A Phase I Pharmacologic and Pharmacodynamic Study of Pyrazoloacridine Given as a Weekly 24-Hour Continuous Intravenous Infusion in Adult Cancer Patients

Jean L. Grem,1 Nancy Harold, Bruce Keith, Alice P. Chen, Viven Kao, Chris H. Takimoto, J. Michael Hamilton, Janet Pang, Marie Pace, Gada B. Jasser, Mary G. Quinn, and Brian P. Monahan

National Cancer Institute-Navy Hematology/Oncology [J. L. G., N. H., B. K., A. P. C., V. K., C. H. T., J. M. H., J. P., G. B. J., M. G. Q.], Department of Internal Medicine [B. P. M.], and Department of Radiology [M. P.], National Naval Medical Center, Bethesda, Maryland 20889

ABSTRACT

Purpose: Pyrazoloacridine (PZA) is an investigational nucleic acid binding agent that inhibits the activity of topoisomerase I and II through a mechanism distinct from other topoisomerase poisons. PZA shows schedule-independent cytotoxicity against tumor cells, whereas host toxicity is greater with shorter infusions. We assessed the clinical toxicities and pharmacologic effects of PZA given as a 24-h i.v. infusion weekly for 3 of 4 weeks.

Experimental Design: Thirty-two adult patients with solid tumors received PZA at five dose levels (100–351 mg/m²). Plasma samples were obtained at the end of the PZA infusion at all of the dose levels, with extended sampling in a cohort treated at the recommended dose.

Results: Dose-limiting granulocytopenia and mucositis occurred in 2 of 6 patients at 351 mg/m², but lower doses were well tolerated. No responses were seen, but 28% had stable disease for >3 months. Plasma levels strongly correlated with the degree of granulocytopenia. Extended pharmacokinetics in 7 patients treated with 281 mg/m² indicated the following averages: maximum plasma level, 1.6 μM; area under the plasma concentration-time curve, 56 μM·h; terminal half-life, 27 h; urinary recovery, 17% over 72 h. DNA fragmentation in post-PZA bone marrow mononuclear cells was seen in 9 of 28 samples (all at ≥281 mg/m²).

Conclusions: Unlike other schedules of PZA, neurotoxicity and thrombocytopenia were not problematic with a weekly 24-h infusion of PZA. The recommended Phase II dose is 281 mg/m², which was well tolerated. Both end of infusion plasma levels and presence of DNA damage correlated with granulocyte toxicity.

INTRODUCTION

PZA1 [9-methoxy-N,N-dimethyl-5-nitropyrazolo-[3,4,5-k-l]acridine-2(6H)-propanamine, monomethanesulfonate; NSC 366140] is an investigational anticancer agent with a tetracyclic structure containing a 9-methoxy substitution and a potentially reducible 5-nitro group ring substitution (1). PZA exerts broad cytotoxicity against tumor cell lines in vitro and in vivo (2–4). Furthermore, PZA retains activity against several drug-resistant cancer phenotypes including cells with overexpression of the multidrug-resistance protein, cells that are resistant to etoposide and doxorubicin through deficient topoisomerase II-mediated DNA damage, and cells that are camptothecin-resistant because of low levels of topoisomerase I (2, 5). PZA exerts cytotoxicity by virtue of its ability to bind to nucleic acids, which interferes with RNA synthesis, DNA synthesis, and DNA repair (5, 6). It interferes with the normal function of both topoisomerase I and II through a mechanism that is distinct from the commercially available topoisomerase I and II-targeting agents (5, 6). PZA potentiates the cytotoxicity of cisplatin in vitro by interfering with removal of platinum-DNA adducts (7). PZA-mediated induction of DNA damage and programmed cell death does not require DNA synthesis (3, 5). Furthermore, in vitro cytotoxicity, inhibition of DNA and RNA synthesis, and DNA damage are each proportional to total drug exposure (AUC; Refs. 4, 5, 8). In vivo studies also demonstrated equivalent antitumor activity with a variety of schedules (1–4).

Initial clinical trials evaluated PZA as a 1-h infusion once every 3 weeks or daily for 5 days (9, 10). Neurotoxicity was prominent with the 1-h infusion schedule, and the duration of infusion was increased to 3 h. The recommended dose in adult patients was 600 mg/m² over 3 h once every 3 weeks; myelosuppression and neurotoxicity were the principal side effects. In murine models, neurotoxicity appeared to be related to peak plasma concentrations of parent drug (11). In contrast, a 24-h infusion was well tolerated in dogs and primates, and produced less myelosuppression (12). Furthermore, the achieved steady-state plasma concentrations were in the range associated with in vitro cytotoxicity.

We reported previously that a 24-h PZA exposure produced near maximum effects in MCF7 breast cancer cells, with 50% and 90% inhibitory concentrations of 0.24 μM and 1.1 μM.
respectively (5). Although a 24-h schedule has not been tested for in vivo antitumor activity, the available data suggest that the anticancer activity of PZA is schedule independent, whereas neurotoxicity is clearly influenced by high peak plasma concentrations. Therefore, we designed a Phase I trial of PZA given as a weekly 24-h continuous i.v. infusion for 3 of 4 weeks to determine whether neurotoxicity could be minimized at the highest tolerated dose by this strategy, and to characterize the pharmacokinetics and pharmacodynamics of PZA with this new schedule.

PATIENTS AND METHODS

Eligibility. Cancer patients who had failed standard therapy or for whom no such therapy was available who had an Eastern Cooperative Oncology Group performance status of 2 or better, adequate marrow (granulocytes ≥2000/µL, platelets ≥100,000/µL), liver (bilirubin ≤1.5 mg/dL, aspartate aminotransferase <3 times the upper limits of normal), and renal function (serum creatinine ≤1.5 mg/dL), and no active neurological disorders were eligible for this trial. Measurable disease was not required. This study had the approval of the local Institutional Review Boards and the Cancer Therapy Evaluation Program, NCI, and all of the patients gave written informed consent.

Treatment Plan. PZA was supplied by the Cancer Therapy Evaluation Program, Division of Cancer Treatment and Diagnosis, NCI, Bethesda, MD. PZA was administered in the outpatient setting as a continuous i.v. infusion over 24 h through a central catheter using a portable infusion pump weekly for 3 weeks followed by a 1-week break. Prophylactic antiemetics or other classes of premedication were not used. The starting dose was 100 mg/m²/24 h. Dose escalation proceeded in 50% increments until grade 2 clinical toxicity attributable to drug was seen (excluding nausea, vomiting, and fatigue), after which escalation proceeded in 25% increments until dose-limiting toxicity was observed in 2 patients at a given level. Dose-limiting toxicity was defined as: ≥grade 2 neurotoxicity (excluding headache), a granulocyte nadir <500/µL at any time, a platelet nadir <50,000/µL at any time, grade 2 nonhematologic toxicity (excluding nausea and vomiting, and alopecia) before completing the three weekly doses of PZA requiring treatment interruption, ≥grade 3 nonhematologic toxicities occurring any time, or the need for a treatment delay of >2 weeks.

The next cycle was repeated 28 days after starting the preceding cycle provided the absolute granulocyte count was ≥1500/µL, the platelet count was ≥75,000/µL, and all of the clinically significant nonhematologic toxicities had resolved. If the previous cycle was stopped early, the next cycle could begin 14 days after the last dose of PZA. Individual patients were allowed to escalate to the next highest dose level provided that no neurotoxicity occurred (except grade 1 headache), other nonhematologic toxicity was ≤grade 1 in severity, hematologic toxicity was ≤grade 2 in severity for the preceding two cycles, and no treatment delays occurred.

Blood counts with WBC differential were obtained twice weekly, and renal and hepatic chemistry panels were obtained weekly. Radiographic studies were repeated every two cycles. Treatment was continued indefinitely until there was evidence of disease progression, provided it was tolerated. Toxicity was assessed by the NCI Common Toxicity Criteria version 1.0. Response assessment was by Eastern Cooperative Oncology Group criteria (13).

Pharmacokinetic Analysis of PZA. In the initial portion of the trial, heparinized venous samples for pharmacokinetic analysis were drawn pretreatment, and twice 1 h apart before the end of the infusion during weeks 1 and 3 of each cycle, and were repeated if the dose of PZA was changed. The samples were placed on ice immediately. After centrifugation at 4°C for 10 min at 800 × g, the plasma was frozen and stored at −70°C. The protocol was amended to permit an additional cohort to be enrolled at the recommended dose of PZA for extended sampling at the following times from the start of the infusion (h): pretherapy, 0.5, 1, 2, 3, 4, 6, 8, 10, 22, 23, 23.5, 24, 24.08, 24.25, 24.5, 25, 26, 27, 28, 30, 32, 34, 48, 41, 54, and 72. Urine was collected for a 72-h period. The urine volume was measured at 24-h intervals, and an aliquot of urine was stored at −70°C.

Plasma samples (0.5 ml) were spiked with 20 µL of N-butylryl-amonafide (0.02 mg/ml), the internal standard. The samples were then loaded onto a 500 mg cyanopropyl endcapped BondElut solid-phase extraction cartridge (Varian) that had been preconditioned with 3 ml of methanol followed by 3 ml 0.1 M potassium phosphate (pH 4.0). After a 3-ml wash, the analytes were eluted with 0.2 ml 0.1 M potassium phosphate (pH 4.0)/acetonitrile (50:50, v/v). The eluate was concentrated to dryness at 40°C using a stream of filtered, compressed air. The samples were reconstituted in the mobile phase buffer [0.1 M potassium phosphate (pH 4.0)/acetonitrile, 78:22, v/v], clarified by centrifugation, and loaded into a Waters (Milford, MA) 717 plus auto-sampler set at 4°C. PZA and the internal standard were resolved on a Waters Radial-Pak CN column (10 micron) with a flow rate of 1.0 ml/min. The absorbance of the internal standard and PZA were recorded at 340 nm and 462 nm, respectively, using a Waters 996 photodiode array detector. The recovery of the internal standard and PZA averaged 93% and 86%, respectively. The retention times were 4.0 ± 0.2 min for the internal standard and 7.6 ± 0.1 min for PZA. The assay was linear over the range of 0.2–10 µM PZA. The average r² values for the standard curves run in conjunction with the patient samples were 0.978 ± 0.017. The within-assay and between-assay coefficients of variation were 3.6% and 7.9%, respectively.

For the extended pharmacokinetic samples, the analytic method was modified to use acridine orange, which has good UV absorption at 462 nm, as the internal standard. A Waters Nova-Pak CN HD column (3.9 × 150 mm) was used; the mobile phase was 0.2 m ammonium acetate (pH 3.5)/acetonitrile (90%:10% v/v) with a flow rate of 1.0 ml/min. The retention times of PZA and the internal standard were 12.3 ± 0.7 min and 24.7 ± 1.6 min, respectively. The assay was linear over the range of 0.1–10 µM PZA. Calibration curves represented by plots of the ratio of peak heights of PZA to the internal standard versus the amount of the calibration sample were generated using weighted (1/y) linear least-squares regression as the mathematical model. The average r² values for the standard curves for the patient samples was 0.994 ± 0.006.

For analysis of the urine samples, 0.4 ml of urine was

Downloaded from clincancerres.aacrjournals.org on April 14, 2017. © 2002 American Association for Cancer Research.
diluted in water to 1.0 ml, spiked with the internal standard (10 µg N-butyl-amonifide), and processed by solid-phase extraction. A standard curve was prepared in urine obtained without PZA therapy. To negate interference from endogenous substances in urine, the mobile phase was changed to 0.2 M ammonium acetate (pH 3.5)/acetonitrile (99:1, v/v). The assay was linear over the range of 0.25–50 µM PZA. The retention times were 11.9 ± 0.6 and 24.5 ± 1.8 min for the internal standard and PZA, respectively. The r² value for the standard curve done in conjunction with the patient samples was 0.998.

Noncompartmental pharmacokinetic analytic methods were used to estimate the AUC by the linear trapezoidal rule using WinNonLin Version 3.1 software package (Pharsight, Mountain View, CA). The terminal elimination half-life was estimated from 3 to 6 points of the terminal portion of the concentration versus time curve.

Assessment of PZA-associated Induction of DNA Damage. Bone marrow aspirates were collected in a heparinized syringe pretherapy and before completion of the 24-h PZA infusion. The aspirates were filtered through a 400-µm mesh filter to remove particulate matter and fat globules, then the mononuclear cells were isolated by Ficoll-Hypaque density centrifugation. Intact cells (100,000/plug) were embedded in 0.5% low melting point agarose plugs, and digested at 50°C for 48 h in a buffer containing 0.5 M EDTA (pH 9.0), 1% sodium lauryl sarcosine, and 0.5 mg/ml proteinase K. The plugs were then washed and stored at 4°C as recommended by the manufacturer (Bio-Rad Laboratories). The samples were analyzed by pulsed field gel electrophoresis using conditions described previously (5). DNA fragmentation was a yes/no dichotomized variable based on a single gel.

Statistical and Graphical Analysis. Graphical analysis was performed with SigmaPlot 2001 for Windows (SPSS, Inc., Chicago, IL), and statistical analysis was performed with SigmaStat for Windows version 2.03 (SPSS, Inc.). The median time to progression was calculated by the Kaplan-Meier survival curve. The strength of linear association between pairs of variables was determined by the Pearson correlation coefficient. The percentage of change in granulocyte nadirs was determined by the following equation: 100 × (baseline value − nadir value) ÷ (baseline value). The relationship between dose and percentage of change in blood counts was analyzed using a sigmoidal maximum effect model.

RESULTS

Patient Characteristics and Clinical Toxicity. Thirty-two patients with metastatic (stage IV) disease were enrolled in this trial (Table 1). The performance status was 0 or 1 in 94% of patients, and the majority had colorectal cancer. All but one patient had received prior chemotherapy (10 with prior irinotecan). Three patients each were entered at each of the first three dose levels between 100 and 225 mg/m² (Table 2). No appreciable toxicity was seen at 150 mg/m². One of the next 3 patients entered at 225 mg/m² experienced grade 1 muscle cramps that were thought to be probably treatment-related. Therefore, subsequent dose escalation proceeded in 25% increments. One of 3 patients treated with 281 mg/m² had a granulocyte nadir of 140/µl. Therefore, 3 additional patients were accrued, and none had dose-limiting toxicity. Eight patients were enrolled at 351 mg/m². One patient required surgical intervention for small bowel obstruction because of malignant disease after receiving only one dose of PZA. The patient had no apparent toxicity from the single PZA dose. A second patient refused additional therapy after receiving ~10 h of the initial dose of PZA because of failing to guess the correct answers during a televised game show (scored as grade 2 neurocognitive impairment). Two of the other 6 patients enrolled at 351 mg/m² had a granulocyte nadir <500/µl; 1 of these also experienced grade 3 mucositis. Therefore, this level was felt to exceed the maximum tolerated dose.

Nine additional patients were then enrolled at 281 mg/m² to additionally characterize the tolerability of the dose and to permit collection of extended pharmacokinetic samples, for a total of 15 patients at this dose. Only 1 of these patients had dose-limiting granulocytopenia during the initial cycle, and 1 patient experienced grade 3 anorexia, mucositis, and fatigue. For cycle one, the granulocyte nadir tended to decrease with increasing dose (Pearson correlation coefficient, r = −0.574; P = 0.0009). Although the median platelet value decreased with increasing PZA dose, the correlation among patients was not significant (P = 0.126). A total of 91 cycles were administered. Two and 3 patients enrolled at 225 and 281 mg/m², respectively, experienced minimal clinical toxicity for two consecutive cycles at their initial dose of PZA, and received one or more dose escalations. The worst toxicity experienced per patient by dose level is shown in Table 3. Granulocytopenia was the most prominent hematologic toxicity, but no patient experienced febrile neutropenia. Only 1 patient experienced grade 3 platelet toxicity. Nausea/vomiting of mild to moderate severity was observed but was well controlled with appropriate medication. At dose levels at or above 225 mg/m², muscle cramps of grade 1 severity represented a possible neurological toxicity. Six of 26 patients treated at dose levels 225–351 mg/m² experienced muscle cramps during one or more cycles. In 2 patients, these did not recur after a PZA dose reduction, whereas 2 patients had no worsening of the muscle cramps despite one or more dose escalations. Other possible neurological effects included transient numbness and tingling of the extremities (grade 1, 2 patients) and a visual field disturbance lasting seconds (grade 1, 1 patient).

<table>
<thead>
<tr>
<th>Table 1 Patient characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Median age (range)</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Performance status</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>Type of cancer</td>
</tr>
<tr>
<td>Colorectal</td>
</tr>
<tr>
<td>Renal</td>
</tr>
<tr>
<td>Gastric</td>
</tr>
<tr>
<td>Other</td>
</tr>
<tr>
<td>Prior chemotherapy: median (range)</td>
</tr>
</tbody>
</table>
One patient experienced extravasation of PZA from a central port into the s.c. tissues and developed an inflammatory reaction (erythema, induration, skin thickening, and discomfort), but this did not result in the formation of an ulcer. No additional PZA was administered to this patient, and the inflammatory reaction gradually improved over a period of weeks.

Three patients developed catheter-associated thrombosis, and 1 patient had an exit site infection, but all were able to keep the catheter with appropriate treatment. No other pump-related malfunctions were noted, and there were no programming errors. Two other patients experienced deep venous thrombosis, one of which was complicated by pulmonary embolus.

No objective responses were seen. The median time to progression was 76 days. Ten patients (31%) had disease progression at the initial restaging at 8 weeks, whereas 9 patients (28%) had stable disease for 3 months or longer (range, 112–315 days). The histologies for the patients with stable disease included colorectal (5), renal (2), pancreas (1), and adenocarcinoma of unknown primary (1).

Pharmacokinetic Data. No interfering endogenous peaks were observed in the pretherapy plasma samples. Two plasma samples were obtained 1 h apart before the end of the 24-h infusion (22.02 ± 0.21 and 23.02 ± 0.33 h into the infusion). Among 75 paired samples, the variation in the two values averaged 6.76% ± 7.59%, and differed by <10% in 81% of samples. Paired blood samples obtained during weeks 1 and 3 were available in 27 patients. No consistent changes were seen in the plasma levels obtained on week 3 compared with the week 1 levels (ratio 1.07 ± 0.36; median, 1.05).

The average PZA plasma concentrations per patient during the initial PZA cycle are shown in Fig. 1. There was a trend for the plasma levels to increase with higher doses (Pearson correlation coefficient = 0.475); however, there was considerable interpatient variability at a given dose and overlap of plasma levels among doses. However, if the plasma levels for 100–225 mg/m² are compared with those for 281–435 mg/m², the distribution of values was significantly different (median 0.53 versus 0.95, rank sum test; \( P = 0.008 \)).
Extended pharmacokinetic sampling was performed in the last 7 patients entered in the protocol after the first weekly dose of 281 mg/m² PZA. For clarity of presentation, the combined data for the ideal time points at which data were available is shown in Fig. 2. Noncompartmental analysis was performed using the actual time of sampling for each of the 7 patients (Table 4). The Cₘₐₓ averaged 1.6 μM; this value and the AUCₜ₋₇₂ h varied by ~6.5-fold. A strong correlation (r = 0.895) was seen between the average of six plasma concentrations taken between 22 and 24.25 h from the start of the PZA infusion and the AUCₜ₋₇₂ h (Fig. 3). Urinary excretion of PZA from 0 to 72 h averaged <20%, and a similar proportion of parent drug was recovered in the urine for each 24-h collection period. There was a good correlation (r = 0.795) between the end of infusion plasma levels of PZA and the percentage of decrease in granulocyte count during the initial cycle (Fig. 4), and the plasma level associated with 50% effect was 0.73 μM. The correlation between dose and granulocyte toxicity was not as strong (r = 0.448).

PZA-mediated Induction of Parental DNA Fragmentation. DNA isolated from paired bone marrow mononuclear cell samples pretherapy and before the end of the initial 24-h infusion of PZA were available in 28 patients. Whereas parental DNA fragmentation was not identified in the pretherapy samples, 9 of the post-PZA samples demonstrated evidence of parental DNA fragmentation (representative patients are shown in Fig. 5). When evaluated according to dose, the proportion of post-therapy samples with DNA fragmentation increased with higher PZA doses (mg/m²): 100–225, 0 of 9; 281, 5 of 13 (38.5%); and 351, 4 of 6 (66.7%). The presence of DNA fragmentation in the post-PZA samples was associated with a higher percentage of decrease in granulocyte count (P = 0.022; Fig. 6). A trend was noted for a higher percentage of decrease in platelet counts with the presence of DNA fragmentation (median 28.1% decrease versus 14.9%; P = 0.105). There was no difference in the median time to treatment failure in the patients whose samples showed no DNA damage (72.5 days; range 15–307) compared with those patients with DNA fragmentation in the post-therapy sample (84 days; range 55–315).

DISCUSSION

Whereas the in vitro cytotoxicity of PZA is related to total drug exposure, acute neurological toxicity in both preclinical toxicology studies and clinical trials was more prominent with shorter infusions. In vitro studies with MCF-7 breast cancer cells indicated that a 24-h PZA exposure produced near maximal cytotoxic effects. Infusion of PZA over 24 h in dogs and primates was associated with less myelosuppression and minimal neurological toxicity while achieving steady-state plasma concentrations associated with in vitro cytotoxicity. Therefore, we designed this trial to evaluate a 24-h infusion of PZA; a weekly for 3 of 4 weeks schedule was chosen in an effort to have lower individual doses given weekly.

The recommended PZA dose given weekly for 3 of 4 weeks, 281 mg/m², was well tolerated. Only 2 of 19 patients (including 15 new patients) who received one or more cycles at this dose level experienced grade 4 granulocytopenia, whereas no patient had worse than grade 2 platelet toxicity. Serious neurological toxicities were not seen. A possible grade 2 neurocognitive toxicity consisted of a patient failing to guess the correct answers while watching a game show, but the causal relationship to PZA-therapy was unclear. Other potential neurological toxicities included muscle cramps, numbness, and tingling of the extremities, and a fleeting visual field disturbance. These symptoms were transient and only of mild severity. With a 1-h infusion of PZA given every 21 days, LoRusso et al. (9) reported restlessness, agitation, anxiety, personality changes, nightmares, and myoclonus in patients treated with 600 or 720 mg/m². Rowinsky et al. observed both neuropsychiatric and neuromotor effects, generally of grade 1 severity, in 9 of 15 patients who received a 1-h infusion of 400 or 600 mg/m² PZA (10). When the infusion duration was increased to 3 h, only 4 of 18 patients treated with 600, 750, or 935 mg/m² PZA had neurotoxicity during the initial cycle (10). Myelosuppression was dose limiting in pediatric patients treated with doses up to 640 mg/m² PZA as a 1-h infusion every 3 weeks (13). In contrast to adults, neurotoxicity was not problematic.

Chemical phlebitis and cellulitis has been reported in other trials when PZA was given through a peripheral vein (9, 10, 14). In the current trial, PZA was administered into a central vein through either an indwelling catheter or a port. One patient had extravasation of PZA when the needle connected to a port was dislodged. Local inflammation occurred, but an ulcer did not form and this gradually improved.

Over the 3–5-fold dose range used in this trial, there was only a moderate trend for the end of infusion PZA plasma levels to increase with higher dose levels, and there was considerable overlap in the PZA levels across doses. At 281 and 351 mg/m², the variation in PZA plasma levels among patients was 4.5- and 3.5-fold, respectively. The reason for the interpatient variability is not clear, but similar findings have been reported in other Phase I studies of PZA (9, 14). Differences in hepatic metabolism likely account for some of the variability, and PZA may also be subject to enterohepatic recirculation (10, 14–16). The very large volume of distribution indicates extensive tissue
binding. Comparison of week 3 and week 1 end of infusion levels within patients showed no significant differences, suggesting that the variability between patients is greater than intrapatient variability. Extended pharmacokinetic sampling at the recommended dose revealed an average AUC over the 72-h sampling period of 56\(\mu\)M\(\times\)h, and the estimated terminal half-life was 20 h. About 17% of the administered dose of PZA was recovered in the urine over a 72-h period, which is higher than that reported for PZA given as a 1- or 3-h infusion (2% of the administered dose was recovered in the urine within 24 h).

There was a strong correlation between PZA plasma concentrations and the percentage of decrease in granulocyte count, whereas the correlation between dose and granulocyte toxicity was not as strong. In the 7 patients who underwent extended pharmacokinetic sampling, a good correlation was also noted between the average of six plasma concentrations obtained between 22 and 24.25 h after the start of the PZA infusion and the AUC\(_{0-72\ h}\), suggesting that a limited sampling strategy might be considered in future studies. At the recommended dose of 281 mg/m\(^2\) PZA, the average plasma concentration during cycle one among 15 patients was 1.1 \(\mu\)M, which approximates the 90% inhibitory concentration we found for a 24-h PZA exposure in MCF-7 breast cancer cells.

**Table 4** Summary of pharmacokinetic parameters from extended pharmacokinetic sampling

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>Range</th>
<th>Ratio Max:Min</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>23.52 ± 1.92</td>
<td>23.05</td>
<td>22.00–27.33</td>
<td>1.24</td>
</tr>
<tr>
<td>(C_{\text{max}}) ((\mu)M)</td>
<td>1.59 ± 1.04</td>
<td>1.73</td>
<td>0.48–3.06</td>
<td>6.38</td>
</tr>
<tr>
<td>AUC(_{0-72\ h}) ((\mu)M(\times)h)</td>
<td>56.08 ± 35.56</td>
<td>66.05</td>
<td>15.55–103.49</td>
<td>6.65</td>
</tr>
<tr>
<td>(V_{ss}) (observed) (liters/m(^2))</td>
<td>488.17 ± 529.30</td>
<td>279.7</td>
<td>102.6–1593.5</td>
<td>15.53</td>
</tr>
<tr>
<td>Clearance (ml/min/m(^2))</td>
<td>192.74 ± 142.56</td>
<td>127.4</td>
<td>60.5–451.3</td>
<td>7.46</td>
</tr>
<tr>
<td>Terminal half-life (h)</td>
<td>26.77 ± 21.60</td>
<td>19.54</td>
<td>10.2–74.8</td>
<td>7.30</td>
</tr>
<tr>
<td>Urinary excretion (% of dose)</td>
<td>0–24 h 5.89 ± 3.20</td>
<td>6.17</td>
<td>1.73–10.55</td>
<td>6.10</td>
</tr>
<tr>
<td>24–48 h 6.35 ± 3.47</td>
<td>7.07</td>
<td>1.87–10.42</td>
<td>5.57</td>
<td></td>
</tr>
<tr>
<td>48–72 h 4.98 ± 2.12</td>
<td>5.32</td>
<td>1.48–7.54</td>
<td>5.09</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3** Correlation between end of infusion PZA plasma concentrations and area under the plasma concentration time curve. The mean of six plasma levels obtained between h 22 and 24.25 after the start of the PZA infusion versus the AUC from 0 to 72 h are shown for the 7 patients who had extended pharmacokinetic sampling. The Pearson correlation coefficient and \(P\) are shown; bars, ± SD.

**Fig. 4** Correlation between PZA plasma levels and granulocyte toxicity. The average end of infusion plasma levels per patient during the initial cycle versus the percentage of decrease in granulocyte count are shown. The data, from 29 patients, were analyzed by nonlinear regression using a 3-parameter Hill sigmoidal equation.

PZA-induced parental DNA fragmentation in MCF-7 cells in a concentration-dependent manner and correlated with loss of viability. Since myelosuppression was predicted to be dose-limiting, we used bone marrow mononuclear cells as a surrogate tissue to assess possible PZA-associated DNA damage. Analysis of the DNA isolated from the mononuclear cells by pulsed field gel electrophoresis suggested dose-dependent induction of DNA fragmentation. DNA damage was not seen in patients treated with 225 mg/m\(^2\) or lower, doses not associated with myelosuppression, whereas high molecular mass DNA fragmentation was seen in 38% and 67% of post-therapy samples taken from patients treated with 281 and 351 mg/m\(^2\) PZA. The degree of granulocyte depression during cycle one was greater in those patients whose post-therapy bone marrow samples showed DNA fragmentation. No such correlation was seen between time to treatment failure and DNA damage, but a heavily pretreated patient population may not be optimal for such an assessment.

In other Phase I studies, one partial and three minor responses were observed in patients with refractory ovarian cancer, and minor responses were also seen in 1 patient each with refractory cervical and colon cancer (10). Phase II studies of PZA given at either 600 or 750 mg/m\(^2\) as a 3-h infusion in patients with previously treated colorectal cancer, pancreatic cancer, transitional cancer of the bladder, germ cell, and renal...
Fig. 5  PZA-associated induction of high-molecular mass DNA fragmentation. DNA isolated from paired bone marrow mononuclear cells obtained before (−) and before completing the 24-h infusion (+) was analyzed by pulsed-field gel electrophoresis. The results from four representative patients are shown.

Fig. 6  Correlation between DNA fragmentation and granulocyte toxicity. Twenty-seven patients had paired bone marrow samples and were assessable for granulocyte toxicity during cycle one. The percentage of decrease in granulocyte counts during cycle one are shown according to whether or not DNA fragmentation was seen in the post-PZA bone marrow sample. The data are presented in box-plot format. The P from a rank sum test is shown; bars, ± SD.

cell cancer have shown no objective responses, whereas myelosuppression was prominent (17–23). In contrast, 1 of 17 patients with hormone refractory prostate cancer was reported to have a 96% decrease in the PSA accompanied by improvement in the bone scan (24). In ovarian cancer, the response rate among 42 patients with platinum-sensitive and 24 patients with platinum-refractory ovarian cancer was 34% and 8%, respectively (25, 26).

In summary, 281 mg/m² PZA administered as a 24-h infusion weekly for 3 of 4 weeks was well tolerated with limited neutropenia and thrombocytopenia, and the use of an ambulatory infusion pump allows this regimen to be given in the outpatient setting. Of importance, the neurotoxicities commonly observed with 1–3-h infusions of PZA were not seen. Additional dose escalation should be feasible with colony-stimulating factor support. Although the Phase II results with the 3-h infusion every 3 weeks have been disappointing, select Phase II studies with the weekly 24-h infusion schedule may be considered in cancers in which some evidence of clinical activity has been seen such as platinum-sensitive ovarian cancer and hormone-refractory prostate cancer. Given the unique mechanism of action of PZA, the ability to inhibit topoisomerases I and II in a manner distinct from that of available topoisomerase-targeting agents, and the capacity to interfere with repair of cisplatin-DNA adducts, combination studies may also be warranted.

REFERENCES


A Phase I Pharmacologic and Pharmacodynamic Study of Pyrazoloacridine Given as a Weekly 24-Hour Continuous Intravenous Infusion in Adult Cancer Patients

Jean L. Grem, Nancy Harold, Bruce Keith, et al.