Pharmacological Disposition of 1-(2-Chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea in Mice

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ABSTRACT

1-(2-Chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU; NSC 95466) is a lipid-soluble nitrosourea that is presently in clinical trial. We have studied the pharmacological disposition of [ethyl-14C]PCNU in mice using an i.v. drug dose of 20 mg/kg animal. Disappearance of total radioactivity from plasma was biphasic with mean half-lives of the two exponential phases of 21.7 min and 27.4 hr, respectively. The plasma half-life of intact drug was 29 min, and levels of intact drug, as measured by thin-layer chromatography, fell below detectable levels by 4 hr. The area under the plasma concentration-time curve for intact drug was 32.72 nmol/hr/ml. Computer analysis of the data for total radioactivity (PCNU equivalents), based upon an open two-compartment model, yielded values of the pharmacokinetic parameters K12, K21, and K10 of 1.49 hr⁻¹, 0.25 hr⁻¹, and 0.19 hr⁻¹, respectively. The highest peak organ level of drug was 168.9 nmol of PCNU equivalents per g tissue in the liver 1 hr after drug administration. Maximum levels in kidney, lungs, heart, and spleen were observed at 5 min, with values of 119.5, 115.4, 80.3, and 66.7 nmol of PCNU equivalents per g of tissue, respectively. A peak high drug level in brain (50.6 nmol/g) agreed with the prediction that PCNU can cross the blood-brain barrier. The levels of intact drug relative to total radioactivity at 30 min were 60% in brain, 55% in heart, and 48% in spleen. The concurrent value in liver was 7% of the total radioactivity, suggesting that metabolism or decomposition of PCNU occurs in this organ. The principal excretory route of [ethyl-14C]PCNU was urinary, with a cumulative excretion of 62% in the first 24 hr.

INTRODUCTION

The chloroethylnitrosoureas are important cytotoxic drugs with clinical activity against a variety of hematological and solid cancers (1). A new member of this class of compounds is PCNUP (Chart 1). Interest in PCNU as a clinically active agent focuses on its high alkylating activity, which is 3- to 4-fold greater than that of CCNU (8), and on its favorable solubility characteristics. Recent studies show close correlation between the antitumor activity of nitrosourea compounds and their alkylating activity (15, 16). While nitrosourea decomposition also produces chemically reactive organic isocyanates that can carbamoylate protein (18), there has been no convincing demonstration that this contributes to either antitumor activity or hematological toxicity (6, 15-17). The solubility of the nitrosoureas also determines, in part, their antitumor activity. The octanol-water partition coefficient P (4) is a critical determinant of the ease with which they cross cell membranes and the blood-brain barrier. A recent study of a large-scale structure-activity relationships between nitrosourea compounds showed a parabolic correlation between antitumor activity and log P, with maximum antitumor activity obtained for values of log P between -0.20 and 1.34 (5, 13). Also, Levine and Kabra (9) studied a series of 6 nitrosoureas and found that PCNU, with a log P of 0.37, had optimal activity against intracerebral rat 9L sarcoma. On the basis of its high alkylating activity and favorable solubility characteristics and activity against intracerebral tumors in test animals (8), PCNU has excellent potential for clinical activity, especially against brain tumors.

PCNU is currently in Phase II clinical trial. The present study of its distribution in mice was performed to complement studies in humans and to clarify its mode of disposition.

MATERIALS AND METHODS

[ethyl-14C]PCNU, specific activity 15.3 µCi/mmol, was obtained through Dr. Robert Engle, Developmental Therapeutics Program, National Cancer Institute, Bethesda, Md. Female BALB/c x DBA/2 F1 (hereafter called CD2F1) mice were kindly supplied by Hazeltan Laboratories (Vienna, Va.) and were maintained on Purina chow and water ad libitum. Silica gel 60 F254 precoated thin-layer chromatography sheets (MC/B, Cincinnati, Ohio) were used for thin-layer chromatography of tissue homogenates and urine. NCS tissue solubilizer and ACS counting scintillant were obtained from Amersham/Searle Corp. (Arlington Heights, Ill.), and Biofluor high-efficiency emulsifier cocktail from New England Nuclear (Boston, Mass.).

Distribution of Radioactivity. The distribution of radioactivity among the organs of the CD2F1 mouse was determined at multiple time points up to 24 hr following a single i.v. dose of PCNU, 20 mg/kg. Each mouse received 2 µCi of radiolabeled drug in the total dose. A solution of 14C-labeled drug was prepared immediately prior to use by dissolving the dry crystals in a small volume of N,N'-dimethylacetamide and diluting to 20 mg/ml with propylene glycol. An appropriate amount of similarly prepared unlabeled PCNU was added to make up the total dose, and the resulting mixture was diluted to volume with physiological saline.

At each time point, a group of 3 mice was sacrificed by cervical dislocation after plasma had been collected from the retroorbital sinus. The organs were rapidly excised, rinsed in PBS, and quickly frozen in a dry ice bath. All samples were stored at -20° until assay. The organs were homogenized in 2 to 4 parts ice-cold PBS using a Polytron homogenizer. Aliquots of the homogenates representing 0.1 to 0.2 g of tissue were transferred to glass scintillation vials, digested overnight in 0.6 ml of NCS, and counted in 20 ml of ACS. The binding of 14C to TCA-precipitable macromolecules was determined by precipitation of small aliquots of tissue homogenates with 5% TCA at 0° for 1 hr. The precipitates were washed twice with 5% TCA and counted as described.

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3 The abbreviations used are: PCNU, 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea; PBS, phosphate-buffered saline (0.01 M phosphate buffer, pH 7.4, 0.85% NaCl); TCA, trichloroacetic acid; AUC∞, area under the plasma concentration-time curve; BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea.

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activity as a function of time after drug administration is shown in Table 2. The level after 2 min was 93 μM, which then decayed in a biphasic fashion. The data for plasma disappearance of total radioactivity were fitted to a 2-compartment open model using the NON-LIN program of Metzler et al. (11). The values of the pharmacokinetic parameters obtained from this analysis are shown in Table 1. The half-lives of the α- and β-phases were 21.7 min and 27.4 hr, respectively. The AUCα—β is 517.61 nmol-hr/ml. The rate of drug transport from the central compartment to the peripheral compartment, K21, is 1.49 hr⁻¹, which is about 6-fold faster than the reverse process, K12.

Chart 3 shows the distribution of radioactivity in various mouse organs at serial time points after PCNU administration. Peak levels were reached within 5 min in most organs. However, the highest levels were in liver and occurred at 1 hr, after which they declined rapidly. The initial peak levels of radioactivity in kidney and lung were also high. Distribution of radioactivity into brain

RESULTS

All results reported in the following sections relate to the administration to individual mice of a single 20- mg/kg i.v. dose labeled with 2 μCi of [ethyl-¹⁴C]PCNU.

Total Radioactivity. In this section, the total radioactivity in plasma and tissues is expressed as the molarity of PCNU equivalents; i.e., the conversion of radioactivity to molarity is based entirely upon the molecular weight of PCNU. Total plasma radio-

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**NOTES**

- **Table 1**
  - Computer-derived pharmacokinetic parameters of [ethyl-¹⁴C]PCNU, based on a 2-compartment open model
  - **C** = 87.01e⁻¹·hr⁻¹ + 11.95e⁻¹·hr⁻¹
  - **A** = 87.01 nmol/ml
  - **B** = 11.95 nmol/ml
  - **α** = 1.91 hr⁻¹
  - **β** = 0.0253 hr⁻¹
  - **t1/2α** = 21.7 min
  - **t1/2β** = 27.4 hr
  - **AUCα—β** = 517.61 nmol-hr/ml
  - **K12** = 1.49 hr⁻¹
  - **K21** = 0.25 hr⁻¹
  - **K12** = 0.190 hr⁻¹
  - **V1** = 0.76 liter/kg
  - **V2** = 5.01 liter/kg
  - **Vss** = 5.77 liter/kg

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**Figure 1**

**Figure 2**

**Figure 3**

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**Chart 1.** Structure of PCNU. Asterisk, position of ¹⁴C.

**Chart 2.** Disappearance of total radioactivity and intact PCNU from plasma of mice given [ethyl-¹⁴C]PCNU, 20 mg/kg. μM/L, μmol/liter.

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**Chart 3.** The distribution of radioactivity in various mouse organs at serial time points after PCNU administration.
Charts. Distribution of radioactivity in mouse organs following [ethyl-14C]PCNU administration. GM, g.

Disposition of PCNU in Mice was rapid and occurred simultaneously with that to visceral organs. The peak level was only slightly below that for other organs. The elimination of radioactivity from brain roughly superimposed the plasma decay curve between 1 and 24 hr, and the plasma concentration:brain concentration ratio in this time period was approximately 1.

Intact PCNU in Plasma and Tissues. The disappearance of intact PCNU from mouse plasma was determined by the colorimetric Bratton-Marshall reaction and by thin-layer chromatography (Chart 2). The computer fit of intact PCNU levels are also shown in Chart 2. The data for the 2 methods were in substantial agreement, but the thin-layer chromatography was more sensitive. The levels of intact N-nitroso group were 45 μM at 2 min, 26.7 μM at 20 min, and 20 μM (limit of detectability) at 1 hr. The intact drug levels, determined by thin-layer chromatography, were 57.4 μM at 5 min, 13 μM at 1 hr, and 0.16 μM at 4 hr. Chart 2 also shows the computerized plasma disappearance of intact PCNU. When the thin-layer data for intact drug were fitted to a 1-compartment model (Table 2), the t1/2 was 28.8 min, the AUCo-∞ was 32.72 nmol-hr/ml, and the volume of distribution of the intact drug was 1.6 liters/kg. The levels of intact PCNU in selected tissues were also determined by thin-layer chromatography 30 min after drug administration. The values of intact drug as a percentage of total tissue radioactivity are shown in Table 3. The lowest intact drug levels (7%) were obtained in liver.

TCA-bound Radioactivity. Chart 4 shows the recovery of radioactivity after TCA precipitation of tissue homogenates. Drug binding increased with time and reached a peak after 4 hr in virtually all tissues examined. Maximum binding was seen in liver at 4 hr, and the value of 13.35 nmol/g represented approximately 28% of total hepatic radioactivity at 4 hr. In heart and brain, the quantities of precipitable drug at 4 hr represented about 50% of the total radioactivity present while, in kidney, lungs, and pancreas, the values were 10.43 nmol/g (22% of total), 7.55 nmol/g (30% of total), and 7.3 nmol/g (10% of total), respectively.

Studies of Urinary and Fecal Excretion. Chart 5 shows the cumulative excretion of radioactive PCNU into urine and feces. Urinary excretion was initially rapid, with up to 20% of administered 14C appearing in the 3-hr urine and 55% by 8 hr. Thereafter, the rate of excretion plateaued and reached 64% at 24 hr. Only a small portion of radioactivity was excreted in feces (4% at 24 hr). To further characterize fecal excretion of PCNU, common bile duct ligation was performed in mice maintained under pentobarbital anesthesia. One hr after drug administration, the whole gall bladder was removed, and the radioactivity of its contents was counted. Recovery was 0.2% of the total injected dose,
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Chart 4. TCA-precipitable radioactivity following [ethyl-14C]PCNU administration. GM, g.

Chart 5. Cumulative urinary and fecal excretion of [ethyl-14C]PCNU showing that biliary excretion of PCNU is minimal compared to urinary excretion.

DISCUSSION

These studies of PCNU distribution in mice confirm the rapid clearance of intact drug from plasma and its appearance in many organs, including brain. The entry of other lipophilic nitrosoureas such as BCNU and CCNU into the central nervous system has been studied previously in animals and man. DeVita et al. (2) described levels of total radioactivity of [14C]BCNU in mouse brain that were comparable to those in lungs, spleen, liver, and heart after i.p. drug administration. Levin et al. (10) have studied the distribution of BCNU and CCNU in the rat after a dose of 40 μmol/kg and observed brain levels of intact drug of 8 nmol/g and 5 nmol/g, respectively, 30 min after drug administration. In the present study, a dose of PCNU (20 mg/kg; 76 μmol/kg) in the mouse has produced brain levels of intact drug of 24 nmol/g. This dose of PCNU is equivalent to 40 μmol/kg in the rat, using a conversion factor of 2, so that the observed brain levels are higher than would be expected for equivalent dosage of BCNU or CCNU. The levels of PCNU equivalents in the mouse liver, 168 nmol/g, are comparable to those found in rats following an equivalent dose of BCNU and CCNU. However, the low hepatic levels of intact PCNU, as compared to other organs, as seen in other studies (10), suggests a rapid conversion of the parent drug to its reactive intermediates in this organ.

The clearance of intact PCNU from mouse plasma fits a single compartment model. The t1/2 for this process is 28.8 min, which is longer than the value of 5 to 10 min reported for CCNU in mice (14). This rapid clearance of intact drug coincides with the initial disappearance of total radioactivity from plasma (Chart 2). The AUC0-∞ values of intact PCNU are 15-fold less than the values for total radioactivity, whereas the volume of distribution of intact PCNU is 3-fold less than the volume of distribution of total radioactivity (Table 1 and 2). The prolonged second phase of disappearance of total radioactivity presumably represents the persistent products of drug metabolism and decomposition which are progressively removed from tissue and excreted by the kidneys. This biphasic elimination of total radioactivity is similar to the patterns for BCNU and CCNU in various animals, including mice, dogs, monkeys, and humans (2, 14). Both BCNU and CCNU are excreted predominantly by the urine. Biliary excretion is a secondary route for BCNU (2), similar to the pattern for PCNU. The water-soluble nitrosourea 1-(2-chloroethyl)-3-glucosyl-1-nitrosourea also exhibits biphasic clearance in mice (12).

The amount of radioactivity associated with TCA precipitation
of tissue homogenates showed a peak at 4 hr after drug administration. The persistence of binding of radioactivity to these macromolecules may influence the prolonged second phase of disappearance of the radioactive metabolites from plasma, with a possible effect on antitumor activity or systemic toxicity.

Mathematical analysis of the data of total radioactivity has been based upon a 2-compartment model to provide pharmacokinetic parameters related to the disposition of radioactive moieties of drug. There is rapid distribution between a relatively small central compartment and a larger peripheral compartment with a high value of $K_{12}$ and a smaller $K_{21}$ (Table 1). The apparent volume of the peripheral compartment $V_2$ is almost 6-fold greater than the central compartment $V_1$, and the volume of distribution,

$$V_{tec} = V_1 + V_2$$

is 5-fold greater than is the actual body volume. These data indicate wide distribution and extensive tissue uptake of parent drug and its radioactive metabolite (Tables 1 and 2).

The importance of these observations to the clinical situation lies in their confirmation of high levels of central nervous system uptake of drug, perhaps in excess of those to be expected for BCNU and CCNU, as well as the clarification of routes of excretion. The pharmacokinetics of PCNU and its radioactive metabolites in the rodent are qualitatively similar to those observed in human trials (19). The animal data on intact drug levels in liver, brain, and other organs extend the observations that can be made in humans. Correlation of both animal and human pharmacokinetic data with clinical efficacy will be of considerable interest in the future.

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