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Pharmacokinetics of tauromustine in cancer patients

Phase I studies

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Summary. The pharmacokinetic properties of tauromustine (TCNU) were studied in 31 cancer patients who participated in phase I trials. The patients received single oral doses of tauromustine in the range of 20–170 mg/m^2. Plasma samples were taken over 24 h after administration and analysed for tauromustine by reversed-phase liquid chromatography. Parent TCNU could be demonstrated in the plasma of all patients. Its absorption was rapid (t_{max} = 38 ± 22 min), the half-life was 57 ± 22 min (mean ± SD), and maximal concentration (C_{max}) and AUC values were linearly related to the dose level. Thus, our study does not indicate dose-dependent pharmacokinetics for the drug in the range of 20–170 mg/m^2. Thrombocytopenia was the dose-limiting toxicity of TCNU; the reduction of platelet counts appeared to be linearly related to the log dose and C_{max} and AUC values. TCNU appears to exhibit pharmacokinetic properties that are different from those of other nitrosoureas, which might be important for the clinical effect of the drug.

Introduction

Tauromustine (TCNU), 1-(2-chloroethyl)-3-[2-(dimethylamino-sulphonyl)ethyl]-1-nitrosourea, LS 2667, (Fig. 1) is a novel nitrosourea compound based on the endogenous amino acid taurine. The drug is a neutral compound; the partition between *n*-octanol and water is described by log $P = 0.6$, which indicates a semi-polar character for TCNU. This drug has shown potent activity against several experimental tumours in vivo and in vitro [2, 7]. When administered orally, it produced a higher therapeutic index than BCNU, CCNU and MeCCNU against Walker 256 carcinosarcoma and L1210 leukemia. The median effective dose (ED_{50}) against Walker 256 carcinosarcoma of TCNU was only one-fourth (0.25 vs 1.0 mg/kg) that of BCNU, CCNU and MeCCNU, whereas TCNU had activity comparable to that of these agents against L1210 leukemia, Harding-Passey melanoma, Lewis lung and colon C26 tumours. Of major interest is its efficacy against nitrosourea-resistant tumours [6]. Its oral bioavailability is about 26% in dogs. C_{max} values are obtained after about 25 min, and the plasma half-life is 15–20 min [2].

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TCNU has recently undergone phase-I clinical trials in cancer patients to determine its toxicity pattern and maximal tolerable dose (MTD) when given orally [9, 10]. During these studies, plasma concentrations of this drug were followed in 31 patients after a single oral dose. The pharmacokinetic parameters were calculated and the relationship between the pharmacokinetics, dose and hematological toxicity of TCNU was evaluated. This pharmacokinetic data is important for the design of optimal therapeutic regimens to be used in subsequent clinical trials.

Materials and methods

Patients. The patients who entered the present study were participating in two clinical trials carried out at the Finsen Institute in Copenhagen [10] and the Western General Hospital in Edinburgh [9]. In all, 22 patients from Copenhagen and 9 from Edinburgh with various malignant diseases joined the pharmacokinetic study. Detailed clinical data are presented elsewhere [9, 10]. Among the inclusion criteria, pretreatment platelet values $\geq 100 \times 10^9$/l and white blood cell counts (WBC) $\geq 3.0 \times 10^9$/l were required, as were normal liver (serum alkaline phosphatase, serum ASAT, serum LDH, serum bilirubin and prothrombin time) and renal (serum-electrolytes and serum creatinine) function tests, with exception being made for abnormal values directly related to the underlying malignant disease [10].

Study design. The pharmacokinetics of TCNU were studied after a single dose. The drug was given orally and the dose range was 20–170 mg/m^2. Two or three patients were included at each dose level. Tablets of 5, 10, 25 and 50 mg were used and the dose was calculated to the nearest 5 mg.

The patients were instructed to come to the hospital after fasting for 8 h. The tablets were swallowed together with 100 ml water, and the patients abstained from food for at least 2 h after the drug was given. An interval of at least 3 weeks since prior antineoplastic therapy, including radiotherapy, was required (6 weeks in the cases of other nitrosoureas and mitomycin C).

At dose levels ≥ 40 mg/m^2 TCNU, patients at the Finsen Institute were treated with i.v. metoclopramide (40–80 mg) about 30 min before receiving TCNU; in cases of nausea and vomiting the dose was repeated at intervals of 2–3 h. Metoclopramide was not given to patients in Edinburgh or to patients C1, C3, C4, C6, C7, C8 and C9 in Copenhagen.

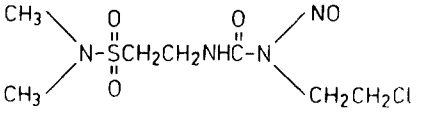

Fig. 1. Structural formula of TCNU

The study was approved by the ethical committee and appropriate government authorities and was carried out according to the Helsinki declaration. All patients gave their informed consent.

Collection of samples. Blood samples were collected in heparinised tubes through an infusion cannula prior to drug administration and subsequently 5, 15, 30, 60, 90 and 120 min and 4, 6, 8, 10 and 24 h thereafter. The samples were at once cooled on ice, and plasma was separated by centrifugation at 1300 *g*, 4°C, for 6 min. The centrifugation was completed within 15 min after sampling, and plasma was immediately frozen and stored below −20°C until analysis.

Analytical method. TCNU concentration in plasma was determined by the method developed by Polacek et al. [5], based on high-performance liquid chromatography with UV detection at 229 nm. The limit of detection was about 6 ng/ml plasma.

TCNU was extracted into a mixture of dichloromethane and chloroform at pH 6, the extract was washed with phosphate buffer (pH 8), evaporated, re-dissolved in methanol-acetic acid (99:1), and chromatographed on a reversed-phase column (Ultrasphere-ODS, 5 µm). The mobile phase was a mixture of acetonitrile and 0.005 *M* phosphate buffer (pH 3.9–4.7) (37 + 63).

Calculations. The area under the plasma concentration-time curve (AUC) was calculated using the linear trapezoidal rule. C_{max} and t_{max} values were determined from the observed plasma concentration-time curve as the maximal concentration and corresponding time. The elimination rate constant, β, was estimated by linear regression analysis of the logarithmic curve. The elimination half-life, $t_{½}$, was determined from its relationship to β ($t_{½} = \ln 2/\beta$). The AUC from zero to infinity, AUC_{inf}, was obtained as the sum of the AUC and the residual area C_z/β. C_z denotes the calculated plasma concentration of the last time-point, t_z, and was obtained from the regression line. Comparisons between groups were made with the help of the Mann-Whitney U-test.

Results

Parent TCNU could be detected in plasma up to 8 h after oral administration. Plasma concentration-time curves for the patients given 130 mg/m² are depicted in Fig. 2. The absorption of TCNU was rapid; peak plasma values were obtained after 38 ± 22 min (mean ± SD). At high dose levels a biphasic plasma elimination curve was found, in contrast to lower dose levels, where the detected elimination appeared monophasic. However, the terminal half-life, appeared to be independent of the elimination phase.

Table 1 summarizes the pharmacokinetics of TCNU in our patients. Its terminal half-life was 57 ± 22 min (mean ± SD). The residual area (AUC_{inf}-AUC) was below 5% ex-

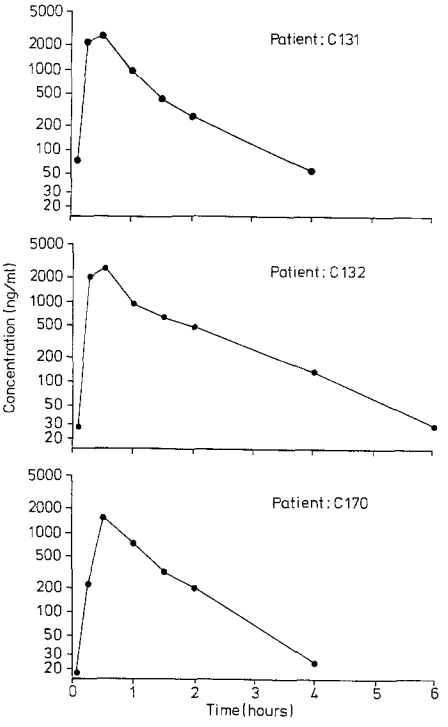

Fig. 2. Plasma concentrations of TCNU after the oral administration of 130 mg/m² to three patients

cept in four patients (patients C3, E14, E31 and E39). The plasma half-life, dose-corrected AUC_{inf} and C_{max} values were calculated; Table 2 shows that previous chemotherapy did not affect TCNU pharmacokinetics. On the other hand, the simultaneous i.v. administration of metoclopramide increased the dose-corrected C_{max} from 16.9 ± 12.7 to 23.0 ± 10.5 (mean ± SD). The difference was statistically significant ($P < 0.05$). The AUC_{inf}/dose value was not changed after concomitant metoclopramide treatment, indicating that metoclopramide increases the rate of TCNU bioavailability. Further details of the effect of metoclopramide on TCNU bioavailability will be published elsewhere (Warrington, personal communication). As can also be seen in Table 2, the half-life and AUC_{inf} values of TCNU tend to be increased in patients with abnormal liver functions.

The variations in pharmacokinetic parameters in the 31 patients are described by the limits for the lower and upper quartile in Table 3; 50% of the patients showed dose-corrected C_{max} and AUC_{inf} values within a two-fold range.

The plasma half-life was not dose-dependent (Fig. 3). The C_{max} values and AUC_{inf} were linearly related to the dose, the correlation coefficients being 0.72 and 0.80, respectively (Fig. 3).

Thrombocytopenia was the dose-limiting toxicity of TCNU. Reductions in platelet counts have been reported elsewhere [9, 10]. We therefore studied the relationship between platelet counts and pharmacokinetic parameters. Irrespective of the platelet level, the percentage of reduction from initial platelet counts was dependent on the dose given, expressed by a linear relation between the reduction and log dose ($r = 0.81$). The reduction in platelet counts appeared to be linearly correlated to log AUC_{inf} and log

Table 1. Pharmacokinetic parameters of TCNU after a single oral dose

Subject	Dose (mg/m^2)	C_{max} (ng/ml)	t_{max} (min)	$t_{1/2}$ (min)	AUC_{obs}[a] (μg × ml/min)	AUC_{inf} (μg × ml/min)
C1[b]	21.1	1150	15	37	26.9	29.7
C3[b]	21.4	104	60	36	7.4	9
C4[b]	30	899	15	51	52.1	54
C6[c]	31.6	832	30	58	79.2	80.3
C7	41.2	382	30	36	32.1	32.7
C8	42.4	305	60	25	20.4	22.2
C9	42.4	1170	30	54	75.8	76.3
C11[d]	50	791	15	33	51.2	51.7
C12[b, d]	50	678	30	67	25	25.9
C14[c, d]	71.1	1870	60	58	141	144
C15[b, d]	69.4	936	60	35	91.6	93.1
C20[b, c, d]	71.4	1760	30	34	114	115
C21[b, d]	70.6	1980	15	77	129	133
C30[b, c, d]	90.6	1780	60	60	217	218
C31[c, d]	90	4110	30	81	301	302
C100[b, d]	88.2	2040	15	49	98.5	101
C131[c, d]	130	2510	30	52	146	150
C132[d]	131.8	2560	30	62	185	188
C170[c, d]	130	1620	30	39	89.1	90.5
C167[c, d]	150	1820	60	60	168	170
C169[d]	150	3880	60	54	269	271
C166[d]	170	7700	30	50	411	413
E14[c]	29.4	300	30	123	24.2	47.2
E20[b, c]	48.3	550	30	79	41.3	47.2
E21[b]	50	415	30	35	39.5	39.8
E23[b, c]	50	651	30	67	48.5	50
E26[b, c]	70.6	1510	15	34	52.8	54.2
E28	69.4	1160	30	46	73.7	74.6
E31	102.9	972	30	87	125	128
E38[c]	100	1030	60	94	130	133
E39[c]	111.1	1130	120	90	211	217

[a] Area computed to the last point > the detection limit.
[b] Prior chemotherapy; [c] Abnormal liver function; [d] i.v. metoclopramide; C, Copenhagen; E, Edinburgh

Table 2. Effect of chemotherapy pretreatment and concomitant i.v. administration of metoclopramide on the pharmacokinetics of TCNU

	C_{max}/dose ($m^2 \times ml^{-1} \times 10^6$)	AUC_{inf}/dose ($min \times m^2 \times ml^{-1} \times 10^3$)	AUC_{inf}/C_{max} (min)	$t_{1/2}$ (min)
All patients ($n = 31$)	19.9 ± 11.9	1.46 ± 0.69	81.3 ± 35.2	56.8 ± 22.3
Chemotherapy pretreatment ($n = 13$)	20.5 ± 12.8	1.32 ± 0.61	69.8 ± 28.1	50.7 ± 17.3
No pretreatment ($n = 18$)	19.4 ± 11.6	1.56 ± 0.73	89.7 ± 38.2	61.2 ± 24.9
Concomitant metoclopramide ($n = 15$)	23.0 ± 10.5*	1.61 ± 0.74	70.8 ± 21.1	54.1 ± 14.7
No metoclopramide ($n = 16$)	16.9 ± 12.7	1.33 ± 0.62	91.2 ± 43.0	59.4 ± 28.0
Normal liver ($n = 17$)	20.8 ± 13.6	1.31 ± 0.59	70.8 ± 24.5	49.0 ± 16.8*
Abnormal liver ($n = 14$)	18.8 ± 9.9	1.64 ± 0.77	94.1 ± 42.5	66.4 ± 25.0

Mean ± SD; * $P < 0.05$

Table 3. Variations in the pharmacokinetic parameters of TCNU

Parameter	Minimal value	Lower quartile	Median	Upper quartile	Maximal value
C_{max}/dose ($m^2 \times ml^{-1} \times 10^6$)	4.9	10.3	16.7	26.3	54.7
AUC_{inf}/dose ($min \times m^2 \times ml^{-1} \times 10^3$)	0.43	1.01	1.34	1.97	3.36

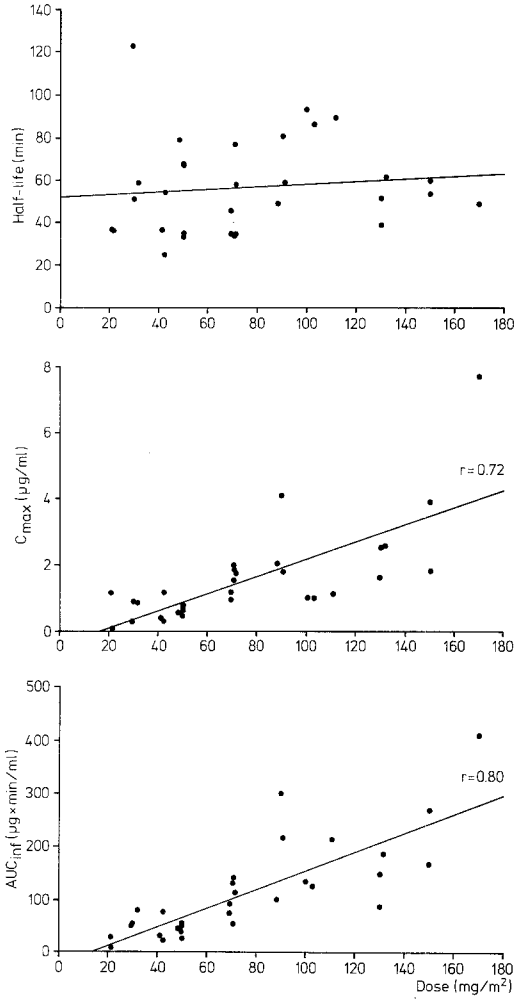

Fig. 3. Relationships between $t_{½}$, C_{max}, AUC and dose after a single dose of TCNU

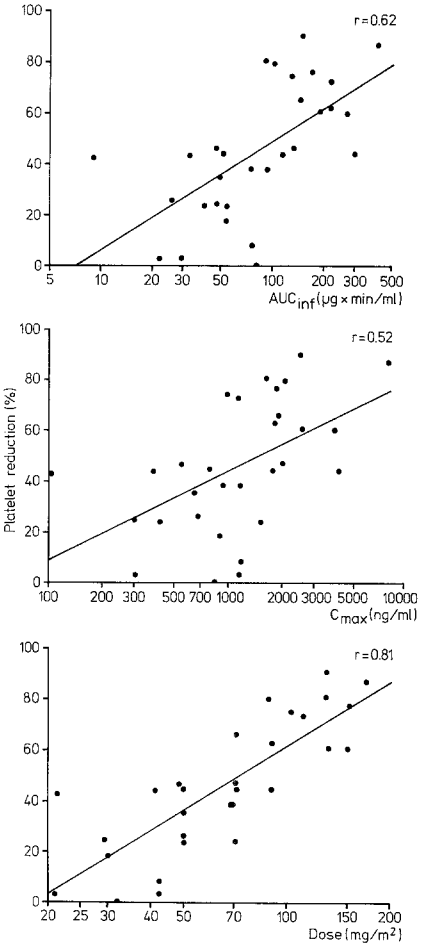

Fig. 4. Relationships between the reduction of platelets and C_{max}, AUC and dose after a single oral dose of TCNU

C_{max} values, but the correlation coefficients were lower than those relating to dose (Fig. 4).

Discussion

By a sensitive, selective analytical method recently developed for TCNU [3], we could detect the parent drug in plasma from all patients in the present study. The compound was detectable in plasma up to 8 h after oral administration; C_{max} values were in th µg/ml range and the plasma half-life was about 60 min.

To our knowledge, there have been only two reports of intact nitrosoureas in human plasma after oral administration. Caddy et al. [1] found MeCCNU in plasma (peak value, 260 ng/ml) from one patient after an oral dose of 100 mg. However, the method used by these authors was not selective for MeCCNU but could determine metabolites with intact nitroso groups. Lemoine and Gouyette [4] demonstrated intact RFCNU (peak value, 13 ng/ml) after 100 mg was given orally to a patient. CCNU, the nitrosourea compound that is most frequently given orally, cannot be detected in plasma, probably due to extensive first-pass metabolism [3].

Therefore, it appears that TCNU has pharmacokinetic properties different from those of other nitrosoureas. The reason for this is at present unknown, but the plasma levels achieved may be explained by either high bioavailability or a low volume of distribution for TCNU. The apparent volume of distribution for nitrosoureas is known to decrease with increasing hydrophilicity [11], and TCNU is a semi-polar compound (log P = 0.6) [5]. In accordance with this finding, its mean volume of distribution has been reported to be in dogs only 1.2 l/kg [2]. One can also speculate that the conjugation of TCNU with a nitrosourea might result in a different pattern of distribution via normal taurine pathways in the body.

The intact chloroethyl nitrosourea compounds are not active against tumours but must be converted to reactive alkylating and carbamoylating species in vivo [11]. Alkylation has been suggested to be most important for anti-tumour activity [3]. Nitrosoureas are degraded non-enzymatically to an identical alkylating species, chloroethyldiazonium ion. Since this compound has a very short half-life, its formation must take place in the target organ. Accordingly, an alteration of the bioavailability or distribution of a nitrosourea may alter its anti-tumour activity.

Several patients participating in the present study were previously treated with other chemotherapeutic agents. Comparisons of $t_{½}$, C_{max} and AUC_{inf} values between chemotherapeutically pretreated and previously untreated pa-

tients revealed similar pharmacokinetic properties for TCNU in both groups. Patients given i.v. metoclopramide together with TCNU tended to absorb the drug faster than those who were not offered antiemetics, which is probably due to accelerated gastric emptying [8].

The wide dose range used in the present pharmacokinetic phase I study provides an opportunity to relate the pharmacokinetic properties of TCNU to the dose level. The plasma half-life for this drug was independent of the dose, and C_{max} and AUC values were linearly related to the dose level; i.e., dose-dependent pharmacokinetics were not observed. Since as many as 31 patients were included in the study, we also tried to relate the pharmacokinetic properties of TCNU to its pharmacological effects, the dose-limiting toxicity. The results of our calculations indicate that the effect of TCNU on the thrombocyte level (percentage of decrease) is linearly correlated with the logarithmic dose level, C_{max} or AUC_{inf}. The highest correlation coefficient was that for the dose, suggesting the possibility of using the pretreatment thrombocyte value for the selection of the dose of TCNU, to reduce the toxicity of the drug. No recommendations can be made at this time, but further evaluation of this aspect is in progress.

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