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Phase I-II study: triciribine (tricyclic nucleoside phosphate) for metastatic breast cancer

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Abstract Triciribine is a purine analogue which inhibits DNA and protein synthesis. We performed two studies to define its activity against metastatic breast cancer. The first study was a phase II study in 14 patients with metastatic breast cancer who had received two or fewer chemotherapy treatments. The treatment schedule was triciribine 20 mg/m^2 per day by 24-h infusion (CI) daily for 5 days every 6 weeks as recommended by a previous open phase I trial. When neither response nor toxicity was seen in the phase II trial, we assumed the starting dose was too low for this group of patients with good performance status and repeated the phase I trial in patients with metastatic breast cancer with good performance status. The starting dose was 35 mg/m^2 per day using the same 5-day CI schedule, and starting doses were increased in subsequent cohorts of three patients in increments of 5 mg/m^2 until toxicity occurred. In the initial (phase II) study, one patient had stable disease for 18 weeks (three courses), the remainder progressed. There were no significant toxic effects. In the subsequent phase I study, ten patients were treated until the study was closed. The maximum dose was 40 mg/m^2. Two patients died, one each at the 35 and 40 mg/m^2 levels, respectively, 3 months and 6 weeks after their last course, one without intervening disease progression. Both had severe hypertriglyceridemia (18- and 21-fold elevation) and severe fatigue. At postmortem examination, one had congestive cardiomyopathy, and the other had severe pancreatitis and hypothyroidism. One patient had severe exacerbation of psoriasis which made her bedridden for more than 30 days. Four patients had hyperglycemia. Plasma pharmacology studies showed erratic drug levels, presumably related to enterohepatic circulation. Postmortem pharmacology studies showed residual drug present as long as 12 weeks after the last dose. We conclude that triciribine is ineffective at all doses tested and at doses of $\geq$ 35 mg/m^2 has unacceptable toxic effects.

Key words Tricyclic nucleoside · Phosphate · Triciribine · Metastatic breast cancer · Hypertriglyceridemia · Hyperglycemia

Introduction

Triciribine, also known as tricyclic nucleoside phosphate (TCN-P), a purine analogue synthesized in 1971, shows activity against murine CD8F1 mammary carcinoma, the human MX1 mammary tumor xenograft, and Novikoff rat hepatoma cells [2, 5, 10, 15]. Triciribine inhibits DNA and protein synthesis [8, 9, 16, 17], and although not polyphosphorylated or incorporated into polynucleotides, it is selectively toxic to S-phase cells. Triciribine inhibits amino acid binding to transfer RNA [8] and accumulates in red blood cells, the pancreas, and the liver [14]. The drug is secreted into the bile, is stored in the gallbladder, and undergoes enterohepatic recirculation, producing recurrent peaks in the plasma levels 1–2 h after a meal [14]. Canine pharmacology studies show a biphasic pattern of elimination with a terminal half-life of 39.4 h, but human studies reveal erratic plasma levels owing to the extensive enterohepatic circulation and repeated interconversion between TCN-P and TCN within cells [4, 12, 14].

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Table 1 Phase I studies performed with triciribine

Site	Reference	Dose and schedule	Recommended phase II dose	Toxicity	Drug-related deaths
Memorial Sloan-Kettering	7	25–350 mg/m^2 every 3 weeks	Not recommended	≥ 250 mg/m^2: nausea, ↑ glucose, hepatic toxicity 350 mg/m^2: irreversible hepatic toxicity in 2 patients, ↓ platelets	1 (liver toxicity)
M. D. Anderson Hospital	3	10–40 mg/m^2/day × 5 continuous infusion every 3–6 weeks	20 mg/m^2/day × 5 continuous infusion every 6 weeks	↑ glucose, hepatic toxicity, ↓ platelets (liver metastases, extensive radiation)	0
Mayo Clinic	6	3–55 mg/m^2/day × 5 every 6 weeks	Not recommended	Hepatic toxicity, ↓ platelets at highest dose	1 (↓ platelets) ?1 (heart failure with liver and renal toxicity)
Wayne State University	14	12–96 mg/m^2 every week × 4	Not recommended	Dose-limiting: nausea, vomiting, diarrhea, ↓ platelets, reversible hepatic toxicity, and ↑ glucose (not dose-dependent)	1 (↑ glucose and ↑ creatinine at 48 mg/m^2)
Brown University[a]		12–92 mg/m^2 every week × 4	Study closed after 10 patients entered; nausea, vomiting, hepatic toxicity	92 mg/m^2: ↑ glucose	0

[a] Personal communication, NCI Drug Monitor, Dr. Hoo Chun

In addition to displaying erratic and prolonged concentrations in the plasma, the drug accumulates in tissues, as demonstrated in two studies of postmortem material [3, 14]. In one study [14] of a patient who had received a single dose of 70 mg/m^2 of triciribine 61 days earlier, high concentrations of triciribine were found in the liver, gallbladder, pancreas, and metastatic lesions in the liver and lung. High concentrations of the nonphosphorylated drug were also found in the gallbladder and in the bile. In a phase I study performed at our institution [3], postmortem examination of four patients who had received 40 mg/m^2 per day of triciribine for 5 days 4–6 weeks earlier found high concentrations of triciribine in the liver and pancreas.

Information from three phase I studies (Table 1) [6, 7, 14] with triciribine at three doses and schedules has suggested that hepatic and pancreatic toxicity are dose limiting and worse in patients with existing liver disease. One study [6] using bolus doses daily for 5 days did not lead to a recommendation for phase II testing. From our phase I study [3] using lower doses infused over 5 days, we were able to recommend that the drug be tested in the phase II setting at a dose of 20 mg/m^2 per day by continuous infusion over 5 days every 6 weeks. Based on that study, this phase II evaluation of patients with metastatic breast cancer was undertaken. To minimize the hepatic and myelosuppressive toxic effects that had been observed in phase I study patients who had liver metastases or extensive prior irradiation of the bone marrow-bearing areas, patients with these characteristics were not eligible for this study. Because no toxic effects were seen at the 20 mg/m^2 level, a new phase I trial was performed with a starting dose of 35 mg/m^2. We present the results of both studies below.

Patients and methods

Patient selection

Patients with histologically confirmed progressive breast cancer who were seen at The University of Texas M. D. Anderson Cancer Center were eligible if they had: two or fewer prior chemotherapy regimens; measurable disease; a UICC performance status of ≤ 2; no evidence of liver or central nervous system metastases; normal liver function tests, including an alkaline phosphatase level of ≤ 110 IU, fasting blood sugar level of ≤ 110 mg/dl, absolute granulocyte count of ≥ 1,500/mm^3, platelet count of ≥ 100,000/mm^3, creatinine level of ≤ 1.3 mg/dl; and no evidence of ascites or pleural effusion. All patients were registered with the central data management office, were advised of the investigational nature of this study, and, prior to treatment, signed an informed consent document approved by our institutional review board.

Treatment and evaluation

In the phase II trial, the triciribine dose was 20 mg/m^2 per day by continuous infusion daily for 5 days every 6 weeks. One patient who had had extensive prior irradiation received 15 mg/m^2 per day. Doses were escalated in the last five patients, as discussed in the Results section. Based on the data from limited dose-escalation in

the phase II trial, in the subsequent phase I trial, the starting dose was 35 mg/m^2 per day by the same schedule; in subsequent cohorts the starting dose was escalated in increments of 5 mg. Patients were examined every 6 weeks in the phase II trial and weekly in the phase I trial. Blood counts and serum glucose levels were determined weekly; in the phase I trial, serum triglyceride levels were also monitored.

Results

Phase II trial

A group of 14 patients were treated with 22 courses from July 1985 through December 1986. All patients were evaluable and their characteristics are shown in Table 2. Most had an excellent performance status and many had disease limited to soft tissue. The one patient, who was treated at 15 mg/m^2 for course one because of concern about excessive toxicity, received 20 mg/m^2 for course two. When neither response nor toxic effects were seen in the first ten patients, five of whom had second courses, the starting dose was increased to 25 mg/m^2 in the next three patients, and to 30 mg/m^2 in the last patient (Table 3). One patient with soft tissue disease was stable for 12 weeks (two courses). The remaining 13 patients had progressive disease. No significant toxic effects were seen. Table 4 shows the hematologic toxic effects.

Phase I trial

A group of 10 patients were treated with 17 courses from January 1988 through November 1989. All patients were evaluable; their characteristics are shown in Table 2. The majority of these patients had visceral- or bone-dominant disease.

The maximum tolerable dose (MTD) was defined as 30 mg/m^2 per day by continuous infusion for 5 days every 6 weeks. Hematologic effects were minimal, as shown in Table 4, but nonhematologic toxic effects,

Table 2 Patient characteristics, overall and by study

Characteristic	Combined results	Phase II results	Phase I results
Number entered	24	14	10
Age (years)			
Median	49.5	50	50
range	33–72	33–72	33–68
UICC performance status			
0	9	7	2
1	12	5	7
2	3	2	1
Prior chemotherapy regimens			
Median	2	2	2
Range	1–3	1–3	1–3
Dominant disease site			
Soft tissue	11	9	2
Bone	7	3	4
Viscera	6	2	4
Median no. disease sites	1	1	1

Table 3 Distribution of doses and number of courses by study and responses

		Phase II study				Phase I study			
Dose (mg/m^2)	Total both studies	Subtotal	Initial	↑/↓[a]	Second[b]	Subtotal	Initial	↑/↓[a]	Second[b]
15	1	1	1	–/–	–	–	–	–/–	–
20	14	14	9	1/–	4	–	–	–/–	–
25	3	3	2	1/–	–	–	–	–/–	–
30	10	3	1	2/–	–	7	4	–/1	2
35	6	1	1	–/–	–	5	3	–/1	1
40	5	–				5	3	–/–	2
Total	39	22	14	4/–	4	17	10	–/2	5
Response									
No change	3	1				2			
Progress	21	13				8			

[a] Dose escalation (↑) or reduction (↓)
[b] Second or later courses

Table 4 Hematologic toxicity by dose (*AGC* absolute granulocyte count $\times 10^3/mm^3$)

Dose (mg/m^2)	No. of patients	No. of courses	Median lowest AGC (day) [range]	Median lowest platelets $\times 10^3/mm^3$ (day) [range]
15	1	1	1.6 (31)	194 (24)
20	10	14	3.4 (15) [1.5–6.5]	266 (22) [175–340]
25	3	3	4.3 (34) [3.1–4.6]	228 (27) [193–273]
30	8	10	2.5 (27) [1.1–5.0]	210 (22) [95–262]
35	5	6	2.4 (21) [1.6–7.7]	111 (27) [32–258]
40	3	5	1.1 (20) [0.9–3.0]	181 (22) [159–211]

Table 5 Grade 3 and 4 nonhematologic toxic effects according to the grading system based on our institution's modification of the UICC toxicity scale [1]: *hypertriglyceridemia* grade 3, 4.4–5.9-fold elevation; grade 4, ≥ 6-fold elevation; *hyperglycemia* grade 3, 2.1–4.3-fold elevation; grade 4, ≥ 4.3-fold elevation; *malaise* grade 3, in bed more than half of each day; *anorexia* grade 3, no significant oral intake; *infection* grade 3, severe organ infection or positive blood cultures without hypotension

Toxic effect	No. patients at dose level (mg/m^2)		
	30	35	40
Hypertriglyceridemia	1	2	–
Hyperglycemia	–	1	3
Malaise	–	2	–
Anorexia	–	1	–
Infection	–	1	–

Table 6 Tissue concentration of TCN and TCN-P (triciribine) in the two patients who died. Patient 1 (*Pt 1*) died 6 weeks after course 2; patient 2 (*Pt 2*) died 13 weeks after course 2. Both patients had received 35 mg/m^2 during course 2

Tissue	Concentration (μg/g tissue) TCN		TCN-P	
	Pt 1	Pt 2	Pt 1	Pt 2
Heart	2.3	–	1.6	–
Lung	4.8	–	3.1	–
Liver	–	2.1	4.9	8.7
Pituitary	–	–	8.7	–
Pancreas	–	5.6	–	4.4
Spleen	–	7.0	–	9.0
Kidney	–	65.9	–	21.3

principally in the pancreas, were dose-limiting (Table 5). Elevated triglyceride levels associated with nausea and vomiting were observed in five patients. Amylase levels were minimally elevated when measured 9 weeks after the second course in one patient, who experienced severe hypertriglyceridemia with an 18-fold elevation of serum triglyceride levels requiring a dose reduction from 40 mg/m^2 to 35 mg/m^2 (patient 2, Table 6). One other patient developed a 21-fold elevation of serum triglycerides after her second course at 35 mg/m^2. This was associated with severe nausea, vomiting, weight loss, hypothyroidism, pancreatitis, and minimal elevation of the serum cholesterol level (patient 1, Table 6). Both of these patients subsequently died after course 2, one patient 6 weeks and one 12 weeks later. Selected organs were obtained and levels of TCN and TCN-P (triciribine) measured (Table 6).

Eight patients had a highest glucose level above 160 mg/dl (grade 2); the maximum was 314 mg/dl (grade 3). Two required oral agents for control of serum glucose levels, and one became insulin dependent. Mild nausea and vomiting occurred in 11 patients. Profound malaise, anorexia, nausea, and vomiting occurred in the two patients who experienced severe hypertriglyceridemia. Infection was minimal, probably because hematologic toxicity was minimal. One patient had a severe exacerbation of psoriasis concomitantly with the institution of treatment with triciribine. She developed severe hypoalbuminemia (2.0 g/dl) and was bedridden for more than 30 days, but recovered.

Pharmacology

TCN and TCN-P levels in plasma and tissue from patients obtained at autopsy were determined according to the method described by Schilcher et al. [13]. Plasma concentrations of TCN and TCN-P revealed an erratic pattern in four patients who were studied (data not shown). Concentrations of TCN at 3 weeks were higher than those at 4–7 days in two patients. Comparison of levels during a second course in two patients showed higher levels in one patient and lower levels in the other. Concentrations of TCN-P were increased 3 weeks after dosing compared with the levels at 1 week. In two patients who had analysis of triciribine levels during the second course, there was evidence of drug accumulation. This was consistent with the autopsy data from two patients, which revealed persistent reservoirs of drug in tissue at 6 and 12 weeks after the last dose of triciribine (Table 6).

Discussion

We saw no responses to triciribine in either the phase II or phase I study. Additionally, triciribine caused excessive toxic effects in a population of patients with good performance status. Preclinical data determined by the 6-day subrenal capsule assay technique suggested that

triciribine was as active against breast cancer as cyclophosphamide when animals were treated until similar degrees of toxicity were reached [2]. However, the early phase I studies suggested that the drug had significant toxic effects. The trials reported here were performed because the earlier phase I trial performed at our institution with a lower dose, continuous infusion schedule suggested that these toxic effects could be ameliorated.

We used this lower dose and continuous infusion schedule in the phase II evaluation. All the patients in the phase II arm had an excellent performance status and tolerated the drug at this lower dose level without any toxic effects. However, because there were no responses to this dose and schedule, we expanded to a phase I study in a similar population of patients with good performance status.

The dose was escalated to a maximum of 40 mg/m^2. Dose-limiting toxicity, consisting of severe hypertriglyceridemia and associated with nausea and vomiting, occurred in two patients, one each at the 35 and 40 mg/m^2 levels, both of whom subsequently died. The mechanism of this hypertriglyceridemia is unknown but was presumably related to toxic effects on the pancreas that inhibited insulin production. Lower insulin levels impair the activity of lipoprotein lipase, resulting in decreased plasma clearance of triglycerides. This effect has also been described for tumor necrosis factor [11].

One patient died because of an acute cardiomyopathy associated with severe hypertriglyceridemia. The relationship of this to triciribine is unclear. However, one patient in the Mayo Clinic phase I trial with multiple other problems died with congestive heart failure.

No responses were seen in the 13 patients treated with doses at or above the MTD, and two patients died. The probability of missing a true 20% response rate with no responses in 13 consecutive patients is less than 5%. Thus, we conclude that triciribine is ineffective at all doses tested, and at doses ≥ 35 mg/m^2 triciribine has unacceptable toxic effects.

The favorable antitumor data obtained with triciribine in mouse tumor models did not predict for clinical efficacy in patients with breast cancer. Conversely, a clearly active drug, paclitaxel, did not have a favorable preclinical profile. Because of these and many other examples of lack predictability, the National Cancer Institute, USA, now uses a screening method employing a large bank of human tumor cell lines. Compounds demonstrating activity against a specific malignancy are then examined in more appropriate in vivo models such as human tumor xenografts in nude mice. Whether this new approach to drug selection and development will result in a better correlation of preclinical efficacy with actual clinical response remains a critically important yet unanswered question.

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