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Chromosome Aberrations and pharmacokinetics in patients receiving tauromustine as either a single or a repeated dose

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Abstract Tauromustine (TCNU) is an exploratory drug that has demonstrated activity in various solid tumors. We examined chromosome aberrations and plasma levels of the drug in two groups of patients receiving either a single dose of 130 mg/m^2 or 40 mg/m^2 on 3 consecutive days. Peak plasma concentrations (mean $\pm$ SD) were obtained at a similar time after both treatments, i.e., at 38 $\pm$ 25, 32 $\pm$ 24, 28 $\pm$ 14, and 40 $\pm$ 26 min after administration of 130 mg/m^2 on day 1 and after that of 40 mg/m^2 on days 1, 2, and 3, respectively. In addition, the cumulative area under the curve (AUC value) determined after administration of 40 mg/m^2 $\times$ 3 was similar to that noted after treatment with a single dose of 130 mg/m^2, i.e., 180 and 179 μg min ml^{-1}, respectively. Both treatments induced chromosome aberrations (CAs) in peripheral blood lymphocytes. A dose-dependent increase in the number of CAs was found, with 40 mg/m^2 producing 5.5% CAs and 130 mg/m^2 yielding 20.9% CAs at 24 h after treatment. In addition, although the drug concentration declined to a level under the detection limit between the daily treatments, drug-induced chromosome damage was cumulative, with the 90-min values increasing from 4.8% on day 1 to 14.3% CAs on day 3. In individual patients, no correlation was found between CAs and kinetic parameters; however, the total mean CA yield was in agreement with the total drug exposure (CAs, 14.3% and 14.6%, AUC 180 $\pm$ 62.8 and 179 $\pm$ 115 μg min ml^{-1}, respectively).

Key words Chromosome aberrations · Pharmacokinetics · Tauromustine · Lung cancer

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Introduction

Initial evaluation of a new chemotherapeutic agent usually entails a determination of its pharmacokinetic properties. These results, although important, are seldom examined in relation to any biological end point. We tried to remedy this by carrying out an analysis of chromosomal aberrations in the peripheral lymphocytes of patients taking part in a pharmacokinetics study of a new nitrosourea, tauromustine.

Tauromustine (TCNU), a water-soluble nitrosourea based on the endogenous amino acid taurine, has demonstrated broad-spectrum antitumor activity in experimental systems [1–3] and has clinical activity in various solid tumors [4–8]. TCNU, like the classic nitrosoureas, is mutagenic and clastogenic, causing a dose-dependent increase both in the number of mutations occurring in *Salmonella typhimurium* TA100 and in the chromosome aberration rate determined in human lymphocytes treated in vitro [9]. In addition, it was found that on treatment of resting lymphocytes (G_0), TCNU unexpectedly produced chromosome-type aberrations, i.e., dicentrics [9]. Thus, it was also of interest to see if chromosome-type aberrations were produced in vivo.

Although several studies have been carried out in which chromosome aberrations have been followed in patients receiving chemotherapy, in general either combination chemotherapy has been given or the sampling time has been delayed for several days or months [10–14]. To our knowledge, only one study has been reported in which plasma levels and chromosome aberrations have been followed in the same patient. This is the study of Nevstad [15], who followed one patient receiving doxorubicin i.v.; although a slight increase in the number of sister chromatid exchanges (SCEs) was found, no increase in the chromosome aberration frequency was observed and, thus, a comparison with the plasma level was impossible.

The pharmocokinetics of TCNU have previously been studied after administration of a single dose of the drug to patients participating in a phase I study [16]. In the present study, blood samples were taken from two groups of miscellaneous lung-cancer patients receiving TCNU. One group of eight patients received 130 mg/m^2 as a single dose, whereas another group of nine patients received 40 mg/m^2 on 3 consecutive days. The blood samples were used to prepare cells for chromosome analysis and to measure the plasma levels of TCNU. This provided us with the opportunity to investigate the correlation between plasma levels and chromosome aberrations and, also, to examine if a dose-dependent increase in chromosomal aberrations would occur in human lymphocytes in vivo after treatment with a nitrosourea.

Patients and methods

Test compound

TCNU, 1-(2-chloroethyl)-3-[2-dimethyl-amino-sulfonyl]ethyl-1-nitrosourea, (Fig. 1), was synthesized by Pharmacia (Helsingborg, Sweden). The drug was given orally as tablets of 10. 25, and 50 mg.

Patients

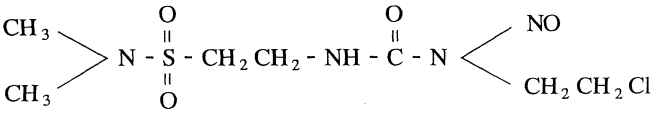
Patients with lung cancer, participating in clinical studies carried out at the Finsen Institute in Copenhagen, made up the present study. Among the inclusion criteria, pretreatment platelet values of 100×10^9/l and a WBC of 3.0×10^9/l were required, as were normal liver- and renal-function tests, exception being made for abnormal values directly related to the underlying malignant disease. The patients had undergone no prior radiotherapy or chemotherapy. All the patients were treated i.v. with 40–80 mg metoclopramide at about 15 min before the ingestion of TCNU.

Study design

The study comprised two parts, the pure pharmacokinetics study and the correlative study between chromosome aberrations and pharmacokinetics. Pharmacokinetics were studied in eight patients after administration of a single dose of 130 mg/m^2 and in nine patients after repeated oral administration on 3 consective days of 40 mg/m^2 TCNU. The correlative study was carried out on six patients receiving 40 mg/m^2 on 3 consecutive days and on eight patients receiving 130 mg/m^2 as a single oral dose. The study was approved by the local ethics committee and appropriate government authorities and was performed according to the Helsinki declaration. All patients gave their informed consent.

Collection of samples

Blood samples were collected in heparinized tubes through an infusion cannula prior to and at 5, 10, 20, 30, 45, 60, and 90 min as well as at 2, 4, 6, 8, 10, and 24 h after administration of 130 mg/m^2 to eight patients and at 15, 30, 45, 60, and 90 min after treatment with 40 mg/m^2 in nine patients. The samples were cooled on ice and the plasma was separated by centrifugation at 1300 g for 6 min and frozen at -20°C for later analysis.



Tauromustine

[1 - (2 - chloroethyl) - 3/2 - (dimethylaminosulfonyl)]- ethyl/- 1 nitrosourea

Fig. 1 Chemical structure of tauromustine (TCNU)

Analytical methods

The samples were analyzed in duplicate according to Polacek et al. [17] for content of TCNU by means of high-performance liquid chromatography (HPLC) with UV detection at 229 nm. The limit of detection was about 6 ng/ml plasma at a signal-to-noise ratio of 3:1.

Calculations

The mean plasma concentrations were used for pharmacokinetic evaluation. The elimination rate constant, 1, was estimated by linear regression analysis of the logarithmic plasma concentration-time curve utilizing the points of the terminal phase. The elimination half-life, $t_{1/2}$, was determined from the relationship $t_{1/2} = \ln 2/\mathrm{l}$. The area under the plasma concentration-time curve, AUC_{0-t}, was calculated using the linear trapezoidal rule. Determinations of half-lives were based upon at least three time points, and AUC values were based upon at least six time points.

Chromosome analysis

Blood samples were taken before treatment and at 1.5 or 2 h and 24 h after treatment from eight patients receiving a single oral dose of 130 mg/m^2 TCNU. In six patients receivng 40 mg/m^2 on 3 consecutive days, blood samples were taken before treatment and at 90 min after treatment on each of the 3 days. From these patients, plasma samples were taken before treatment and at 5, 10, 20, 45, 60, 90, 120, 240, and 360 min for analysis of TCNU in accordance with the details described above.

Lymphocyte cultures and cytogenetics

Venous blood from a healthy donor was cultivated in McCoy's 5A medium supplemented with 25% fetal calf serum, 1% glutamine, and 1.6% phytohemagglutinin. After the culture period the cells were spun down and first exposed to 0.075 mol KCl/l for 10 min and then fixed three times in 3:1 (v/v) methanol/acetic acid, air-dried, and stained with 2% Giemsa. Colcemid (10 μg/ml) was added at 1 h before harvest [18]. Duplicate cultures for fixation at 48, 72, and 96 h were set up for all samples. In the single-dose study, cultures were fixed at 48 and 72 h; however, since no mitotic cell was found at 48 h in the majority of patients, the data given are derived from 72-h cultures for all patients except patient 8, where sufficient metaphases were present in the 48-h cultures. In the split-dose study, no mitotic cell was found at 48 h in any patient; therefore, all data are derived from 72-h cultures except for patient 3 on day 3, where 96-h cultures were required to obtain sufficient metaphases

for analysis. Chromosome analysis was carried out on 100 metaphases (50/culture) at each time piont in the individual patients. Chromosome aberrations were classified according to Buckton and Evans [19]. The quantity of abnormal cells includes cells with only gaps, whereas these are omitted in the total aberration yield. The mitotic index is based on 4000 cells per individual and time point.

Results

Administration of a high single dose 130 mg/m^2 of TCNU

The mean plasma concentration-time curve generated for TCNU in eight patients given a single oral dose of 130 mg/m^2 is depicted in Fig. 2. The peak plasma concentrations, C_{max}, were obtained after 38 $\pm$ 25 (mean $\pm$ SD) min, with the mean plasma level being 2500 $\pm$ 1280 μg/ml (Table 1). The plasma concentration declined rapidly in a monophasic manner with a terminal half-life of 53 $\pm$ 17 min. Individual C_{max} and AUC values are presented in Table 3. On repeated administration of 130 mg/m^2 at least 5 weeks later (data not shown), the pharmacokinetic parameters did not significantly change.

Administration of a low oral dose, 40 mg/m^2 on 3 consecutive days

Nine patients received 40 mg/m^2 TCNU on 3 consecutive days. The mean values for the pharmacokinetic parameters of these patients are found in Tabel 1. The mean C_{max} value was obtained after 32 $\pm$ 24, 28 $\pm$ 14, and 40 $\pm$ 26 min on days 1, 2, and 3, respectively. It can be seen from Table 1 that the pharmacokinetic parameters did not change after the second and third doses. Half-lives could not be detemined as the sampling intervals were too short.

A comparison of the AUC values recorded after single versus repeated administration indicates that the pharmacokinetics of TCNU are linear in cancer patients. The AUC value resulting from a single dose of 130 mg/m^2 (180 μg min ml^{-1}) was similar to the cumulative AUC value recorded during 3 days of treatment with the lower dose, 40 mg/m^2, which was 179 μg min ml^{-1}.

Chromosome aberrations

Single dose

Treatment with 130 mg/m^2 TCNU did not affect the mitotic index in six of eight patients, whereas in two cases (patients 9, 14) it showed a 2-fold decrease as shown in Fig. 3. The total aberration yield before the start of treatment was found to lie within the range found for our normal individuals [20]. However, at 2 h after treatment the total aberration yield demonstrated a 6.5-fold increase and the number of gaps, a 3-fold increase (see Table 2). Both of these abnormalities exhibited a further increase at the 24-h sampling time, the total aberration yield having increased 9-fold and gaps, 4-fold from the pretreatment values. The

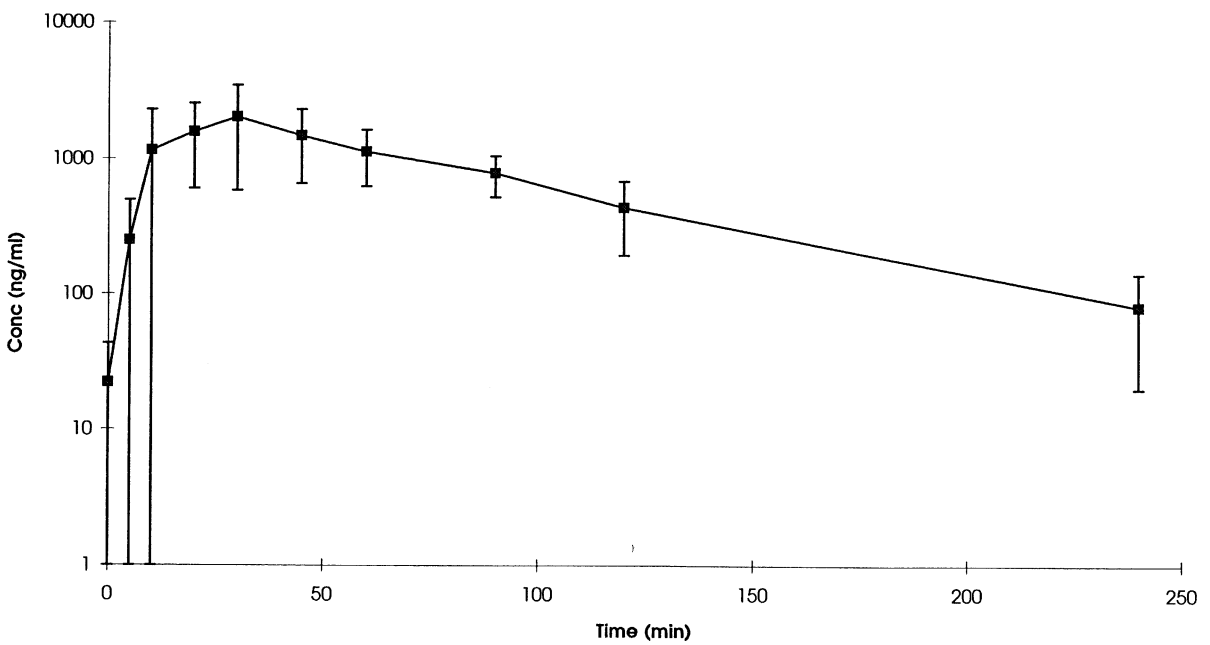


Fig. 2 Mean plasma concentration of TCNU determined after administration of a single oral dose of 130 mg/m^2 to 8 cancer patients (mean $\pm$ SD)

Table 1 Pharmacokinetic parameters (mean ± SD) of TCNU determined after administration of a single dose of 130 mg/m^2 ($n = 8$) and three consecutive daily doses of 40 mg/m^2 ($n = 9$) TCNU to lung-cancer patients

Dose (mg/m^2)	C_{max} (ng/ml)	T_{max} (min)	$AUC_{0-90\,min}$ (μg min ml^{-1})	$AUC_{0-360\,min}$ (μg min ml^{-1})	$t_{1/2}$ (min)
130	2500 ± 1280	38 ± 25	112 ± 46.5	180 ± 62.8	53 ± 17
40 Day 1	1160 ± 840	32 ± 24	45.6 ± 31.2	58.1 ± 40.5[a]	–
40 Day 2	894 ± 520	28 ± 14	42.0 ± 23.7	60.1 ± 40.7[a]	
40 Day 3	987 ± 667	40 ± 26	41.1 ± 24.2	61.0 ± 34.5[a]	
40 Sum days 1–3	–	–	–	179 ± 115[a]	

[a] $n = 4$

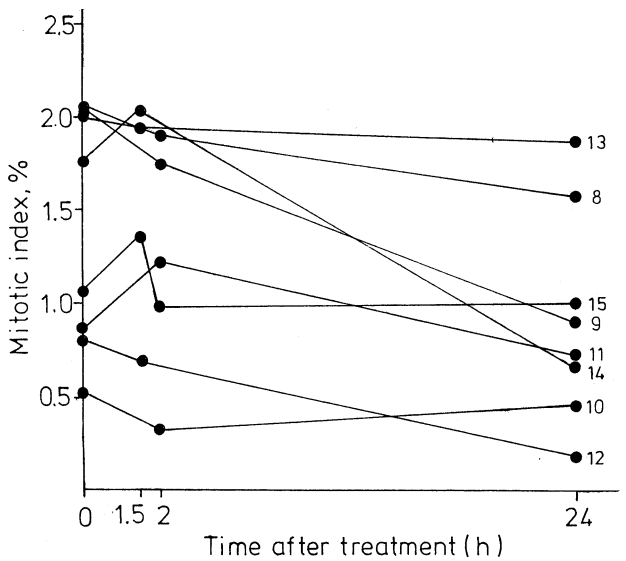


Fig. 3 Mitotic index determined in 8 patients before and after administration of a single dose of 130 mg/m^2 TCNU

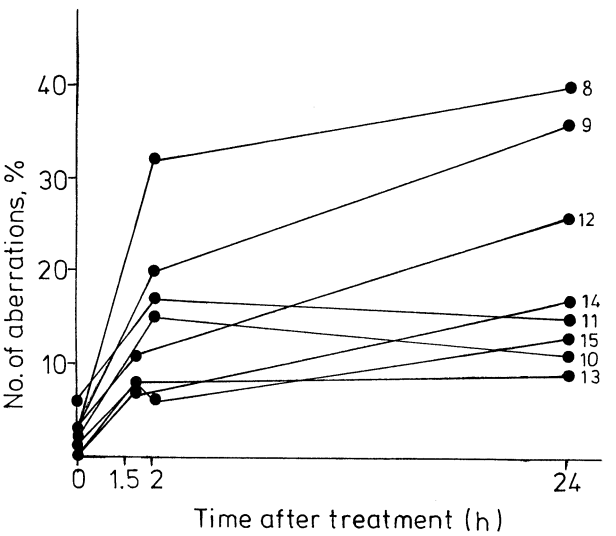


Fig. 4 Chromosomal aberrations found in 8 patients before and after administration of a single dose of 130 mg/m^2 TCNU

additional increase from the 2-h time point to the 24-h sampling time was not found for all patients; as can be seen from Fig. 4, some patients remained at the same level. All aberration types except chromosome-type exchanges (in this case, dicentrics) increased after treatment with TCNU (Table 2). Two individuals (patients 12, 13) were also sampled at 4 weeks after treatment. In both patients the spectrum of aberrations and the total aberration yield remained unchanged, i.e., at a level similar to that found for the 24-h sampling time: for

Table 2 Spectrum of aberrations found in 8 patients receiving 130 mg/m^2 TCNU as a single oral dose[a] *B′* Chromatid break, *B″* isochromatid break, *E′* chromatid exchange, *E″* dicentric chromosome exchange

Stage of therapy	Number of metaphases analyzed	Number of abnormal cells	Gaps	Breaks B′	Breaks B″	Exchanges E′	Exchanges E″	Minutes	Total number of aberrations without gaps	Aberrations (%)
Before treatment	800	39	20	11	3	1	2	1	18	2.3
2 h after treatment	800	140	59	50	45	11	1	10	117	14.6
24 h after treatment	800	190	81	73	62	27	3	2	167	20.9

[a] Numbers of aberrations represent the sum for all patients

Table 3 Pharmacokinetic parameters and chromosome-aberration yield determined after a single oral dose of 130 μg/m² TCNU

Patient number	C_{max} (ng/ml)	AUC_{0-24h} (μg min ml^{-1})	Maximal number of aberrations	Maximal number of aberrations + gaps
8	2130	155	40	61
9	3200	288	36	45
10	1840	167	15	22
11	2660	95.6	17	36
12	2630	244	26	30
13	5120	240	9	15
14	1460	124	17	24
15	960	165	13	20

patient 12, 9 and 13 aberrations at 24 h and 4 weeks later, respectively; for patient 13, 26 and 21 aberrations at 24 h and 4 weeks later, respectively. No correlation was found between the peak plasma levels or AUC values recorded after administration of a single dose of 130 mg/m² TCNU and the maximal chromosome aberration yield in individual patients (Pearson's correlation -0.70, $P > 0.05$; Table 3).

Repeat dosing

It can be seen from Fig. 5 that the mitotic index (MI) in individual patients either remained unchanged throughout the treatment period (patients 3, 5–7) or fluctuated as shown for patients 2 and 4, with no consistent pattern being visible. In contrast, the aberration yield increased day by day in all patients (Fig. 6). This is even more evident in Table 4, which shows that this day-by-day increase applies not only to the total aberration yield but also to all individual aberration types except minutes. The repeat-dosing schedule caused an increase in chromosome-type aberrations as demonstrated by an increase in the number of dicentrics and isochromatid breaks, all of which in this study were of the nonunion type and, therefore, possible chromosome-type aberrations. The total number of gaps also demonstrated a significant increase from day 1 to day 3. The split-dose data also confirm the findings from the single-dose study that the aberration yield is higher at 24 h after dosing than at 90 min after dosing; i.e., for day 1 at 90 min and day 2 at time zero (24 h later) the yield was 29 and 33 aberrations, respectively, and for day 2 at 90 min and day 3 at time zero it was 46 and 74 aberrations, respectively. It is also clear that repeat dosing causes damage to cells that have not been damaged by a previous dose, as the number of abnormal cells increased from 47 on the 1st day to 102 on day 3, demonstrating a dose-response relationship. The dose-response relationship is also evident when one considers the total aberration yield, i.e., at 24 h after administration of 40 mg/m² the aberration yield was 33

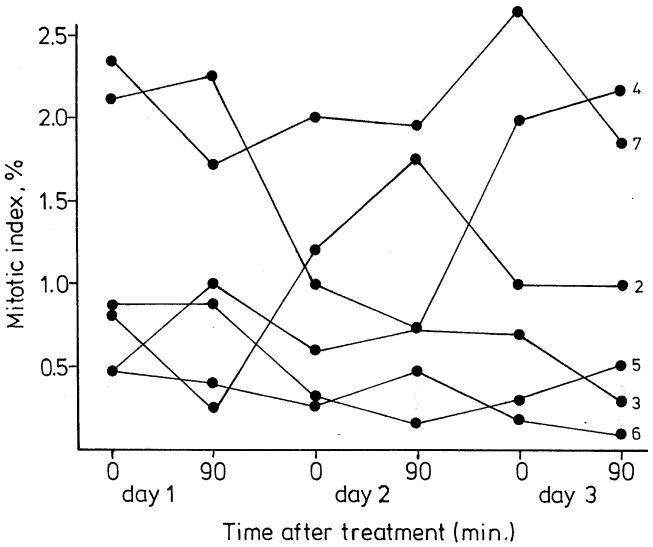


Fig. 5 Mitotic index determined in 6 patients before and after administration of 40 mg/m² TCNU per day for 3 consecutive days

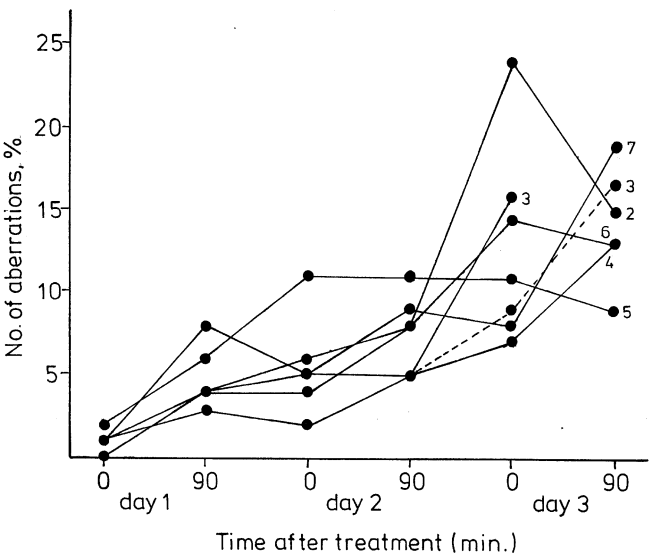


Fig. 6 Chromosomal aberrations found in 6 patients before and after administration of 40 mg/m² TCNU per day for 3 consecutive days

and after treatment with 80 mg/m² (2×40 mg/m²) 74 aberrations were found. The dose-dependent effect is also seen from the data demonstrating that after treatment with the three daily doses amounting to 120 mg/m² the aberration yield at 1.5 h was 14.3%, which is almost identical to the 14.6% value found at 2 h after administration of 130 mg/m² as a single dose.

The pharmacokinetic data recorded for the individual patients, in similarity with those noted for the larger patient group, did not demonstrate any accumulative increase for either the C_{max} or the AUC value during the 3-day treatment period (Table 5), but the

Table 4 Spectrum of aberrations found in patients receiving 40 mg/m^2 TCNU on 3 consecutive days[a] *B′* Chromatid break, *B″* isochromatid break, *E′* chromatid exchange, *E″* dicentric chromosome exchange

Stage of therapy	Number of metaphases analyzed	Number of abnormal cells	Gaps	Breaks B′	Breaks B″	Exchanges E′	Exchanges E″	Minutes	Total number of aberrations without gaps	Aberrations (%)
Day 1										
Before therapy	600	31	21	4	2	0	0	0	6	1.0
90 min after therapy	600	47	20	13	13	2	1	0	29	4.8
Day 2										
Before therapy	600	57	24	17	11	1	1	3	33	5.5
90 min after therapy	600	77	45	23	15	0	2	6	46	7.7
Day 3										
Before therapy	600	102	51	34	25	10	3	2	74	12.3
90 min after therapy	600	101	33	30	35	9	6	6	86	14.3

[a]Numbers of aberrations represent the sum for all patients

Table 5 Pharmacokinetics and total number of chromosome aberrations determined in patients receiving 40 mg/m^2 TCNU on 3 consecutive days (*CA* Chromosome aberrations)

Patient number	Day 1 C_{max} (ng/ml)	Day 1 $AUC_{0-360\,min}$ (μg min ml^{-1})	Day 1 CA	Day 2 C_{max} (ng/ml)	Day 2 $AUC_{0-360\,min}$ (μg min ml^{-1})	Day 2 CA	Day 3 C_{max} (ng/ml)	Day 3 $AUC_{0-360\,min}$ (μg min ml^{-1})	Day 3 CA
2	504	24.5[a]	4	531	36.5[a]	8	336	40.8[a]	15
3	1090	31.6[a]	3	533	42.8[a]	5	534	48.1[a]	17
4	220	20.4	4	92	17.1	5	172	29.3	13
5	620	70.2	6	714	47.2	11	398	60.3	9
6	1630	110	4	1540	110	8	1290	109	13
7	674	31.8	8	596	39.2	9	913	45.4	19

[a]AUC extrapolated to 360 min

sum of the AUC values recorded after 3 days was similar to that noted after treatment with the single dose, i.e., 179 ± 115 and 180 ± 62.8, respectively Table 1). No correlation was found between plasma levels and aberration yields in individual patients receiving 40 mg/m^2 on 3 consecutive days. (Pearson's correlation: day 1 0.49, day 2 − 0.1, day 3 0.32; $P > 0.05$).

Discussion

The search for a biological end point with which we could correlate plasma drug levels led us to the choice of chromosomal aberrations. Chromosomal damage such as breaks, gaps, and sister chromatid exchanges are recognized as a sensitive biological end point for exposure to mutagenic and carcinogenic compounds, but only a few studies have been reported involving patients undergoing cytostatic therapy with nitrosoureas [10, 11, 21] and even fewer have involved treatment with only one cytostatic [13, 22]. Thus, this study is the first systematic investigation of the appearance of chromosome aberrations in the peripheral lymphocytes of patients during ongoing monotherapy with a nitrosourea. We found that chromosomal aberrations induced by TCNU in vivo are dose-dependent, and although the plasma concentration of the drug declines to a level under the detection limit after each treatment, drug-induced chromosome damage is cumulative. However, no correlation between the plasma level of drug and chromosomal damage could be ascertained in the individual patients.

An initial finding in these studies was that in the 48-h cultures from these patients, no divisions were present. This is not uncommon, as other authors have also failed to find mitoses in 48-h cultures of lymphocytes taken from cancer patients [13] or have shown that

even 72-h cultures have a low level of division as compared with controls [10]. Therefore, the majority of our data are derived from 72-h cultures or, in the case of the split-dose treatment on day 3, even from 96-h cultures. However, as on all occasions we checked the 48-h and the 72-h cultures and found no divisions, we are confident that the metaphases analyzed are first divisions. Thus, the generally accepted criterion that cells should be in their first division when analyzed has been fulfilled.

We found that at as early as 90 min after treatment, chromosome aberrations were manifested. The likelihood of these being due to drug remaining in the plasma and causing these effects during the incubation period is negligible, as at 90 min after treatment the mean plasma level was 838 ± 317 ng/ml, which is below the 3.25 mg/ml concentration of TCNU that we have previously shown is necessary to cause an increase in the chromosome aberration yield even after 24 h exposure in vitro [9].

Nitrosoureas have previously been shown to be clastogenic in vivo [10, 11, 21], but these studies were confounded by the observation that all but six patients in Vyas et al.'s, study had received various combinations of drugs. In addition, the earliest sampling time was after the first 5 days of therapy, which precludes a direct comparison of that investigation with the present study. However, an increase in the aberration rate over that recorded before therapy was found [21], and in Gebhart et al.'s study [10] a further increase after each round of therapy was also observed. Thus, the findings obtained in the present study confirm and extend this observation.

Apart from the present data, no information has been reported on chromosome aberrations using different doses of the same drug in patients. However, it is well known that X-rays cause a dose-dependent induction of chromosome aberrations both in vitro and in vivo and, therefore, as we have previously shown that TCNU causes a dose-dependent increase in human lymphocytes in vitro, the present results are not unexpected. The higher incidence noted for chromatid aberrations in relation to chromosome type is in agreement with that found by Gebhart et al. [10] using carmustine (BCNU), and the presence of chromosome-type aberrations supports our in vitro data indicating an induction of chromosome-type aberrations in G_0 lymphocytes.

The lack of correlation between individual chromosome aberration yields and plasma drug levels probably reflects the inherent heterogenicity found between individuals with regard not only to drug absorption but also to the induction of chromosomal aberrations. However, consideration of the group means reveals a similarity between the sum of the AUC levels and the level of chromosome aberrations. This is evident from a comparison of the sum of the respective AUC value (mean $\pm$ SD) found after administration of a single dose and after the 3-day dosing, 180 ± 62.8 and 179 ± 115 μg min ml^{-1} and the mean aberration yield, 14.3% and 14.6%, respectively. This demonstrates that the aberration level in peripheral blood lymphocytes is an expression of the total drug level to which the patient is exposed.

In conclusion, the present study demonstrates that a dose-dependent increase in chromosomal aberrations is found in the peripheral lymphocytes of patients receiving repeated doses of oral TCNU and that cumulative chromosomal damage occurs despite a return of plasma drug concentrations to levels below the detection limit between the daily doses. However, the mean level of aberrations appears to represent the total drug exposure, irrespective of the dose schedule.

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References

1. Hartley-Asp B, Christensson PI, Gunnarsson K, Gunnarsson PO, Jensen G, Polacek J, Stamvik A (1988) Anti-tumour, toxicological and pharmacokinetic properties of a novel taurine-based nitrosourea (TCNU). Invest New Drugs 6:19–30
2. Fergusson RJ, Anderson LE, Macpherson JS, Robins P, Smyth JF (1988) Activity of a new nitrosourea (TCNU) in human lung cancer xenografts. Br J Cancer 57:339–342
3. Phillips RM, Bibby MC, Double JA (1988) In vitro and in vivo responses of a panel of murine colon tumours to TCNU: a positive correlation. Eur J Cancer Clin Oncol 24:1365–1371
4. Vibe-Petersen J, Bork E, Møller H, Hansen HH (1987) A phase I clinical evaluation of 1-(2-chloroethyl)-3-[2-(dimethylaminosulphonyl)ethyl]-1-nitrosourea (TCNU). Eur J Cancer Clin Oncol 23:1837–1843
5. Smyth JF, MacPherson JS, Warrington PS, Kerr ME, Whelan JM, Cornbleet MA, Leonard RCF (1987) Phase I study of TCNU, a novel nitrosourea. Eur J Cancer Clin Oncol 23:1845–1849
6. Vibe-Petersen J, Bach F, Pedersen AG, Smyth J, Hansen HH (1989) A phase II trial of TCNU in patients with squamous, adeno and large cell carcinoma of the lung. Eur J Cancer Clin Oncol 25:1881–1885
7. Smyth JF, Gundersen S, Renard J, Pinedo HM (1989) Randomized phase II trial of TCNU versus mitozolomide in malignant melanoma. Eur J Cancer Oncol 25:755–757
8. Taal BG, Ten Bokkel Huinink WW, Rodenhuis S (1993) Tauromustine (TCNU) combined with 5-fluorouracil and leucovorin in the treatment of advanced colon cancer. Eur J Cancer, 29A:1916–1917
9. Hartley-Asp B (1992) Genotoxicity of tauromustine, a new water soluble taurine-based nitrosourea. I. Mutagenic and clastogenic activity of tauromustine in vitro. Mutagenesis 7:427–431
10. Gebhart E, Lösing J, Wopner F (1980) Chromosome studies on lymphocytes of patients under cytostatic therapy. Hum Genet 55:53–63
11. Harrod EK, Cortner JA (1968) Prolonged survival of lymphocytes with chromosomal defects in children treated with 1,3-bis(2-chloroethyl)-1-nitrosourea. J Natl Cancer Inst 40:269–282
12. Musilova J, Michalová K, Urban J (1979) Sister-chromatid exchanges in chromosomal breakage in patients treated with cytostatics. Mutat Res 67:289–294

13. Reeves BR, Casey G, Harris H, Dinning WJ (1985) Long-term cytogenetic follow-up study of patients with uveitis treated with chlorambucil. Carcinogenesis 6:1615–1619
14. White AD, Jones BM, Clark RE, Jacobs A (1992) Chromosome aberrations following cytotoxic therapy in patients in complete remission from lymphoma. Carcinogenesis 13:1095–1099
15. Nevstad NP (1978) Sister chromatid exchanges and chromosomal aberrations induced in human lymphocytes by the cytostatic drug adriamycin in vivo and in vitro. Mutat Res 57:253–258
16. Gunnarsson PO, Vibe-Petersen J, Macpherson JS, Warrington PS, Polacek J, Ellman M, Hansen HH Smyth JF (1989) Pharmacokinetics of tauromustine in cancer patients. Cancer Chemother Pharmacol 23:176–180
17. Polacek J, Gunnarsson PO, Brandin S (1988) Determination of tauromustine, a nitrosourea-based antitumour agent, in plasma by high-performance liquid chromatography. J Chromatogr 425:424–428
18. Hartley-Asp B, Hyldig-Nielsen F (1984) Comparative genotoxicity of nitrogen mustard and nor-nitrogen mustard. Carcinogenesis 5:1637–1640
19. Buckton KE, Evans JH (1973) Methods for the analysis of human chromosome aberrations. WHO, Geneva
20. Hartley-Asp B (1984) Variations in mitotic index and chromosomal aberration rates in women. In: Pero R, De Serres F (eds) Individual susceptibility to genotoxic agents in the human population. Plenum, New York, pp 235–242
21. Vyas RC, Adhvaryu SG, Shah VC (1988) Effects of CCNU therapy on human chromosomes. Mutat Res 206:163–166
22. Lambert B, Holmberg K, Einhorn N (1984) Persistence of chromosome rearrangements in peripheral lymphocytes from patients treated with melphalan for ovarian carcinoma. Hum Genet 67:94–98