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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 Rat Organs and Intracerebral Tumors

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Summary. *To obtain a clearer definition of the relationship between the structure of BCNU and CCNU and their antitumor activity, we determined the uptake, distribution, and tissue/plasma partition ratios of both compounds in normal organs and intracerebral (ic) 9L tumors in rats. Greater uptake, distribution, and tissue/plasma partition ratios were obtained for parent CCNU in fat, liver, and brain, and for parent BCNU in kidney. CCNU distributes more rapidly and extensively than BCNU only in fatty tissues. BCNU distributed more extensively in kidney and liver. Rats received bolus IV injections of ^{14}C-labeled BCNU or CCNU in increasing doses; measurements taken 30 min after injection showed that three- to fourfold more BCNU than CCNU was bound to nucleic acids in brain and ic 9L tumor tissue. Because the chloroethyl group is the alkylating moiety for both drugs, these findings implied that BCNU biotransformed to its reactive intermediate more rapidly than did CCNU.*

These observations, together with previous findings, indicate that one reason for the greater effectiveness of BCNU than CCNU against ic 9L tumors is its superior ability to form an intermediate that can bind to ic 9L tumor cell nucleic acids.

Introduction

BCNU (NSC-409962) and CCNU (NSC-79037) have shown in vivo antitumor activity in experimentally induced intracerebral (ic) brain tumors in animals (Levin et al., 1970; Levin and Kabra et al., 1974; Barker et al., 1973; Geran et al., 1974), as well as in clinical trials of central nervous system tumors in humans (Wilson et al., 1970; Fewer et al., 1972). Although the mechanism of this action is not fully understood, the accumulating evidence suggests that these nitrosoureas decompose nonenzymatically to yield reactive intermediates that are capable of alkylation (Montgomery et al., 1967; May et al., 1974; Colvin et al., 1976) and carbamoylation (Montgomery et al., 1967).

Hansch analyses of nitrosourea antitumor activity against ic L1210, ic 9L, and Lewis lung tumors have shown a correlation of antitumor activity with log P values[1] (Hansch et al., 1972; Levin and Kabra, 1974; Wheeler et al., 1974). In an earlier study of six substituted nitrosoureas, we found that antitumor activity against ic 9L tumors was dependent on log P, was maximal at log $P \simeq 0.4$, and decreased with increasing and decreasing log P values (Levin and Kabra, 1974). Antitumor activity was in proportion to in vitro alkylating activity and the rate of spontaneous nonenzymatic transformation, and was inversely related to in vitro carbamoylating activity (Levin and Kabra, 1974; Wheeler et al., 1974).

In an effort to explain the known differences between the antitumor activities of BCNU and CCNU, we analyzed the in vivo pharmacokinetics, tissue/plasma partitioning, organ uptake and distribution, and dose-dependent uptake of ^{14}C-CCNU and ^{14}C-BCNU in brains and tumors of normal and ic 9L tumor-bearing rats.

Materials and Methods

Isotopically Labeled Compounds

^{14}C-CCNU and ^{14}C-BCNU labeled in the chloroethyl moiety and ^{14}C-cyclohexyl-labeled CCNU were generously supplied by Dr. Ro-

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[1] Log P = log (octanol/water partition coefficient)

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bert Engle of the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute.

The synthesis of these compounds unambiguously established the position of the label, as shown by the synthesis[2] of ^{14}C-BCNU. Condensation of 2-chloroethylisocyanate with 2-chloro[$^{14}C_2$]ethylamine gave the precursor to BCNU, 1-N-(2-chloro[$^{14}C_2$]ethyl)-2-N-(2-chloroethyl)urea. Nitrosation of this compound gave 1-N-(2-chloro[$^{14}C_2$]ethyl)-2-N-(2-chloroethyl)-2-N-nitrosourea (Compound 1 in Fig. 5) and 1-N-(2-chloro[$^{14}C_2$]ethyl)-2-N-(2-chloroethyl)-1-N-nitrosourea (Compound 2 in Fig. 5). Any scrambling of the labeled chloroethyl group under the reaction conditions would not produce bis-labeled compound in excess of 1% of the total reaction products. CCNU was selectively labeled in either the cyclohexyl or chloroethyl moieties by similar series of reactions.

Specific activities of the isotopes were 52 μCi/mg for CCNU, and 47 μCi/mg for BCNU. Purity was reconfirmed by thin-layer chromatography and high-pressure liquid chromatography (HPLC) (purity > 98% for CCNU; > 94% for BCNU).

Animals

Male Fischer 344 rats weighing 180–250 g received either ^{14}C-CCNU or ^{14}C-BCNU by the IV route. Rats with ic 9L tumors received 0.5 ml of 2% trypan blue IP 24 h before study. All animals were anesthetized with pentobarbital (60 mg/kg) before study.

Techniques of Injection and Sampling

Approximately 20–25 μCi ^{14}C-CCNU or ^{14}C-BCNU and varying amounts of unlabeled CCNU and BCNU were solubilized in 0.1–0.2 ml absolute alcohol. This solution was further diluted with 0.5–0.6 ml saline before injection, and each animal received 1–2 ml/kg body weight in a saphenous vein via either a 27-gauge needle or surgically placed PE10 tubing. Three experimental groups were established:

Group 1. Two groups of normal animals were used: Seven animals received 40 μm/kg of ^{14}C-chloroethyl-CCNU and nine others received 40 μm/kg of ^{14}C-BCNU via an IV catheter. Animals were sacrificed at timed intervals, 2–41 min after a 20-s bolus of drug. At sacrifice, arterial blood was obtained by catheter; each animal was decapitated and the head frozen in liquid nitrogen. Duplicate liver, kidney, lung, omental fat, and skeletal muscle samples were rapidly removed, placed into tared vials, and weighed. The frozen head was cut with a Stryker cast saw and samples of cortex and subcortex were removed. Tissue and plasma samples were frozen on dry ice and stored at −70° C until analyzed.

Group 2. Twenty-four rats with ic 9L tumors received injections of varying doses of ^{14}C-BCNU labeled in the chloroethyl moiety or ^{14}C-CCNU labeled in the chloroethyl or cyclohexyl moiety. Injections were administered over a 20-s period via an indwelling IV catheter. Animals were sacrificed at 30 min. The head was frozen in liquid nitrogen for 45 s, after which duplicate samples of tumor, contralateral cortex, and subcortex were removed for analysis.

Group 3. An IV infusion schedule was developed to maintain relatively constant levels of parent CCNU or BCNU over a period of 95–120 min. Two groups of two normal rats each received approximately 40 μm/kg either ^{14}C-BCNU or ^{14}C-CCNU labeled in the chloroethyl moiety. Arterial samples were taken at three or four time intervals to verify the plasma steady state. The animals were sacrificed at intervals between 95 and 120 min after initiation of the infusions. Duplicate samples of brain, liver, kidney, lung, ometal fat, and muscle were obtained and analyzed, and the tissue/plasma ratios of total drug, dichloromethane (DCM) soluble drug, and parent CCNU and BCNU were calculated.

[2] John Montgomery, Southern Research Institute, and John Kepler, Research Triangle Institute, personal communications, 1977

CCNU and BCNU Extraction and Chromatography

The frozen tissues from all rats were finely minced between glass slides and all tissue was transferred to 10-ml separating funnels. Both tissues and plasma were extracted three times with 5 ml of DCM to remove the free drug. Recovery of CCNU and BCNU was 99%.

All DCM extracts were pooled and evaporated at 37° C in vacuo. The DCM-soluble residue was redissolved in DCM to a volume of 2 ml. Duplicate 100 μl samples were counted for radioactivity in a Beckman LS-250 scintillation spectrometer (Beckman Instruments, Fullerton, CA). Portions of the remaining DCM fraction were analyzed by HPLC (Chromatronix 3510, Mt. View, CA) with Bio-Sil A columns (Bio-Rad, Richmond, CA), 50 cm × 2.1 mm, and the elutants were counted to determine free CCNU- and BCNU-associated radioactivity.

Free CCNU was eluted with isooctane/chloroform (2 : 1, v/v) at a flow rate of 1 ml/min. The CCNU peak retention time was 4 min. Free BCNU was eluted with isooctane/chloroform (1 : 1, v/v) at a flow rate of 1.2 ml/min. The BCNU peak retention time was 4.5 min. Total peak elutants for both CCNU and BCNU were counted for radioactivity. Sample collections taken before and after free CCNU and BCNU peaks did not demonstrate significant radioactivity above background. HPLC analysis of *cis*-2- and *trans*-4-hydroxylated ^{14}C-CCNU compounds (obtained from the Southern Research Institute, Birmingham, AL) failed to show radioactivity in the region of the CCNU peak.

All samples of tumor, brain, and plasma counted for radioactivity were corrected for sample quench by external standardization. The samples were corrected for dilution; radioactivity was normalized to DPM/g wet weight; then, based on the standards, the quantity of drug was computed in micrograms per gram or nanograms per gram.

Binding of ^{14}C-CCNU and ^{14}C-BCNU to Nucleic Acids and Proteins

The binding of ^{14}C-CCNU or ^{14}C-BCNU or their derivatives to DNA, RNA, and protein was determined by a minor modification of the Schmidt-Thannhauser technique (Schmidt and Thannhauser, 1945; Cheng et al., 1972). After the extraction of free CCNU and BCNU, the tissues were successively extracted three times with 3 ml ether and ethanol. Each fraction was counted for radioactivity. The tissue was then precipitated and washed three times with 2 ml 0.2 *N* perchloric acid (PCA) in the cold. The acid-soluble fraction was counted for radioactivity; the remaining precipitate was hydrolyzed with 4 ml 0.3 *N* KOH at 37° C for 1 h. The hydrolysate was treated with 4 ml 0.4 *N* PCA at 0–4° C to precipitate DNA and protein. The soluble fraction was counted for RNA-bound radioactivity. The precipitate was treated twice with 4 ml 10% NaCl at 100° C; the soluble fraction was counted for DNA-bound radioactivity. The remaining precipitate was digested with NCS tissue solubilizer (Amersham-Searle, Chicago) and the radioactivity bound to protein was determined.

To test the efficiency of DNA separation, ic 9L cells were grown for 14 h in culture medium containing ^{3}H-thymidine: 97% of the tissue-bound radioactivity was associated with the DNA fraction and 3% with the RNA fraction.

Because the PCA precipitation conditions are rather harsh, nonspecific depurination could occur. Therefore, the absolute value of DNA and RNA (nanomoles per gram) may be slightly in error. The *relative* differences between BCNU and CCNU nucleic acid binding, however, should be valid.

Results

Group 1. Figure 1 shows the uptake and distribution of ^{14}C-CCNU and ^{14}C-BCNU (both labeled in the chloroethyl moiety) and their respective radioactivity levels in the organic soluble fraction (DCM, ether, ethanol, and ethanol-ether washes), the PCA soluble fraction, RNA, DNA, protein, and total radioactivity in kidney, brain, liver, lung, omental fat, and skeletal muscle, respectively. Despite their limited statistical significance (only one animal was evaluated per time point, in most cases), these data show trends in the organ-specific distribution and metabolism of CCNU and BCNU.

Figure 1a shows a dramatic difference between CCNU and BCNU levels in kidney: the levels of parent BCNU and organic-soluble, PCA-soluble and RNA-bound BCNU radioactivity are markedly higher.

There are greater amounts of parent CCNU than of

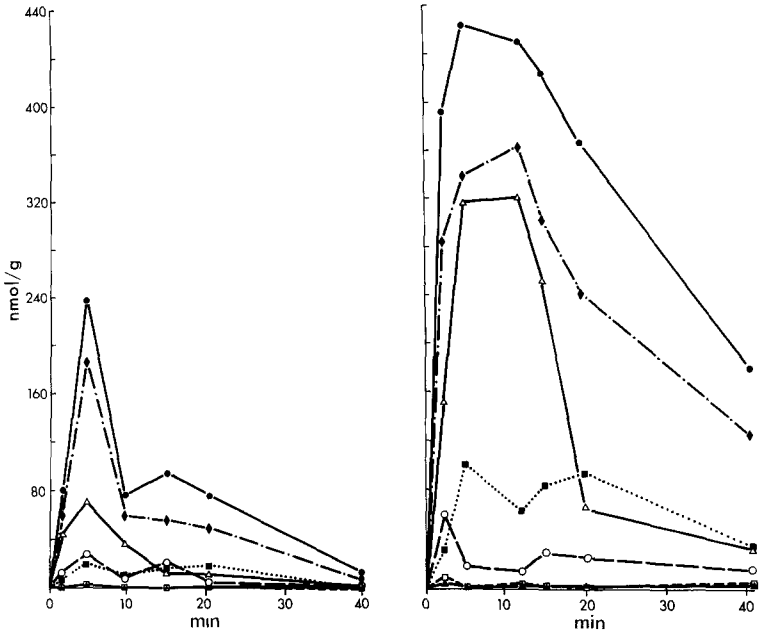

Fig. 1a

Fig. 1a–f. Uptake and distribution of ^{14}C-CCNU- (left), and ^{14}C-BCNU-derived (right) radioactivity in **a** rat kidney, **b** omental fat, **c** liver, **d** brain, **e** lung, and **f** striated muscle, following an IV bolus of 40 μm/kg body weight. Results are presented as nmoles/gram of wet tissue. Each point represents one animal, except for the 40-min CCNU and the 15-, 20-, and 40-min BCNU points, which are averaged for two animals. - · -◆- · - organic soluble; ····■···· PCA-soluble; - - ○ - - RNA; —▲— DNA; - - □ - - protein; —△— parent species; —●— total ^{14}C

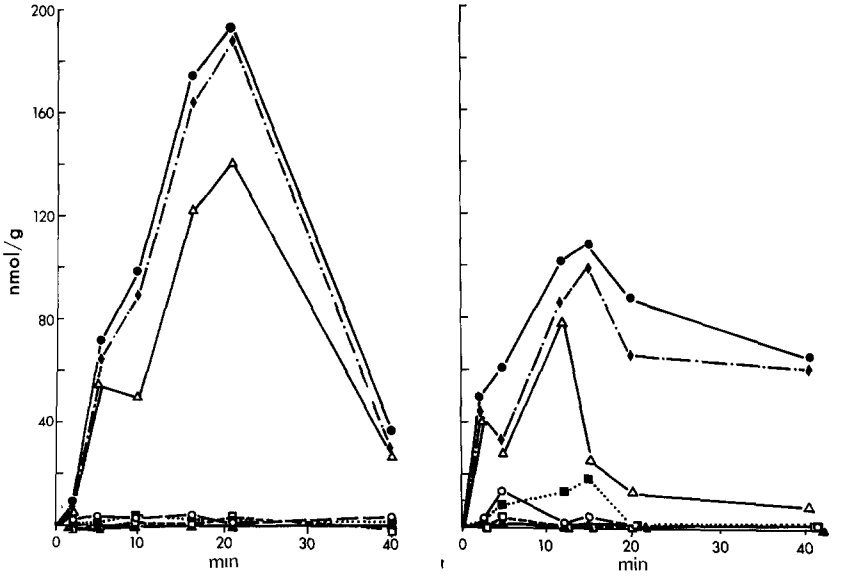

Fig. 1b

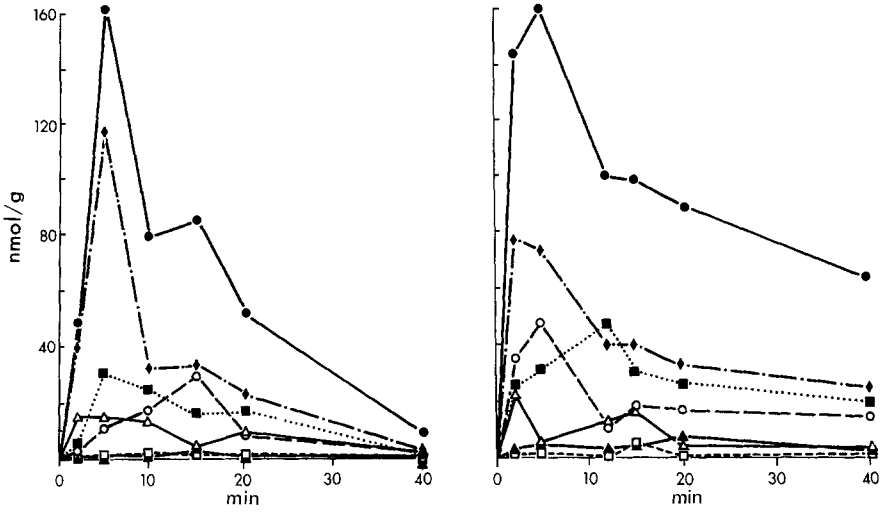

Fig. 1c

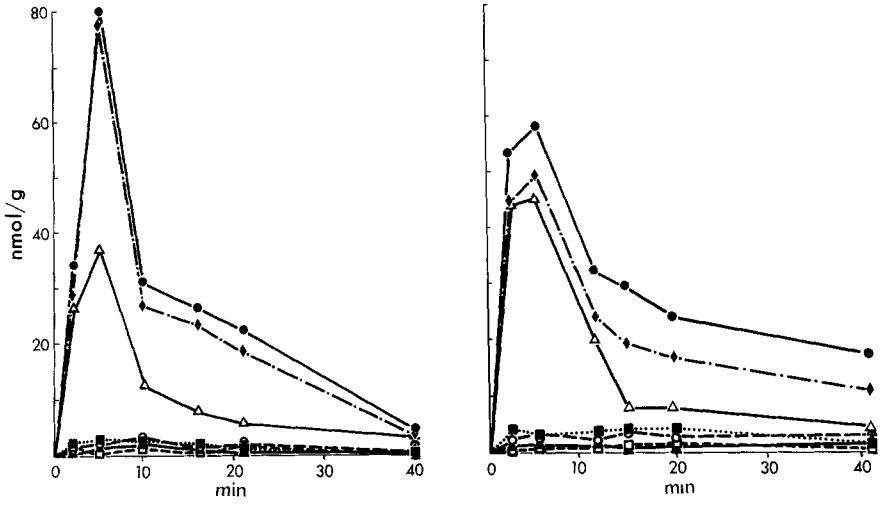

Fig 1d

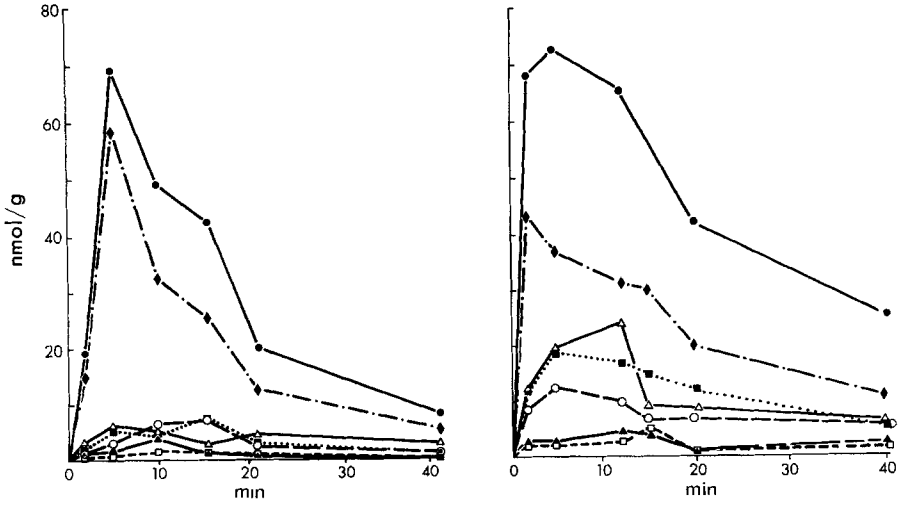

Fig. 1e

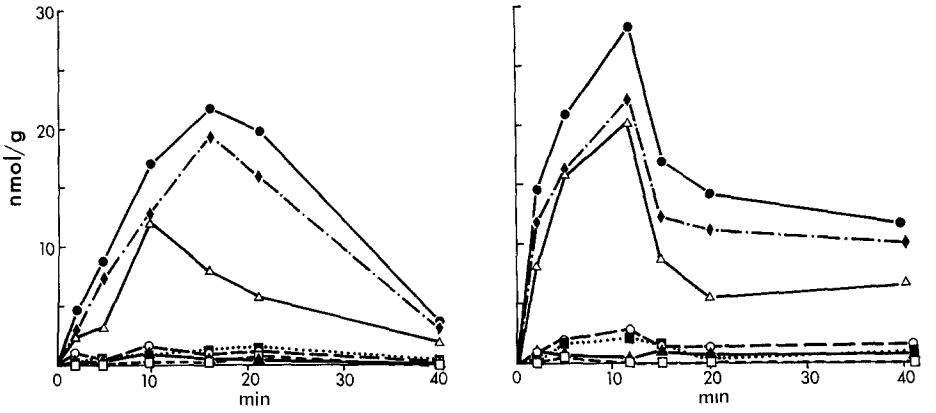

Fig. 1f

parent BCNU in omental fat (Fig. 1b). In addition, the amounts of organic-soluble CCNU radioactivity and parent CCNU in omental fat are quite closely parallel, whereas less parent BCNU is associated with this fraction after 20 min. Little DNA or RNA binding was seen for either BCNU or CCNU in fat.

In the liver (Fig. 1c), little difference was seen between the levels of the free BCNU and CCNU parent species. There was little intact parent species for either drug, because of the conversion of the parent species to their reactive intermediates. Total radioactivity was greater for BCNU than for CCNU, as a result of the persistence of PCA-soluble, RNA-bound, and non-BCNU organic-soluble radioactivity.

Figure 1d shows greater persistence of total radioactivity for BCNU than for CCNU in brain, and greater uptake of PCA-soluble and RNA-bound BCNU.

Comparison of the levels of the two drugs in the lung (Fig. 1e) shows that the animals receiving BCNU achieved higher total radioactivity levels, more PCA- and RNA-associated drug, and higher parent drug levels.

In muscle (Fig. 1f), BCNU showed more prolonged total radioactivity, and more organic-soluble and intact parent drug.

Group 2. Figures 2–4 compare the uptake of BCNU- and CCNU-derived radioactivity into brain and ic 9L tumor as a function of the dose administered. Table 1 summarizes these comparisons in terms of slope, intercept, standard error (SE) of estimate, standard deviation (SD) of slope, and correlation coefficient. Figure 2 shows that the levels of BCNU in brain and tumor are comparable, and that the levels of CCNU in brain and tumor are also comparable. However, there is twofold more BCNU than CCNU in brain and tumor. In addition, there is a linear increase in soluble drug uptake as a function of dose administered.

Figures 3 and 4 show the dose-dependent uptake of DNA- and RNA-bound radioactivity in brain and ic 9L tumor after BCNU and CCNU administration, respec-

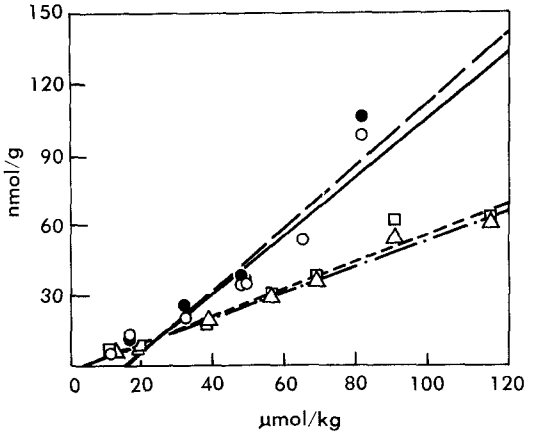

Fig. 2. Total amount of ^{14}C-BCNU and ^{14}C-chloroethyl-CCNU radioactivity in rat brain (——○ BCNU; – – –△ CCNU) and ic 9L tumor (– – –● BCNU; – · –□ CCNU) following increasing doses (μm/kg) of each drug. Each point represents one animal sacrificed 30 min after injection. The lines were fit by the method of least squares

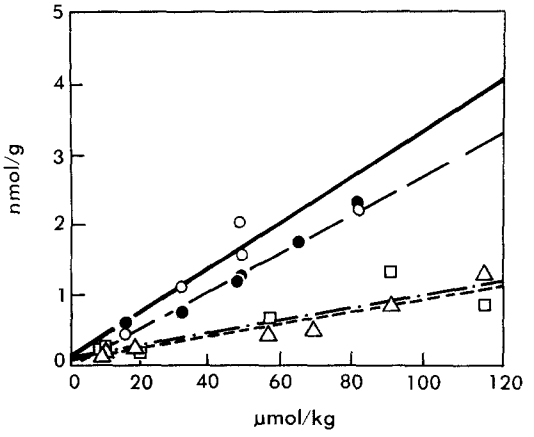

Fig. 3. Amount of ^{14}C-BCNU and ^{14}C-CCNU radioactivity bound to DNA of rat brain (——○ BCNU; – – △ CCNU) and ic 9L tumors (– – –● BCNU; – · –□ CCNU) following increasing doses (μm/kg) of each drug. Each point represents one animal sacrificed 30 min after injection. The lines were fit by the method of least squares

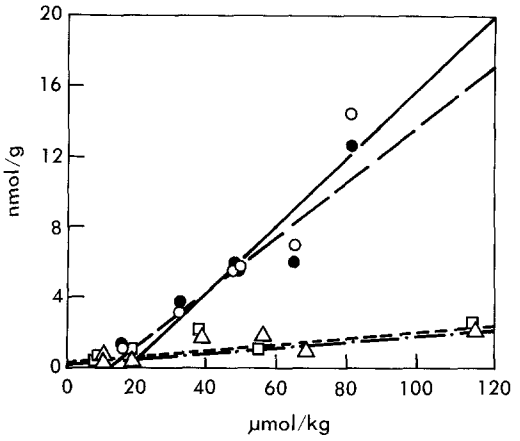

Fig. 4. Amount of ^{14}C-BCNU and ^{14}C-CCNU radioactivity bound to RNA of rat brain (——○ BCNU; – – –△ CCNU) and ic 9L tumors (– – –● BCNU; —·—□ CCNU) following increasing doses (μm/kg) of each drug. Each point represents one animal sacrificed 30 min after injection. The lines were fit by the method of least squares

tively. DNA binding is three times as great for BCNU as for CCNU, and this binding is reasonable linear with increasing doses for both drugs (Fig. 3). The same relationship holds for drug bound to RNA (Fig. 4), except that there is nine- to tenfold more BCNU than CCNU bound to RNA. Again, the binding of BCNU and CCNU to RNA is also reasonable linear with increasing dose, although the correlation coefficients for CCNU binding to brain and tumor RNA are < 0.80.

Group 3. Following the infusion of ^{14}C-CCNU and ^{14}C-BCNU, partition ratios were computed (nmoles of tissue/nmoles of plasma) for the drug found in DCM solvent, for parent drug, and for total drug (Table 2). The DCM drug fraction contained the parent nitrosourea as well as certain intermediates and metabolites (e.g., hydroxylated CCNU, certain amines). Since the plasma steady state for the parent nitrosourea and DCM fraction (± 5%) was better than that for the total drug radioactivity (± 15%), the DCM and parent drug partition ratios are more reliable. For CCNU, the highest DCM ratios were obtained in fat and kidney and the lowest in muscle. Maximum ratios for parent CCNU also were obtained in fat and kindey. All other CCNU ratios were approximately 1.

For BCNU, the DCM ratios were highest in kidney and fat. Parent BCNU ratios paralelled DCM ratios. A comparison of BCNU and CCNU ratios (BCNU/CCNU) showed dramatic differences for kidney (4.9 for DCM; 3.2 for parent species), fat (0.4 for parent species), brain (0.4 for parent species), and liver (1.8 for DCM; 0.2 for parent species).

Discussion

In an earlier study (Levin and Kabra, 1974), we evaluated the antitumor activity of six nitrosoureas of differing log P against ic 9L tumors and found that: (1) BCNU was 1.8 times more active than CCNU (μmole/kg basis) in extending median survival time; (2) 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU; NSC-95466), the most active compound, had a log $P \simeq 0.4$; and (3) antitumor activity in ic 9L tumors was positively correlated to alkylating activity and rapid spontaneous nonenzymatic transformation, corroborating the findings of Wheeler et al. (1974). Although we concluded that low lipophilicity, high alkylating activity,

Table 1. Slopes, intercepts, standard error of estimate, standard deviation of slopes, and correlation coefficients for Figures 2–4

Experiment	Intercept	Slope	SE Estimate	SD Slope	Correlation Coefficient
Total drug					
BCNU: brain	− 19.13	1.25	11.61	0.228	0.94
BCNU: tumor	− 21.01	1.35	13.15	0.259	0.93
CCNU: brain	− 1.78	0.56	2.77	0.027	0.99
CCNU: tumor	− 2.72	0.59	5.46	0.054	0.98
DNA-bound					
BCNU: brain	0.07	0.033	0.476	0.0094	0.87
BCNU: tumor	− 0.10	0.028	0.122	0.0024	0.99
CCNU: brain	0.08	0.009	0.285	0.0028	0.80
CCNU: tumor	0.05	0.009	0.248	0.0025	0.85
RNA-bound					
BCNU: brain	− 3.10	0.190	1.63	0.032	0.95
BCNU: tumor	− 1.82	0.155	1.50	0.029	0.93
CCNU: brain	0.34	0.016	0.52	0.006	0.79
CCNU: tumor	0.38	0.016	0.57	0.006	0.76

and rapid chemical transformation were necessary for optimal antitumor activity in ic 9L tumors, the relationship of chemical factors, such as log *P*, alkylating activity, and the rate of transformation, to in vivo antitumor activity still remained problematical. Therefore, we planned our present study to evaluate BCNU and CCNU with respect to their in vivo pharmacokinetics, nucleic acid binding, and tissue/plasma partitioning activities.

Since it has been suggested that the primary antitumor action of nitrosoureas is related to alkylating activity, and since one mole of BCNU or CCNU will decompose to yield one mole of alkylating species derived from the chloroethyl moiety (BCNU will also form a small amount of the alkylating species 2-chloroethylamine), we hypothesized that chloroethyl moieties from BCNU and CCNU reflect the antitumor activity of these drugs.

Unfortunately, the radioactive BCNU used in this study was a mixture of 1-N-(2-chloro[$^{14}C_2$]ethyl)-2-N-(2-chloroethyl)-2-N-nitrosourea (1) and 1-N-(2-chloro[$^{14}C_2$]ethyl)-2-N-(2-chloroethyl)-1-N-nitrosourea (2), in which 50% of the radioactivity is associated with the chloroethyl group attached to the urea nitrogen bearing the nitroso group. While the precise mechanism of BCNU antitumor activity is not fully understood, it has been suggested that the alkylating moiety is attached to the urea nitrogen bearing the nitroso group (Cheng et al., 1972; Colvin et al., 1976). On the basis of the mode of decomposition suggested by Colvin et al. (1976), Figure 5 shows that the BCNU mixture used in this study would decompose to equimolar amounts of four compounds. The potential alkylating agents 3 and 6, or reactive species derived from them, are identical in every respect, with the exception that 6 is labeled; the same is true for the potential carbamoylating agents, where 4 contains the label. Thus, 50% of BCNU chloroethyl moieties that could alkylate nucleic acids by this route will contain radioactivity, and the total nmoles of BCNU chloroethyl groups bound to nucleic acid can be approximated by multiplying the nmoles of *radioactive* chloroethyl groups bound to nucleic acids by a factor of 2. Since BCNU is a poor carbamoylating agent (Wheeler et al., 1974), the contribution of labeled carbamoylating moieties to total bound radioactivity should be negligible.

This interpretation, however, does not take into account the fact that the chloroethyl group not associated with the nitroso group can form the potential alkylating species 2-chloroethylamine (Weinkam et al., in press), which is not as reactive an alkylating species as the diazohydroxide formed from BCNU. If we considered it a possible source of nucleic acid bound radioactivity, it might be more appropriate to multiply the number of nmoles of radioactivity bound to nucleic acid by 1.5 instead of 2. But even then BCNU nucleic acid binding would exceed that for CCNU (Figs. 3, 4, 6, and 7).

To verify experimentally that it is the drug labeled in the chloroethyl moiety that accounts for the major portion of the CCNU and BCNU radioactivity bound to nucleic acid, we performed a comparison trial to determine the dose-dependent binding of ^{14}C-cyclohexyl-labeled CCNU to DNA and RNA, using six of the animals in Group 2. Figures 6 and 7 show that the CCNU labeled in the cyclohexyl moiety did not bind to RNA and DNA as effectively as did the CCNU labeled in the chloroethyl moiety; the lower portions of the graphs represent the additive nucleic acid binding of the chloroethyl and cyclohexyl moieties of CCNU. The upper portion of these graphs represents the extrapolation of comparative BCNU binding (Figs. 3 and 4). Under these circumstances, we found that ^{14}C-BCNU binds threefold more to DNA and fourfold more to RNA than does ^{14}C-chloroethyl-CCNU. This, together with the extrapolation based on the foregoing assumptions, appears to support the contention that chloroethyl-labeled BCNU binds to nucleic acid more effectively than chloroethyl-labeled CCNU.

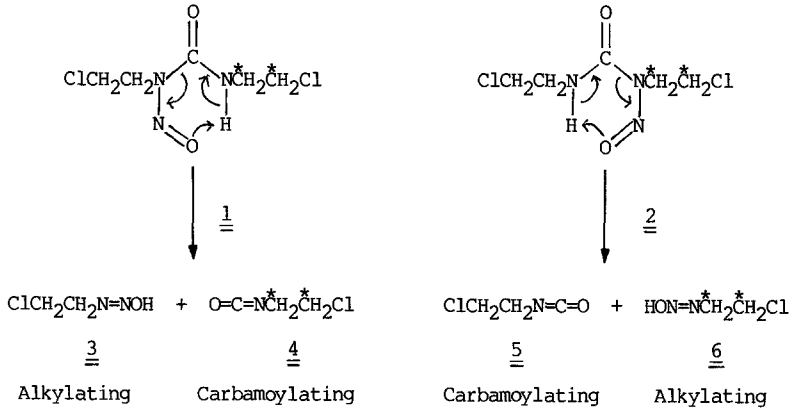

Fig. 5. Probable mode of decomposition of ^{14}C-BCNU used in this study, after Colvin (1976)

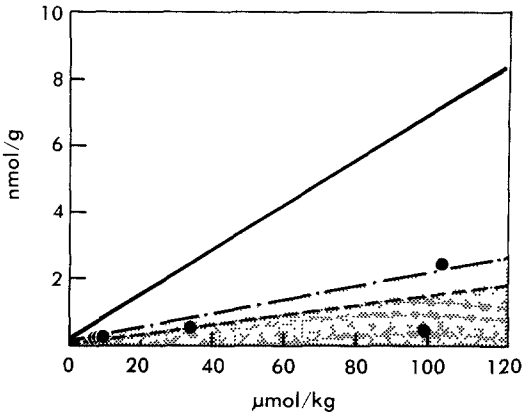

Fig. 6. Comparison of total ^{14}C-BCNU (theoretically twice that shown in Fig. 3) binding to brain DNA (——) and the total ^{14}C-CCNU (– – –● cyclohexyl; — · — chloroethyl) binding to DNA, each as a function of increasing doses of drug. The ● points represent one animal at each point. The lines were fit by the method of least squares

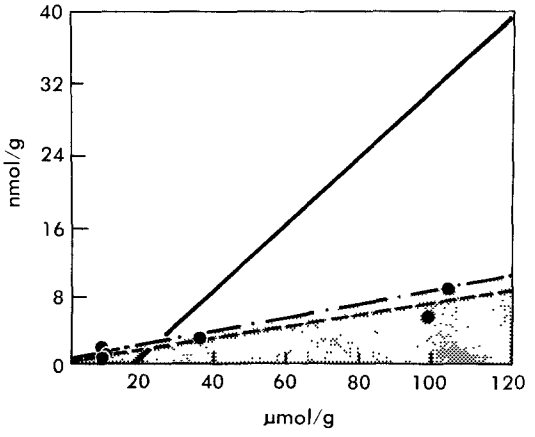

Fig. 7. Comparison of total ^{14}C-BCNU (theoretically twice that shown in Fig. 3) binding to brain RNA (——) and the total ^{14}C-CCNU (– – –● cyclohexyl; — · — chloroethyl) binding to RNA, each as a function of increasing dose of drug. The ● points represent one animal at each point. The lines were fit by the method of least squares

From organ uptake data presented in Fig. 1, the major differences between CCNU and BCNU appear to be their relative distributions in kidney and fat. In kidney, more total radioactivity, organic-soluble drug, PCA-soluble drug, and parent species were found in the animals treated with BCNU. This is no doubt a consequence of the greater aqueous solubility of BCNU. In fat, however, there is more total radioactivity, organic-soluble drug and parent species for the animals treated with CCNU. This also is to be expected, since CCNU is more lipophilic (log $P = 2.8$) than BCNU (log $P = 1.5$). The finding that CCNU and BCNU peak in fat after 15–20 min, whereas they peak in all other tissues but muscle at 5 min, can be explained by the facts that both CCNU and BCNU are limited in their distribution and transport in the body by blood flow, and that the blood flow to fat and nonexercising muscle is considerably slower (on a ml/g/s basis) than to other organs.

Because the liver receives a high blood flow and is the site of many chemical reactions, it would be expected to have relatively high levels of both BCNU and CCNU. Figure 1a–c shows that levels of both the drugs in the liver, while lower than in kidney and fat, are higher than in all other organs. Since there are relatively large amounts of PCA-soluble and RNA-bound drug in the liver, conversion to reactive intermediates is also rapid.

Very little intact CCNU or BCNU is present in the liver, where CCNU is metabolized rapidly to hydroxylated intermediates ($T_{1/2} = 5$ min) (Hilton and Walker, 1975), which may partially explain the CCNU findings. It does not explain the data for BCNU, for which the nonenzymatic breakdown in liver supernatants and microsomes is quantitatively greater and more rapid than metabolic breakdown (Weikam et al., 1978).

Kinetic studies of CCNU and BCNU in brain (Fig. 1a) show modest differences between them in the uptake and distribution of total radioactivity and organic-soluble drug over a 40-min period. However, total radioactivity and organic-soluble radioactivity diminish more rapidly in the CCNU animals than in those treated with BCNU. In addition, although the differences obtained for PCA-soluble and nucleic acid-bound drug appear to be slight, they are statistically significant. With increasing doses, more BCNU than CCNU bound to DNA and RNA (Figs. 3 and 4), even after correction for cyclohexyl-labeled CCNU binding (Figs. 6 and 7). Parent CCNU levels in brain were lower than parent BCNU levels. Considering the amount of organic-soluble drug present, the parent CCNU levels were lower than would be expected, no doubt because much of the CCNU had been converted to hydroxylated species that were not observed in our HPLC separations for CCNU.

The tissue/plasma partition ratios (Table 2) corroborate the finding that CCNU attains higher parent drug levels and organic-soluble drug product(s) levels in fat, and higher parent drug levels in liver and brain. When the total drug (C) in the various organs (Fig. 1) was computed over a period of 40 min (T), all organs that we evaluated, other than fat, had BCNU levels equal to or greater than CCNU. Table 3 summarizes the $C \times T$ for striated muscle, fat, liver, brain, kidney, and lung with computations based on an 8-μmol dose delivered to a 200-g rat. Using this model, the maximum drug exposure over a 40-min period would be 320 μmol. The greater lipophilicity of CCNU results in its greater uptake in fat: this alone accounts for its diminished relative organ uptake in the remaining tissues. The greater aqueous solubility of BCNU, which is reflected in its

Table 2. Tissue/plasma partition ratios following 95–120 min at a constant plasma drug level[a, b]

Tissue	CCNU			BCNU			BCNU : CCNU		
	DCM	CCNU	Total	DCM	BCNU	Total	DCM	Parent	Total
Striated muscle	0.6	1.4	0.5	0.6	1.4	0.5	1.0	1.0	1.0
Fat	3.1	13.0	1.8	2.9	5.6	2.1	0.9	0.4	1.2
Liver	0.8	0.9	2.5	1.4	0.2	4.1	1.8	0.2	1.6
Brain	0.8	1.4	0.6	0.8	0.6	0.9	1.0	0.4	1.5
Kidney	2.3	3.2	3.5	11.3	10.0	11.5	4.9	3.1	3.3
Lung	1.0	1.4	1.1	1.0	1.3	1.7	1.0	0.9	1.5

[a] Two animals per drug and two tissues per animal
[b] Parent drug and dichloromethane (DCM)-soluble drug product levels were maintained constant (± 10%) during the infusion period. Total drug radioactivity fluctuation was greater (± 20%)

Table 3. Organ-specific concentration × time (C × T)[a] for total CCNU and BCNU following a single 40-μmol/kg dose in 200-g Fisher 344 rats

Organ	Weight[b] (g/200 g)	μmol × 40 min		BCNU : CCNU
		CCNU	BCNU	
Striated muscle	100.0	53.2	64.0	1.2
Fat	50.0	224.7	156.6	0.7
Liver	6.7	16.2	25.2	1.6
Brain	2.4	2.6	2.8	1.1
Kidney	2.2	6.9	29.5	4.3
Lung	1.6	1.9	3.0	1.6
Total	162.9 (81%)	305.5 (95%)	281.2 (88%)	

[a] Percentage of total drug C × T in 40 min (e.g., 8 μmol × 40 min)
[b] Spector, 1956; Bischoff, 1971

uptake in kidney, does not seem to influence its distribution in other organs as greatly.

Our conclusion that BCNU breaks down more rapidly than CCNU in vivo is based on the observations that (1) there are more free parent CCNU and organic soluble products (probably hydroxylated CCNU) than free BCNU in fat; (2) there is more nucleic acid-bound BCNU in brain, ic 9L tumor, and liver; and (3) there is more total BCNU radioactivity in muscle, liver, kidney, and lung. These observations parallel in vitro findings with aqueous solutions (Montgomery et al., 1967; Wheeler et al., 1974; Hilton and Walker, 1975).

Thus, the greater extent of BCNU binding than CCNU binding to nucleic acids can be attributed to its lesser lipophilicity and more rapid spontaneous nonenzymatic breakdown to a reactive intermediate. The findings presented here support our hypothesis that the relative in vivo nucleic acid-binding affinities of BCNU and CCNU are related to their proven antitumor activities (Levin and Kabra, 1974).

As a corollary to this study, we examined the possibility that the relationship between log P and the tissue/plasma ratio might be helpful in predicting the organ site tumors that would benefit most from treatment with nitrosoureas of specific log P values. However, we found that very little information from this experiment can be applied without the study of additional nitrosoureas and other extracerebral tumors. On the basis of our studies of the ic 9L tumor, it is unlikely that the relationship is direct, since the rate of chemical transformation to reactive intermediates is probably as important as the extent of partitioning in various organs. Investigations must be carried out in individual tissues and tumors before a relationship can be established.

It is reasonable to view nitrosoureas such as BCNU and CCNU as pro-drugs. The extent of pro-drug distri-

bution is dependent on log *P*. High log *P* drugs, such as CCNU, are distributed more extensively in body fat, while the less lipophilic drugs such as BCNU have a smaller distribution volume and are therefore likely to accumulate in greater amounts in body organs other than fat. The ultimate cytotoxicity and fate of the nitrosourea pro-drugs will depend on their rate of intracellular biotransformation to reactive intermediates with alkylating capability. From these studies, it appears that the rate of intracellular transformation of such alkylating agents as the nitrosoureas may be more important than log *P*.

The comparative pharmacokinetic data obtained for BCNU and CCNU in our study should be supplemented with data from similar studies of additional nitrosoureas. The cumulative data would allow more precise correlation of chemical factors (log *P*, alkylating activity, rate of chemical transformation) with in vivo tumor cell kill, drug pharmacokinetics, and nucleic acid binding. From these data, it may be possible to construct an appropriate 'structure/activity' relationship that could be directly applicable to clinical chemotherapy of solid tumors.

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