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SHORT COMMUNICATION

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A phase II pharmacodynamic study of pyrazoloacridine in patients with metastatic colorectal cancer

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Abstract *Purpose*: To perform a phase II trial of pyrazoloacridine (PZA), a novel DNA intercalator, in patients with metastatic colorectal carcinoma and no previous therapy. *Methods*: PZA was administered at a dose of 750 mg/m^2 intravenously over 3 h every 21 days. Pharmacokinetic studies to determine PZA plasma concentrations were performed. *Results*: No responses were seen in 14 response-evaluable patients. Patients received a median of two cycles of PZA (range 1–6). Toxicity included neutropenia and neurologic side-effects, which were ≥ grade III in 73% and 14%, respectively. High plasma concentrations of PZA (C_{max}) correlated with low neutrophil counts ($P = 0.04$). *Conclusions*: PZA is inactive at this dose and schedule in colorectal cancer, and produces moderately severe toxicity.

Key words Colorectal cancer · Metastatic · Pyrazoloacridine · Phase II trial

Introduction

Colorectal carcinoma represents a leading cause of cancer-related deaths in the United States, with more than 135,000 cases diagnosed annually [4]. In patients who relapse following initial surgery and/or adjuvant therapy, or present with metastatic disease, therapy remains inadequate. The antimetabolite 5-fluorouracil (5-FU) with leucovorin represents the current standard therapy for metastatic colorectal cancer. The majority of responses to this combination are partial, and the overall response rate is about 20% [9]. A recent meta-analysis of randomized trials did not indicate improvement in survival for this combination over 5-FU alone [2]. In view of these results, new agents are needed for therapy of patients with this neoplasm.

Novel thymidylate synthase inhibitors such as Tomudex [21], and fluorouracil prodrugs such as UFT [18] and capecitabine [16] have been investigated in this patient population. Although these drugs may be less toxic, they present the same cytotoxic activity as 5-FU. The topoisomerase I inhibitor CPT-11 has reported activity in 5-FU refractory patients, but has significant toxicity [8]. The synthetic acridine derivative pyrazoloacridine (PZA), as well as other new agents, is of interest given its preclinical activity against resistant solid tumors and its activity versus tumor cells with the multidrug resistance phenotype [20]. The mechanisms of action of this agent are unknown, but it binds DNA by intercalation, inhibits both DNA and RNA synthesis, and produces single and double DNA strand breaks [12]. Antitumor activity in patients with cisplatin-resistant ovarian cancer and fluorouracil-resistant colon cancer have been reported [19].

Phase I trials of PZA [6, 15, 19] have been completed and have utilized several schedules, including daily for 5 days [19], and 3- and 24-h infusions [6]. Dose-limiting toxicity included myelosuppression and neurologic side-effects [19]. A single-dose schedule of 750 mg/m^2 over 3 h every 3 weeks was recommended from these studies, owing to the toxicity profile. The present study was initiated to evaluate the response rate to PZA therapy in patients with metastatic colorectal carcinoma, further characterize the toxicity of this agent, and investigate its pharmacodynamics.

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Materials and methods

The study was initiated on 17 March 1994 and closed to patient accrual on 1 November 1995. PZA (NSC 366149) was supplied by the National Cancer Institute (NCI) as a lyophilized formulation (100- and 500-mg vials) that was reconstituted with sterile water for injection, USP. PZA was administered at a dose of 750 mg/m^2 as a 3-h infusion through a central catheter, diluted in 100 ml of 5% dextrose and water, on day 1, and repeated every 21 days.

Patient eligibility

Patients with histologically confirmed unresectable metastatic colorectal carcinoma were eligible. Measurable disease was defined as one of the following: bidimensionally measurable lung lesions on chest X-ray or CT scan, palpable lymphadenopathy containing tumor $\geq 2 \times 2$ cm, abdominal masses or liver lesions $\geq 2 \times 2$ cm quantifiable by CT scan or MRI, or palpable hepatomegaly with the liver edge >5 cm below the costal margin accompanied by a CT scan or MRI consistent with metastatic disease. No prior systemic therapy for metastatic disease was permitted, and ≤ one prior adjuvant chemotherapy regimen was allowed. The following were also required: ECOG performance status (P.S.) ≤1, age ≥18 years, absolute neutrophil count (ANC) ≥1500/μl, white blood count (WBC) ≥3500/μl, platelet count ≥100,000/μl, serum creatinine ≤1.5 mg/dl, total bilirubin ≤2.0 mg/dl, and SGOT/alkaline phosphatase ≤1.5 times institutional normal values. Exclusion criteria included the following: brain metastases, history of a seizure disorder requiring anticonvulsants, history of a second malignancy within the past 5 years (exceptions: cervical carcinoma in situ, basal/squamous cell carcinoma of the skin), severe cardiac disease (New York Heart Association Class ≥III), pregnancy, and major surgery within 21 days. Informed consent in accordance with federal and institutional guidelines was obtained, and all sexually active patients were required to be surgically sterile or practicing contraception.

Study parameters

The following were obtained prior to study entry and every 21 days: history, physical examination, radiographic studies required for tumor measurements (if CT scan utilized every 6 weeks), carcinoembryonic antigen, urinalysis, and biochemical studies (creatinine blood urea nitrogen, LDH, SGOT, alkaline phosphatase, total bilirubin, calcium, phosphorus, albumin, total protein, glucose, uric acid, sodium, potassium, chloride, CO_2). Complete blood counts and differential counts and toxicity evaluations were performed weekly.

Treatment plan

Patients received PZA until evidence of progressive disease or unacceptable toxicity was seen. Dose levels of PZA were modified for hematologic toxicity based on nadir values as follows: ANC 500–999/μl, or WBC 1000–1999/μl or platelets 25,000–49,000/μl – decrease 25%; ANC <500/μl, or WBC <1000/μl, or platelets <25,000/μl – decrease 50%. Grade III or IV nonhematologic toxicity required a 25% decrease in PZA dose. In this situation, therapy was reinstituted when the toxicity returned to ≤ grade I. Recurrence of an identical grade III/IV toxicity required removal from study.

Study definitions

Standard response criteria were utilized [17]. NCI common toxicity criteria (version 1.0) were employed to grade side-effects.

Pharmacokinetic studies

The first 13 patients treated had plasma levels of PZA monitored during cycle 1 of therapy. One-milliliter aliquots of plasma were obtained from a peripheral vein at the following time intervals: 0, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 24, 48, 72 and 96 h from the start of PZA infusion. PZA levels in plasma were measured utilizing high-performance liquid chromatography (HPLC) analysis by a previously described method [10]. Blood samples collected in heparinized vacutainer tubes were centrifuged at 1000× *g* for 10 min at 4 °C and plasma stored at −20 °C. Extraction of plasma was carried out by vortexing 0.5 ml of the plasma with 10 μl of 0.35 m*M* amsacrine (internal standard) in 1.5 ml of methanol. The extract was centrifuged for 10 min at 25,000× *g* and the supernatant transferred to an autosampler vial for analysis. Analysis by HPLC was carried out using a Beckman ultrasphere CN column (5 μm, 15 × 0.46 cm) using isocratic elution with 85% ammonium acetate buffer:15% acetonitrile at a flow rate of 1.2 m/min. Normal plasma samples containing known amounts of PZA and internal standard were used to generate a standard regression line plot of PZA peak area/amsacrine (internal standard) peak area. The ratio of the PZA peak area/amsacrine (internal standard) peak area from patient samples was used to determine the concentration of PZA from the regression equation generated from the standard calibration plot. The area under the curve (AUC) was calculated by the trapezoidal method for the area from time 0 to the time of last blood sample.

Statistical considerations

The study was designed as a two-stage phase II trial. A response rate of 5% or less was considered uninteresting, and a response of 20% or greater was considered promising. Fourteen eligible and evaluable patients were to be accrued in the first stage; if one or more responses were observed, 12 additional patients were to be accrued in the second stage. If no responses were observed in the first 14 patients, the study was to be terminated. The significance level and power of this test were 13% and 90%, respectively.

Pearson's correlation coefficient [2] was used to assess the relationship between neutrophil nadir and both C_{max} and estimated AUC. Survival was estimated via the Kaplan-Meier method [14]. The Jonckheere-Terpstra test was used to determine whether liver function or C_{max} was associated with grade of selected toxicities. All statistical tests were two-sided; $P < 0.05$ was used to indicate statistical significance.

Results

A total of 15 patients (11 with colon carcinoma and four with rectal carcinoma) were treated with PZA, and 14 were evaluable for response. Three individuals had received prior adjuvant therapy. The one inevaluable patient developed bradycardia and hypotension during the initial administration of PZA and was removed from study. The majority of individuals were male ($n = 9$). ECOG P.S. was 0 in eight patients, and 1 in the remaining seven. The mean age was 59 years (range 41–71 years). A median of two cycles of PZA were administered (range 1–6), and dose reductions for toxicity were required in 11 patients. In the 14 evaluable patients, no partial or complete response was noted, and accrual was terminated. Median survival of the 15 patients receiving PZA was 15 months.

Toxicity related to PZA was moderately severe and is summarized in Table 1. Grade III or IV neutropenia developed in 11/15 patients and required dose reductions. In most patients ($n = 10$), this was seen during cycle 1 of treatment. Thrombocytopenia was noted in 4/15 patients,

Table 1 Cumulative toxicity of PZA in 15 patients with colorectal carcinoma

Type of toxicity	Grade	
	I/II (% patients)	III/IV (% patients)
Nausea/vomiting	53	–
Hematologic		
Anemia	87	7
Neutropenia	13	73
Thrombopenia	27	–
Hepatic:		
SGOT elevation	47	–
Alkaline phosphatase elevation	67	–
Neurologic		
Neurocortical	47	7
Cerebellar	27	–
Motor	13	7
Mood changes	40	–
Vision	7	–
Hypotension	7	–
Bradycardia	7	–

but was generally mild. Neurologic toxicity was reported in 13/15 patients and was grade I in the majority. Agitation, irritability, dizziness, and mood alterations were the most common problems reported. Six individuals developed grade II or III neurologic toxicity. In the four individuals with grade II neurologic side-effects, irritability ($n = 1$), insomnia ($n = 1$), dizziness ($n = 1$), and confusion ($n = 1$) were noted. The last-mentioned patient also developed an unsteady gait related to the PZA infusion. In the two individuals with grade III neurologic toxicity, hallucinations were reported by one, and cerebellar symptoms including involuntary extremity extension by the other. In all patients, these toxicities were transient and resolved completely.

The pharmacokinetics of PZA were investigated in 13 patients. The mean peak plasma level was $2.68 \pm 1.26\ \mu M$. The absolute nadir neutrophil counts were then correlated with the peak plasma concentrations of PZA in these patients. Higher C_{max} values were significantly correlated with lower neutrophil nadirs ($r = -0.57$, $P = 0.04$). The estimated AUC showed a similar correlation with the severity of neutrophil nadirs ($r = -0.62$, $P = 0.10$), but data were only available in eight patients so the correlation, although clinically relevant, is not statistically significant. Abnormalities of liver function (bilirubin, SGOT, and alkaline phosphatase) and the occurrence of neutropenia and neurologic toxicity were also investigated. No significant associations were found.

Discussion

The present study again demonstrates that the major toxicities of PZA when administered as a 3-h infusion are myelosuppression and neurocortical side-effects. These findings resemble those reported by other investigators utilizing a similar dose and schedule of this agent [15, 19]. No evidence of antitumor activity in patients with colorectal cancer was found, similar to the results reported by Zalupski et al. [22].

In the same dose schedule, PZA has been shown to be inactive in pancreatic [23] and renal cell cancer [7]. Additional phase II trials of PZA are ongoing in lung, breast, and ovarian cancer.

The hematologic toxicity of PZA consists predominantly of neutropenia. This was generally short in duration, and was not associated with febrile episodes. Thrombocytopenia, in contrast, was mild. The severity of neutropenia significantly correlated with peak concentrations of PZA. Neutropenia had a similar correlation with the AUC of PZA, but data were only available in eight patients, and this correlation did not reach statistical significance.

The neurologic toxicity resembled that reported by other investigators [14, 15, 19]. Like the neutropenia, these side-effects were noted during cycle 1, and in the 13 patients receiving more than one cycle, did not appear cumulative. The etiology of these side-effects is unclear, but the findings were transient and reversed completely after the PZA infusion had been completed. Previous investigators [19] reported that neurologic toxicity was correlated with peak PZA levels. In the present trial, this was not found.

The pharmacokinetics of PZA reported by other investigators [19] demonstrated significant interpatient variability. Multiple peaks and troughs in the concentration versus time curves have been noted, and in the present trial C_{max} varied from 1.0 to $>5.0\ \mu M$. These data are consistent with other studies and may reflect complex drug sequestration as might occur with enterohepatic recirculation [1]. The concentrations achieved in patients appeared similar to mean IC_{50} values ($\leq 0.38\ \mu M$) for a variety of solid tumor cell lines [13] and those inhibiting cell growth ($\geq 1\ \mu M$) and producing DNA damage ($\geq 5\ \mu M$) in MCF-α human breast carcinoma cells [11].

In conclusion, PZA has broad activity in solid tumors in vitro; however, the present study did not demonstrate antineoplastic activity in patients with previously untreated colorectal carcinoma. The toxicity of the 3-h infusion schedule was moderately severe, and dose reductions were required in 73% of patients. The lack of activity did not appear related to the type of patient treated, since all individuals had an ECOG performance status of 0 or 1, and the majority were previously untreated except for 5-FU based adjuvant therapy ($n = 3$). Despite the lack of responses, the median survival of this patient group was 15 months. The two studies in colorectal cancer patients suggest PZA is inactive at the dose and schedule utilized.

References

1. Adjei A (1999) Current status of pyrazoloacridine as an anticancer agent. Invest New Drugs 17: 43

2. The Advanced Colorectal Cancer Meta-Analysis Project (1992) Modulation of fluorouracil by leucovorin in patients with advanced colorectal cancer: evidence in terms of response rate. J Clin Oncol 10: 896
3. Altman DG (1991) Relation between two continuous variables. In: Practical statistics for medical research. Chapman & Hall, London, p 277
4. American Cancer Society, Cancer Facts & Figures (1995) Atlanta: American Cancer Society
5. Berg W, McCaffrey J, Schwartz H, Mariani T, Mazumdar M, Motzer R (1999) A phase II study of pyrazoloacridine in patients with advanced renal cell carcinoma. Invest New Drugs 16: 337
6. Berg SL, Blaney SM, Adamson PC, O'Brien M, Poplack DG, Arndt C, Blatt J, Balis FM (1998) Phase I trial and pharmacokinetic study of pyrazoloacridine in children and young adults with refractory cancers. J Clin Oncol 16: 181
7. Berg WL, McCaffrey J, Schwartz LH, Mariani T, Mazumdar M, Motzer R (1999) A phase II study of pyrazoloacridine in patients with advanced renal cell carcinoma. Invest New Drugs 16: 337
8. Cohen AM, Minsky BD, Schulsky RL (1997) Cancer of the colon. In: DeVita VT, Hellman S, Rosenberg SA (eds) Cancer principles and practice of oncology. Lippincott-Raven, Philadelphia, p 1144
9. Erlichman C, Fine S, Wong A, Elhakin T (1998) A randomized trial of fluorouracil and folinic acid in patients with metastatic colorectal cancer. J Clin Oncol 6: 469
10. Foster B, Wiegand R, LoRusso P (1995) Pharmacokinetics of 9-methoxy-*N*,*N*-dimethyl-5-nitropyrazolo [3,4,5-*k*] acridine-2 (6*H*)-propanamine (PZA, PD 115394, NSC 366140) in mice: guidelines for early clinical trials. Clin Cancer Res 1: 831
11. Grem JL, Politi PM, Berg SL, Benchekroun NM, Patel M, Balis FM, Sinha BK, Dahut W, Allegra CJ (1996) Cytotoxicity and DNA damage associated with pyrazoloacridine in MCF-7 breast cancer cells. Biochem Pharmacol 51: 1649
12. Jackson RC, Leopold WR, Sebolt JS (1988) Biochemical and experimental chemotherapy studies of the pyrazoloacridines. Proc AACR 29: 536
13. Jackson RC, Sebolt JS, Shilis JL, Leopold WR (1990) The pyrazoloacridines: approaches to the development of a carcinoma-selective cytotoxic agent. Cancer Invest 8: 39
14. Kaplan EL, Meier P (1958) Nonparametric estimation from incomplete observations. J Am Stat Assoc 53: 457
15. LoRusso P, Foster BJ, Poplin E (1995) Phase I clinical trial of pyrazoloacridine NSC 366140 (PD 115934). Clin Cancer Res 1: 1487
16. Mackean M, Planting A, Twelves C, Schellens J, Allman D, Osterwalder B, Reigner B, Griffin T, Kaye S, Verweiu J (1998) Phase I and pharmacologic study of intermittent twice-daily oral therapy with capecitabine in patients with advanced and/or metastatic cancer. J Clin Oncol 16: 2977
17. Miller A, Hoogstroten B, Stoquet M, Winkler A (1981) Reporting results of cancer treatment. Cancer 47: 20
18. Pazdur R, Lassere Y, Rhodes V, Ajani JA, Sugarman SM, Patt YZ, Jones DV Jr, Markowitz AB, Abbruzzese JL, Bready B (1994) Phase II trial of uracil and tegafur plus oral leucovorin: an effective oral regimen in the treatment of metastatic colorectal carcinoma. J Clin Oncol 12: 2296
19. Rowinsky E, Noe D, Grochow L, Sartorious S, Bowling M, Chen T, Lubejko B, Kaufmann S, Donehower R (1995) Phase I and pharmacologic studies of pyrazoloacridine, a novel DNA intercalating agent, on single-dosing and multiple-dosing schedules. J Clin Oncol 13: 1975
20. Sebolt J, Havlick M, Hamelehle K, Nelson J, Leopold W, Jackson R (1989) Activity of the pyrazoloacridines against multidrug-resistant tumor cells. Cancer Chemother Pharmacol 24: 219
21. Zalcberg JC, Cunningham D, Green G, Francois E, van Cutsem E, Schornagel J, Adenis A, Seymour L, Azab M (1995) The final results of a large phase II study of the potent thymidylate synthase inhibitor Tomudex in advanced colorectal carcinoma. Proc ASCO 14: 204a
22. Zalupski MM, Philip PA, LoRusso P, Shields AF (1997) Phase II study of pyrazoloacridine in patients with advanced colorectal carcinoma. Cancer Chemother Pharmacol 40: 225
23. Zalupski M, Shields A, Philip P, Kraut M, LoRusso P, Heilbrun L, Vaitkevicius V (1998) Evaluation of pyrazoloacridine in patients with advanced pancreatic carcinoma. Invest New Drugs 16: 93