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Phase I and pharmacokinetic study of tiazofurin (NSC 286193) administered by 5-day continuous infusion

D. Raghavan, J. Bishop, D. Sampson, J. Grygiel, R. Woods, A. Coates, and R. Fox

Royal Prince Alfred Hospital and Ludwig Institute for Cancer Research, Sydney; The Cancer Institute, Melbourne; and Royal North Shore Hospital, Sydney, Australia

Summary. A phase I and pharmacokinetic study of tiazofurin (NSC 286193), a C-nucleoside that inhibits IMP dehydrogenase, has been completed. The drug was administered by continuous infusion over 5 days. The maximum tolerated dose was 1650 mg/m^2 per day, neurological toxicity being the dose-limiting factor. Gastrointestinal and hematological toxicity were mild. A definite relationship exists between dosage and steady-state levels. The plasma clearance was 29.13 (±SD 4.05) ml/min per m^2. No complete or partial remissions were demonstrated among the 18 patients treated at five dose levels between 550 mg/m^2 and 2200 mg/m^2 per day.

Introduction

Tiazuofurin (2-β-D-ribofuranosyl thiazole-4-carboxamide; NSC 286193) is a water-soluble C-nucleoside, synthesized as part of a research programme to develop antiviral agents [14]. Although structurally related to the antiviral agent ribavirin, tiazofurin has little such activity. However, it has been shown to possess substantial antitumour activity against several murine cancers, including Lewis lung carcinoma and P388 and L1210 leukaemias over a broad dose range [13].

The mechanism of action of this drug has not been fully defined; it inhibits inosine monophosphate dehydrogenase (IMPD), depleting guanosine pools, with an associated inhibition of DNA and RNA synthesis. Resistance to growth inhibition in strains of P388 cell lines has been inversely correlated with the cells' ability to manufacture an anabolite of tiazofurin in which the nicotinamide moiety of nicotinamide adenine dinucleotide has been replaced by thiazole-4-carboximide [3, 4, 6, 7]. In the murine models there has been evidence of schedule dependency, with a 'daily ×5' schedule yielding greater antitumour activity than a single bolus.

Animal toxicology studies performed in mice and beagle dogs have shown a wide range of toxicity, including prostration and lethargy, a watery ocular discharge, corneal oedema, fever, emesis, weight loss, mild leukopoenia, and mild elevation of renal and liver function tests. The toxicities are dose-related and are reversible at lower dose schedules. The mouse equivalent lethal dose at the 10% level (MELD 10) on a daily ×5 bolus schedule is 5489 mg/m^2.

Phase I evaluation of tiazofurin has been initiated by the U. S. National Cancer Institute with schedules including 5-day bolus and continuous infusion. We have evaluated a phase I schedule of 5-day continuous infusion, on the basis of a rationale analogous to that for the administration of 5-fluorouracil: it has been shown that the haematological and gastrointestinal toxicity of 5-fluorouracil for a specified dose can be reduced by the use of a continuous infusion schedule [9]. Compared with bolus doses, this schedule allows the use of larger total doses of 5-fluorouracil without reducing the antitumour efficacy of the drug [12].

The aims of the study included:

1. Definition of the maximum tolerated dose (MTD) of tiazofurin when administered by 5 day continuous infusions;
2. Documentation of drug-related toxicities;
3. Demonstration of antitumour activity (if present);
4. Assessment of possible relationships between toxicity and steady-state drug levels.

Materials and methods

Selection of patients. Patients were eligible for inclusion in the study if they had histologically proven metastatic or recurrent solid malignancies for which no conventional treatment was proven or disease that had become resistant to established forms of treatment. Characteristics of patients are summarized in Table 1.

Drug delivery. Tiazofurin was supplied by the Cancer Therapy Evaluation Program, National Cancer Institute, as a lyophilized dosage form which was reconstituted to yield a final concentration of 1 mg/ml. The drug was administered as 5-day continuous infusion via an I-med continuous infusion pump. Treatment was repeated every 21 days, if indicated.

Because of the inhibition of IMPD, resulting in an elevation of IMP pools, it seemed likely that serum uric acid levels would be increased by the drug [8]. Accordingly, all patients received allopurinol (300 mg daily) beginning the day before the commencement of treatment.

Offprint requests to: D. Raghavan, Dept. of Clinical Oncology, Royal Prince Alfred Hospital, Camperdown, NSW 2050, Australia

Table 1. Details of patients

Number			19
Age (years)	Mean (standard deviation)		52.5 (9.5)
	Range		30–63
Sex		M:F	11:8
ECOG performance status		0–2	14
		3	5
Prior treatment:	radiotherapy		14
	chemotherapy		15
	no treatment		0
Tumour types:	Non-small cell lung cancer		4
	SCC, head and neck		4
	AdenoCA, colon		3
	AdenoCA, pancreas		2
	Renal cell carcinoma		2
	TCC, renal pelvis		1
	Hodgkin's disease		1
	Fibrosarcoma		1
	Large cell undifferentiated CA with unknown primary site		1

Abbreviations: SCC, squamous cell carcinoma; TCC, transitional cell carcinoma; AdenoCA, adenocarcinoma; CA, carcinoma

The planned dose escalation schedule (mg/m^2 per day $\times 5$) was 550–1100–1650–2200–3300–5000–6250. At least three patients were to be treated at each dose level; dose escalations were not permitted within individual patients. Treatment was to be discontinued upon the development of unacceptable toxicity or tumour progression, or at the patient's request. If no severe side effects occurred after treatment of three patients at a particular dose level the dose was escalated by one level for the next set of patients. Routine antiemetic medication was not administered unless a patient developed severe nausea or vomiting.

Pharmacokinetic study. Blood samples were collected from patients immediately before treatment and at 24, 48, 72, 96, and 120 h after commencement of the infusion; samples were centrifuged immediately at 4 °C, and aliquots of serum and red blood cells were frozen. In one case samples of ascitic fluid were also stored. No attempt was made to study the rate of decline of serum tiazofurin levels after the discontinuation of treatment, as the index of the pharmacokinetic study was steady-state levels.

Red blood cell haemolysate was prepared by freezing and thawing packed red cells; the mixture was centrifuged and 5 µl haemolysate was injected onto the chromatography column. Plasma and ascitic fluid were centrifuged to remove any particulate material and 5 µl was injected onto the column. Standards of 25, 50, 75, 100 and 250 µmol/l were injected after every 12 injections of patient samples to confirm assay reproducibility. If necessary, 500- and 750-µmol/l standards were also injected. The standard plot was linear up to at least 4 nmol injected onto the column.

A Waters high-pressure liquid chromatographic (HPLC) system was used, consisting of a model 710A autoinjector, a 6000A solvent delivery system, and a model 450 variable wavelength ultraviolet detector set at 235 mm (maximum absorption wavelength for thiazofurin). The column was 300 mm long, with a 4-mm internal diameter filled with microBondapak C18 reverse-phase packing, and preceded by a guard column. The mobile phase was glacial acetic acid : acetonitrile : distilled water (1 : 2.5 : 96.5) with a flow rate of 0.8 ml/min and column back-pressure of 1000–2500 psi, depending on the age of the column. The guard column was repacked when the back-pressure reached 2500 psi, usually after about 30 injections of red cell haemolysate or 80–100 injections of plasma.

For plasma samples with levels of 20, 70, 137, and 186 µmol/l, the coefficients of variation were 3.5%, 2.9%, 2.1%, and 1.8%, respectively.

Indices of clinical study. Prior to the commencement of treatment, each patient underwent full history taking and physical examination, and a series of baseline investigations, including haematological and biochemical screening, assay of CPK, electrocardiography, documentation of performance status and measurement of tumour masses.

Standard criteria of toxicity and of response were used [11]. Patients were examined daily and were questioned regarding symptoms of toxicity daily during the infusion and at 1 week and 2 weeks after and then before commencement of the next infusion. Haematological and biochemical screens were repeated on days 3 and 5 and at 1, 2 and 3 weeks after the infusion.

Results

Treatment

Nine patients received only one course of tiazofurin (including four removed from study because of rapidly progressive tumour); seven patients received two courses; and three patients received three courses. The doses were escalated to 2200 mg/m^2, as shown in Table 2. For each dose level between 1100 and 2200 mg/m^2 inclusive at least three patients received two courses of the drug, allowing evaluation of delayed and cumulative toxicity. However, the assessment of the dose level 2000 mg/m^2 (representing a dose reduction after the toxicity found at 2200 mg/m^2) was terminated early because of the side effects, and each patient only received one cycle.

Toxicity

The pattern of side effects is summarized in Table 3. In general, the toxicity was sporadic and unpredictable at daily doses less than 2000 mg/m^2. There was, however, clear evidence of an increased prevalence and severity of side effects at the higher doses. The dominant side effects included neurotoxicity, atypical chest pain (which resembled pleuro-pericarditis), conjunctivitis and anaemia.

There was a broad spectrum of neurological toxicity, which included depression, emotional lability, epileptic fits, headache and severe sedation. The development of grand mal fits (with one case of status epilepticus and one case associated with a cardiac arrest and death), and an additional patient who suffered grade 4 sedation, made 'neurotoxicity' the dose-limiting side effect at 2200 mg/m^2 per day.

An unusual syndrome resembling pleuro-pericarditis was seen in two patients. Each of these patients experienced severe, stabbing chest pain without features of angi-

Table 2. Doses and plasma levels

Dose rate (mg/m² per dx5d)	No. of patients	No. of courses	Css_b	Cl_b	Episodes of grade IV toxicity
550	3	6	55	26.6	0
1100	3	8	83–130	22.6–35.3	0
1650	6	9	139–181	24.3–31.6	1
2200	5	7	182–197	29.7–32.3	3
2000	2[a]	2	–	–	1

Cl, rate of clearance of tiazofurin from plasma (ml/min per m²)

[a] Discontinued early (see text); dose reduction because of toxicity at 2200 mg/m² per day

[b] Css, mean plasma tiazofurin concentration at steady state. Steady state was assumed when tiazofurin concentrations in plasma collected at 24 h intervals on 3 consecutive days were within 10% of each other. In such circumstances, a mean plasma tiazofurin concentration was determined (μm)

na. During the pain, there were no ischaemic changes on electrocardiogram, although one patient subsequently developed atrial fibrillation. Cardiac enzymes remained normal. The pain was not relieved by simple analgesics and required narcotics and cessation of infusion in each instance. As noted above, one patient suffered a cardiac arrest and an associated grand mal epileptic fit during the second course of treatment (2200 mg/m² per day). The patient had a past history of Wolff-Parkinson-White syndrome; however, serial electrocardiograms during the first

Table 3. Spectrum of toxicity

Toxicity	Dosage schedule (mg/m²)				
	550	1100	1650	2000	2200
	No. 3	3	6	2	5
Alopecia	0	0	0	0	0
Cardiac					
Cardiac arrest	0	0	0	0	1
Arrhythmia	0	0	1	1	0
Chest pain	0	0	1	0	1
Conjunctivitis	2	3	3	1	4
Cutaneous					
Rash	0	0	1	1	2
Desquamation	2	1	1	2	2
Fever	0	0	0	0	2
Gastrointestinal					
Nausea & vomiting	0	3	2	1	3
Diarrhoea	1	0	0	0	2
Stomatitis	0	0	0	0	1
Anal discomfort	0	0	1	0	0
Hematemesis	0	0	1	0	0
Hypertension	0	1	1	1	0
Infection					
Herpes simplex (lip)	0	0	1	0	0
Urinary tract infection	0	0	1	0	0
Local/injection site	0	2	0	0	1
Myelosuppression					
Anaemia	2	0	3	–	3
Leukopenia	1	0	1	0	1
Thrombocytopenia	1	0	2	0	0
Neurological					
Depression	0	0	1	1	1
Emotional lability	1	0	1	0	1
Epileptic grand mal fit	0	0	0	0	2
Myoclonic episode	0	0	1	0	0
Hallucinations	0	0	0	0	1
Sedation	0	0	0	1	3
Headache	0	0	1	0	2
Agitation	0	0	0	0	1
Photophobia	0	0	0	0	1

course and the first 2 days of the second course of tiazofurin showed no ectopic beats or ischaemic changes. Autopsy did not reveal the cause of death; specifically no evidence was found of myocardial ischaemia or infarction, or of atheromatous changes or other myocardial disease. Histological examination of the brain was not performed.

Although not severe, the commonest side effect of the drug was conjunctivitis (13 patients), with grittiness of the eyes or a watery ocular discharge. In four instances a Schirmer's test (use of blotting paper to measure production of tears) was positive, suggesting the possibility of an immunological syndrome. Furthermore, eight patients showed evidence of a mild desquamative eruption of the palms and soles. However, there was no clear evidence of immunological dysfunction in such parameters as DNA binding, antinuclear factor, or complement; rheumatoid factor was positive in sporadic patients (including conversion to positive in two cases after treatment). T- and B-lymphocyte subsets were not studied.

In general, the haematological toxicity was mild, with anaemia being the most prevalent feature (8 patients). Only three patients had leukopenia (2 grade IV, 1 grade II) and three had thrombocytopenia (1 grade IV, 2 grade I). No complications resulted from the myelosuppression. With the doses studied haematological toxicity did not appear to be dose-related and was only transient.

Gastrointestinal symptoms were mild (predominantly nausea with occasional vomiting or diarrhoea), and there was no evidence of drug-induced alopecia. Four patients experienced transient elevation of serum CPK (not the myocardial isoenzyme). Other sporadic side effects included fever (1 patient), injection site irritation (2 patients) and hypertension (2 patients). The details of patients who experienced grade 4 toxicity are summarized in Table 4.

Pharmacokinetic study

A definite relationship was demonstrated between administered dosage and steady-state levels (Table 2). Although there was some variability between patients, the mean plasma clearance was 29.13 ml/min per m^2 ($\pm$SD 4.05).

In all patients studied, the level of tiazofurin assayed in red blood cell haemolysate exceeded that demonstrated in the plasma, with ratios in the range of 1.2–28; in most cases an increased ratio was seen towards the end of the 5-day infusion. Too few patients were studied in detail to allow a relationship to be assessed between red blood cell levels and the development of anaemia.

In one patient, synchronous measurement of tiazofurin in red blood cells, plasma and ascites revealed ratios of 1.4:1.3:1.0, 0:1.4:1.0 and 2.4:1.3:1.0 at 24 h, 48 h, and 96 h, respectively, from the commencement of infusion.

Over the dose range investigated the disposition of plasma tiazofurin obeyed linear kinetics in that the steady-state plasma concentrations achieved were proportional to the dose rate. Furthermore, interindividual variability at each dose rate was less than two-fold; intraindividual variation between courses was negligible.

Response to treatment

No objective complete or partial responses were demonstrated. In four patients (pancreatic cancer, non-small cell lung cancer, colonic cancer), stabilization of disease [11] of 2–3 months' duration was achieved, but was of little clinical significance. In one other patient with metastatic colonic cancer, who had previously been treated with 5-fluorouracil and mitomycin C, objective regression (less than partial response) was demonstrated in a lymph node metastasis, with stable disease in lung and bone. This patient underwent autopsy (having sustained a cardiac arrest during the second course, as noted above), which revealed extensive areas of tumour necrosis histologically in lung and lymph nodes.

Discussion

Tiazofurin can be administered by continuous infusion over a 5-day period with relative safety in doses less than 2000 mg/m^2 per day. The clinical experience with this drug appears to reflect the preclinical toxicology, with such side effects as sedation, lacrimation and conjunctivitis, and only mild haematological toxicity. Although it is difficult to define an exact maximum tolerated dose from our data, because of the sporadic and unpredictable nature of the side effects, it appears to be approximately 1650 mg/m^2 per day for 5 days. For phase II studies in fit patients, we would recommend a dose by continuous infusion of 1500 mg/m^2 per day. Our patient population could be regarded as a 'good risk' group – most patients were relatively young, with an ECOG performance status less than or equal to 2, and without heavy prior treatment. Therefore, it may be necessary to modify our recommended doses for unfit or heavily pretreated patients.

There was no evidence of saturation of the elimination mechanisms for tiazofurin in these selected patients. However, caution must be excercised with the use of this drug, particularly in patients with impaired hepatic and/or renal function, until such time as the major excretion mechanisms are identified.

Table 4. Episodes of grade IV toxicity

Dose (mg/m^2)	Toxicity	Episodes/courses/patients
550	–	0/6/3
1100	–	0/8/3
1650	Neutropenia, thrombocytopenia	1/9/5
2000	Status epilepticus	1/2/2
2200	Neutropenia	1/7/5
	Grand mal fit, cardiac arrest	1/7/5
	Coma	1/7/5

Neurotoxicity was the dose-limiting side effect, and included epileptic fits, severe prostration and lethargy at doses of 2000 mg/m^2 per day or more. Other lesser features included emotional lability, depression, headache and hallucinations. It should however, also be emphasized that there may be a risk of life-threatening cardiotoxicity at the highest dose used, and ongoing cardiac evaluation should be recommended for phase II studies.

Our pharmacological studies have demonstrated a clear correlation between dose and plasma drug concentrations and, in general, a relationship between blood levels (or dose) and toxicity. There also appears to be good penetration into third spaces, as indicated by the levels found in ascitic fluid in one patient. Penetration into cerebrospinal fluid was not assessed in our study, although the presence of high levels of tiazofurin in cerebrospinal fluid in monkeys has previously been documented [5].

We have not defined an association between renal dysfunction and toxicity, which has previously been reported by others [1]. Futhermore, the plasma levels demonstrated in our study appear higher at each dose level than those reported elsewhere [2]. Although a randomized comparison between schedules of administration has not been carried out, there is no obvious difference in doses administered or in the spectrum of toxicity when tiazofurin is administered according to our own schedule or via 5-day bolus doses [10].

Although this study was not designed to assess the efficacy of treatment, it is of interest to note clinical and histological evidence of tumour regression in one patient (less than partial response) who had previously been treated with 5-fluorouracil and mitomycin C. However, the paucity of objective responses reported in other phase I studies is of concern. Nevertheless, further studies may be of interest in view of the mild gastrointestinal and haematological toxicity associated with this drug. Caution will be needed in view of the cardiac and neurological side effects at higher doses.

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