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Phase I Study of TCNU, a Novel Nitrosourea

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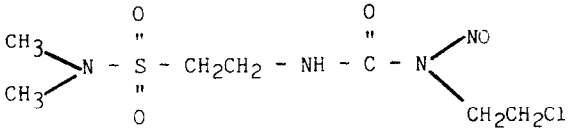
Abstract—*TCNU is a chloroethyl nitrosourea based on the endogenous amino acid taurine. This paper reports its first evaluation in man. Eighty-four patients with refractory cancer received 12 dose escalations from 10–150 mg/m² TCNU administered orally every 6 weeks. Clinical side-effects were predominantly gastro-intestinal but dose-limiting toxicity was thrombocytopenia. Pharmacokinetic monitoring with an HPLC assay sensitive to the nanogram range demonstrated unchanged TCNU in plasma for up to 8 h following administration. The mean half-life was 60 min. Clinical responses were seen in melanoma (four patients), lung cancer (two squamous, one small cell) and one patient each with renal and stomach cancer. These responses, together with the unusual pharmacokinetic profile of TCNU, warrant exploration in disease-orientated phase II studies at a recommended dose of 130 mg/m² p.o. q 5 weeks*

INTRODUCTION

TCNU (tauromustine) [1-(2-chloroethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea] was synthesized to provide a chloroethyl nitrosourea structurally related to CCNU and BCNU but with greater hydrophilicity, and linked to a naturally occurring amino acid [1]. TCNU is based on the endogenous substance taurine but with the sulphonic acid group replaced by a dimethylaminosulphonyl group and the amino group nitrogen forming one of the nitrogens of the nitrosourea. The structure of TCNU is shown as follows.



Scheme 1.

In preclinical screens, TCNU was found to be active against a wide variety of murine and human tumour models. *In vitro*, TCNU is active against the BE and HT-29 human colon carcinomas to the same order of activity as that shown by BCNU and CCNU. *In vivo*, TCNU is active against the Walker 256 carcinosarcoma in the rat with a higher therapeutic index than either BCNU or CCNU (4× and 2×, respectively). TCNU is also active against the L1210 mouse leukaemia, the Harding–Passey melanoma and the Lewis lung carcinoma [1]. In xenograft studies TCNU has been shown to have activity against human lung cancers of both small cell and non-small cell histological types [2].

Toxicological studies in mice, rats and dogs show the dose-limiting toxicity to be myelosuppression with the typically delayed pattern observed with other nitrosoureas. In the mouse the LD_{10} is 127 mg/m² whereas the dog was more sensitive with the maximum tolerated dose being 45 mg/m². Pharmacokinetic studies in dogs demonstrated that following oral administration, unchanged TCNU is detectable in plasma with time to maximum concentration of 25 min and a plasma half life of 15–20 min. Thus TCNU had interesting *in vitro* and *in vivo* activity in preclinical screening, and unusual pharmacokinetic properties in dogs. For these reasons it was decided to proceed to phase I clinical trials in man.

METHODS

Phase I study design

TCNU was administered orally every 6 weeks for a minimum of two courses of treatment. Based on the animal toxicology the starting dose was 10 mg/m². Every patient had a histologically confirmed diagnosis of cancer that was either refractory to conventional therapy or of a type known to be unresponsive to conventional cytotoxic drugs. Eligibility criteria included: age between 16 and 75 years, performance status of 0–3 (ECOG), all previous anti-cancer therapy to have been withdrawn

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for a minimum of 3 weeks (6 weeks for nitrosoureas or mitomycin C), a white blood cell count $> 3000\ mm^3$ and platelet count $> 100{,}000\ mm^3$ prior to entry to this protocol. All patients gave informed consent to their participation in the study. At each dose level a minimum of three patients received two or more courses of TCNU. Patients were monitored throughout the study by weekly history, physical examination and measurement of the following: haemoglobin, haematocrit, white blood cell count, platelet count, differential and reticulocytes, electrolytes and urea, calcium, phosphate, uric acid, creatinine, bilirubin, SGOT, GT, total protein, albumin and urine analysis. For patients participating in the pharmacokinetic part of the study, TCNU was administered after an overnight fast and for these patients no anti-emetic therapy or concurrent medication was given.

TCNU assay

1-(2-Chloroethyl)-3-[2-(dimethylaminosulphonyl)-ethyl]-1-nitrosourea (TCNU, LS 2667) and 4-hydroxy benzoic acid isopropyl ester (isopropagin) were synthesized and supplied by Leo AB, Helsingborg, Sweden.

Organic solvents acetonitrile, chloroform dichloromethane and methanol (HPLC grade), and glacial acetic acid (HPLC grade) were obtained from Rathburn Chemicals Ltd, Peebles, Scotland. 2-Propanol (Analar grade) was obtained from Koch-Light Laboratories Ltd. Organic solvents and HPLC mobile phase were vacuum filtered through a FHUP-047 Millipore filter.

Blood samples (5–10 ml) were collected at times 0, 15, 30, 45, 60, 90 min, 2 h and hourly thereafter until 8 h following treatment. Plasma was separated by centrifugation within 10 min of collection and stored at −20°C until analysed.

TCNU was analysed by high pressure liquid chromatography using the method of Polacek *et al.* [3] but with a modified mobile phase of 0.1% glacial acetic acid in water/acetonitrile 63/67.

Pharmacokinetic calculations

C_{max}, the maximum plasma concentration, and the corresponding time t_{max}, were the observed values from the logarithmic–linear plasma concentration time curve, and the elimination rate constant β was calculated by linear-regression analysis of the curve.

The half-life was calculated from the equation

$$t_{1/2} = \frac{\ln 2}{\beta} = \frac{0.693}{\beta}.$$

The total AUC was calculated using the linear trapezoidal rule from zero to the last measured time point and then by extrapolation to infinity.

Table 1. Patient characteristics

Number	84		
Sex	Male	48	
	Female	36	
Age	Mean	56	
	Range	17–74	
Diagnoses	Lung carcinoma		31
		Squamous	17
		Small cell	8
		Adenocarcinoma	5
		Other	1
	Melanoma		19
	Renal carcinoma		8
	Colon carcinoma		5
	Miscellaneous		21

Table 2. Dose escalations

Dose of TCNU (mg/m^2)	No. of patients
10	3
20	5
30	6
40	5
50	6
70	15
90	5
100	9
110	2
120	8
130	16
150	6
Total	86*

*Two patients initially treated at 20 mg/m^2 were subsequently treated at 40 mg/m^2 and 110 mg/m^2, respectively.

RESULTS

The characteristics of the 84 patients entered into this study are summarized in Table 1. Forty-three of these patients had not received any prior chemotherapy before receiving TCNU. From the starting dose of 10 mg/m^2 it was necessary to perform 12 escalations before the maximum tolerated dose of 150 mg/m^2 was reached (see Table 2). Eleven patients are unevaluable for haematological toxicity since they died of progressive disease within the first 6 weeks following TCNU. None of these deaths was attributable to the investigational agent. The data presented concern 161 courses of TCNU administered.

TCNU was well tolerated by the majority of patients, the only significant clinical side-effect being dose-dependent nausea and vomiting. This is

Table 3. Gastrointestinal toxicity

Dose of TCNU (mg/m²)	No. patients	Worst toxicity for individual patients WHO grade 0	1	2	3	4
10	3	3				
20	5	5				
30	6	3		3		
40	5	1		4		
50	6	1	1	2	1	1
70*	15	1	1	8	5	

*At doses higher than 70 mg/m², all patients were treated prophylactically with anti-emetics.

summarized in Table 3. When present, nausea and vomiting started 2–3 h after taking TCNU and rarely persisted beyond 4 h from the time of TCNU administration. Having demonstrated the pattern of gastrointestinal disturbance all patients (except those volunteering to participate in the pharmacokinetic studies) receiving TCNU at doses greater than 70 mg/m² received prophylactic anti-emetics usually with metoclopramide. The only other clinical side-effect reported was of pain in the site of the tumour by two patients with malignant melanoma. There was no alopecia and no neurological toxicity observed. No significant changes in hepatic or renal function were detected but the dose-limiting toxicity, as predicted by preclinical toxicology, was haematological—predominantly thrombocytopenia. This was first observed at doses of 70 mg/m² where one patient each experienced WHO grade 3, grade 2 and grade 1 toxicity. At 70 mg/m² two patients had grade 2 and two patients grade 1 white cell count haematological toxicity. The haematological toxicity for patients treated at 100 mg/m² and above is summarized in Table 4. The nadir of the platelet count occurred between 1 and 2 weeks earlier than the white cell count. Thrombocytopenia always recovered by 6 weeks, but for white blood count this was sometimes delayed for up to 8 weeks. It should be noted that for white cells, the median nadir at 100 and 130 mg/m² did not occur until week 6. Although data on the cumulative toxicity are limited by the nature of the protocol design, in patients receiving more than two courses of TCNU there was a tendency towards cumulative haematological toxicity. In Table 5, the data on patients receiving three or more cycles of TCNU are shown for thrombocytopenia.

Table 4. Haematological toxicity

No. of Patients	Dose of TCNU (mg/m²)		Nadir Week Median	(Range)	Count × 10⁹/l Median	(Range)
9	100	WBC	6	(1–6)	3.55	(2.7–6.6)
		PLATS	4	(3–5)	108	(65–183)
8	120	WBC	5	(1–8)	3.15	(1.3–5.9)
		PLATS	4	(1–6)	95	(23–165)
16	130	WBC	6	(3–8)	2.7	(1.3–8.9)
		PLATS	4	(3–6)	54	(23–199)
6	150	WBC	4	(3–6)	2.0	(1.0–6.3)
		PLATS	3	(3–4)	35	(23–138)

Pharmacokinetic studies

Twelve patients were studied in detail for the pharmacokinetic analysis of TCNU. The results are summarized in Table 6. Following oral administration, TCNU was rapidly absorbed with maximum concentrations being achieved between 15 min and 2 h from administration (mean 45 min). The maximum concentrations achieved following doses of 70–150 mg/m² ranged from 0.5–2.9 μg/ml (mean 1.3 μg/ml). The mean half life was 60 min. Unchanged TCNU was detectable in plasma for up to 8 h following administration. Figure 1 illustrates the plasma decay of three patients treated at doses of 120–150 mg/m² of TCNU. In parallel with a similar phase I study of TCNU conducted in Copenhagen [4], the pharmacokinetic data pertaining to a larger series of patients have been studied and are reported separately [5].

Clinical response

Nine patients showed a response to TCNU in this phase I study. One complete remission (durable 8+ months off treatment) and three partial remissions were seen in patients with malignant melanoma (receiving doses of 100, 110 and 130 mg/m² TCNU). The responses were all in soft tissue or nodal metastases, and 3/4 responding patients had been previously treated with vindesine ± DTIC. There were three responses in previously untreated patients with lung carcinoma (two with squamous histology [one complete remission], and one patient with small cell lung cancer receiving doses of 100, 150 and 130 mg/m²). Partial remissions were

Table 5. Thrombocytopenia for patients given ≥ 3 cycles of TCNU

	Previous chemotherapy	Nadir of thrombocytes × 10^9/l TCNU course No.							Delay in treatment dose due to low thrombocytes	
		1	2	3	4	5	6	7	(Weeks)	(Course)
70 mg/m²	+	208	241	210	176	236	195	157	0	
	+	230	188	164					0	
	+	238	275	225					0	
	+	177	138	31					0	
	+	33	35	27	21				1	(3)
100 mg/m²	+	136	65	89					0	
	+	100	108	82	183				0	
	−	114	128	177					0	
120 mg/m²	−	60	38	30	29	16			1	(4)
	−	128	108	143	29	52*			2	(4/5)
130 mg/m²	+	41	44	37	38				0	
	−	144	59	53	48	86*			3	(2/3/4)
	−	53	37	25	51*				0	
	−	72	107	15					0	
150 mg/m²	−	53	35	38*	103*				0	
	−	46	26	47					0	

*Dose reduced due to toxicity (↓ WBC or plats).

Table 6. Summary of pharmacokinetic characteristics

Dose of TCNU (mg/m²)	Sex	Diagnosis	T_{max} (min)	C_{max} (ng TCNU/ml)	$t_{1/2\beta}$ (min)	AUC (μg × min/ml)
70	Female	Squamous lung	60	957	62	85.9
	Male	Melanoma	30	1570	67	130.8
	Female	Alveolar cell	15	1780	54	81.8
120	Male*	Adeno lung	30	2913	58	213.6
130	Male	Melanoma	45	1498	61	173.7
	Male*	Melanoma	45	1420	71	153.1
	Male	Melanoma	60	668	45	63.7
	Male	Melanoma	120	861	70	153.0
	Male	Small cell lung	30	1339	75	151.2
	Female	Melanoma	15	1092	40	65.0
150	Female*	Adeno lung	60	513	75	102.3
	Female*	Adeno small bowel	30	1202	46	99.5

*No previous chemotherapy.

obtained in a patient with renal cell carcinoma, and a patient with carcinoma of the stomach, neither of whom had received prior chemotherapy.

DISCUSSION

As a class of cytotoxic drugs, the nitrosoureas have been conspicuous for the discrepancy between good activity in experimental tumour systems and disappointing results in man. Several aspects of the phase I study of TCNU suggest that this nitrosourea has properties different from other analogues. The most noticeable difference is the fact that TCNU is detectable in plasma for up to 8 h following administration. Although the rapid metabolism of CCNU has prevented detailed pharmacokinetic studies of this nitrosourea in the past, Lee *et al.* have recently reported a study of the pharmacokinetics of CCNU administered orally at 130 mg/m² [6]. Using reverse phase high performance liquid chromatography, CCNU was not detected in the plasma of any of the four patients studied due to its conversion to monohydroxylated metabolites during the first pass through the liver and gastrointestinal tract. Two monohydroxylated metabolites of CCNU were found at high concentration 2–4 h after administration. Little is known at present of the metabolism of TCNU but the atypical pharmacokinetics probably result from the taurine moiety

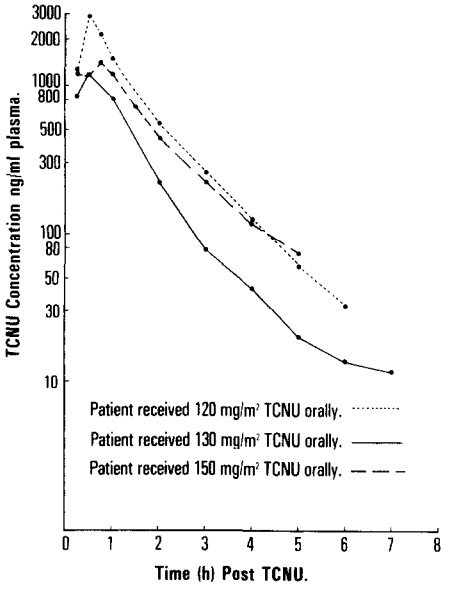

Fig. 1. Plasma decay of TCNU in three patients treated orally at doses of 120–150 mg/m^2.

of the compound. The role of taurine in mammalian systems is complex. Free taurine is found in millimolar concentrations in a number of tissues particularly the brain and the retina; it is also found in lymphocytes, platelets and in cardiac muscle. The major role of taurine is thought to be that of protecting cells both by membrane stabilization and as an anti-oxidant [7, 8]. The availability of a sensitive assay for TCNU will facilitate the study of TCNU metabolism in future studies to determine individual variation in drug handling and the effect of other drugs on the distribution and metabolism of TCNU.

The toxicity of this new nitrosourea was predictably thrombocytopenia but the compound was well tolerated clinically although anti-emetics were required prophylactically at doses in excess of 70 mg/m^2. Due to the marked species variation in maximum tolerated dose between dogs and rodents noted in preclinical toxicology studies, the dose escalations in man were deliberately cautious. This resulted in 12 escalations before the maximum tolerated dose in man was determined. Although the pharmacokinetic parameters were linearly related to the dose level [5], and haematological toxicity was generally dose-dependent, it should be noted that all but one of the clinical responses observed in this phase I study occurred at dose levels below the maximum tolerated dose which was 150 mg/m^2. For phase II studies 130 mg/m^2 is recommended to avoid exposing patients to the possibility of severe thrombocytopenia. Although the nadir of white cell counts occurred at 6 weeks, this is not dose-limiting and for phase II studies the drug is recommended to be repeated at 5 week intervals to optimize dose intensity [9, 10]. Given the patient population studied, the responses seen in refractory cancers such as non-small cell lung cancer and malignant melanoma were unusual. The contribution that the slower plasma decay of TCNU secondary to the inclusion of an endogenous amino acid makes to this clinical activity remains to be established, but based on the encouraging results and unusual properties of TCNU seen in this phase I study, phase II studies are now in progress to evaluate the therapeutic activity of this drug against a wide range of malignant diseases.

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