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In vitro evaluation of a new nitrosourea, TCNU, against human small cell lung cancer cell lines*

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Summary. The cytotoxic activity of a new nitrosourea, TCNU, was compared with that of BCNU in five human small cell lung cancer cell lines in vitro. TCNU was found to be equivalent or inferior to BCNU when compared on a microgram to microgram basis. If the potential of in vitro phase II trials for selection of new drugs can be validated, it can be concluded that TCNU is not superior to other nitrosoureas for the treatment of SCCL.

Introduction

Since the introduction of the nitrosourea compounds into clinical oncology in the late 1960s, both BCNU (1.3- bis (2-chloroethyl)-1-nitrosourea) and CCNU (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) have proved to be active cytostatic agents useful in a variety of malignancies. Both are characterized by delayed hematologic toxicity, making it difficult to use these agents in combination chemotherapy. Consequently, a great deal of effort has been invested in the development of new nitrosoureas with a different pattern of toxicity and antineoplastic activity.

The aminosulfonicacide taurine, a degradation product of cysteine, has become the basis of one such new nitrosourea, TCNU [1-(2-chloroethyl)-3/2-(dimethylaminosulfonyl] ethyl/-1-nitrosourea). A higher therapeutic index has been found in Walker 256 carcinosarcoma in rats for this more hydrophilic compound than for BCNU and CCNU [10].

Validation of in vitro methods for assessing the activity of new drugs against a given type of tumor might conceivably diminish the need for drug testing in patients [9]. Since the potential of a panel of human small cell lung cancer cell lines (SCCL) for comparison of drug analogues has been described elsewhere [7, 8], this panel was used to compare the activity of TCNU and BCNU against SCCL.

Materials and methods

The cell lines used and their source, maintenance, and monitoring have been described elsewhere [8]. The cell lines used were NCI-H69, NCI-N592, OC-TOL, OC-NYH, and OC-ROL, all of which were maintained in Roswell Park Memorial Institute medium 1640 with 10% fetal calf serum and in a 7.5% CO_2 humidified atmosphere. The cell lines were free of mycoplasm contamination, and flow-cytometric DNA analysis showed that they had stable DNA content.

Drugs. BCNU (Bristol Laboratories) was dissolved in ethanol and subsequently diluted with tissue culture medium. The cells were exposed to a maximum of 0.03% ethanol in the experiments, a concentration yielding no cell kill.

TCNU (Aktiebolaget Leo, Sweden) was dissolved in dimethylsulfoxide (DMSO) and subsequently diluted with tissue culture medium. Since the maximum DMSO concentration (0.1%) had a slight cytotoxic effect, 0.1% DMSO in tissue culture medium was used as the control solution for the TCNU experiments. All solutions were freshly made just before use.

Clonogenic assay. Cell survival was assessed by colony formation in soft agar as described previously [8]. A single-cell suspension was exposed to one of the drugs for 1 h, washed twice and plated in soft agar on top of a bottom layer. After solidification of the agar 1 ml medium was added to prevent drying. Each concentration was set up in triplicate. The plates were incubated at 37 °C in a 7.5% CO_2 humidified atmosphere. After 3 weeks, the colonies were counted using a dissecting microscope, and the surviving fractions were calculated by dividing the number of colonies on the treated plates by the number of colonies on the control plates. As the number of plated cells and the number of colonies were not in proportion, the surviving fractions should ideally be corrected with simultaneously performed dilution experiments [8]. However, since the present investigation compared analogues in simultaneously performed experiments, the correction would affect the dose-response curves for both analogues in an identical manner and has therefore not been done [8].

Since previous experiments showed that the sensitivity patterns are dependent on the bottom layer used [8], the present experiments were performed with two different bottom layers, one containing both sheep red blood cells (SRBC) and mercaptoethanol and one without.

Determination of drug-induced cell-cycle perturbations by flow-cytometric DNA analysis. The cell lines were exposed

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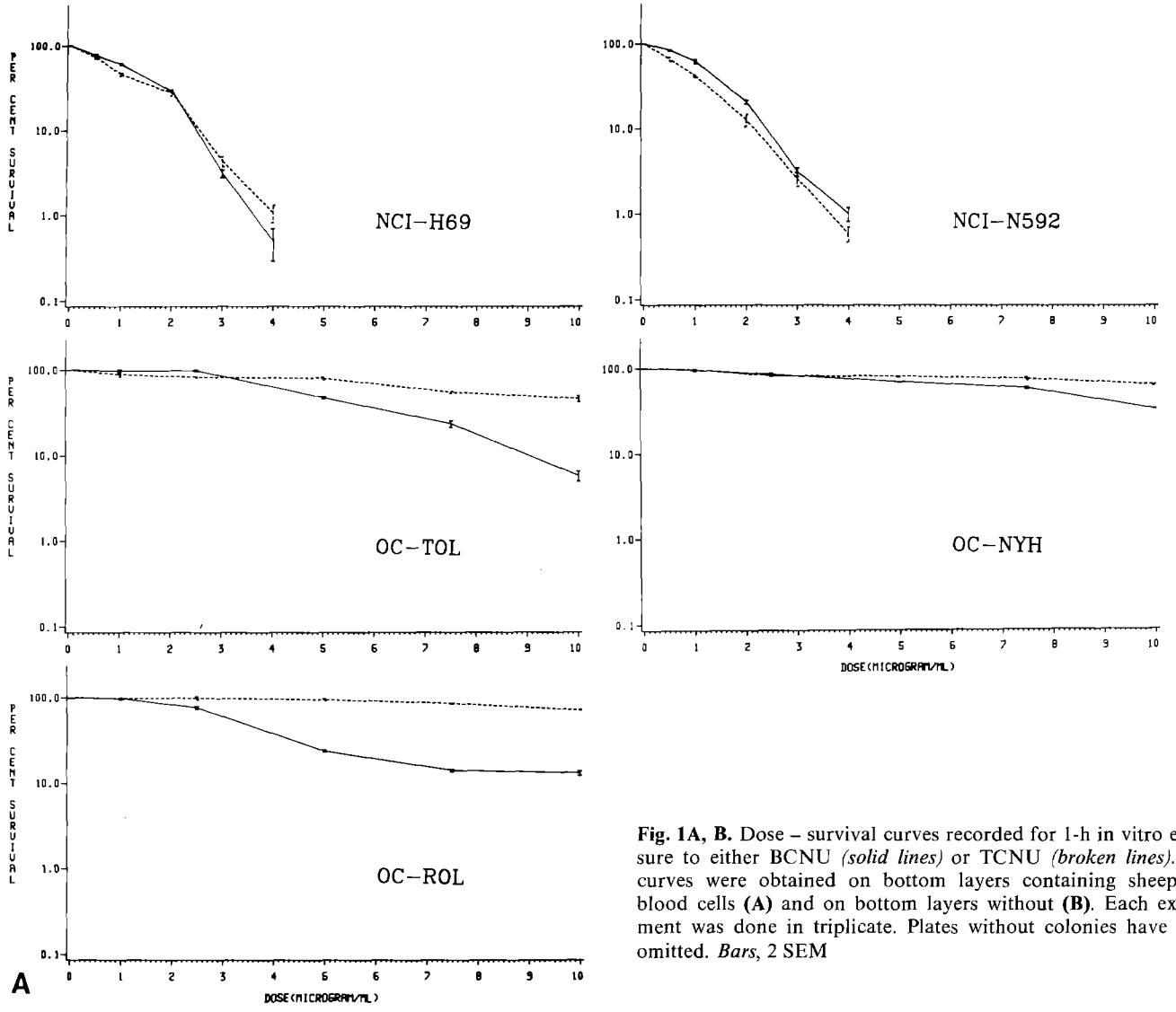
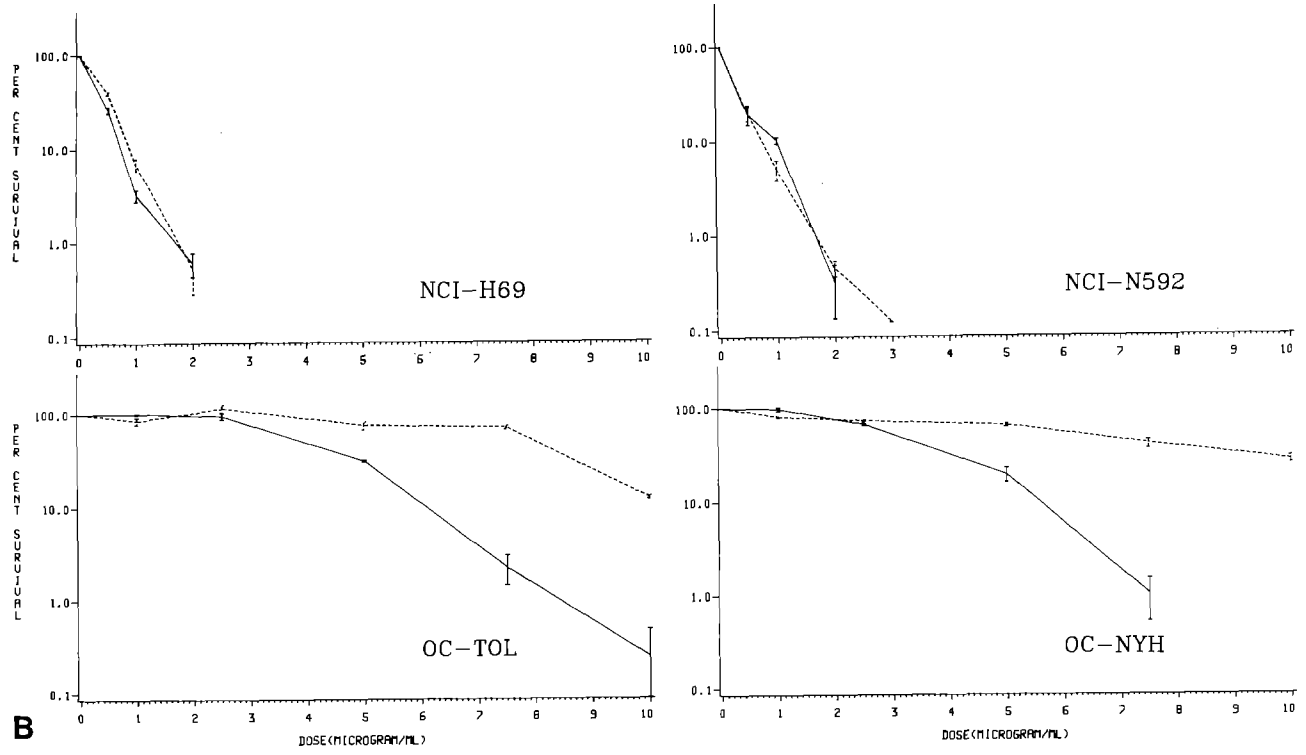


Fig. 1A, B. Dose – survival curves recorded for 1-h in vitro exposure to either BCNU *(solid lines)* or TCNU *(broken lines)*. The curves were obtained on bottom layers containing sheep red blood cells **(A)** and on bottom layers without **(B)**. Each experiment was done in triplicate. Plates without colonies have been omitted. *Bars*, 2 SEM



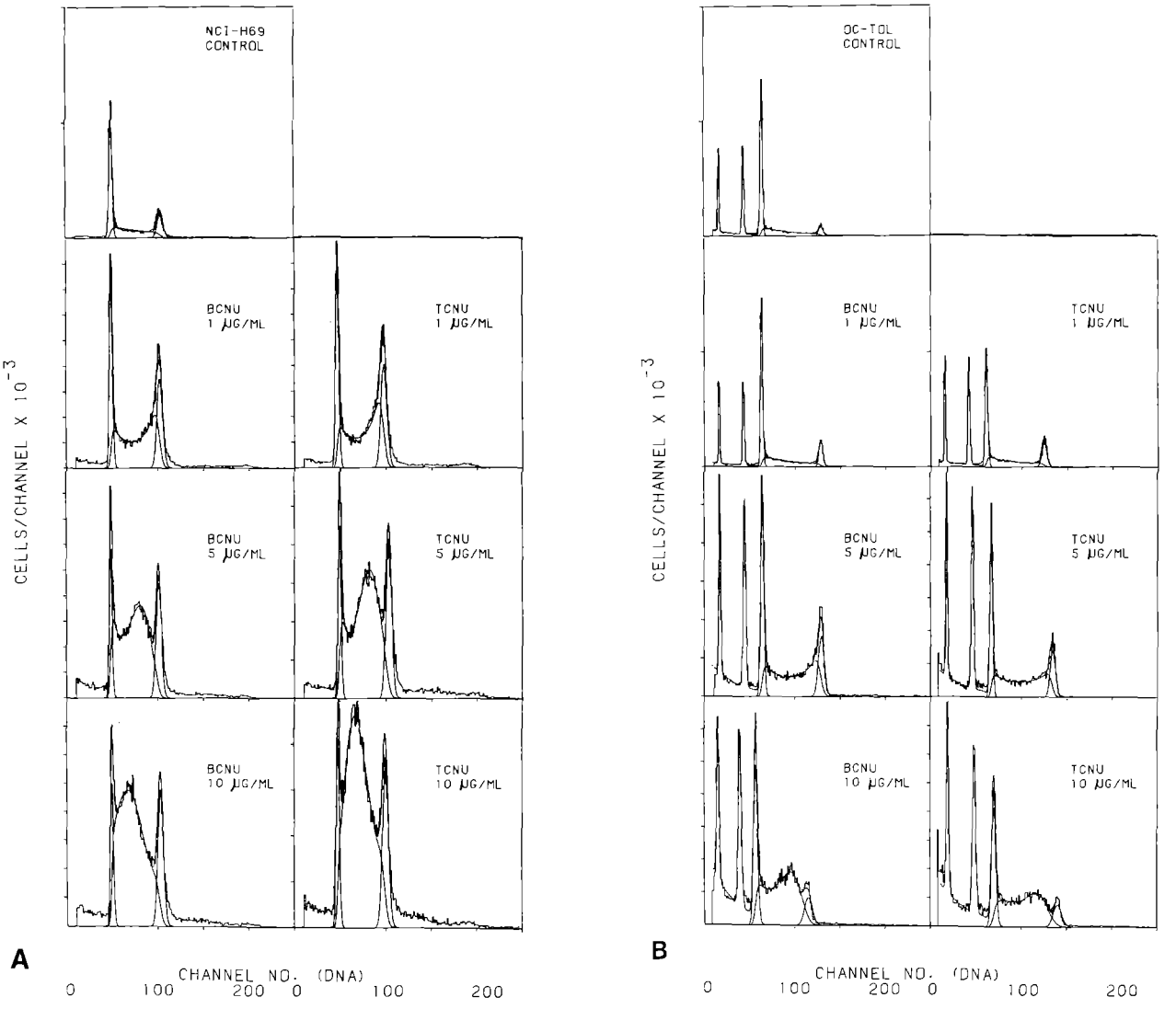


Fig. 2A, B. Flow cytometric DNA histograms obtained after exposure of NCI-H69 **(A)** and OC-TOL **(B)** to the indicated BCNU or TCNU concentrations for 24 h. The first two peaks in **B** represent internal references (chicken and trout erythrocytes) used to calculate the DNA index. The internal reference has been omitted in **A** since the trout peak merge into the G_1 peak

to the drugs for 24 h in tissue culture flasks. After centrifugation the cells were resuspended in citrate buffer, frozen on ethanol with dry ice, and stored at −80 °C until analysis [12]. The samples were prepared and stained with propidium iodide as described elsewhere [13] before analysis in a FACS III (Becton Dickinson, Sunnyvale, Calif). The percentage of cells in the cell-cycle phases were determined by statistical analysis of the DNA distribution [2].

Results

The in vitro sensitivity of each of the five SCCL cell lines tested simultaneously with BCNU and TCNU is shown in Fig. 1. The sensitivity patterns were obtained on bottom layers with and without SRBC in most cases (Fig. 1). OC-ROL was only tested on SRBC, since it does not form colonies when grown without [8]. The cells were seen to be more resistant to the drugs on bottom layers with SRBC than on bottom layers without. Despite these differences, the relative sensitivity was identical in the two growth conditions.

It appears that three cell lines were highly resistant to BCNU (OC-TOL, OC-ROL, and OC-NYH). For the three most resistant cell lines, the sensitivity to TCNU was lower than the sensitivity to BCNU on a microgram to microgram basis.

The two drugs produced almost identical dose – response curves in the two more sensitive cell lines (NCI-N592 and NCI-H69).

Figure 2 shows the DNA histograms obtained by flow cytometry after exposing a sensitive (NCI-H69, Fig. 2a) and a resistant cell line (OC-TOL, Fig. 2b) to BCNU and TCNU for 24 h. The cell-cycle perturbations induced by the two drugs appeared to be identical. The perturbations took the form of an accumulation of cells in S-phase and a concomitant decrease of the fraction of cells in G_1. With increasing dose, an increasing fraction of cells accumulated in the S-phase; in the test conditions used the accumulated cells were detected earlier in the S-phase with higher doses (Fig. 2). Furthermore, the perturbations were more pronounced, especially at low concentrations, in the sensitive than in the resistant cell line.

Discussion

This investigation has shown that the cytostatic activity of TCNU against SCCL cell lines in vitro is equivalent or inferior to that of BCNU when compared on a microgram to microgram basis. The inferiority of TCNU is not accounted for by the use of DMSO in the control plates, since calculation of the surviving fractions by dividing by the number of colonies on the control plates exposed to tissue culture medium (data not shown) would change the results only insignificantly.

Neither can differences in in vitro stability of the two drugs account for the reduced cell kill obtained by TCNU, because the half-life of BCNU in Ringer's solution at pH 7.4 and 37 °C is 51.4 min [6] and that of TCNU in aqueous media at 37 °C and pH 7.5 is 48 min (unpublished data from AB Leo).

BCNU interferes with the synthesis of DNA as an alkylating agent [1]. This is consistent with the changes in the cell-cycle distribution demonstrated by flow cytometric DNA analysis (Fig. 2). The induction of identical changes by TCNU suggests a similar action of this drug.

The potential of flow cytometric DNA analysis in sensitivity testing has previously been described for doxorubicin [4], melphalan [5] and epipodophyllotoxin derivatives [7]. The applicability of the method in sensitivity testing of nitrosoureas is indicated by the demonstration of dose-related cell-cycle perturbations and by the agreement in the differential effect among the cell lines obtained in the clonogenic assays.

The results of the present investigation suggest a low activity of TCNU against SCCL. However, if the maximum TCNU concentration that can be achieved in the tumors greatly exceeds the maximum tumor concentration of BCNU attainable, TCNU could still be a superior nitrosourea in the treatment of SCCL. However, such a concentration advantage of TCNU would require radically altered pharmacokinetics, since the maximum tolerable dose (MTD) of BCNU exceeds that of TCNU (250 mg/m^2 v 150 mg/m^2) [3, 11]. Information on the in vivo activity of TCNU on SCCL can be obtained by treating the tumor lines after xenografting into nude mice with equitoxic doses of the two analogues.

Although the final conclusion must await clinical trials, the combined results of the in vitro and preclinical in vivo investigations will provide a sound basis for evaluation of the potential usefulness of TCNU as against BCNU in the treatment of SCCL. Furthermore, as data accumulate on the correlation between the results of in vitro and of clinical phase II trials, it will become apparent to what extent in vitro trials have predictive value.

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