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Anti-tumour Activity of TCNU in a Panel of Transplantable Murine Colon Tumours

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Abstract—*The novel nitrosourea, 1-(2-chloroethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea (TCNU) is active against a panel of three transplantable murine adenocarcinomas of the colon of varying growth characteristics and morphology (MAC system). It shows greater activity than previously tested nitrosoureas in this system and the routes of administration of TCNU do not significantly alter its anti-tumour activity. These data suggest that TCNU may be useful in the management of large bowel cancer as the MAC system has been previously shown to be a good model of human disease.*

INTRODUCTION

THE NITROSOUREAS have shown a broad spectrum of anti-tumour activity against experimental tumours and have established clinical activity for a broad spectrum of human malignancies. Although at present the nitrosoureas have not lived up to expectations in the clinic due to delayed bone marrow toxicity, the alkylating activity of these compounds is still thought to have great potential as the 'bullet' to be used in the treatment of cancer if an accurate delivery system can be achieved. To this end, 1-(2-chlorethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea (TCNU) has been developed. TCNU (Fig. 1) is based on the naturally occurring amino acid taurine, a metabolite of cysteine that is involved in the synthesis of bile salts [1] and also acts as an inhibitory amino acid neurotransmitter in the cerebellum having similar actions to glycine on motor neurones [2]. Due to this taurine moiety, TCNU has high solubility in aqueous solution.

TCNU has recently undergone phase I clinical trials using the oral route and was shown to produce an objective response in the treatment of small cell and large cell adenocarcinoma of the lung, mesothelioma and breast cancer, following oral administration [3]. TCNU has now entered phase II clinical trials.

The mouse adenocarcinoma of the colon (MAC) series of transplantable tumours has been shown to have a similar spectrum of histology and chemosensitivity to human large bowel cancer [4]. The purpose of this study was therefore to assess the activity of TCNU in this system and also to examine the influence of the route of administration of this drug on its anti-tumour activity.

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MATERIALS AND METHODS

Pure strain NMRI mice from the inbred colony at the Clinical Oncology Unit, University of Bradford, aged 6–8 weeks were housed in cages in an air conditioned room, where regular alternate cycles of 12 h light and darkness were maintained. Animals were supplied with a pellet diet (CRM, Labsure, Croydon, U.K.) and water *ad libitum*.

TCNU was a gift from Leo Laboratories, Helsingborg, Sweden and was dissolved in sterile 0.9% saline and administered either by oral gavage, intraperitoneally, or intravenously by tail vein injection. 1-(2-Chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU) was used as a positive control compound.

The transplantable tumours were developed from primary tumours induced by 1,2-dimethylhydrazine [5]. The tumours show no strong antigenic properties as solid tumours are retransplantable following surgical excision. The differing morphology and growth characteristics of the tumour line necessitated the use of different chemotherapy protocols.

MAC 13 tumour fragments from donor animals were implanted subcutaneously into the left flank

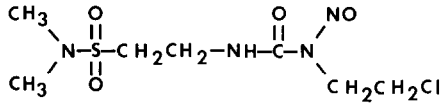

Fig. 1. Structural formula of TCNU.

of female mice by means of a trocar. Two days after transplantation when tumours are approximately 2 mm^3 (day 0) the mice were allocated into groups by restricted randomization prior to treatment. Chemosensitivity was assessed by tumour weight 21 days later and tumours were fixed for histological evaluation.

MAC 26 tumour fragments were implanted into the left flank of male mice and the animals allocated into groups by restricted randomization. As MAC 26 is a slow growing tumour (tumour volume doubling time 4.5 days), chemotherapy was commenced when the tumours had reached a size which could be accurately measured (tumour volume approximately 40 mm^3) (day 0), approximately within 17 days of implantation. Chemosensitivity was assessed by twice weekly caliper measurements of the tumour. Tumour volumes were determined by the formula $(a^2 \times b)/2$ where a is the smaller and b is the larger diameter of the tumour [6]. Semi-log plots were drawn of relative tumour volume (RTV) against time. On completion of the experiment, 30 days later for treated groups, mice were sacrificed and the tumours removed, weighed and fixed for histology.

MAC 15A was implanted into male mice by injecting 1×10^6 cells suspended in 0.2 ml of 0.9% saline (day 0) either intraperitoneally or intravenously via the tail vein. The animals were allocated into groups and treated on day 2 as previously. Anti-tumour activity was determined by comparing the lifespan of the treated and control groups. Deaths were recorded and the median survival time (MST) determined.

Activity scores for TCNU and control compounds were allocated as in Table 1.

Table 1.

Solid s.c. tumours		MAC 15A (ascitic and systemic tumours)	
Percentage inhibition*	Score	T/C%†	Score
<25	0	<125	0
25–49	1+	125–149	±
50–74	2+	150–200	1+
75–90	3+	>200	2+
>90	4+	<50% cures	3+
		>50% cures	4+

*Solid tumours assessed by weight or volume inhibition.
†MST of treated animals, over MST of control animals × 100.

Table 2. Influence of route of administration on activity of TCNU against MAC 15A

Dose (mg kg^{-1})	Route	T/C%	Activity score
40	p.o.	275	4+*
30	p.o.	170	1+
20	p.o.	175	1+
10	p.o.	120	0
5	p.o.	112	0
2.5	p.o.	112	0
30	i.p.	210	2+
20	i.p.	210	2+
10	i.p.	150	1+
30	i.v.	210	2+
20	i.v.	108	0
10	i.v.	109	0

*>50% cures.

All tumours were fixed in Bouin's fluid and then processed on a Shandon Automatic tissue processor. Tissues were then embedded in wax blocks and sections (5 μm) were cut on a Leitz microtome. All sections were then stained with haematoxylin and eosin.

RESULTS

The influence of route of administration on the response to TCNU at different doses is presented in Table 2. Percentage T/C represents the ratio of median survival times of treated groups to control groups. The maximum tolerated dose of TCNU by the i.p. and i.v. routes in these male MAC 15A tumour bearing mice is 30 mg kg^{-1}. These mice tolerated 40 mg kg^{-1} by the oral route (p.o.) with increased anti-tumour effects. Significant responses were achieved down to 10 mg kg^{-1} i.p. but not by the other routes.

Activity against systemic MAC 15A at maximum tolerated dose by the i.p. route is presented in Table 3. TCNU showed significantly greater activity at maximum tolerated dose than the positive control compound MeCCNU.

MAC 13 tumours were highly sensitive to TCNU by all three routes examined with greater than 90% inhibition of tumour weight at day 21 following a dose of 30 mg kg^{-1}. Activity was demonstrated down to 2.5 mg kg^{-1} by the oral route (Table 4).

Treatment of mice bearing MAC 26 tumours with TCNU at all the doses and routes used caused a significant delay in tumour growth as compared to controls (Table 5). Best responses were seen at a dose of 30 mg kg^{-1} (Fig. 1). Administration of TCNU at a dose of 40 mg kg^{-1} orally produced a significant delay in tumour growth but at the expense of host toxicity.

Histological examination of MAC 13 tumours showed the presence of viable tumour cells in all

Table 3. Activity of TCNU against systemic tumours produced by intravenous inoculation of MAC 15A tumour cells

Drug	Dose (mg kg^{-1})	Median survival time (days)	T/C%	Cures	Activity score
Control	—	17	—	—	—
TCNU	30	>200	>200	9/10	4+
MeCCNU	20	105	618	0/10	2+

Table 4. Influence of route of administration on activity of TCNU against MAC 13

Dose (mg kg^{-1})	Route	T/C%	% inhibition (100−T/C)	Activity score
40	p.o.	0.6	99.4	4+
30	p.o.	4.7	95.3	4+
20	p.o.	16.0	84	3+
10	p.o.	12.1	87.9	3+
5	p.o.	17.0	83.0	3+
2.5	p.o.	54.0	46.0	1+
30	i.p.	5.8	94.2	4+
20	i.p.	6.6	93.4	4+
10	i.p.	30.4	69.6	2+
30	i.v.	2.0	98	4+
20	i.v.	4.6	95.4	4+
10	i.v.	10.1	89.9	3+
5	i.v.	27.0	73.0	2+
2.5	i.v.	88.0	12.0	0

Table 5. Activity of TCNU against MAC 26

Dose (mg kg^{-1})	Route	Survivors	T/C%	Activity score
40	p.o.	5/6	—	—
30	p.o.	6/6	23	3+
20	p.o.	6/6	36	2+
30	i.p.	6/6	19.0	3+
30	i.v.	6/6	18.0	3+
20	i.v.	6/6	44	2+

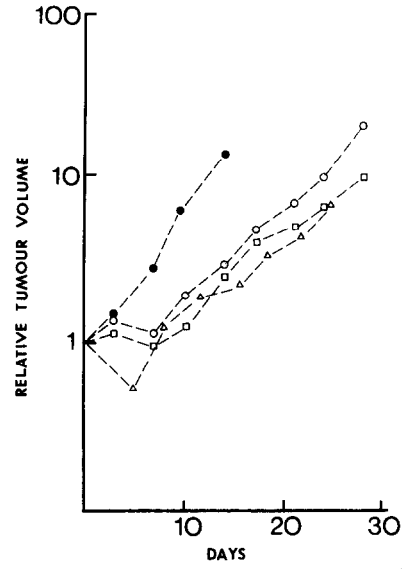

Fig. 2. Anti-tumour activity of TCNU at 30 mg kg^{-1} *against MAC 26 (● control, △ intraperitoneal administration, ○ oral administration, □ intravenous administration).*

tumours excised following treatment with TCNU except for those treated with 30 mg kg^{-1} i.v. where viable tumour cells were found only in three of five tumours examined.

DISCUSSION

Previous use of nitrosoureas in the MAC system of tumours has shown marked differences in response with good anti-tumour effects being only achieved at or close to maximum tolerated dose. One of the series, MAC 26, a well differentiated slow growing adenocarcinoma [7], is resistant to therapy with standard nitrosoureas. The present study indicates that TCNU has equal or greater anti-tumour activity in this system compared to previously studied standard nitrosoureas.

The presence of the more water soluble group attached to the reactive chloroethylnitroso moiety may allow a greater drug delivery to the tumour and therefore enhance anti-tumour effects. However, merely increasing the water solubility of nitrosoureas has not greatly increased experimental anti-tumour activity compared to standard nitrosoureas, none being more effective than methyl-CCNU and some showing long term toxic effects [8]. The possibility that the taurine-like moiety acts as a carrier group in a similar fashion to streptozotocin which possesses a glucose-amine carrier group and shows good effect in the treatment of pancreatic tumours [9], may explain the enhanced activity of TCNU. Taurine is known to be actively taken up by certain tumours and also by normal tissues [10] and therefore in TCNU, the taurine-like group may allow a rapid uptake of drug into the tumour.

This study has demonstrated that anti-tumour effects of TCNU are not significantly altered by route of administration although marginally better responses are achieved at lower doses by the i.p. route in MAC 15A and by the i.v. route against MAC 13.

TCNU was highly active against systemic MAC 15A disease with 9/10 cures being achieved at maximum tolerate dose and also against MAC 13

where 2/5 cures were achieved when the drug was administered i.v.

The results of this study suggest that TCNU may be useful in the management of human large bowel cancer as the MAC system has been shown to be a good model of human disease with responses to standard agents only normally seen close to maximum tolerated dose. However, recent experimental studies have demonstrated that even though TCNU is less haematoxic than carmustine it still demonstrates significant residual bone marrow impairment [11]. Recent clinical studies in breast cancer have also demonstrated that TCNU has significant marrow toxicity and side effects that prohibit dose escalation [12]. It remains to be seen therefore whether there is a sufficiently large therapeutic window to allow for anti-tumour effects in specific cancer types without prohibitive host toxicity.

REFERENCES

1. Huxtable RJ, Lippincott SE. Sources and tumour rates of taurine in newborn, weanling, and mature rats. In: Huxtable RJ, Pasantes-Morales H, eds. *Taurine in Nutrition and Neurology: Advances in Experimental Medicine and Biology* 1982, New York, Plenum Press, Vol. 139, 23–47.
2. Hosli L, Hosli E. Electrophysiological and autoradiographic studies of GABA and glutamate neurotransmitters at the cellular level. In: Hertz L, Kramme E, McGeer EG, Schousboe A, eds. *Glutamine, Glutamate and GABA in the Central Nervous System: Neurology and Neurobiology*. New York, Alan R Liss, Vol. 7, 441–455.
3. Vibe-Petersen J, Hansen HH. A phase I clinical evaluation of TCNU—a new water soluble nitrosourea compound. Fifth NCI/EORTC Symposium on New Drugs in Cancer Therapy, 22–24 October 1986, Free University, Amsterdam, The Netherlands.
4. Double JA, Ball CR. Chemotherapy of transplantable adenocarcinoma of the colon in mice. *Cancer Chemother Rep* 1975, **59**, 1083–1089.
5. Haase P, Cowan DM, Knowles JC, Cooper EH. Evaluation of dimethylhydrazine induced tumours in mice as a model system for colorectal cancer. *Br J Cancer* 1973, **24**, 530.
6. Geran RI, Greenberg NH, MacDonald MM, Schumacher AM, Abbot BJ. Protocols for screening chemical agents and natural products against tumours and other biological systems (third edition). *Cancer Chemother Rep* 1972, **3**, 1–103.
7. Double JA, Bibby MC. Evaluation of the activity of *N*-[*N'*-(2-chloroethyl)-*N'*-nitrosocarbamoyl(CNC)]-alanine and derivatives against a slow growing, well differentiated adenocarcinoma of the mouse colon. Fifth NCI/EORTC Symposium on New Drugs in Cancer Therapy, 22–24 October 1986, Free University, Amsterdam, The Netherlands.
8. Johnson JP, Montgomery JA. Relationship of structure to anticancer activity and toxicity of the nitrosoureas in animal systems. *Cancer Treat Rep* 1986, **70**, 13–30.
9. Mitchell EP, Schein PS. Contributions of nitrosoureas to cancer treatment. *Cancer Treat Rep* 1986, **70**, 31–41.
10. Baskin SI, Wakayama K, Knight T, Jepson JH, Besa EC. The sulfur-containing amino acid pathway in normal and malignant cell growth. In: Huxtable RJ, Pasantes-Morales H. *Taurine in Nutrition and Neurology—Advances in Experimental Medicine and Biology*. New York, Plenum Press, Vol. 139, 127–143.
11. Molineux G, Schofield R, Testa NG. Haematopoietic effects of TCNU in mice. *Cancer Treat Rep* 1987, **71**, 387–841.
12. Ernst P, Balslev I, Movridsen T. Second line chemotherapy with tauromustine in metastatic breast cancer in postmenopausal women: a phase II study. *Cancer Treat Rep* 1987, **71**, 987–988.