients were randomly assigned to the oral route for the first treatment course.

Blood Collection. Blood (10 ml) was collected from an indwelling needle or catheter at various times from 5 min to 24 h after drug treatment and mixed immediately with 1 ml of a solution of 0.4 M semicarbazide (pH 7.4) in capped tubes and centrifuged (2000 rpm for 3 min). Plasma was separated, immediately frozen in capped, graduated tubes, and stored at -20°C until analysis.

Preparation of Metabolite Fractions. Plasma samples were allowed to thaw and their volume was recorded. Extraction of CPA and of 4-hydroxy-CPA, stabilized as aldophosphamide semicarbazone, was accomplished with chloroform (3 X 5 ml). Chloroform extracts were dried over sodium sulfate, filtered, concentrated to a small volume in a stream of nitrogen, and stored at -20°C. Extracted plasma was immediately frozen and lyophilized, and the residue in methanol was treated with excess ethereal diazomethane (prepared from Diazald, Aldrich Chemical Co.), allowed to stand 5 min at room temperature, and evaporated to dryness in vacuo. The residue was triturated with acetone and centrifuged, and the solution was separated, concentrated to a small volume in a stream of nitrogen, and stored at -20°C. This procedure serves to stabilize and isolate PM as its methyl ester. The extraction procedures have previously been shown to remove approximately 95% of the radioactivity present in blood or plasma of CPA-treated experimental animals (8). In order to determine the conversion and extraction efficiency for the aldophosphamide semicarbazone, [3H]-4-hydroxy-CPA, prepared by our reported procedure (9) from [3H]CPA, was added to plasma. Semicarbazide solution was added and mixed, and extraction with chloroform resulted in recovery of the semicarbazone with acceptable efficiency in four determinations (82, 85, 82, and 90%).

Analysis of Extracts. The two extracts were fractionated separately in triplicate by co-TLC with appropriate synthetic standards on Analtech (Newark, DE) precoated silica gel G thin-layer plates (250 μm thickness) in chloroform:methanol (9:1, v/v). Standards used with the 4-hydroxy-CPA fraction were CPA and aldophosphamide semicarbazone, as reported previously (9, 10), and standards used with the PM
fraction were PM methyl ester and carboxyphosphamide methyl ester, prepared as reported (11). Thin-layer plates were activated before TLC by heating 1 h at 100°C and storing in a dessicated cabinet. PM methyl ester fractions were fractionated on plates additionally heated 15 min at 140°C just prior to analysis; plates were allowed to cool to room temperature before sample application. This additional plate activation is necessary to effect efficient separation of the methyl esters of PM and carboxyphosphamide, which is also present in this fraction. Detection of metabolites and synthetic standards was accomplished by spraying plates with 1% 4-(p-nitrobenzyl)pyridine in acetone, heating 10 min at 140°C, and spraying with 3% potassium hydroxide in methanol. This technique visualizes the synthetic standards as blue spots. Individual alkylating spots (CPA and aldophosphamide semicarbazone on one set of TLC plates and PM methyl ester and carboxyphosphamide methyl ester on another set) were collected, transferred to scintillation vials, treated with 1 ml of methanol, and radioassayed. Radioassay data were converted into plasma levels.

Pharmacokinetic Analysis. The trapezoidal rule was used to determine areas under the CPA and metabolite plasma decay curves (AUC). When plasma concentration levels were greater than 0 after 24 h, the AUC from 24 h to infinity was computed using the terminal slope of the plasma concentration curve. Curves were plotted for CPA, 4-hydroxy-CPA (as aldophosphamide semicarbazone), and PM from appropriate plasma level values ± SD (obtained from triplicate analysis of each plasma sample). Paired t tests were used to compare p.o. versus i.v. AUC data since each patient served as his or her own control by receiving the drug by both administration routes.

RESULTS

Data were obtained for seven patients for two routes of drug administration and for blood collection times ranging from 5 min to 24 h. Illustrative values are shown in Table 1 for patient 7. As expected, high levels of CPA were observed immediately after i.v. administration followed by a fairly regular decline whereas maximum levels after oral treatment were attained after 0.25–3 h. A typical plot of plasma CPA concentration versus time is shown in Fig. 2 and was derived from patient 3.

Although somewhat variable, the oncolytic metabolites 4-hydroxy-CPA and PM reached peak levels at approximately 2–4 h after both routes of administration. A plot of plasma 4-hydroxy-CPA concentration versus time in patient 2 is shown in Fig. 3, wherein little difference was observed for levels of this metabolite by the two routes of treatment, and a plot of plasma concentration versus time for PM is shown in Fig. 4 for patient 1, also with little difference found between the two routes.

Plasma level data for CPA, 4-hydroxy-CPA, and phosphor-
amide mustard for the seven patients were used for calculation of plasma AUC values for the two metabolites, and results are shown in Table 2, revealing appreciable interpatient variation but less intrapatient differences. These p.o. versus i.v. plasma AUC data were compared for the group of seven patients with respect to the parent compound and individual metabolites. $P$ values from these comparisons are shown in Table 3. Although there were no significant differences in systemic availability of

<table>
<thead>
<tr>
<th>Patient</th>
<th>Initial route</th>
<th>Dose (mg/m²)</th>
<th>CPA</th>
<th>HOCPA*</th>
<th>PM</th>
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<tbody>
<tr>
<td>1</td>
<td>i.v.</td>
<td>600</td>
<td>344.90</td>
<td>203.63</td>
<td>1.15</td>
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<tr>
<td>2</td>
<td>p.o.</td>
<td>600</td>
<td>161.67</td>
<td>143.70</td>
<td>0.50</td>
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<tr>
<td>3</td>
<td>i.v.</td>
<td>1000</td>
<td>249.67</td>
<td>214.74</td>
<td>0.47</td>
</tr>
<tr>
<td>4</td>
<td>p.o.</td>
<td>1000</td>
<td>214.89</td>
<td>168.20</td>
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</tr>
<tr>
<td>5</td>
<td>p.o.</td>
<td>1000</td>
<td>270.22</td>
<td>213.90</td>
<td>0.32</td>
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<tr>
<td>6</td>
<td>p.o.</td>
<td>1000</td>
<td>178.65</td>
<td>126.89</td>
<td>0.28</td>
</tr>
<tr>
<td>7</td>
<td>p.o.</td>
<td>1000</td>
<td>210.33</td>
<td>148.10</td>
<td>1.08</td>
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</table>

* Corrected for dose differences.

Table 3: Comparison of mean AUC values for CPA, 4-hydroxycyclophosphamide, and PM

<table>
<thead>
<tr>
<th>Compound</th>
<th>i.v.</th>
<th>p.o.</th>
<th>(i.v. − p.o.)</th>
<th>$P$ value</th>
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</thead>
<tbody>
<tr>
<td>CPA</td>
<td>203.958 (55.872)</td>
<td>154.174 (48.151)</td>
<td>49.783 (23.027)</td>
<td>0.0012</td>
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<tr>
<td>HOCPA*</td>
<td>0.531 (0.289)</td>
<td>0.513 (0.212)</td>
<td>0.017 (0.166)</td>
<td>0.79</td>
</tr>
<tr>
<td>PM</td>
<td>0.507 (0.309)</td>
<td>0.490 (0.232)</td>
<td>0.017 (0.222)</td>
<td>0.84</td>
</tr>
</tbody>
</table>

* Numbers in parentheses ± SD.

DISCUSSION

In our previous, related studies (7, 12), 4-hydroxy-CPA was trapped as aldophosphamide cyanohydrin (13). However, because of improved stability of aldophosphamide semicarbazone in comparison with the cyanohydrin, semicarbazide was used in this study, and 4-hydroxy-CPA was found to be efficiently converted to aldophosphamide semicarbazone and extracted into chloroform (82–90%). An additional advantage of trapping 4-hydroxy-CPA as the semicarbazone is that its TLC mobility in the system used in these experiments results in wider separation than aldophosphamide cyanohydrin from CPA and other nonpolar metabolites of CPA extractable from plasma with chloroform. The greater polarity of the semicarbazone results in an $R_f$ value of 0.1, which is appreciably less than the $R_f$ value of CPA, 4-keto-CPA, N-dechloroethyl-CPA, and aldophosphamide in this TLC system.

The time required for 4-hydroxy-CPA and PM to reach peak levels (2–4 h) after both routes of drug treatment (Figs. 3 and 4) is consistent with results from an earlier study on plasma levels of PM in patients treated i.v. with CPA (3).

The results of these studies as summarized in Table 3 suggest that the major oncolytic metabolites of CPA are equally available after i.v. versus p.o. dosing and are present in approximately equal quantities. In contrast, CPA availability after p.o. dosing is significantly reduced as a result of first-pass hepatic metabolism to both active and inactive metabolites. These investigations document that i.v. and p.o. administration of cyclophosphamide are equally efficacious. Our study results are obviously at variance with those of Juma et al. (1), but we believe that our data are more precise in that they are restricted specifically to the metabolites of CPA that have demonstrated antitumor activity against experimental tumors in vivo (9). We conclude from these data that the i.v. and p.o. routes of CPA administration can be used interchangeably in the clinic. Other than specific drug protocol considerations, the major determinant of the preferred CPA administration route depends on patient reliability and tolerance to its acute gastrointestinal side effects.
side effects. Obviously, i.v. drug administration negates the need for such patient compliance concerns.

For those interested in establishing the superiority of one of the two cytotoxic metabolites as the major mediator of the antitumor effect of CPA in vivo, our data from this study lend some support to the arguments of others (13-17), who by application of various data and rationale have concluded that 4-hydroxy-CPA fulfills this role. The support from this study for their arguments arises from the greater cytotoxicity of 4-hydroxy-CPA versus PM against many (although not all) fresh or established tumor cells in vitro (16, 18). Since plasma levels of the two metabolites in this study were similar, it would be reasonable to conclude that 4-hydroxy-CPA would have dominated the total cytotoxic potential in plasma available for delivery to tumor cells in various tissues. However, plasma level data and AUC data for these metabolites in patients, as reported by others, can also be invoked to support a major role for PM (3, 4, 16, 17, 19).

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