Cancer Chemother Pharmacol (1983) 11: 147–152

Cancer Chemotherapy and Pharmacology

Therapeutic evaluation of five nitrosoureas in a human melanoma xenograft system

Rainhardt Osieka, Peter Glatte, Rita Pannenbäcker, and Carl Gottfried Schmidt

Innere Klinik (Tumorforschung), West German Tumor Center, Universitätsklinikum Essen, Hufelandstrasse 55, D-4300 Essen 1, Federal Republic of Germany

Summary. *The development of nitrosoureas has switched from more lipophilic derivatives to congeners with higher water-solubility, since this property was presumably associated with a decrease in myelosuppression. We have compared the therapeutic efficacy of clinically well-known lipophilic nitrosoureas BCNU, CCNU, and MeCCNU with the recently introduced water-soluble nitrosoureas chlorozotocin (CZT) and hydroxyethyl-CNU (HeCNU), using a human melanoma xenograft system. There were considerable differences in tumor-inhibitory activity, with HeCNU ranking first and CZT last, and the rank order was similar for drug-induced lethality or bone marrow damage (in terms of reduced cellularity or macromolecular DNA damage). When the doses are expressed as percentages of the corresponding* $LD_{10/30}$ *values, CZT ranks last and HeCNU low among conventional nitrosoureas. We conclude that water-solubility is not associated with reduced myelosuppression and that other guidelines will have to be adopted for rational development of nitrosoureas.*

Introduction

The nitrosoureas are often used as an example to illustrate the rational design and development of drug analogs according to structure-activity relationships. Thus lipophilicity, expressed by pK values, guided the development of the first series of nitrosourea derivatives, since this property was associated with the ability to penetrate so-called pharmacologic sanctuaries. BCNU, CCNU, and methyl-CCNU have been ranked among the most active antineoplastic agents on the basis of their curative potential against a wide spectrum of transplanted tumors [16, 17]. In addition, the nitrosoureas were reported to act synergistically with several important classes of antineoplastic agents and no cross-resistance was observed with most other alkylating agents [17].

Despite such enthusiastic endorsement from laboratory investigations, the success of the nitrosoureas at the clinical level has been limited, with overall response rates of 27% for BCNU [4] and 17% for CCNU [24] among patients with the 10 most actively studied tumor types. Myelosuppression was found to be dose-limiting in patients, and studies were therefore initiated to circumvent this toxicity.

Streptozotocin, a naturally occurring methylnitrosourea with a sugar moiety, displayed reduced myelotoxicity and, subsequently, a series of water-soluble sugar-substituted nitrosoureas were synthesized [19].

Reprint requests should be addressed to R. Osieka

The preclinical ranking of nitrosoureas remains controversial with quite divergent conclusions, depending on the animal tumor system and the toxicity data used by each investigator. The disparity between the excellent activity against some conventional murine tumor systems and the modest clinical usefulness has not been explained. We have therefore used a nitrosourea-sensitive melanoma of human origin that had been established as a xenograft in congenitally athymic (nu/nu) mice to compare the tumoricidal properties of established nitrosoureas such as BCNU, CCNU, and methyl-CCNU with water-soluble derivatives assumed to be less myelosuppressive, such as chlorozotocin and HeCNU [5].

Therapeutic ranking requires considerations of tumor-inhibitory effects and toxicity. Lethality and damage to the hematopoietic system were used as indicators of drug toxicity. Bone marrow cellularity and DNA damage in terms of DNA interstrand cross-links were assayed after exposure of normal mice with equi-tumoricidal doses of each nitrosourea. The relevance of these preclinical data to the clinical rating of nitrosoureas will be discussed, since for CZT an assessment can be made as to its rank among clinically established nitrosoureas and recently a preliminary report has been published about the clinical use of HeCNU.

Materials and methods

Drugs

Methyl-CCNU (MeCCNU; NSC-95441), chemical name 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea, and chlorozotocin (CZT; NSC-178248) were obtained from Dr Engle at the National Cancer Institute, USA.

CCNU (NSC-79037), chemical name 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea) and

HeCNU, chemical name 1-(2-hydroxyethyl)-3-(2-chloroethyl)-3-nitrosourea were obtained from Prof. Dr G. Eisenbrand, University of Kaiserslautern, FRG.

BCNU (NSC-409962), chemical name 3-bis(2-chloroethyl)-1-nitrosourea) was obtained commercially (Carmubris, Bristol-Myers Company, FRG).

BCNU was dissolved in 3% ethanol and 97% physiological saline. CZT and HeCNU were dissolved in water, whereas CCNU and MeCCNU were dissolved in 10% ethanol, suspended in 10% emulphor and 80% physiological saline. All drugs were made up in concentrations allowing injection of 0.01 ml/g body weight IP for any treatment.

Tumor source. The Str xenograft line was established from a neurosurgically removed brain metastasis of a disseminated malignant melanoma. The female donor patient had previously achieved a partial remission with the combination of cisplatin and ifosfamide and, subsequently, a complete remission during treatment with DTIC, but had never been exposed to nitrosoureas. Histology and karyotype of the xenograft line were quite similar to those of the original donor patient's tumor specimen. All experiments were performed on transplant generations 10–17.

Animals. Congenitally athymic (nu/nu) mice (NIH-Swiss background, random bred) were kept in sterile cages placed in laminar air flow racks. All mice received sterile water and the protein-rich diet Altromin no. 1440 (Altromin, Lage/FRG). For toxicity studies random-bred males NMRI mice weighing 20–25 g were used and housed under SPF conditions.

Transplantation and tumor measurements. Tumor fragments about 1–2 mm in diameter were transplanted SC in the flank of about 4-week-old (nu/nu) mice by a trocar without a suture [14].

Treatments were begun about 3 weeks later with tumor sizes ranging from 60 to 600 mg. Caliper measurements (length L and width B) were taken from then on every other day and tumor volumes (V) were calculated according to the formula:

$$V = L \times B \times B \times 0.5.$$

Tumor volumes were set at 1 at the beginning of treatment and tumor volume-doubling times were calculated by linear interpolation. Growth delay (GD) was calculated from the formula:

$$GD = Tt - Tc/Tc$$

where Tc is the median tumor volume-doubling time of untreated controls and Tt the corresponding value for treated animals.

Toxicology. Male NMRI mice (25 g) were caged in groups of nine or 11 animals and received increasing single doses of each nitrosourea, evenly spaced on a logarithmic scale. Lethality was recorded daily for 30 days. No late lethality was observed after single doses of any nitrosourea. Data were subjected to probit analysis using the SAS computer program.

Bone marrow cellularity was assessed 3 days after exposure to drug doses causing equal growth delay by flushing both femurs and counting nucleated cells per femur with a Coulter Counter model ZB I. Data were expressed as percentages of untreated control values.

DNA damage to mouse bone marrow was assessed 24 h after exposure to multiples of doses causing equal growth delay. Proteinase K-resistant DNA interstrand cross-links were assayed by the alkaline filter elution method adapted to unlabeled cells according to Erickson et al. [6]. Cross-link factors were calculated according to Kohn [13].

Results

Changes in relative tumor volumes have been plotted for individual tumor-bearing mice receiving increasing doses of HeCNU (Fig. 1). While there is a clear indication of a dose-response relationship, some heterogeneity with respect to tumor shrinkage and regrowth is evident within each treatment group. This was apparent for all five nitrosoureas tested.

In Fig. 2 mean relative tumor volumes are plotted for each treatment group. With increasing dose, tumor regression became more prominent, and for the highest dose tumor volume-doubling times were not reached by some animals. These animals died of causes unrelated to tumor progression.

Growth delay is assumed to depend on dose as a linear function [21], and therefore linear regression curves were calculated for each nitrosourea. In Fig. 3 growth delay is plotted as a linear function of dose (in mg/kg of body weight) for each of the five nitrosoureas under study.

As apparent from the slopes of these dose-response curves, HeCNU stands out as the most effective derivative,

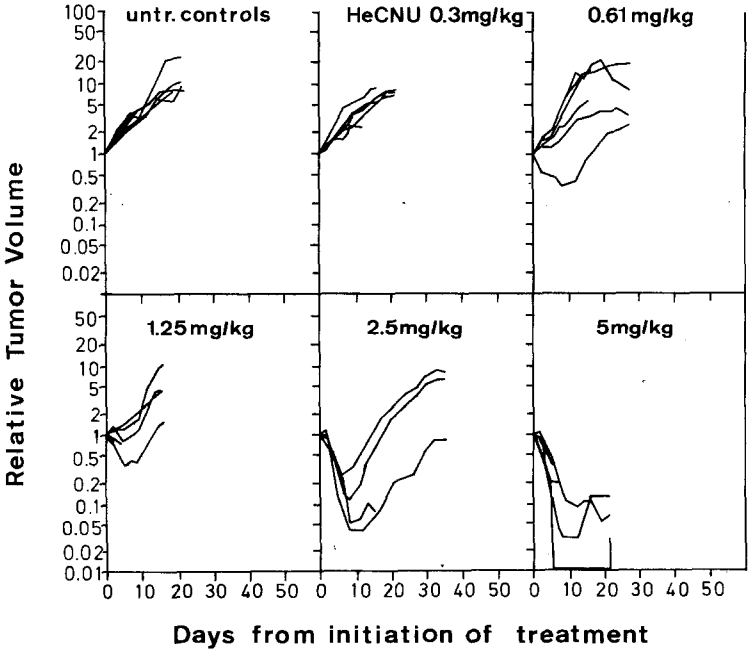

Fig. 1. Treatment results after increasing single doses of HeCNU with human melanoma xenograft line Str, Note the heterogeneity in shrinkage and regrowth of tumor volumes. A *dashed line* indicates the regrowth of a tumor that was not measurable transiently

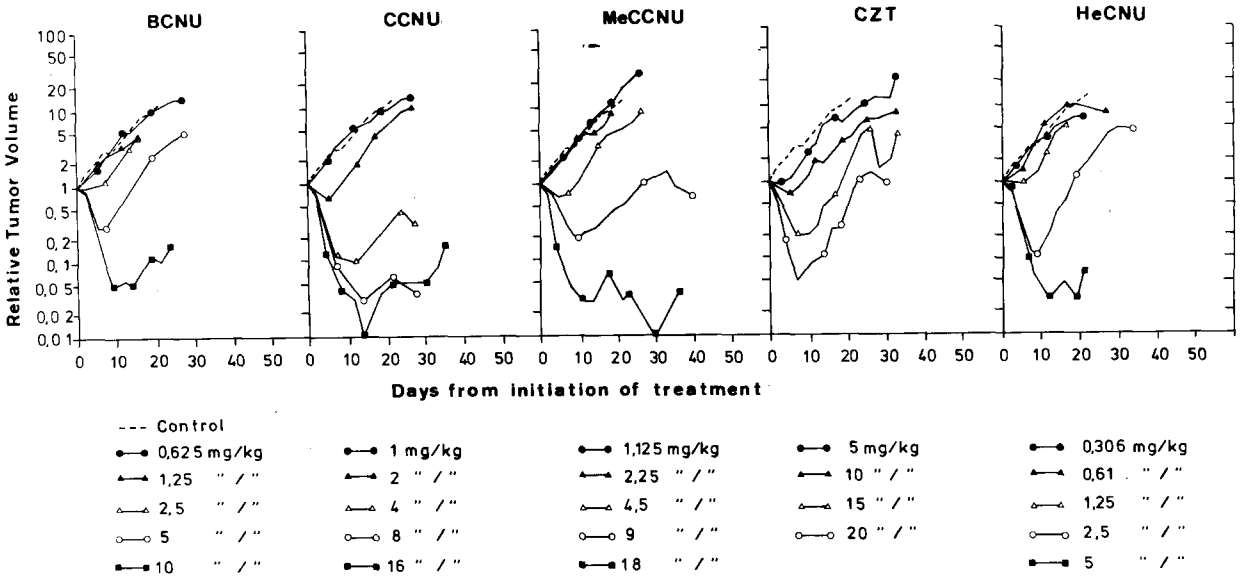

Fig. 2. Response of mean tumor volumes to increasing doses of nitrosourea derivatives. At the highest doses some tumors did not recover to their original volume at initiation of treatment

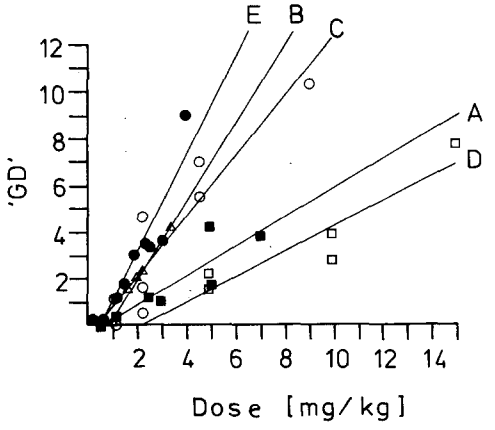

Fig. 3. Growth delay (*GD*) as a function of dose in mg/kg body weight for all five nitrosoureas under study. *Straight lines* were fitted by linear regression. *A* (■): BCNU; *B* (△): CCNU; *C* (○): MeCCNU; *D* (□): CZT; *E* (●): HeCNU

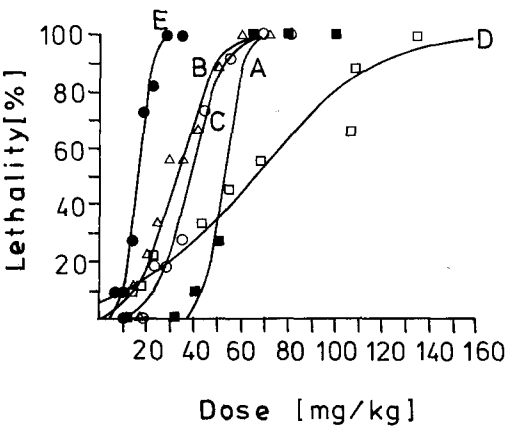

Fig. 4. Probability of death after single doses of nitrosoureas. Actual data were subjected to probit analysis by use of the SAS program and then replotted on a linear scale. *Symbols* as in Fig. 3. *A* BCNU; *B* CCNU; *C* MeCCNU; *D* CZT; *E* HeCNU

whereas CZT appears to be the least effective agent. (This order is not reversed if doses are expressed in micromoles/kg.)

Tumoricidal activity, however, should be seen in the context of host toxicity. Lethality is a crude indicator of toxicity that lends itself easily to construction of dose-response curves.

The results obtained are not always in accordance with published preclinical toxicity data. In Fig. 4 probability of death is plotted as a function of dose for each nitrosourea. HeCNU stands out as most toxic and CZT as the least toxic agent, as judged from the position of the dose-response curves.

Thus the differences observed among the nitrosoureas tested may partially be explained as changes in drug potency. Compared with the other four nitrosoureas tested, the dose-response curve for CZT-induced mouse lethality has a flatter slope.

If doses exceeding the respective $LD_{10/30}$ values are used for comparing antineoplastic activities, CZT might appear in an unduly favorable light. After single doses of nitrosoureas no late deaths were observed. This, of course, does not exclude cumulative toxicity, which is thought to be a major clinical problem.

In order to illustrate our findings with respect to both antineoplastic and toxic effects, growth delay values achieved by treatment with each nitrosourea derivative were plotted as a function of dose expressed as percent of the respective $LD_{10/30}$ values.

Figure 5 shows the dose-response curves for all nitrosoureas in this comprehensive manner. There were more than two points for construction of curve D (CZT). These data were

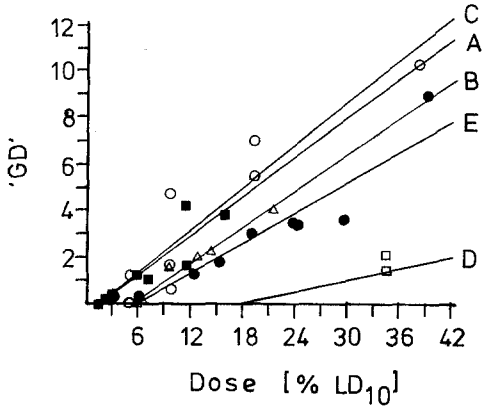

Fig. 5. Growth delay (*GD*) as a function of doses expressed as percentage of the respective $LD_{10/30}$ for each nitrosourea derivative. *Symbols* as in Fig. 3. *A* BCNU; *B* CCNU; *C* MeCCNU; *D* CZT; *E* HeCNU

Table 1. Mean reduction (R) of mouse bone marrow cellularity 3 days after nitrosourea treatment

Drug	Dose (mg/kg)	*GD*	R (%)	SE (%)
BCNU	3.8	2	72.4	13.4
	8.6	5	63.0	11.8
	16.6	10	52.1	14.1
CCNU	2.0	2	72.2	13.8
	3.8	5	68.9	8.9
	6.7	10	68.7	10.1
MeCCNU	2.4	2	71.6	5.1
	4.6	5	61.6	7.8
	8.2	10	60.2	8.5
CZT	6.1	2	69.3	10.4
	11.6	5	54.4	9.3
	20.9	10	55.7	11.9
HeCNU	1.5	2	69.7	10.9
	2.9	5	55.5	6.5
	5.3	10	38.5	6.1

Table 2. Macromolecular DNA damage expressed by cross-link factors (CF) 24 h after nitrosourea treatment

Drug	Dose (mg/kg)	*GD*[a]	*P* (*L*)[b]	CF
MeCCNU	18	22	4%	0.031
	30	38	25%	0.085
	50	63	85%	0.138
CZT	40	20	27%	0.022
	70	36	55%	0.120
	100	53	80%	0.187
HeCNU	11	21	14%	0.055
	18	36	60%	0.107
	30	61	100%	0.173

[a] Extrapolated values
[b] Probability of drug-induced death (cf. Fig. 4)

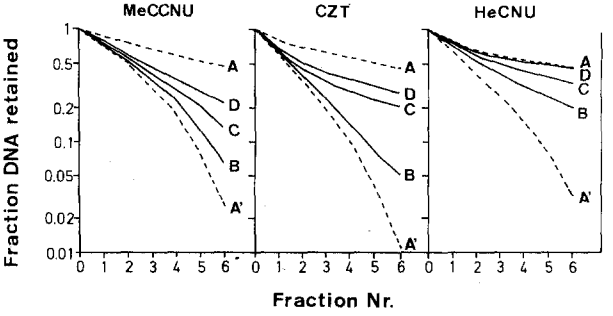

Fig. 6. Alkaline elution patterns of mouse bone marrow DNA 24 h after exposure to multiples of doses causing equal growth delay. *Dashed lines* indicate the elution patterns of DNA from untreated mouse bone marrows receiving 0 Gy (*upper line: A*) or 6 Gy (*lower line: A'*). Doses for MeCCNU were: *B*: 18 mg/kg; *C*: 30 mg/kg; *D*: 50 mg/kg. Doses for CZT were: *B*: 40 mg/kg; *C*: 70 mg/kg; *D*: 100 mg/kg. Doses for HeCNU were: *B*: 11 mg/kg; *C*: 18 mg/kg; *D*: 30 mg/kg. See Table 2 for corresponding probabilities of death and extrapolated *GD* values

subjected to analysis of covariance. Significant differences were detected for growth delay values depending on the drug employed and on dose. Additionally there was a significant interaction for drug and dose, indicating different slopes for the corresponding dose-response curves.

The poor clinical performance of CZT is well reflected in the xenograft model. According to our data, HeCNU ranks last in therapeutic activity among the remaining nitrosoureas.

In order to substantiate our comparison we investigated the damage incurred to mouse bone marrow at doses causing equivalent growth delay. With a similarly tumor-inhibiting dose of each drug, there should be less bone marrow toxicity in animals receiving drugs with superior therapeutic activity. Reduction of bone marrow cellularity was measured 3 days after a single dose of nitrosourea treatment and the results expressed as percentages of values measured in normal untreated mice. The data are given in Table 1. Statistical analysis reveals significant differences for bone marrow cellularity values, depending on drug as well as dose, but not for interaction between drug and dose (drug + dose). Both water-soluble derivatives, HeCNU and CZT, are at least as myelosuppressive as lipophilic nitrosoureas, when applied in doses causing equal growth delay.

Bone marrow cellularity data were obtained at drug levels that remained below the $LD_{10/30}$ level. Macromolecular DNA damage to mouse bone marrow was assessed 24 h after drug exposure with the alkaline filter elution technique. The use of much higher doses was necessary, since after low-dose in vivo drug exposure DNA damage was not detectable with the presently available techniques. DNA damage was apparent in mouse bone marrow cells for all nitrosoureas tested. In Table 2 the extrapolated growth delay values and the corresponding probability of death are contrasted with the cross-link factors obtained from analysis of mouse bone marrow cells 24 h after drug exposure. Using independent methodology we have shown that the two water-soluble nitrosoureas cause more damage to the murine bone marrow at equi-tumoricidal doses than conventional lipophilic nitrosoureas (Fig. 6). This supports the contention that CZT and HeCNU display inferior therapeutic activity in our xenograft model.

Discussion

The therapeutic ranking of nitrosoureas has remained controversial even in well-defined experimental tumor systems. This may be due to widely divergent definitions of LD_{10} values in mice, ranging from 24 to 44 mg/kg for BCNU, 15 to 50 mg/kg for CCNU, 23 to 42 mg/kg for MeCCNU, and 10.2 to 20 mg/kg for CZT [12, 15, 16, 20, 21; data from this work].

In several rat tumors, such as Walker 256 and L5222, but not in DMBA-induced rat tumors [5, 7, 8, 26]. HeCNU was ranked best among the nitrosoureas under study. An exception to this is a report about activity of nitrosoureas in rat leukemia L5222 [27], where HeCNU is ranked first.

In many murine tumor systems (L1210 leukemia, B16 melanoma, colon carcinoma 26, and mammary carcinoma 16) HeCNU was judged to be superior to commonly used nitrosoureas, but in the Lewis lung carcinoma it ranked below MeCCNU [20].

It should be emphasized that in several of these studies doses well above the LD_{10} were used for therapeutic evaluations [5, 7, 8, 11, 20, 26, 27]. Such high doses may be irrelevant to the clinical situation [16].

CZT performed well only against mouse L1210 leukemia and not against experimental solid tumors, where it ranked consistently below MeCCNU [10, 12].

We have been able to demonstrate dose-response relationships after in vivo treatment in the xenograft model. The heterogeneity of response among individual tumor-bearing mice is a feature shared with conventional murine tumor systems [18]. No influence of initial tumor volume on subsequent response to treatment was observed for tumors within the size range studied.

At high dose levels some tumors do not regrow in the xenograft model. This phenomenon has been discussed in depth elsewhere [25] and may lead to underestimates of tumor-inhibitory effects. Since there are unaccounted deaths in any colony of congenitally athymic mice, we have refrained from defining permanent cures and any correction procedures based upon the incidence of such 'cures' [25].

The data elaborated with the human Str melanoma line reflect the clinical inferiority of CZT, and preclude significant therapeutic superiority of HeCNU.

Judged from these experiments molecular modifications in the nitrosoureas under study have led to changes in drug potency rather than improved therapeutic efficacy.

This point of view is strengthened by the data on bone marrow toxicity. At doses (below the $LD_{10/30}$) leading to equal growth delay all nitrosoureas studied reduced mouse bone marrow cellularity. At very high doses MeCCNU, CZT, and HeCNU all lead to macromolecular DNA damage that persists for at least 24 h. The complete kinetics of expression and removal of DNA interstrand cross-links are the subject of further investigations intended to determine at what time-point DNA damage correlates best with cytotoxicity.

Recently it has been reported [2] that at equimolar doses HeCNU causes much higher damage to the bone marrow in terms of stem cell reduction and of macromolecular DNA damage than does the conventional nitrosourea BCNU.

Thus at equal risk of lethality the water-soluble nitrosoureas have not yielded superior tumor-inhibitory effects, and conversely there was no reduction of acute bone marrow toxicity at nearly equi-tumoricidal doses. This confirms the report of Panasci et al. [15] that no reduction of host toxicity is conferred by improved water-solubility.

Only BCNU, CCNU, MeCCNU, and CZT have undergone extensive clinical trials. While all agents display definite antitumor activity, CZT has failed to fulfill promises based on data with mouse L1210 leukemia. Its inferior activity against human colon cancer xenografts [14] was confirmed by clinical phase-II trials [3], as was its inferiority to other nitrosoureas in malignant melanoma [1]. It has definite marrow toxicity in human patients when employed in doses causing clinical antitumor responses [1, 3, 22].

HeCNU was tested in 59 patients with a variety of neoplasms. The overall response rate was 11%, which is not superior to response rates reported for BCNU or CCNU [4, 24]. There appears to be considerable myelosuppression, but this may be due to heavy pretreatment of some patients [9].

So far, the predictions of the xenograft system have been borne out well in clinical studies. Studies with human tumor xenografts from a wide range of donor patients have in some instances demonstrated antineoplastic activity of nitrosoureas, but their overall activity in the xenograft model does not rank them among the most active antineoplastic agents available to the clinician [14, 21].

Studies with tumors of human origin may therefore help to determine the true value of the nitrosoureas more clearly than investigations with murine or other conventional transplantation tumor systems, which yield conflicting or even contradictory results.

Our work has not dealt with repeated drug administrations ('chronic schedules'), since there often are unexplained deaths in congenitally athymic mice. Artificially conditioned mice do not suffer from this disadvantage, but late recovery of immune functions may simulate long-term drug-induced tumor inhibition.

It has also been difficult to obtain data on chronic toxicity of the nitrosoureas under clinical circumstances. Only in brain tumor patients is single-agent therapy with a nitrosourea justified by tumor remissions of sufficient duration. In addition, such patients are not usually compromised by pretreatment with other myelosuppressive drugs, irradiation of major hematopoietic sites, or neoplastic bone marrow infiltration [23].

Since for the nitrosoureas overall clinical response rates range between 10% and 20%, very large numbers of patients would have to enter phase-II–III trials if superiority of any derivative were to be demonstrated with statistical significance. Simultaneous testing of drug derivatives in human tumor xenografts may become a useful adjunct to clinical trials designed to identify drug derivatives with improved therapeutic index.

Acknowledgements. RO was supported by grant no. SFB 102 from the Deutsche Forschungsgemeinschaft and PG and RP by award PTB 8215 from the Bundesministerium für Wissenschaft und Forschung.

We thank Dipl.-Math. H. Hirche (Institut für Medizinische Informatik und Biomathematik, GHS Essen) for statistical analysis and helpful discussions.

The gift of HeCNU from Prof. G. Eisenbrand, University of Kaiserslautern, Federal Republic of Germany, is gratefully acknowledged.

References

1. Al-Sarraf M (1981) Clinical use of nitrosoureas in patients with malignant melanoma: A review. In: Prestayko AW, Baker LH, Crooke ST, Carter SK, Schein PS (eds) Nitrosoureas: Current

status and new developments. Academic Press, New York London Toronto Sydney San Francisco, p 301
2. Bedford P, Berger M, Eisenbrand G, Schmähl D (1983) Relationship between DNA damage and the progenitor cells in mice after chloroethylnitrosoureas. In: Das Resistenzproblem bei der Chemo- und Radiotherapie maligner Tumoren. Grundlagen und Klinik. Paper presented at a Symposium in Regensburg (W. Germany), June 9–11, 1983
3. Bleiberg H, Rozencweig M, Michel J, Clavel M, Longeval E, Feremans W, Bondue H, Lardinois J, Crespeigne N, Kenis Y (1981) Phase II trial with chlorozotocin in advanced colorectal cancer. Eur J Cancer Clin Oncol 17: 863
4. Carter SK (1973) An overview of the status of nitrosoureas in other tumors. Cancer Chemother Rep [3] 4, No 3: 35
5. Eisenbrand G, Fiebig HH, Zeller WJ (1976) Some new congeners of the anticancer agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Synthesis of bifunctional analogs and water-soluble derivatives and preliminary evaluation of their chemotherapeutic potential. Z Krebsforsch 86: 279
6. Erickson LC, Osieka R, Sharkey NA, Kohn KW (1980) Measurement of DNA damage in unlabeled mammalian cells analyzed by alkaline elution and a fluorometric DNA assay. Anal Biochem 106: 169
7. Fiebig HH, Eisenbrand G, Zeller WJ, Deutsch-Wenzel T (1977) Water-soluble derivatives and bifunctional analogs of the anticancer agent 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Eur J Cancer 13: 937
8. Fiebig HH, Eisenbrand G, Zeller WJ, Zentgraf R (1980) Anticancer activity of new nitrosoureas against Walker carcinosarcoma 256 and DMBA-induced mammary cancer of the rat. Oncology 37: 177
9. Fiebig HH, Schuchhardt C, Henss H, Eisenbrand G, Löhr GW (1983) Phase I study of the water-soluble nitrosourea 1-(2-hydroxyethyl)-3-(2-chloroethyl)-3-nitrosourea (HeCNU). Proc Am Assoc Cancer Res 24: 139 Abstr 548
10. Fujimoto S, Ogawa M (1982) A new nitrosourea derivative TA-077, 1-(2-chloroethyl)-3-isobutyl-3-(β-maltosyl)-1-nitrosourea. I. Comparative study on antitumor activity. Cancer Chemother Pharmacol 9: 134
11. Habs M, Reinbold H, Eisenbrand G, Bhide S, Goga-Ionescu S, Schmaehl D (1981) Antitumor activity of new nitrosoureas on Yoshida sarcoma ascites cells implanted into the colon wall of rats. J Cancer Res Clin Oncol 101: 285
12. Hayat M, Bourut C, Chenu E, Montero JL, Imbach JL, MacDonald JS, Mathé G (1979) Comparative pharmacology of three new nitrosourea analogues: RFCNU, RPCNU, and chlorozotocin. I. Oncostatic effects in mice. Cancer Chemother Pharmacol 3: 217
13. Kohn KW, Erickson LC, Laurent G, Ducore J, Sharkey N, Ewig RA (1981) DNA crosslinking and the origin of sensitivity to chloroethylnitrosoureas. In: Prestayko AW, Baker LH, Crooke ST, Carter SK, Schein PS (eds) Nitrosoureas: Current status and new developments. Academic Press, New York London Toronto Sydney San Francisco, p 69
14. Osieka R, Houchens DP, Goldin A, Johnson RK (1977) Chemotherapy of human colon cancer xenografts in athymic nude mice. Cancer 40: 2640
15. Panasci LC, Green D, Nagourney R, Fox P, Schein PS (1977) A structure-activity analysis of chemical and biological parameters of chloroethylnitrosoureas in mice. Cancer Res 37: 2615
16. Schabel FM Jr (1976) Nitrosoureas: A review of experimental antitumor activity. Cancer Treat Rep 60: 665
17. Schabel FM Jr, Trader MW, Laster WR Jr, Corbett TH, Griswold DP Jr (1979a) *cis*-Dichlorodiammineplatinum(II): Combination chemotherapy and cross-resistance studies with tumors of mice. Cancer Treat Rep 63: 1459
18. Schabel FM Jr, Griswold DP Jr, Corbett TH, Laster WR, Mayo JG, Lloyd HH (1979b) Testing therapeutic hypotheses in mice and man: observations on the therapeutic activity against advanced solid tumor of mice treated with anticancer drugs that have demonstrated or potential clinical utility from treatment of advanced solid tumors of man. In: DeVita VT Jr, Busch H (eds) Cancer drug development, part B. Methods in cancer research, Vol 17. Academic Press, New York San Francisco London, pp 3–51
19. Schein PS, McMenamin MG, Anderson T (1973) 3-(Tetraacetyl glucopyranos-2-yl)-1-(2-chloroethyl)-1-nitrosourea, an antitumor agent with modified bone marrow toxicity. Cancer Res 33: 2005
20. Spreafico F, Filippeschi S, Falautano P, Eisenbrand G, Fiebig HH, Habs M, Zeller V, Berger M, Schmähl D, van Putten LM, Smink T, Csanyi E, Somfai-Relle S (1981) In: Prestayko AW, Baker LH, Crooke ST, Carter SK, Schein PS (eds) Nitrosoureas: Current status and new developments. Academic Press, New York London Toronto Sydney San Francisco, p 27
21. Steel GG, Courtenay VD, Peckham MJ (1983) The response to chemotherapy of a variety of human tumour xenografts: A review. Br J Cancer 47: 1
22. Ungerleider RS (1980) Antitumor activity of chlorozotocin (NSC-178748). Cancer Treat Rev 7: 191
23. Walker MD (1973) Nitrosoureas in central nervous system tumors. Cancer Chemother Rep [3] 4, No 3: 21
24. Wasserman TH, Slavik M, Carter SK (1974) Review of CCNU in clinical cancer therapy. Cancer Treat Rev 1: 131
25. Wheldon TE, Brunton GF (1982) Relation of growth delay to cure for experimental tumour systems conforming to Poisson curve statistics. Br J Cancer 45: 1
26. Zeller WJ, Eisenbrand G, Fiebig HH (1978) Chemotherapeutic activity of new 2-chloroethylnitrosoureas in rat L5222 leukemia: Comparison of bifunctional and water-soluble derivatives with 1,3-bis(2-chloroethyl)-1-nitrosourea. J Natl Cancer Inst 60: 345
27. Zeller WJ, Eisenbrand G, Fiebig HH (1979) Examination of four newly synthesized 2-chloroethylnitrosoureas in comparison with BCNU, CCNU, MeCCNU, chlorozotocin, and hydroxyethyl-CNU in preterminal rat leukemia L5222. J Cancer Res Clin Oncol 95: 43

Received March 1, 1983/Accepted August 3, 1983

Appendix

An analysis of covariance performed on the *GD* values, the drugs and doses (mg/kg and percentages of $LD_{10/30}$) revealed a significant difference among the mean *GD* values for doses (in mg/kg or as percent of $LD_{10/30}$) and for drugs, and notably, a significant interaction between drug and dose (drug + dose), which indicates a different slope for the corresponding dose-response curves. Under a predetermined error type I of $2\alpha = 0.05$ the empirical error p was found to be less than 0.001.

The same type of analysis was performed on the values for relative bone marrow cellularity, drugs and doses (expressed as *GD* values achieved). Mean values for relative bone marrow cellularity depend significantly on dose or drug, but there is no significant interaction between drug and dose; cf. above for levels of significance.