ABSTRACT

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide), a new nucleoside antimetabolite, was evaluated in a phase I trial involving children with refractory cancers. The drug was administered i.v. as a 10-min infusion daily for 5 consecutive days repeated at 3-week intervals. The dose ranged from 550 to 3300 mg/sq m/day. Seventeen patients received 23 courses and were evaluable for toxicity. The maximally tolerated dose was 2200 mg/sq m/day. The major dose-limiting toxicities were nonhematological. Neurotoxicity, including headache, drowsiness, and irritability, was common and was the principal dose-limiting toxicity at the higher doses. Severe myalgias were also dose limiting in one patient. Other side effects were mild, reversible elevations in serum transaminases; nausea, vomiting, and diarrhea; mild hypertension; dysphagia; and exfoliative dermatitis of the hands and feet. Myelotoxicity was not significant. The pharmacokinetics of tiazofurin was studied in 16 patients. Plasma disappearance following administration was triphasic with half-lives of 9.7 min, 1.6 h, and 5.5 h. Clearance was dose related, ranging from 120 ml/min/sq m at 550 mg/sq m/day to 70 ml/min/sq m at 3300 mg/sq m/day. The primary route of elimination was renal with 85% of the drug recoverable in the urine as the parent compound in the 24 h following administration.

INTRODUCTION

Tiazofurin (2-β-D-ribofuranosylthiazole-4-carboxamide) is a new nucleoside antimetabolite which has demonstrated activity against the murine leukemias P388 and L1210 and the usually refractory Lewis lung carcinoma (1). The mechanism of action of tiazofurin has been studied extensively in vitro and in vivo (2-5). It is metabolized intracellularly to thiazole carboxamide adenine dinucleotide, an analogue of the coenzyme NAD*. Thiazole carboxamide adenine dinucleotide inhibits the NAD*-dependent enzyme inosine-5'-monophosphate dehydrogenase, inhibiting the conversion of inosine to guanine and thus depleting the intracellular pool of guanine nucleotides. The oncolytic activity of tiazofurin in murine tumor systems was noted over a broad dosage range (1) and is schedule dependent. The multiple daily dosing schedules produced the greatest increase in the life span of tumor-bearing mice. Preclinical toxicological studies were performed in mice and dogs. Lethargy and prostration, resembling anesthesia, were noted in the mouse lethality studies. Other clinical signs of toxicity observed in dogs included vomiting, diarrhea, and anorexia. Mild, reversible, dose-dependent myelotoxicity, nephrotoxicity, and hepatotoxicity were also noted.

In this report we present the results of a multi-institutional phase I trial and pharmacokinetic study of tiazofurin administered by rapid i.v. infusion, daily for 5 consecutive days to pediatric patients with refractory cancers.

MATERIALS AND METHODS

Patient Eligibility. Patients 21 years of age and younger with histologically confirmed cancer (with the exception of two patients with the clinical diagnosis of brain stem glioma) refractory to conventional forms of therapy were eligible for entry into this trial. Patients were required to have a life expectancy of at least 8 weeks and they must have recovered from the toxic effects of prior therapy. Patients with solid tumors (without bone marrow involvement) were required to have adequate peripheral blood counts (granulocyte count, >1500/μl, platelet count, >150,000/μl), and all patients had a total bilirubin <2.0 mg/dl, serum transaminases <1.5 times normal, and normal serum creatinine (for age), electrolytes, blood sugar, and uric acid.

Prior to entry on study informed consent was obtained from the patient or his/her parent in accordance with the individual institutional policies.

Study Design. The primary objective of the study was to determine the toxicities and maximally tolerated dose of tiazofurin in children when administered as an i.v. bolus daily for 5 days. Courses of therapy were repeated every 21 days. A minimum of three patients evaluable for toxicity were treated at each dosage level, and at least two of the patients entered at each dose level had to have adequate bone marrow function to evaluate hematological toxicity. The starting dose for this study was 550 mg/sq m/day, which was one-tenth of the mouse equivalent 10% lethal dose. The dose was escalated until dose-limiting toxicity was observed. The dose escalation followed the modified Fibonacci search scheme with escalations to 1100, 2200, and 3300 mg/sq m/day. After the second patient treated with 3300 mg/sq m/day experienced severe neurotoxicity, subsequent patients were entered at a dose level of 2800 mg/sq m/day.

Patients also received allopurinol at a dose of 300 mg/sq m/day during the 5-day course of tiazofurin to prevent hyperuricemia that had been observed in some patients treated on earlier adult trials (6, 7).

Patients were monitored with complete blood counts, electrolytes, creatinine, calcium, phosphorus, uric acid, blood sugar, liver function tests, CPK, and urinalysis on days 1, 3, and 5 and then weekly. In patients with measurable disease other laboratory or radiological examinations pertinent to tumor response were measured at the end of the 21-day cycle. Patients were also monitored closely for clinical signs of toxicity.

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Individual patients were removed from the study if they experienced unacceptable toxicity or if objective disease progression occurred after one or more courses of tiazofurin. The phase I trial was terminated when consistent dose-limiting toxicity was identified at a dose level.

Drug Formulation and Administration. Tiazofurin was supplied by the National Cancer Institute as a sterile lyophilized powder in 10-ml vials containing 1000 mg. The drug was reconstituted with 4.6 ml of sterile water (200 mg/ml) and the appropriate dose was further diluted in 25 ml of 5% dextrose in water and infused i.v. over 10 min.

Pharmacokinetics. Patients were studied following the first dose of tiazofurin. Blood samples were obtained at the end of the 10-min infusion and then 5, 10, 15, 20, 30, 45, and 60 min and 2, 4, 6, 12, and 24 h after the dose. Samples were also obtained 24 h after the second through the fifth doses. The blood samples were collected in heparinized tubes and placed on ice until the plasma was separated by centrifugation. Plasma samples were stored frozen at −20°C. All urine was collected in 24-h aliquots during the 5-day treatment course. CSF was obtained from two patients 2 h after the dose of tiazofurin.

Tiazofurin in plasma, CSF, and urine was measured by a high-pressure liquid chromatography assay modified slightly from a previously described method (8). 2-β-O-Ribofuranosylthiazole-5-carboxamide, a structural isomer of tiazofurin, was used as an internal standard. Tiazofurin was first extracted and concentrated from plasma standards and patient samples using C18 Sep-Pak cartridges (Waters Associates, Milford, MA). The extracted samples were then injected into a 10-μm C8 Radial-Pak column (Waters Associates). The mobile phase contained 40% acetic acid and 1.5% methanol pumped at 1.5 ml/min. The eluent was monitored with a UV detector at a wavelength of 240 nm. Retention times for the 2-β-O-ribofuranosylthiazole-5-carboxamide and tiazofurin were 7.5 and 8.5 min, respectively, and the lower limit of sensitivity (with a 4-fold concentration) was 0.1 μM.

Plasma concentration-time data were fit to both biexponential (n = 2) and triexponential (n = 3) equations

\[ C(t) = \sum_{i=1}^{n} A_i e^{-\lambda_i t} \]

using MLAB, a nonlinear curve-fitting program (9). The best fit was determined by using Akaike’s information criterion (10). The half-life for each phase of elimination was calculated by dividing 0.953 by the rate constant (λ) for that phase. Other pharmacokinetic parameters were calculated using model-independent methods. Area under the plasma concentration-time curve was derived by the trapezoidal method (11) and extrapolated to infinity by adding the quotient of the plasma concentration at 24 h divided by the terminal rate constant (λt); total clearance (CLt) was the dose divided by the area under the plasma-concentration-time curve; and the volume of distribution at steady state (Vss) was calculated using the area under the moment curve (12).

RESULTS

Phase I Trial. Twenty-two patients were entered on study. Seventeen were evaluable for toxicity, and of these 11 were evaluable for hematological toxicity (i.e., had no bone marrow involvement with tumor). Of the five patients ineligible for toxicity three had acute lymphoblastic leukemia with rapidly progressive disease necessitating a change in therapy before the end of either the treatment or the required observation period, and two other patients developed sepsis during the 5-day treatment course resulting in the death of one patient and discontinuation of tiazofurin after three doses in the other. These five ineligible patients did not experience any unusual or severe toxicities from tiazofurin while on the study. The characteristics of the 17 evaluable patients are described in Table 1. Table 2 lists the number of patients and cycles at each dose level and the number with dose-limiting toxicity.

The major toxicities on this study were nonhematological. Neurotoxicity consisting of headache (onset within minutes after the dose), drowsiness, and irritability occurred in 12 of 14 patients at doses of 1100 mg/sq m/day and higher. Although difficult to objectively quantitate, the severity of these symptoms appeared to be dose related. At 1100 and 2200 mg/sq m/day the neurological symptoms were tolerable while at higher doses neurotoxicity was dose limiting. A single patient at a dose of 2800 mg/sq m/day had headaches severe enough to discontinue tiazofurin after the first dose. Another patient treated with 3300 mg/sq m/day became obtunded, developed seizures, and had an electroencephalographic pattern consistent with an encephalopathy shortly after the fifth dose of tiazofurin. The seizures were focal and initially refractory to anticonvulsant therapy. Over the subsequent 2 weeks the patient’s mental status improved and the frequency of seizures decreased. The patient had no history of a seizure disorder and no evidence of central nervous system spread of tumor. Computer-assisted tomographic scan and CSF examination performed after the onset of seizures were both normal.

Myalgias also occurred and were severe enough in one patient at the 2800-mg/sq m/day dosage level to result in discontinuation of the drug after the third dose. The serum CPK in this patient was normal. Two patients, one treated with 550 mg/sq m/day and the other with 1100 mg/sq m/day, had transient elevations in serum CPK (skeletal muscle origin) to 7760 and 2940 units/liter, respectively, during the 5-day treatment course. However,
both patients were asymptomatic.

The maximally tolerated dose of tiazofurin administered daily for 5 consecutive days in pediatric patients was 2200 mg/sq m/day. At 2800 mg/sq m/day two of three patients had dose-limiting toxicity including severe headache in one patient and myalgias in the other. At 3300 mg/sq m/day one of two patients had dose-limiting toxicity consisting of seizures and obtundation.

Other toxicities observed in order of frequency were: mild, reversible elevations in serum transaminases at all dose levels; severity not dose related (11 patients); mild nausea, vomiting, or diarrhea (10 patients); mild hypertension (2 patients); dysphagia (2 patients); exfoliative dermatitis involving the hands and feet (2 patients); mucositis (1 patient); arthralgias (1 patient); and chest pain (1 patient). Hematological toxicity was not significant. No patient with adequate bone marrow function became granulocytopenic (<1000/µl) or thrombocytopenic (<100,000/µl). With the prophylactic allopurinol therapy hyperuricemia also did not occur.

No responses were seen in the 20 patients who were evaluable for response.

Pharmacokinetic Study. Sixteen patients had blood samples obtained following the first dose of tiazofurin. Plasma disappearance of the drug was best described by a triexponential equation (Chart 1) with half-lives of 9.7 ± 7.8 (SD) min, 1.6 ± 0.9 h, and 5.5 ± 1.9 h. Plasma levels drawn 24 h after the second through fifth doses demonstrated no significant accumulation of the drug over the 5-day treatment schedule, even at the highest dose levels administered. C/TB for each dose level is given in Table 3. A statistically significant (P < 0.05) correlation exists between dose and C/TB with a linear correlation coefficient of 0.54. The linear relationship between dose and C/TB derived by the method of least squares is described by the equation

\[ C/TB = 129 - 0.154 \times \text{dose} \]

The mean V_{ss} for all doses was 29.3 ± 7.6 liters/sq m.

CSF was obtained from two patients treated at the 550- and 1100-mg/sq m/day doses. CSF tiazofurin concentrations were 19.9 and 18.5 µM, respectively, or 42 and 37% of the concurrent plasma levels.

Complete urine collections were available in 11 patients. Over the 24-h period following a dose of tiazofurin, 85 ± 13% was excreted unchanged in the urine, indicating that renal excretion is the primary route of elimination for tiazofurin.

The patient who developed seizures following the 5-day course of tiazofurin had plasma levels significantly higher than expected and a markedly decreased C/TB of 22 ml/min/sq m (data not included in Table 3 or regression analysis of dose versus C/TB). Serum creatinine was normal before the first dose of tiazofurin and remained normal throughout the 5-day treatment course. However, the patient did develop ureteral obstruction from a pelvic tumor requiring percutaneous nephrostomy for drainage on the third day of tiazofurin therapy. Creatinine clearance before tiazofurin was 62 ml/min/sq m and fell to 46 ml/min/sq m on the second day of treatment. Following the nephrostomy the creatinine clearance increased to 86 ml/min/sq m.

**DISCUSSION**

Tiazofurin is an interesting compound because of its unique mechanism of action and its curative activity in the Lewis lung carcinoma murine tumor model. A number of phase I trials evaluating different dosing schedules have been reported (6, 7, 13, 14). In this multiinstitutional pediatric phase I trial of tiazofurin administered as an i.v. bolus daily for 5 days, the dose-limiting toxicities were nonhematological. The dose recommended for phase II trials using this schedule is 2200 mg/sq m/day. The maximally tolerated dose in adult trials using the same dosing schedule ranged from 1650 mg/sq m/day (6) to 4100 mg/sq m/day for a study which is still entering patients (7). Since the major dose-limiting toxicities (neurotoxicity and myalgias) of tiazofurin occurred shortly after the first dose, shortening the course would probably not permit higher doses to be used.

As in our study nonhematological toxicities including severe headache and myalgias (14) have also been dose limiting in several adult trials (6, 14). In our trial neurotoxicity (headache, drowsiness, irritability) appeared to be dose related; the severity increased with each dose level. The patient who received the highest dose (3300 mg/sq m/day) and had delayed clearance experienced life-threatening seizures and encephalopathy. Neurotoxicity may be related to the ability of this drug to gain access to the central nervous system in significant concentrations, as demonstrated previously in monkeys (15) and confirmed in two patients on this study.

Although myelotoxicity did not occur in our trial it has been reported in adult trials (16) and was dose limiting in a trial administering tiazofurin by continuous infusion for 5 days (13). However, myelosuppression from tiazofurin in general has been sporadic and does not appear to be dose related (16). If the drug...
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has antitumor activity in future phase II trials, its minimal myelotoxicity would make it useful in combination chemotherapy with more myelosuppressive agents.

The pharmacokinetic behavior of tiazofurin does not appear to be significantly different in children compared to adults. The terminal half-life in our patients fits into the wide range of 4 to 13 h reported in other trials (6, 7, 13, 17). Reported values for $C_{\text{TB}}$ are lower in adults, ranging from 54 to 84 ml/min/sq m. A relationship between dose and clearance was not noted in these reports; however, the patients studied were treated over a smaller dose range.

Renal excretion was the primary route of drug elimination accounting for over 80% of the administered dose. The single patient with renal dysfunction treated on this study had delayed clearance and experienced severe neurotoxicity. In addition a review of patients treated on other trials revealed a relationship between thrombocytopenia and an increase in serum creatinine, presumably a result of tiazofurin nephrotoxicity (16). In designing phase II studies dosage adjustment should be considered for patients with compromised renal function.

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Pediatric Phase I Trial and Pharmacokinetic Study of Tiazofurin (NSC 286193)


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