Cancer Chemother. Pharmacol. 4, 243–248 (1980)

Cancer Chemotherapy and Pharmacology

© Springer-Verlag 1980

Original Articles

Distribution of Bratton-Marshall-positive Material in Mice Following Intravenous Injections of Nitrosoureas

Prasad Kari, William R. McConnell, Joe M. Finkel, and Donald L. Hill

Kettering-Meyer Laboratory Southern Research Institute, 2000 Ninth Avenue South, Birmingham, Alabama 35205, USA

Summary. *As determined by a colorimetric assay measuring parent compounds plus ether-extractable, nitroso-containing metabolites, N,N'-bis(2-chloroethyl)-N-nitrosourea (BCNU) disappeared more rapidly than N-(2-chloroethyl)-N'cyclohexyl-N-nitrosourea (CCNU) and N-(2-chloroethyl)-N'-(4-trans-methylcyclohexyl)-N-nitrosourea (MeCCNU) and their products from the tissues of mice injected IV. Assay of selected samples by high-pressure liquid chromatography revealed that the colorimetric assay for BCNU was specific in that the two assays gave equivalent results. Following IV-injections of CCNU and MeCCNU, however, levels of the parent compounds decreased much more rapidly than the total, color-producing material.*

Of seven tissues, the largest Cxt values for BCNU, as determined by the colorimetric assay, were noted for blood (442 μg-min/ml) and large intestine (285 μg-min/g). Liver (29 μg-min/g) had the lowest Cxt value, reflecting rapid metabolism of the compound by this organ. Color-producing material related to CCNU disappeared from the solid tissues of mice in a manner generally parallel to that for blood. Of the Cxt values for this compound and its products, those for lung (1753 μg-equivalents-min/g), kidney (1633 μg-equivalents-min/g), and small intestine (1557 μg-equivalents-min/g) were highest. Consistent with its slower rate of metabolism, MeCCNU and its color-producing metabolites remained in most tissues of mice for 12 h following injection. Except for brain (1434 μg-equivalents-min/g), Cxt values for this nitrosourea and its metabolites in tissues were higher than those of blood (5485 μg-equivalents-min/ml), with kidney (15,324 μg-equivalents-min/g), liver (12,921 μg-equivalents-min/g), and large intestine (11,501 μg-equivalents-min/g) being highest. For each nitrosourea, a fair correlation was observed between the Cxt values for tissues and the toxic and/or antitumor effects at those sites.

Reprint requests should be addressed to: Donald L. Hill

Introduction

A large number of nitrosoureas have activity against leukemia L1210 in mice [26]. Of these, *N,N'*-bis(2-chloroethyl)-*N*-nitrosourea (BCNU), *N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea (CCNU), and *N*-(2-chloroethyl)-*N'*-(4-*trans*-methylcyclohexyl)-*N*-nitrosourea (MeCCNU) have been widely used in clinical tests [5, 8, 36]. These compounds decompose chemically through reactive intermediates and are metabolized to derivatives that, in some cases, retain anticancer activity [21, 35].

Although studies involving distribution of radioactivity from labeled BCNU, CCNU, and MeCCNU have been accomplished [6, 16, 24, 30, 34], little is known about the distribution of intact nitrosoureas and their reactive, nitroso-containing metabolites in animals. In previous investigations, intact BCNU and CCNU were measured in several rat tissues at times up to 40 min following an IV injection [16], and BCNU was measured in human blood [17].

The present study was undertaken to investigate the possibility of a relationship between distribution of BCNU, CCNU, and MeCCNU and their reactive products in mice and the biological activity of these compounds. A preliminary report of this work has appeared [13].

Materials and Methods

Emulphor-EL620 (polyethoxylated vegetable oil) was obtained from General Aniline and Film Corp., New York, NY. Sulfanilamide dihydrochloride and *N*-(1-naphthyl)ethylenediamine dihydrochloride were purchased from Eastman Organic Chemicals,

0344-5704/80/0004/0243/$ 01.20

Rochester, NY. Hydroxylated derivatives of CCNU were supplied by Dr T P Johnston of Southern Research Institute.

For injection, nitrosoureas were dissolved in an Emulphor-EL620/ethanol (1 : 1, v/v) mixture and diluted with 4 volumes of 0.9% saline to final concentrations of 5, 3, and 5 mg/ml for BCNU, CCNU, and MeCCNU, respectively [3]. These solutions were stable for up to 90 min at room temperature. Male BDF_1 mice, 7–8 weeks old and weighing between 23 and 28 g, were fasted for 24 h before administration of drugs.

A colorimetric method utilizing the Bratton-Marshall reaction [19] was used for measurement of nitrosoureas. Mice, four per group, received IV injections of BCNU or MeCCNU, 50 mg/kg, or CCNU, 25 mg/kg; controls received vehicle only. The 50 mg/kg doses are equivalent to the IV LD_{10} values for BCNU and MeCCNU; the 25 mg/kg dose of CCNU is equivalent to 0.6 of the LD_{10} dose (FM Schabel, unpublished results). At various times after treatment, 0.5 ml blood from each animal was drawn by heart puncture with a heparinized syringe and needle. The blood was diluted with an equal volume of 0.1 *M* phosphate-buffered saline, pH 7.4, and extracted immediately with 4 ml cold diethyl ether. Control blood both with and without BCNU, CCNU, or MeCCNU (20 μg/ml) was treated similarly. Portions (3 ml) of the supernatants from animals of the same group were pooled and evaporated to dryness in vacuo. The dry residue was dissolved in 1.5 ml sulfanilamide reagent (0.5% in 2 *N* HCl) and incubated at 52° for 45 min. After the mixture was quickly brought to room temperature, 0.1 ml 0.3% *N*-(1-naphthyl)ethylenediamine was added. In 10 min a colored complex formed, and the solutions were filtered by suction through an F sintered glass funel. After appropriate dilution of the solutions, their absorbance was measured at 540 nm. Absorbance values of controls without added nitrosoureas were subtracted; and values were corrected for extractability of added nitrosoureas (10 μg/ml), which varied from 80% to 96% depending on the compound and the tissue. Recoveries from blood were 96%, 92%, and 92%, respectively, for BCNU, CCNU, and MeCCNU. All assays were performed in duplicate, and all determinations were made in three separate experiments.

In tissue distribution studies, six mice per group were given BCNU, CCNU, or MeCCNU as described above. At various times after treatment, the animals were killed by exsanguination, tissues were removed, blotted on filter paper, pooled according to type, and frozen immediately in solid CO_2. At the time of tissue collection, contents of the intestines were removed. Cold 0.1 *M* phosphate-buffered saline, pH 7.4, was added (5 ml/g) to the thawed samples; and the pooled tissues were homogenized with a Brinkman Polytron equipped with a P-10 generating head (Brinkman Instruments, Westbury, NY). Portions (5 ml) of each tissue homogenate were extracted immediately with 20 ml cold diethyl ether and centrifuged at 1,640 *g* for 3 min. A portion (15 ml) of the ether extract was evaporated to dryness and analyzed as described above with the same type of controls.

Analysis of selected blood samples was accomplished by high-pressure liquid chromatography with an instrument from Waters Associates (Milford, Mass.). The instrument was equipped with two pumps (Model 6000 A), a solvent flow programmer (Model 660), a Partisil ODS-2 column preceded by a PXS 10/25 pre-column, a UV detector (Model 440) set at 254 nm, and a Hewlett-Packard 3380 A integrator. Methanol : water solvents of the following compositions were used: 1 : 1, solvent A; 7 : 3, solvent B; 2 : 3, solvent C; and 13 : 7, solvent D. For separation of CCNU and its metabolites, a 16-min gradient was used, starting with 90% A and 10% B and reaching 100% B as the final condition. Isocratic elutions with solvents C and D were used for BCNU and its products and MeCCNU and its products, respectively. Samples were prepared as described above, except that the dried ether extract was dissolved in ethanol. A 10-μl portion was used for injection into the HPLC instrument. *Trans*-4-hydroxy-CCNU and *cis*-3-hydroxy-CCNU were identified in extracts of blood by their characteristic retention times.

Half-lives for BCNU were calculated by fitting a polyexponential equation to the data through the use of a nonlinear least-squares program, NONLIN [23]. Initial parameter estimates were obtained by exponential stripping [28]. The number of exponential terms required was determined by means of a pseudo F test. For calculation of the Cxt values, a minimum value for cytotoxicity of 1 μg/ml, based on in vitro studies [29], was used.

Results and Discussion

In general, BCNU (Table 1) disappeared from the tissues of mice more rapidly than CCNU (and its extractable metabolites) (Table 2), and much more rapidly than MeCCNU (and its extractable metabolites) (Table 3). At 1 h after injection, BCNU in tissues of the animals was 1 μg/g or less, except for large intestine (2 μg/g) (Table 1). At 45 and 60 min, the concentration of BCNU in brain, lung, small intestine, and kidney was similar to that for blood. The BCNU concentration in liver was low, even at 5 min after injection, and was below detection at 45 min. The rapid drop of BCNU concentration in this organ probably resulted from metabolism, since BCNU is a substrate for a denitrosating enzyme [12] and for a glutathione *S*-transferase [11] present in mouse liver. The shape of the curve for blood levels of BCNU in mice is similar to that derived for rats given an IV dose of 1 mg/kg [18]. Assay by high-pressure liquid chromatography of blood samples taken at 5 and 15 min after injection gave results similar to those derived by the colorimetric assay (Table 1).

As judged by killing of leukemia cells injected at various times after BCNU, the biological half-life of this nitrosourea was between 15 and 30 min [2], and, as determined by a colorimetric procedure similar to that used in the present study, the half-life of BCNU in the blood of dogs was less than 15 min [20]. Our experiments indicated a half-life of 15 min for BCNU in the blood of mice (Table 4). In tissues, the values for $t_{1/2\beta}$ ranged from 15 min (liver) to 91 min (lung).

When BCNU, labeled either in the chloroethyl groups or in the carbonyl carbon, was injected IP to mice or hamsters, the highest concentrations of radioactivity were found in liver, kidneys, and lungs [34]. At 1 h after an IP injection or oral dose, 13% of the radioactivity from ethyl-labeled BCNU was in the small intestine; 8% was in the liver; and 14% was in the remaining carcass of mice [6]. Our results, which show that liver and small intestine have relatively low Cxt values for BCNU (Table 4),

Table 1. Levels of Bratton-Marshall-positive material in tissues of mice following an IV dose of 50 mg BCNU/kg

Minutes	Tissue[a]						
	Blood	Brain	Kidney	Lung	Liver	Sm. intestine	Lg. intestine
5	22.9 ± 3.7 (22.3)[b]	8.0 ± 0.7	5.0 ± 0.6	6.1 ± 0.2	2.1 ± 0.1	5.2 ± 0.2	6.7 ± 0.5
10	12.2 ± 2.3						
15	8.5 ± 1.8 (10.7)	3.4 ± 0.2	2.5 ± 0.2	2.7 ± 0.6	1.0 ± 0.1	1.9 ± 0.1	3.9 ± 0.2
30	3.9 ± 0.4	1.6 ± 0.2	1.5 ± 0.4	1.5 ± 0.1	0.6 ± 0.1	1.6 ± 0.0	3.2 ± 0.2
45	1.6 ± 0.5	1.0 ± 0.1	0.9 ± 0.2	0.9 ± 0.2	0.3	1.0 ± 0.1	3.1 ± 0.1
60	1.0 ± 0.1	0.7 ± 0.0	0.7 ± 0.1	0.9 ± 0.0		0.8 ± 0.1	2.1 ± 0.2

[a] Values are averages (± standard errors) for three separate experiments, expressed as BCNU equivalents/g or ml
[b] The numbers in parentheses represent the amount of intact BCNU present, as determined by HPLC

Table 2. Levels of Bratton-Marshall-positive material in tissues of mice following an IV dose of 25 mg CCNU/kg

Minutes	Tissue[a]						
	Blood	Brain	Kidney	Lung	Liver	Sm. intestine	Lg. intestine
5	24.7 ± 1.2	16.7 ± 2.5	21.7 ± 3.5	23.0 ± 1.0	21.0 ± 3.6	17.7 ± 4.0	9.3 ± 0.8
15	16.3 ± 3.2 (4.1)[b]	11.0 ± 1.0	22.3 ± 4.0	23.7 ± 5.9	13.7 ± 3.1	20.7 ± 6.1	11.7 ± 1.5
30	11.7 ± 2.1	6.6 ± 1.2	14.0 ± 3.0	16.7 ± 3.0	7.5 ± 0.1	16.7 ± 2.3	8.6 ± 1.0
45	9.3 ± 1.5	4.9 ± 0.1	11.3 ± 2.3	12.3 ± 2.5	5.3 ± 0.9	11.3 ± 1.5	8.2 ± 1.1
60	5.3 ± 0.7	4.4 ± 0.9	10.4 ± 1.4	10.1 ± 3.5	4.2 ± 0.8	11.5 ± 1.7	7.6 ± 0.6
120	2.6 ± 0.1 (0.2)	0.7 ± 0.1	4.4 ± 1.0	5.3 ± 1.1	1.1 ± 0.2	5.7 ± 0.6	3.7 ± 0.6
180	1.5 ± 0.3	0.3 ± 0.1	2.3 ± 0.3	2.1 ± 0.1	0.5 ± 0.0	1.7 ± 0.2	1.0 ± 0.3

[a] Values are averages (± standard errors) for three separate experiments, expressed as CCNU equivalents/g or ml
[b] The numbers in parentheses represent the amount of intact CCNU present, as determined by HPLC

Table 3. Levels of Bratton-Marshall-positive material in tissues of mice following an IV dose of 50 mg MeCCNU/kg

Minutes	Tissue[a]						
	Blood	Brain	Kidney	Lung	Liver	Sm. intestine	Lg. intestine
5	52.0 ± 6.5						
10	45.7 ± 5.6 (15.0)[b]	23.7 ± 1.9	93.1 ± 2.7	65.7 ± 3.7	150 ± 6.5	75.5 ± 3.8	47.4 ± 2.8
15	40.2 ± 4.5						
20	34.6 ± 5.2	18.7 ± 1.4	73.9 ± 3.0	47.9 ± 3.8	74.4 ± 4.4	62.0 ± 2.7	51.9 ± 3.6
30	29.2 ± 4.0	10.2 ± 0.4	65.6 ± 3.1	41.3 ± 2.3	57.4 ± 3.7	50.5 ± 2.2	44.4 ± 3.2
45	26.7 ± 3.3	7.4 ± 0.7	58.3 ± 2.1	35.4 ± 3.0	49.3 ± 3.0	38.1 ± 3.0	37.6 ± 3.5
60	22.7 ± 3.0	7.2 ± 0.6	45.3 ± 2.6	29.7 ± 1.0	45.7 ± 2.4	34.5 ± 3.3	34.4 ± 2.6
120	15.7 ± 2.8 (1.0)	3.0 ± 0.5	32.2 ± 0.2	21.2 ± 2.0	27.7 ± 2.9	22.2 ± 3.1	27.1 ± 1.6
180	10.2 ± 1.1	2.6 ± 0.4	25.4 ± 2.8	16.6 ± 0.4	21.7 ± 1.8	17.0 ± 0.6	22.2 ± 0.8
240	8.2 ± 0.1	1.0 ± 0.0	24.2 ± 2.8	11.9 ± 0.3	14.3 ± 0.7	11.9 ± 0.8	15.9 ± 0.7
480	1.5 ± 0.4		8.8 ± 0.7	2.6 ± 0.2	3.5 ± 0.4	2.0 ± 0.3	6.5 ± 0.6
720	0.5 ± 0.1		3.6 ± 0.4	0.7 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	3.2 ± 0.8

[a] Values are averages (± standard error) for three separate experiments, expressed as MeCCNU equivalents/g or ml
[b] The numbers in parentheses represent the amount of intact MeCCNU present, as determined by HPLC

demonstrate that studies on the distribution of intact BCNU can be considerably different from those on distribution of radioactivity. In another study, high concentrations of intact BCNU were found in the kidneys shortly after an IV dose of 8.5 mg/kg to rats; lower concentrations appeared in brain, fat, muscle, liver, and lung [16].

Of the Cxt values derived in the present study for BCNU, that for blood was greatest, although-large intestine had a high value (Table 4). The value for kidney was relatively low. For both mice and rats, BCNU caused signs of gastrointestinal toxicity [32] and hepatotoxicity [31]. It appears that high concentrations of intact BCNU are not required for the

Table 4. Pharmacokinetic values for BCNU, CCNU, MeCCNU

	BCNU			CCNU[a]	MeCCNU[a]
	$t_{1/2\alpha}$[b]	$t_{1/2\beta}$[b]	Cxt[c]	Cxt[d]	Cxt[d]
Blood	3	15	442	983	5,485
Kidney	5	32	197	1,633	15,324
Liver	ND[e]	14	29	741	12,921
Brain	5	32	158	599	1,434
Lung	6	91	124	1,753	8,109
Small intestine	1	29	125	1,557	8,458
Large intestine	ND	41	285	925	11,501

[a] Plus ether-extractable, nitroso-containing metabolites
[b] Minutes
[c] μg-min/ml or μg-min/g from C_0 to 1 μg/ml or 1 μg/g
[d] μg-equivalents-min/g from C_0 to 1 μg-equivalent/ml or 1 μg-equivalent/g
[e] ND, not detected

observed toxicity to liver (Table 1). Some therapeutic effect of BCNU has been observed for cancer of the colon in humans [33]. In patients given BCNU, there were gastrointestinal disturbances, occasional hepatic and renal damage, and occasional pulmonary edema [1, 15].

As indicated above, a single ether extraction of various biological samples allowed recovery of 80%–96% of the parent nitrosourea present. We also determined that, by our procedure, 60%–70% of *cis*-4-hydroxy-CCNU and *trans*-2-hydroxy-CCNU, two monohydroxylated metabolites of CCNU, were extracted. Only 25% of added 2,6-dihydroxy-CCNU, however, was recoverable.

At 15 min after injection of CCNU, the total amount of extractable, nitroso-containing material in blood was 16.4 μg-equivalents/ml. Of this, 4.1 μg/ml was CCNU (Table 2), 7.7 μg/ml was *trans*-4-hydroxy-CCNU, and 4.6 μg/ml was *cis*-3-hydroxy-CCNU (not shown). At 90 min, only *trans*-4-hydroxy-CCNU could be identified. The presence of monohydroxylated metabolites in extracts of tissues of mice treated with CCNU [21] does not alter our interpretation of the results, for they have toxicity and antitumor activity equivalent to or slightly greater than that of CCNU [35]. Since the extractabilibity of hydroxylated derivatives is somewhat less than that for CCNU, the values reported here for CCNU plus monohydroxylated derivatives are low, to some extent. Very probably, the monohydroxylated derivatives of MeCCNU would have activity and extractability similar to that of corresponding derivatives of CCNU. No nitroso-containing metabolite of BCNU is known.

The concentration of CCNU (and its ether-soluble, nitroso-containing metabolites) in mouse serum dropped below the level of detection after 2 h (Table 2). As determined by high-pressure liquid chromatography, the concentration of intact CCNU in blood dropped from 4.1 μg/ml (25% of the total material present) at 15 min to <0.2 μg/ml at 2 h (Table 2). The half-life of intact CCNU in mice and rats is only 5–6 min [24, 25]. Its biological half-life, however, is reported to be 94 min [2]. Following doses of CCNU (Table 2), nitroso-containing material persisted in mouse tissues for longer periods than after BCNU, even though the dose administered was smaller. There were no noteworthy differences between the disappearance from blood and that from other tissues. In general, the concentration, which was 10–20 μg-equivalents/g or ml at early time points, dropped at similar rates to about 1 μg-equivalent/g at 3 h.

Of the Cxt values for CCNU (and its ether-soluble metabolites), those for the lung, kidney, and a small intestine were greatest, being considerably higher than the Cxt value for blood (Table 4). It is noteworthy that CCNU is superior to BCNU and MeCCNU in the treatment of human lung cancer [33]. Nephrotoxicity, hepatotoxicity, and pulmonary edema were evident in dogs [1, 10, 27], and kidney lesions were prominent in monkeys to which CCNU had been administered (LH Schmidt, unpublished results). In mice and rats, CCNU caused signs of hepatic and gastrointestinal toxicity [32]. The toxic effect on the gastrointestinal tract correlates with the relatively large Cxt value for the small intestine (Table 4). The relatively low Cxt value for large intestine is consistent with the lack of effect of CCNU on human colon cancer [33].

MeCCNU (and its ether-extractable, nitroso-containing metabolites) remained in blood and most other tissues of mice during the 12 h in which assays were made (Table 3). At 10 min after injection, concentrations ranged from 24 μg-equivalents/g in brain to 150 μg-equivalents/g in liver. Except for brain, in which the level had dropped to 3 μg-equivalents/g, the tissue concentrations at 2 h were in the range of 20–30 μg-equivalents/g. Also except for brain, tissue concentrations of color-producing material were higher than those in blood. The concentration of intact MeCCNU in blood decreased from 15 μg/ml at 10 min to 1.0 μg/ml at 120 min (Table 2). We have previously reported [12] that MeCCNU is metabolized by mouse liver microsomes at a rate lower than that for CCNU.

Cxt values were large for kidney, liver, lung, and intestine of mice injected with MeCCNU (Table 4). For this compound, antitumor and/or toxic effects have been noted for each of these organs. MeCCNU has been widely used in the treatment of gastroin-

testinal tumors of humans, and is superior to CCNU and BCNU in its therapeutic effect [33]. It also has activity against human lung cancer [33] and a remarkable effect on Lewis lung carcinoma of mice [22]. Both MeCCNU and CCNU are active against a transplantable renal sarcoma of mice [14]. MeCCNU is reported to cause kidney and gastrointestinal damage in mice [4], humans [9], and dogs and monkeys (LH Schmidt, unpublished results). All three nitrosoureas enter the brain, but concentrations there never reach those in the blood (Tables 1–3). All three compounds have activity against human brain tumors [33].

It has been reported that considerably more Bratton-Marshall-positive material appears in the metastases than in the primary of Lewis lung tumor following an IV injection of *N*-methyl-*N*-nitrosourea [7]. These metastases are known to be more responsive to chemotherapy than the primary tumor. Perhaps nitrosoureas accumulate in sensitive normal and tumor tissues as a prerequisite for their effect on these tissues. It is possible that distribution studies on other nitrosoureas could allow prediction of organ sites for toxicity and/or antitumor activity.

Acknowledgements. We are grateful to Dr. Harris H. Lloyd for calculating the pharmacokinetic values. This work was supported by Contract N01-CM-87162 from the Division of Cancer Treatment, National Institutes of Health, Department of Health, Education, and Welfare.

References

1 Carter SK, Newman JW (1968) Nitrosoureas: 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC 409962; BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (NSC-79037; CCNU) – clinical brochure. Cancer Chemother Rep 1(3): 115–152

2 Chirigos MA, Humphreys SR, Goldin A (1965) Duration of effective levels of three antitumor drugs in mice with leukemia L1210 implanted intracerebrally and subcutaneously. Cancer Chemother Rep 49: 15–19

3 Davignon JP, Yang KW, Wood HB Jr, Cradoch JC (1973) Formulation of three nitrosoureas for intravenous use. Cancer Chemother Rep 4(3): 7–11

4 Denine EP, Harrison SD, Peckham JC (1977) Qualitative and quantitative toxicity of sublethal doses of methyl-CCNU in BDF_1 mice. Cancer Treat Rep 61: 409–417

5 DeVita VT, Carbone PP, Owens AB, Golo GL, Krant MJ, Edmondson J (1965) Clinical trials with 1,3-bis(2-chloroethyl)-1-nitrosourea. NSC 409962. Cancer Res 25: 1876–1881

6 DeVita VT, Denham C, Davidson JD, Oliverio VT (1967) The physiological disposition of the carcinostatic 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in man and animals. Clin Pharmacol Ther 8: 566–577

7 Donelli MG, Colombo T, Broggini M, Garattini S (1977) Differential distribution of antitumor agents in primary and secondary tumors. Cancer Treat Rep 61: 1319–1324

8 Hansen HH, Selawry OS, Muggia FM, Walker MD (1971) Clinical studies with 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. NSC 79037. Cancer Res 31: 223–227

9 Harmon WE, Cohen HJ, Schneeberger EE, Grupe WE (1979) Chronic renal failure in children treated with methyl CCNU. N Engl J Med 300: 1200–1203

10 Henry MC, Davis RD, Schein PS (1977) Hepatotoxicity of 1-(2-chloroethyl-3-cyclohexyl-1-nitrosourea (CCNU) in dogs: use of serial percutaneous liver biopsies. Toxicol Appl Pharmacol 25: 410–417

11 Hill DL (1976) *N,N'*-bis(2-Chloroethyl)-*N*-nitrosourea (BCNU), a substrate for glutathione (GSH) *S*-transferase. Proc Am Assoc Cancer Res/ASCO 17: 52

12 Hill DL, Kirk MC, Struck RF (1975) Microsomal metabolism of nitrosoureas. Cancer Res 35: 296–301

13 Hill DL, Kari P, McConnell WR (1979) Distribution of nitrosoureas in mice. Proc Am Assoc Cancer Res/ASCO 20: 33

14 Hrushesky WJ, Murphy GP (1974) Evaluation of chemotherapeutic agents in a new murine renal carcinoma model. J Natl Cancer Inst 52: 1117–1122

15 Lessner HE, Vogler WR (1974) Toxicity study of BCNU (NSC-409962) given orally. Cancer Chemother Rep 58: 407–411

16 Levin VA, Kabra PA, Freeman-Dove MA (1978a) Relationship of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) pharmacokinetics of uptake, distribution, and tissue/plasma partitioning in rats organs and intracerebral tumors. Cancer Chemother Pharmacol 1: 233–242

17 Levin VA, Hoffman W, Weinkam RJ (1978b) Pharmacokinetics of BCNU in man: a preliminary study of 20 patients. Cancer Treat Rep 62: 1305–1312

18 Levin VA, Stearne J, Byrd A, Finn A, Weinkam RJ (1979) The effect of phenobarbital pretreatment on the antitumor activity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU). J Pharmacol Exp Ther 208: 1–6

19 Loo TL, Dion RL (1965) Colorimetric method for the determination of 1,3-bis(2-chloroethyl)-1-nitrosourea. J Pharm Sci 54: 809–810

20 Loo TL, Dion RL, Dixon RL, Rall DP (1966) The antitumor agent 1,3-bis(2-chloroethyl)-1-nitrosourea. J Pharm Sci 55: 492–497

21 May HE, Boose R, Reed DJ (1974) Hydroxylation of the carcinostatic 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) by rat liver microsomes. Biochem Biophys Res Commun 57: 426–433

22 Mayo JG, Laster WR Jr, Andrews CM, Schabel FM (1972) Success and failure in the treatment of solid tumors. III. "Cure" of metastatic Lewis lung carcinoma with methyl-CCNU and surgery-chemotherapy. Cancer Chemother Rep 56: 183–195

23 Metzler CM, Elfring GL, McEwen AJ (1974) A package of computer programs for pharmacokinetic modeling. Biometrics 30: 562–563

24 Oliverio VT, Vietzke WM, Williams MK, Adamson RH (1970) The absorption, distribution, excretion, and biotransformation of the carcinostatic 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea in animals. Cancer Res 30: 1330–1337

25 Reed DJ (1975) Pharmacokinetics of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in the rat. Proc Am Assoc Cancer Res/ASCO 16: 92

26 Schabel FM Jr, Johnston TP, McCaleb GS, Montgomery JA, Laster WR, Skipper HE (1963) Experimental evaluation of potential anticancer agents. VIII. Effects of Certain nitroso-

ureas on intracerebral L1210 leukemia. Cancer Res 23: 725–733

27 Schaeppi U, Fleischman RW, Phelan RS, Ethier MF, Luthra YK (1974) CCN(NSC-79037). Preclinical toxicologic evaluation of a single intravenous infusion in dogs and monkeys. Cancer Chemother Rep 5(3): 53–64

28 Sedman AJ, Wagner TG (1976) CSTRIP, a Fortran IV computer program for obtaining initial poly-exponential parameter estimates. J Pharm Sci 65: 1006–1010

29 Skipper HE, Schabel FM Jr, Mellett LB, Montgomery JA, Wilkoff LJ, Lloyd HH, Brockman RW (1970) Implications of biochemical, cytokinetic, pharmacologic, and toxicologic relationships in the design of optimal therapeutic schedules. Cancer Chemother Rep 54: 431–450

30 Sponzo RW, DeVita VT, Oliverio VT (1973) Physiologic disposition of 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) and 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU) in man. Cancer 31: 1154–1156

31 Thompson GR, Larson RE (1969) The hepatotoxicity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) in rats. J Pharmacol Exp Ther 166: 105–112

32 Thompson GR, Larson RE (1972) A toxicologic comparison of the potency and activity of 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (CCNU) in mice and rats. Toxicol Appl Pharmacol 21: 405–413

33 Wasserman TH, Comis RL, Goldsmith M, Handelsman H, Penta JS, Slavik M, Soper WT, Carter SK (1975) Tabular analysis of the chemotherapy of solid tumors. Cancer Chemother Rep 6(3): 399–419

34 Wheeler GP, Bowdon BJ, Herren TC (1964) Distribution of C^{14} from C^{14}-labeled 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC-409962) in tissues of mice and hamsters after intraperitoneal administration of the agent. Cancer Chemother Rep 42: 9–12

35 Wheeler GP, Johnston TP, Bowdon BJ, McCaleb GS, Hill DL, Montgomery JA (1977) Comparison of the properties of metabolites of CCNU. Biochem Pharmacol 26: 2331–2336

36 Young RC, Walker MD, Canellos GP, et al (1973) Initial clinical trials with methyl CCNU 1-(2-chloroethyl)-3-(4-methylcyclohexyl)-1-nitrosourea (MeCCNU). Cancer 31: 1164–1169

Received November 13, 1979/Accepted May 27, 1980