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Clinical report

Phase I trial of i.v. administered tirapazamine plus cyclophosphamide

Paulo M Hoff,[1] Everardo D Saad,[1] Farhad Ravandi-Kashani,[1] Elizabeth Czerny[2] and Richard Pazdur[3]

[1]Department of Gastrointestinal Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA. [2]Sanofi Research Division, Malvern, PA 19355, USA. [3]Division of Oncology Drug Products, Center for Drug Evaluation and Research, Food and Drug Administration, Rockville, MD 20857, USA.

Our objective was to determine the maximum tolerated doses of tirapazamine and cyclophosphamide given i.v. in combination. Eligible patients had advanced solid tumors refractory to conventional treatment. Tirapazamine (escalated from 80 to 390 mg/m^2) was given i.v. over 2 h and followed by cyclophosphamide over 1 h. The cyclophosphamide dose was fixed at 1000 mg/m^2 until the tirapazamine dose of 390 mg/m^2 was reached. Once that dose of tirapazamine was reached, the cyclophosphamide dose was escalated to 1250 and 1500 mg/m^2. Twenty-eight patients were enrolled. The dose-limiting toxicity was granulocytopenia. One patient had transient deafness for 2 days. Four other patients had grade 1 ototoxicity. Grade 1 and 2 muscle cramps were observed at all dose levels. Other toxic effects observed included fatigue, nausea, vomiting, headache, diarrhea, drug fever, elevated transaminases and elevated creatine phosphokinase. Three patients had stable disease and the longest time to progression was 5 months. The combination of tirapazamine and cyclophosphamide is feasible, and the dose-limiting toxicity is granulocytopenia. The use of growth factors could possibly allow escalation of tirapazamine doses in future phase II trials. Without growth factor support, the recommended doses of tirapazamine and cyclophosphamide when administered in this schedule are 260 and 1000 mg/m^2, respectively. [© 2001 Lippincott Williams & Wilkins.]

Key words: Cyclophosphamide, hypoxia, phase I trial, solid tumors, tirapazamine.

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Correspondence to PM Hoff, Department of Gastrointestinal Medical Oncology, 1515 Holcombe Boulevard, Box 426, Houston, TX 77030, USA.
Tel: (+1) 713 792-0601; Fax: (+1) 713 745-116;
E-mail: phoff@mdanderson.org

Introduction

Solid tumors are characterized by accelerated, disorganized growth, leading to abnormal vasculature and cell hypoxia within the tumor.[1–4] Hypoxic cells are more resistant to radiation and chemotherapy agents than adequately oxygenated cells.[5,6] Recent research efforts have attempted to capitalize on the special characteristics of poorly oxygenated cells, designing therapies that examine oxygen-mimetic sensitizers used in combination with radiotherapy and chemotherapy.[7,8]

In 1986, Zeman *et al.*[9] described a potent benzotriazine di-*N*-oxide with potent cytotoxic activity against hypoxic cells, tirapazamine (3-amino-1,2,4-benzotriazine-1,4-di-*N*-oxide). *In vitro*, tirapazamine demonstrated a 50% inhibitory concentration for aerobic cells that was several-fold higher than those for hypoxic cells. *In vivo* studies using nude mice with transplanted solid tumors demonstrated that tirapazamine was toxic to hypoxic tumor cells and had little systemic toxicity.[9,10] Tirapazamine and radiation, as well as DNA-reactive cytotoxic drugs, including cyclophosphamide, nitrosoureas, cisplatin and melphalan, were demonstrated to have synergistic activity in this model.[11–16]

The development of tirapazamine was extended to combine it with other active drugs, based on *in vitro* synergy. Langmuir and colleagues examined the combination of tirapazamine with cyclophosphamide in nude mice bearing human breast xenografts.[14] Following the work of Dorie and Brown,[17] cyclophosphamide was administered 2 h after tirapazamine. The combination showed a synergistic effect; the authors postulated that synergy resulted from enhancement of DNA damage, inhibition of DNA repair

or induction of apoptosis. Tirapazamine's action is attributed to its bioreduction under low oxygen tension conditions, releasing free radicals that induce DNA breaks.[18–20] Cytochrome P450 reductase is the principal bioreductive enzyme causing the activation of tirapazamine.[18,21–23]

Other combinations are under investigation. Phase I trials combining tirapazamine with cisplatin among patients with various types of malignancies have been done.[24–27] Johnson *et al.* recommended 260 mg/m^2 of tirapazamine when it was combined with full doses of cisplatin; nausea and vomiting were the most important toxic effects.[26]

On the basis of pre-clinical data we designed the present phase I trial, combining i.v. tirapazamine with cyclophosphamide. The aim of the study was to determine the maximum tolerated dose (MTD) of both drugs in combination.

Patients and methods

Eligible patients had pathologically proven advanced solid tumors that were refractory to therapy of proven efficacy, age $\geqslant$18 years and a Karnofsky performance status of $\geqslant$60%. Patients were required to have adequate renal, hepatic and bone marrow function (leukocyte count $\geqslant$4000/μl and platelet count (100 000/μl) and an estimated life expectancy of at least 3 months. Exclusion criteria were active infections, pregnancy or lactating state, use of any chemotherapy or immunotherapy agent within 30 days of entry into the study (60 days for nitrosoureas or mitomycin C) and evidence of congestive heart failure (New York Heart Association class II–IV). Fertile patients were required to use adequate contraceptive methods, and had to give informed consent in accordance with federal, state and institutional guidelines. The protocol was approved by the Institutional Review Board.

Tirapazamine was supplied by Sterling Winthrop Inc in glass ampules. Each ampule contained 20 ml (0.7 mg/ml) of tirapazamine in an isotonic citrate buffer. The total dose of tirapazamine for each patient was transferred from the supplied ampules directly into a chemotherapy bag without use of diluent. The drug was than administered via an infusion pump over 2 h. Cyclophosphamide was administered i.v. over 1 h, 60 min after completion of tirapazamine. Both drugs were repeated every 3 weeks. All treatment was given on an outpatient basis at the MD Anderson Cancer Center. Antiemetics were used according to usual practice.

The cyclophosphamide dose for each subject was fixed at 1000 mg/m^2 until the MTD for tirapazamine was reached. This dose was selected due to the expected good tolerance and potential antitumor activity. The starting tirapazamine dose was 80 mg/m^2. This dose equaled 20% of the highest single-dose level tested at the time this study was initiated. The dose was escalated progressively until the tirapazamine dose of 390 mg/m^2 was reached. Once the MTD for tirapazamine was achieved, the cyclophosphamide dose was escalated to 1250 and 1500 mg/m^2. Three patients were enrolled at each dose level and no intra-patient dose escalation was allowed. If at least one grade 3 or higher toxicity was observed at a dose level, three additional patients were entered at the same level. If three or more instances of grade 3 toxicity were observed at a given level, the escalation was be stopped. The MTD of tirapazamine was defined as the dose level below the one in which at least three out of six patients experienced grade 3 or 4 toxicity. Even though there was no dose escalation or reduction of tirapazamine in individual patients, a reduction of cyclophosphamide dose was allowed for patients receiving more than 1000 mg/m^2 of cyclophosphamide. Courses were repeated every 3 weeks. Patients with stable disease or objective response could be treated for up to six additional courses at the same dose level.

Baseline evaluation included a complete history and physical examination, which were repeated before every subsequent course. A full chemistry panel [including creatine phosphokinase (CPK)], liver enzymes and complete blood count were obtained weekly. Imaging studies of the tumor were done at baseline and with each course. A full eye exam, electrocardiogram and audiogram were performed at baseline and 28 days after study removal.

Results

Twenty-eight patients were enrolled in eight dose levels and received a total of 57 courses of the combination. All patients were evaluable for toxicity and 26 were evaluable for response. The patient characteristics are shown in Table 1. Twenty-two patients had colorectal adenocarcinoma and two patients had squamous cell carcinoma. The remaining patients had different tumor types: one hepatocellular carcinoma, one leiomyosarcoma, one melanoma and one mesothelioma. One patient had no prior therapy, while 27 had prior chemotherapy, nine had prior radiotherapy and seven had prior immunotherapy.

Eighteen patients had received three or more chemotherapy agents.

The toxicity profile for the combination is shown in Table 2. The most significant toxic effect observed was granulocytopenia. At level 1 (tirapazamine 80 mg/m^2 plus cyclophosphamide 1000 mg/m^2), grade 2 granulocytopenia was noted, with a median nadir granulocyte count of 2.1/μl (range 1.4–2.6). At level 2 (tirapazamine 130 mg/m^2 plus cyclophosphamide 1000 mg/m^2), only one patient had significant granulocytopenia (grade 4) and the median nadir count was 1.7/μl (range 0.2–1.8). Grade 3 or 4 granulocytopenia was not seen at level 3 (tirapazamine 195 mg/m^2 plus cyclophosphamide 1000 mg/m^2), in which a median nadir count of 1.5/μl (range 1.1–2.4) was recorded. At level 4 (tirapazamine 260 mg/m^2 plus cyclophosphamide 1000 mg/m^2), two patients had grade 3 granulocytopenia and the median nadir count was 1.1/μl (range 0.5–1.3). At level 5 (tirapazamine 330 mg/m^2 plus cyclophosphamide 1000 mg/m^2) hematologic toxicity was pronounced, with three out of five courses resulting in grade 4 granulocytopenia and a median nadir count of 0.4 (range 0.1–1.4). At level 6 (tirapazamine 390 mg/m^2 plus cyclophosphamide 1000 mg/m^2), all patients experienced grade 3 or 4 neutropenia. The median nadir count on this level was 0.7 (range 0.1–3.9).

Levels 7 and 8 consisted of a fixed tirapazamine dose of 390 mg/m^2 with escalating doses of cyclophosphamide. Again, the majority of courses resulted in grade 3 or 4 granulocytopenia. At level 7 (cyclophosphamide 1250 mg/m^2) the median granulocyte nadir count was 0.7 (range 0.0–1.9) and at level 8 (cyclophosphamide 1500 mg/m^2) it was 0.8 (range 0.0–2.1). The nadir count was achieved on days 14–16 and none of the patients required admission due to neutropenic fever. All patients recovered the granulocyte count and the length of time to recovery varied from 6 to 14 days. There was a slight tendency towards longer periods for recovery at higher dose levels. Significant anemia or thrombocytopenia was not seen.

Other significant toxic effects included one episode of transient deafness that lasted 2 days before the patient recovered to baseline; four other patients developed grade 1 ototoxicity. Mild nausea was common. Grade 3 nausea was seen in two patients, while grade 1 and 2 nausea was recorded in 15 and 10 patients, respectively. Vomiting was seen in 22 patients, but in only two patients it was grade 3. Standard anti-emetics were effective and no patient discontinued the treatment because of nausea or vomiting. Muscle cramps were seen at all dose levels. Cramps were tolerable and were classified as grade 1 (22 courses) and grade 2 (12 courses). Fatigue has been frequently reported with tirapazamine. Although present at all dose levels, fatigue was not dose limiting in any patient. Seventeen patients noted grade 1 fatigue and nine described grade 2. Only one patient had greater than four courses of the combination.

Other toxic effects observed included grade 2 headache in four patients, grade 1 and 2 drug fever in eight patients, elevated transaminases (grade 2 in three patients and grade 3 in one patient). CPK

Table 1. Patient characteristics

Total no. of patients	28
Age (median)	30–74 (58)
Males/females	17/11
Histology	
adenocarcinoma	22
squamous cell carcinoma	2
leiomyosarcoma	1
hepatocellular carcinoma	1
melanoma	1
mesothelioma	1
Performance status (%)	
100	9
90	12
80	4
unknown	3
Prior chemotherapy regimens	
0	1
1	3
2	11
3	5
$\geqslant$4	8

Table 2. Dose levels and number of patients enrolled

Level	Tirapazamine (mg/m^2)	Cyclophosphamide (mg/m^2)	Patients	Courses
1	80	1000	3	10
2	130	1000	3	5
3	195	1000	4	10
4	260	1000	3	6
5	330	1000	3	5
6	390	1000	5	9
7	390	1250	5	8
8	390	1500	3	4

elevation was seen in three patients, but only at level 1. Grade 1 alopecia was noted in eight patients and grade 2 in one patient. Grade 2 diarrhea was seen in seven patients and grade 3 in one patient. Nine patients had grade 1 stomatitis.

The dose-limiting toxicity was granulocytopenia, which was more pronounced when the cyclophosphamide dose was escalated. No growth factors were used in our study. The MTD of this combination according with the protocol was 260 mg/m^2 of tirapazamine with 1000 mg/m^2 of cyclophosphamide. However, because the granulocytopenia resolved rapidly and no patient developed neutropenic fever, the dose of tirapazamine continued to be escalated up to the targeted 390 mg/m^2.

Neither complete nor partial responses were observed in this heavily pretreated population. Only disease stabilization was observed. Three patients (two with colorectal carcinoma and one with mesothelioma) had stable disease as their best response and the longest time to progression was 5 months. Twenty-three patients progressed and two were not evaluable for response. One patient refused treatment after enrollment and one was lost to follow-up after the first course.

Discussion

We demonstrated in this phase I trial that the combination of cyclophosphamide and tirapazamine can be safely administered to patients with solid tumors. Using a fixed dose of cyclophosphamide of 1000 mg/m^2, we escalated the dose of tirapazamine up to 390 mg/m^2, which has been used in combination therapies in previously untreated patients.[28] When the target dose of tirapazamine was achieved, the cyclophosphamide dose was escalated to 1250 and 1500 mg/m^2.

The dose-limiting toxicity was granulocytopenia, which become more pronounced after the dose of tirapazamine was escalated beyond 260 mg/m^2 (level 4). Despite the granulocytopenia, no patient experienced granulocytopenic fever, thus allowing continued tirapazamine escalation to 390 mg/m^2. Up to dose level 6 (tirapazamine 390 mg/m^2) thrombocytopenia was not observed. After the cyclophosphamide dose was escalated, mild to moderate thrombocytopenia was noted, with the lowest nadir being 66 000 platelet/μl. No patient required red cell transfusions during the treatment. Granulocytes are more sensitive than the other cell lineages, providing a rational for the use of growth factors to further escalate tirapazamine and cyclophosphamide.

During initial studies, ototoxicity was seen after single-agent treatment with tirapazamine.[29] In our study, four patients had minimal hearing impairment, determined by routine audiometry. One patient treated at dose level 6 was transitorily deaf, but recovered to baseline after 2 days. Overall, there seems to be no direct correlation between tirapazamine dose and ototoxicity. Ototoxicity appeared to be reversible.

Other tirapazamine clinical trials found fatigue to be a significant toxicity.[24,27] However, we did not observe this toxicity to be dose limiting. Few patients were treated with repeated courses and fatigue may be cumulative. Nausea also was easy to control, indicating that the severe nausea noted in other studies was possibly related to other agents used in combination regimens.[26] A common symptom was muscle cramping.[30] At dose level 1, CPK elevation was observed, but this abnormality did not correlate with muscle cramping. Cramping and fatigue may be explained by tirapazamine's action on energy metabolism rather than muscle destruction. Ara and colleagues[25] have demonstrated that when breast cancer cells are incubated *in vitro* with tirapazamine at high doses (500 μmol/l) an uncoupling of oxidative phosphorylation results in oxygen consumption without adenosine triphosphate generation.

In these heavily treated patients, neither partial nor complete responses were seen. Three patients had stable disease (two patients with colorectal cancer and one with mesothelioma). One patient received six courses before progressing. The use of non-invasive imaging methods to detect hypoxic cell markers may, in the future, help to select individual patients with high likelihood of response.[31,32]

In summary, we conducted a phase I trial of tirapazamine in combination with cyclophosphamide, and determined the doses of 260 mg/m^2 of tirapazamine and 1000 mg/m^2 of cyclophosphamide as the most suitable for phase II trials. Doses of tirapazamine up to 390 mg/m^2 are well tolerated with full doses of cyclophosphamide, but would possibly require the addition of a granulocyte growth factor. Studies targeting different tumors where cyclophosphamide has established antitumor activity could be pursued with this combination.

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