Eur J Cancer Clin Oncol, Vol. 24, No. 8, pp. 1355–1360, 1988.
Printed in Great Britain

0277-5379/88$3.00+0.00

Effects of Routes of Administration of TCNU on its Plasma, Tissue and Tumour Concentrations

J.A. DOUBLE,* M.C. BIBBY,* P.M. LOADMAN* and J.C. BLOOMER†

Clinical Oncology Unit and †School of Biomedical Sciences, University of Bradford, Bradford, West Yorkshire BD7 1DP, U.K.

Abstract—*Tissue and plasma samples were taken from normal and tumour bearing mice at various time intervals following a dose of* 30 mg kg^{-1} *TCNU given by various routes. TCNU levels were measured by HPLC.*

The results show that the route of administration influences plasma concentrations, bioavailability and tissue and tumour concentrations. Intravenous administration gave 100% *plasma bioavailability but only* 15% *of this level was seen following oral administration with consequently lower levels in tissues and tumours. The effect of gastric contents played a major role in this reduced biovailability and plasma levels increased to* 65% *following overnight fasting.*

The differences in concentrations of TCNU observed here in plasma, tumour and normal tissues after oral and intravenous administration may have important clinical implications and may influence both anti-tumour activity and toxicity.

INTRODUCTION

FACTORS which alter the concentration of a drug at its primary site of action or the period of time which a drug is available for activity at the biological receptors must be considered in the use of cancer chemotherapy [1]. In order to achieve its effect a drug must first be presented in a suitable form at an appropriate site of administration. It must then be absorbed and distributed through the body to the site of action. For the effects to diminish, the drug must almost always be metabolized and or excreted and the residues must be removed from the body [2]. Therefore, although the outcome of cancer chemotherapy may depend in the large part on the inherent sensitivity of the tumour under treatment, pharmacokinetic factors such as drug absorption, metabolism and elimination are extremely important in determining the dose schedule and route of drug administration [3].

A drug enters the circulation by being injected there directly, or by absorption from depots where it has been placed. The most obvious advantage of the intravenous route is that the drug is placed in the circulation with minimal delay. The commonest depot is the gastrointestinal tract, the drug having been taken orally [4]. Most drugs are absorbed from the gastrointestinal tract by lipid diffusion of non-ionized molecules. The intraperitoneal route is commonly used in animal experiments where diffusion into the blood is rapid owing to the large surface area of the absorbing surface. Careful selection of the route of administration may improve the anti-tumour effect of a given drug by allowing it to reach high concentrations in the areas of clinical need [1].

The nitrosoureas are well absorbed and disappear rapidly from plasma but their metabolites may persist for several days [5]. The major toxicity of the nitrosoureas is delayed bone marrow suppression commonly occuring 4–6 weeks after a given dose of drug [6]. The most noteworthy effects in humans have been against brain tumours and lymphoma with lesser activity seen in lung cancer, melanoma, myeloma and cancer of the colon [7]. 2-Chloroethyl-*N*-nitrosoureas are extremely effective anti-cancer agents in experimental tumour systems [7]. Unfortunately their clinical value has been limited by their pronounced and delayed toxicity especially to the bone marrow. The development of new analogues with equally good anti-tumour activity but with reduced toxicity would provide a major contribution to cancer chemotherapy.

TCNU (1-(2-chloroethyl)-3-[2-(dimethyl-aminosulphonyl)ethyl]-1-nitrosourea) is a new water soluble nitrosourea. We have previously reported preliminary results on the activity of TCNU given intraperitoneally in a panel of transplantable adenocarcinomas of the mouse colon (MAC) with varying sensitivities to standard nitrosoureas [8]. TCNU

Accepted 1 March 1988.
This work was supported by the Whyte Watson/Turner Cancer Research Trust, Bradford, West Yorkshire, U.K. and by a mini project funded by the Screening and Pharmacology Group of the EORTC.

was active against all tumour lines tested, representing an improvement over standard nitrosoureas. The activity against MAC 26 was particularly interesting as this tumour is unresponsive to standard nitrosoureas. The latter improvements may indicate that the water solubility of TCNU may alter pharmacokinetics which may have therapeutic implications. TCNU given orally has undergone Phase I evaluation and objective responses were seen in squamous cell, adenocarcinoma and large cell carcinoma of the lung as well as mesothelioma and breast cancer. It is well tolerated with thrombocytopenia being the dose limiting factor [9]. Phase II evaluation in non small cell lung cancer, melanoma, breast cancer and colorectal cancer is now in progress.

Experimental studies in this laboratory have demonstrated that TCNU is sufficiently water soluble to be administered intravenously at therapeutic doses [10]. The aim of this study was to determine the pharmacokinetic profiles and tissue disposition of TCNU following oral, intraperitoneal and intravenous administration.

MATERIALS AND METHODS

Animals

The animals used were pure strain male NMRI mice (average weight 30 g and aged 8–12 weeks) from the colony of the Clinical Oncology Unit, University of Bradford. With the exception of one group of mice that was starved overnight, the animals were fed and watered *ad libitum*.

Tumours

The development of several adenocarcinomata of the large bowel in NMRI mice from primary tumours induced by 1,2-dimethylhydrazine has been described elsewhere [11]. MAC 26 tumours were transplanted into male mice by subcutaneous implantation of tumour fragments into the flank.

Test compound

1-(2-Chloroethyl)-3-[2-(dimethylaminosulphonyl)-ethyl]-1-nitrosourea (TCNU) was a gift from Leo laboratories, Helsingborg, Sweden.

At all times TCNU was protected from light and kept at 4°C to prevent breakdown.

Reagents

All solvents were at HPLC grade (May and Baker, Dagenham, England) and other reagents were of analytical grade. *n*-Propyl-*p*-hydroxybenzoate (Sigma Chemical Co., Poole, Dorset) and triple distilled water were used.

Drug regimes

TCNU was given orally by gavage or injected by the intravenous or intraperitoneal route in 0.9% (w/v) saline (30 mg kg^{-1} body wt).

Preparation of plasma samples

Blood samples were taken by cardiac puncture under ether anaesthesia, collected into heparinized tubes containing 0.5 ml acetate buffer (0.2 M, pH 4) to stabilize the TCNU. The samples were centrifuged at 2000 ***g*** and 4°C for 10 min. The plasma was removed and frozen and stored at −20°C until analysed.

Preparation of tissue specimens

Mice were sacrificed and samples were taken of liver, kidney, testes and tumour (MAC 26) and frozen in liquid nitrogen and stored at −20°C until analysis. They were homogenized in distilled water (10% w/v) with 1 N HCl (20 μg/ml) added to stabilize the TCNU. Samples (0.5 ml) of homogenates were taken for protein estimation in duplicate. The remainder was centrifuged at 2500 ***g*** for 15 min and supernatants were extracted for HPLC analysis.

Sample extraction

TCNU was extracted from plasma and tissues using solid phase chromatography. Two hundred microlitres of internal standard (*n*-propyl-*p*-hydroxybenzoate 5 μg/ml in phosphate buffer pH 6.0) was added to the plasma/tissue sample (50–200 μl). Bond Elut cartidges containing particles of C_{18} coated silica (Analytichem International) were activated by passing ethanol (1 ml) then phosphate buffer (1 ml) through under negative pressure. Plasma and tissue specimens were added to the Bond Elut cartridges. The cartridges were then washed with buffer and air dried. Two hundred microlitres of 1% glacial acetate acid in methanol was passed through the cartridges and the eluents collected.

Chromatography

TCNU was measured by HPLC. A Lichrosorb RP-18 column was used (Merck-BDH, Poole, Dorset) with a C_{18} pre column. An isocratic mobile phase of 0.1% glacial acetic acid in water/acetonitrite (60/40) was pumped at a constant flow of 1.5 ml/min using a Waters (Milford, MA, U.S.A.) 6000A solvent delivery system. The injection was 10 μl and detection was at 229 nm using a Waters Lambda-Max 480 LC spectrophotometer. For measuring TCNU in tissues the isocratic mobile phase was altered to 65/35 water/acetonitrile and the flow rate decreased to 1.2 ml/min to enable separation of the TCNU peak from an interfering peak. A standard curve was prepared by the addition of TCNU to control mouse plasma and

plotting the ratios of peak areas of the compound to the internal standards against drug concentration. Peaks were traced and integrated with an Isaac Model 42A data module (Cyborg Corporation, U.S.A.), an Apple II E computer (Apple Computers Inc. U.S.A.) and Appligration II software (Dynamic Solutions Co-op, U.S.A.). The curve was linear over a range of 0.1–20 μg/ml. Overall recovery time was 95% and the detection limit was approx. 5 ng/ml. The coefficent of variation for replicate samples at a concentration of 1 μg/ml was 7.8%.

Quantification of drug and metabolites in biological specimens

The peak area of TCNU on the chromatograph trace was measured and expressed as a ratio with that of the internal standard and concentration estimated from the calibration curve. Each value was adjusted to take into account the volume of plasma analysed and the dilution of whole blood with acetate buffer. Concentrations of TCNU in tissue specimens were adjusted to take account of the 10% dilution of the tissue and the volume of homogenate analysed. Final tissue concentrations were expressed as a ratio to protein content.

In vitro *stability studies*

Twenty microlitres TCNU (500 μg/ml) was added to 200 μl of solvent and incubated at 4°C and 20°C. The solvents used were acetate buffer, acetate buffer and mouse plasma and saline. Twenty microlitres of each solution was added to 20 μl HCl (to stop the reaction) and 200 μl internal standard at time intervals up to 2 h. TCNU was then extracted using the Bond Elut cartridge and 10 μl of the eluent injected into the HPLC.

Data analysis

The AUC was calculated for the blood level time curves obtained with the various routes of administration of TCNU.

Bioavailability

This is the ability of a drug to reach its target site in an active form. The bioavailability of TCNU after oral and i.p. dosing was calculated using the following equation

$$\text{Bioavailability} = \frac{(\text{AUC})_{\text{i.p. or oral}}}{(\text{AUC})_{\text{i.v.}}} \times \frac{x^{\circ}_{\text{i.v.}}}{x^{\circ}_{\text{i.p. or oral}}}$$

x° = concentration.

Statistical methods

Mean values and standard errors were calculated for experiments repeated in triplicate. The differences between TCNU concentrations after various routes of administration were tested for significance using Student's *t*-test.

RESULTS

The breakdown of TCNU in various media at 4°C and 20°C after 60 and 120 min is shown in Table 1. Degradation was temperature and pH dependent, being greatest in mouse plasma at 20°C but this could be significantly reduced by the addition of acetate buffer at pH 4.0. Analysis of normal mouse plasma showed no interfering peaks in the region of TCNU.

Figure 1 shows the plasma levels of TCNU following a dose of 30 mg kg^{-1} given by three routes. It also demonstrates the effect of gastric contents on the bioavailability of TCNU given by the oral route, although absorption from both the gastrointestinal tract and peritoneum are still rapid, with significant levels being detected in the plasma 2 min after administration (the shortest time that it was logistically possible to obtain blood by cardiac puncture). The presence of food in the stomach increased the peak plasma time to 5 min compared with 2 min by the other routes and in standard mice with access to food also produced the greatest degree of individual variation. The associated changes in bioavailability are summarized in Table 2.

It can be seen that there are considerable differences in peak plasma concentrations, the highest levels being after i.v. administration and the lowest following oral administration of fed mice. These differences were significant ($P < 0.0025$) as was the difference between fed and fasted mice ($P < 0.01$). Comparison of the four AUCs consequently showed that in this experiment the absolute systemic bioavailability varied from 14.5% after oral administration to fed mice to 78.7% after i.p. injection, when compared to i.v. bioavailability.

Table 1. *Stability of TCNU*

	Percentage TCNU remaining			
	4°C		20°C	
	60 min	120 min	60 min	120 min
Acetate buffer and mouse plasma	94.4	87.0	87.1	74.1
Acetate buffer	74.8	74.3	68.6	41.9
Mouse plasma	84.7	83.5	44.5	13.4
Saline	97.5	95.0	69.9	60.8

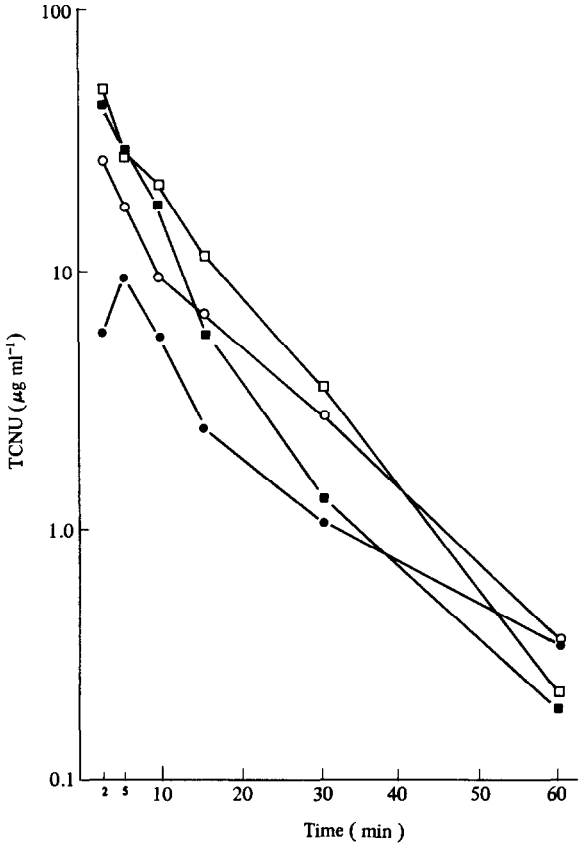

Fig. 1. Mouse plasma levels of TCNU following 30 mg/kg^{-1} *administered by different routes* (□ *i.v.*; ■ *i.p.*; ○ *oral fasted and* ● *oral fed*). *Points represent mean values obtained from three mice.*

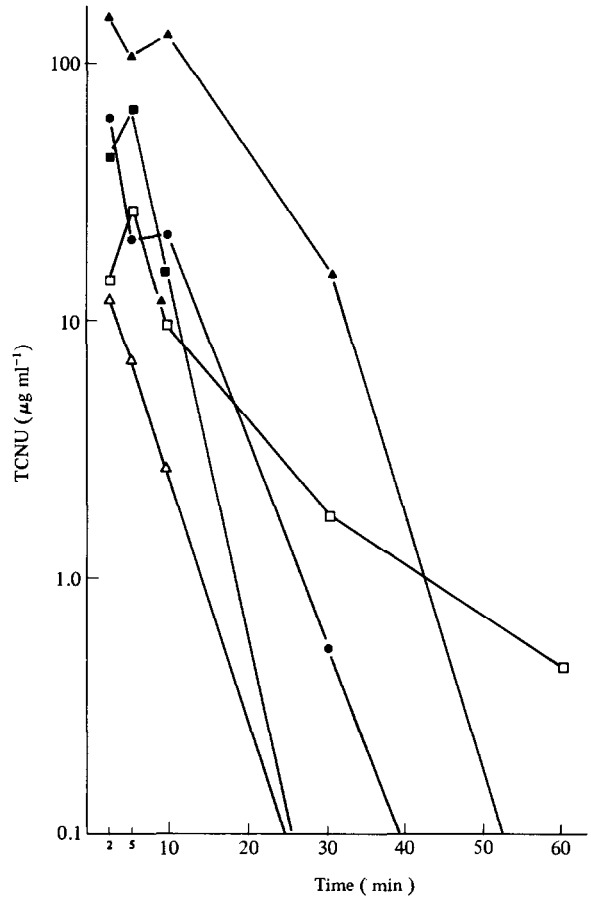

Fig. 2. Mouse tissue and plasma levels of TCNU following a dose of 30 mg/kg^{-1} (*i.v.*) (● *plasma*; ■ *liver*; ▲ *kidney*; △ *testis*; □ *tumour*).

Figure 2 shows the levels of TCNU in plasma and various tissues following an intravenous dose of 30 mg kg^{-1}. Peak levels probably occur in less than 2 min in plasma, kidney and testes and at 5 min in liver and tumour. Figure 3 shows similar data following oral administration. As might be expected peak plasma levels are lower and the time to reach peak levels with the exception of the liver is longer, being as long as 10 min in the kidney and tumour. The rapid uptake by the liver is indicative of good gastric uptake and entry into the gastric venous circulation.

Table 3 shows the levels of TCNU with time in various tissues in fed mice bearing the solid MAC 26 tumour following a dose of 30 mg kg^{-1}. The differential bioavailability between the oral and intravenous route is clearly apparent but this does not seem to result in any selective uptake by the various tissues studied as the ratios of TCNU in tissue and plasma are essentially similar by either route. Similarly with the ratios of AUCs with the possible exception of that in the tumour, route of administration appears to have little influence.

DISCUSSION

The choice of the route of administration is hopefully based on the pharmacokinetic assessments of bioavailability and the feasibility of formulating an acceptable dose preparation. An acceptable dose is one which will achieve prolonged plasma levels above a minimal anti-tumour concentration but

Table 2. Peak plasma concentrations, AUCs and bioavailability of TCNU in plasma following i.v., i.p. and oral administration of 30 mg kg^{-1}

Route of administration	Peak plasma concentration (μg/ml)	AUC (μg h ml^{-1})	Bioavailability (%)
i.v.	61.1	8.46	100
i.p.	41.49	6.66	78.7
Oral (fasted)	26.51	5.47	64.7
Oral (fed)	6.6	1.23	14.5

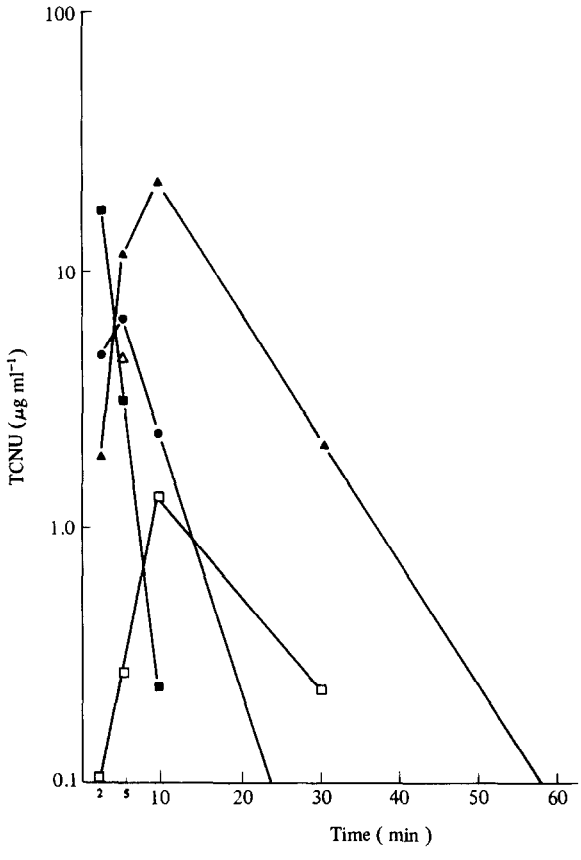

Fig. 3. Mouse tissue and plasma levels of TCNU following a dose of 30 mg/kg^{-1} *(oral)* (● *plasma*; ■ *liver*; ▲ *kidney*; △ *testis*; □ *tumour*).

below levels toxic to the host [3]. This study has shown how the route of administration can influence the bioavailability of TCNU and its subsequent concentrations in tissues and tumour.

Pharmacokinetic studies on many anti-cancer agents have been limited by their instability. Nitrosoureas decompose nonenzymatically at a relatively rapid rate when exposed to varying conditions of pH, temperature and media. They also undergo base catalysed decomposition to generate the alkylating chloroethyldiazonium hydroxide entity and metabolic transformation [5]. The preliminary studies were necessary to confirm the validity of the results obtained. The addition of acetate buffer to plasma and maintaining samples at 4°C reduced initial variations seen when measuring TCNU.

The results clearly show how the route of administration effects the concentration of TCNU in plasma and tissues. The low and more varied bioavailability of TCNU following oral administration may have some therapeutic significance. However we have shown [10] that this has little effect on anti-tumour activity but slightly higher doses can be given by the oral route.

The differing bioavailability seen between oral and intravenous administration seems to have only quantitative rather than selective influence on normal tissue disposition but in the case of tumour there is a hint of selectivity as shown by the ratios of the AUCs by the two routes. This perhaps indicates that prolonged exposure or repeated lower doses may have a therapeutic advantage. There is evidence to suggest that in experimental systems repeated doses are well tolerated and not associated with the conventional cumulative toxicity. Anti-tumour activity experiments along these lines are currently in progress.

Although this study has shown that the bioavailability of TCNU is significantly reduced by the oral route compared with intravenous administration, high levels of drug still rapidly reach the circulation indicating good gastric absorption. This is emphasized by the short peak concentration time in the liver. This finding suggests that TCNU possesses high lipid solubility like other nitrosoureas, facilitating rapid transport into cells and across membranes [5]. However, the water solubility of TCNU at therapeutic doses may confer disposition properties not seen with standard nitrosoureas. This may be a significant factor in its improved experimental therapeutic activity.

Table 3. TCNU tissue levels (μg/g *tissue*)

30 mg kg^{-1} TCNU	MAC 26									
	Plasma		Liver		Kidney		Testis		Tumour	
	Oral	i.v.	Oral	i.v.	Oral	i.v.	Oral	i.v.	Oral	i.v.
2 min	4.76	61.1	17.4	43.5	2.0	169	<0.8	10.9	0.06	14.5
5 min	6.6	20.1	3.21	68.7	12.6	108	4.4	7.0	0.28	27.2
10 min	2.41	21.0	0.24	15.1	20.2	132	<0.8	2.51	1.32	9.3
30 min	<0.27	0.53	<0.2	<0.2	2.14	14.5	<0.8	<0.8	0.24	1.84
60 min	<0.27	<0.27	<0.2	<0.2	<0.4	<0.4	<0.8	<0.8	0.073	0.44
AUC (μg.h/ml)	1.23	8.46	1.05	9.40	6.0	47.6	0.35	1.75	0.41	5.23
AUC $\frac{\text{tissue}}{\text{plasma}}$	1.0	1.0	0.79	1.11	4.5	5.62	0.26	0.21	0.31	0.47
AUC $\frac{\text{i.v.}}{\text{oral}}$	6.36		8.95		7.9		5.0		12.6	

REFERENCES

1. Haskell CM. *Principles of Cancer Chemotherapy. Cancer Treatment*, 2nd edn, Ch. 3. Philadelphia, Saunders, 1985.
2. Curry SH. *Disposition and Fate. Drug Disposition and Pharmacokinetics: with a Consideration of Pharmacological and Clinical Relationships*, 3rd edn, Ch. 2. Oxford, Blackwell Scientific, 1980.
3. Chabner BA. *The Role of Drugs in Cancer Treatment. Pharmacological Principles of Cancer Treatment*, Ch. 1. Philadelphia, Saunders, 1982.
4. Goldstein A, Aronow L, Kalman SM. *Principles of Drug Action: the Basis of Pharmacology*, 2nd edn. New York, Wiley, 1975.
5. Olivero UT. Pharmacology of the nitrosoureas: an overview. *Cancer Treat Rep* 1976, **60**, 709–713.
6. Wasserman TH. The nitrosoureas: an outline of clinical schedules and toxic effects. *Cancer Treat Rep* 1976, **60**, 709–713.
7. Schabel FM. Nitrosoureas: a review of experimental antitumour activity. *Cancer Treat Rep* 1976, **60**, 665–699.
8. Bibby MC, Double JA. Anti-tumour activity of TCNU in transplantable colon tumours in NMRI mice. *Br J Cancer* 1987, **56**, 199.
9. Vibe-Petersen J, Hansen HH. *Proceedings of the Vth NCI/EORTC Symposium on New Drugs in Cancer Therapy*, Amsterdam, 1986.
10. Bibby MC, Double JA, Morris CM. Anti-tumour activity of TCNU in a panel of transplantable murine colon tumours. *Eur J Cancer Clin Oncol* 1988, **24**, 1361–1364.
11. Double JA, Ball CR. Chemotherapy of transplantable adenocarcinoma of the colon in mice. *Cancer Chemother Rep* 175, **59**, 1083–1089.