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ACKNOWLEDGMENTS

Abstracted from a thesis submitted by M. M. Karkaria to the Massachusetts College of Pharmacy in partial fulfillment of the Master of Science degree requirements.

The authors express their appreciation to The Gillette Co. for financial assistance.

Biodistribution of ^{14}C-Lomustine in an Animal Tumor Model

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Received May 14, 1979, from the *Department of Radiology, Massachusetts General Hospital, Boston, MA 02114.* Accepted for publication August 13, 1979.

Abstract □ A formulation of ^{14}C-lomustine in propylene glycol–ethanol (4:1) was administered intravenously to rats infiltrated with glioma tumors of the astrocytic series (RT6). The organ and tumor distribution of this agent was followed at 1, 4, 12, and 24 hr. Rapid blood disappearance (0–1 hr) of the label concomitant with an increase in all organs except the lung, muscle, and brain was observed. Only the blood, liver, and muscle contained >1% of the dose after 24 hr. The bladder, liver, small bowel, and kidneys concentrated the highest percentages throughout the study. The distribution of ^{14}C-lomustine in the tumor relative to the brain, muscle, and blood showed a maximum 4–12 hr after administration.

Keyphrases □ Lomustine, ^{14}C-labeled—biodistribution, rat tumor model □ Antineoplastic agents—^{14}C-lomustine, biodistribution, rat tumor model □ Biodistribution—^{14}C-lomustine, rat tumor model

Early detection of malignant tumors serves as a basis for improving cancer control. One experimental approach is to label minute quantities of antineoplastic drugs with appropriate radionuclides and to follow their biodistribution in a suitable animal tumor model. One example involved the *N*-nitrosoureas, a class of chemotherapeutic drugs for various malignant tumors (1). The structures of several *N*-substituted nitrosoureas are shown in Table I, where the chlorine- or fluorine-substituted haloethyl derivatives are the most active, with the methyl-substituted nitrosourea possessing the least chemotherapeutic activity (2).

The agent investigated in this study, lomustine [1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea[1], CCNU, I], is an asymmetrical *N*-substituted nitrosourea used for the palliative treatment of primary and metastatic brain tumors and for Hodgkin's disease (3). This investigation followed the radiopharmacodynamics of ^{14}C-I in tumor-bearing rats for 24 hr after intravenous injection.

EXPERIMENTAL

Materials and Methods—The *N*-nitrosoureas are lipophilic agents whose solubility characteristics require formulations with hydroalcoholic vehicles (4). Propylene glycol–ethanol (4:1) was the vehicle chosen for intravenous administration of ^{14}C-I[1] (5).

The ^{14}C-I [1-(2-chloroethyl-U-^{14}C)-3-cyclohexyl-1-nitrosourea] had a specific activity of 12.156 mCi/mmole (52.017 μCi/mg; 1.0195 mCi in 19.6 mg) and a radiochemical purity of 99.7%. The ^{14}C-I was labeled at the chloroethyl moiety (Table I). Prior to the animal studies, the ^{14}C-I was added to 10 ml of the propylene glycol–ethanol mixture.

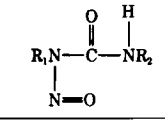
Tissue Distribution—The rat tumor model (RT6) was a brain malignancy induced by repeated intravenous injections of *N*-nitrosomethylurea (6). The induced tumors were gliomas of the astrocytic series, the histology of which did not vary significantly with serial passage through tissue culture, subcutaneous implantation, or freezing (6). This model was used previously for the determination of the tumor affinity of various ^{99m}Tc-labeled compounds (7). Early detection of this type of tumor in patients with glioblastoma multiforme would be advantageous since the current survival estimate for this pathology 2 years after surgery is ~10% (8).

To determine the pharmacodynamics of ^{14}C-I, 0.2 ml was injected intravenously *via* the tail vein in adult male rats. Three rats were sacrificed at 1, 4, 12, and 24 hr postadministration. Each 0.2 ml of solution had 0.385 mg (1.65 μmoles) of ^{14}C-I (20 μCi of ^{14}C).

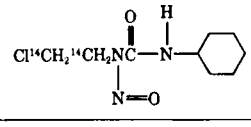
At the time of sacrifice, the blood (7% of the body weight), brain, liver, spleen, pancreas, lungs, small bowel, kidneys, heart, bladder, bone marrow, muscle, and tumor fluid, capsule, and necrotic center were isolated from each animal, and their radioactivity content was measured. The dose percentage per gram of tissue, the dose percentage per organ, and the mean ± standard deviation (n = 3) were calculated.

Activity Measurement—Approximately 100 mg of each tissue was placed in a glass scintillation vial containing 2 ml of tissue solubilizer (9).

Table I—Structures of Several *N*-Substituted Nitrosoureas



R_1	R_2	Name
Methyl	H	1-Methyl-1-nitrosourea
Fluoroethyl	Fluoroethyl	1,3-Bis(2-fluoroethyl)-1-nitrosourea
Chloroethyl	Chloroethyl	1,3-Bis(2-chloroethyl)-1-nitrosourea
Chloroethyl	Cyclohexyl	1-(2-Chloroethyl-U-^{14}C)-3-cyclohexyl-1-nitrosourea



[1] Synthesized by the Monsanto Chemical Co. under contract with the National Institutes of Health and provided by Dr. R. Engle of the National Cancer Institute (sample NSC-79037,350-4H).

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Table II—Biodistribution of ^{14}C-I in Rat Tumors (RT6) (n = 3)

Organ	Dose Percent after 1 hr		Dose Percent after 4 hr		Dose Percent after 12 hr		Dose Percent after 24 hr	
	Mean	±*SD*	Mean	±*SD*	Mean	±*SD*	Mean	±*SD*
Blood	7.851	3.031	2.899	1.450	2.461	0.229	2.218	1.764
Brain	0.112	0.005	0.056	0.020	0.091	0.003	0.009	0.003
Liver	6.133	1.550	6.150	1.700	1.763	0.846	1.017	0.346
Spleen	0.320	0.003	0.422	0.143	0.129	0.073	0.040	0.022
Pancreas	0.289	0.207	0.208	0.124	0.282	0.138	0.075	0.057
Lungs	0.204	0.006	0.154	0.046	0.114	0.016	0.033	0.009
Small bowel	1.432	0.711	2.789	0.288	0.316	0.228	0.231	0.028
Kidney cortex	1.230	0.205	1.883	0.301	0.339	0.074	0.074	0.055
Kidney medulla	0.964	0.150	0.995	0.230	0.181	0.066	0.011	0.015
Heart	0.091	0.030	0.045	0.006	0.019	0.002	0.007	0.002
Bone marrow	0.198	0.088	0.051	0.089	0.099	0.012	0.023	0.016
Muscle	13.602	3.980	6.568	0.798	1.459	0.132	1.156	0.084
Bladder	0.072	0.041	0.165	0.016	0.030	0.020	0.051	0.017
Tumor fluid	0.726	0.851	5.460	0.548	1.678	0.232	0.523	0.015
Tumor capsule	0.718	0.419	3.560	0.621	1.463	1.073	0.482	0.443
Tumor necrotic	0.641	0.474	0.310	0.149	0.189	0.129	Background	Background

Each sample was minced with a scissors, and the capped vials were stored at room temperature until dissolution occurred. A toluene-based scintillation solution [4.2 mg of 2,5-diphenyloxazole/ml and 52.5 mg of 1,4-bis[2-(5-phenyloxazolyl)]benzene/ml], 15 ml, was added to each vial, and the radioactivity content was determined for each sample (10).

RESULTS

The initial biodistribution (1 hr) and subsequent translocation data for 24 hr for ^{14}C-I are shown in Tables II and III and are illustrated in Figs. 1–3. The data are displayed *versus* time as the mean dose percentage per organ (±*SD*) (Table II), the log of the mean dose percentage per gram (Figs. 1–3), and the dose percent per gram ratio of the tumor to the brain, muscle, and blood (Table III). Each point represents the mean for three determinations.

After intravenous administration, the whole body blood level of ^{14}C-I dropped rapidly from ~8% after 1 hr to <3% after 4 hr (Table II); therefore, the amount of tracer in the vascular compartment remained constant through the termination of the experiment at 24 hr. Concomitant with its clearance from the blood, the ^{14}C-I activity showed an increased concentration in all organs except for the brain, lungs, and muscle. Thereafter, the remaining organs exhibited a tracer concentration decrease. The muscle, liver, small bowel, and kidneys concentrated the highest dose percentage after 1 hr whereas the blood, liver, and muscle contained >1% of the dose after 24 hr (Table II).

The bladder and kidney cortex contained the greatest quantity of activity per gram (Fig. 1), emphasizing the rapid kidney excretion of ^{14}C-I. In fact, the bladder activity remained maximum up to 24 hr whereas the other organs decreased to a greater extent. Figure 2 illustrates the ^{14}C-I change per gram in the blood, muscle, and brain with time. The vascular compartment remained constant after 4 hr (protein binding), but the muscle and brain decreased rapidly up to 24 hr.

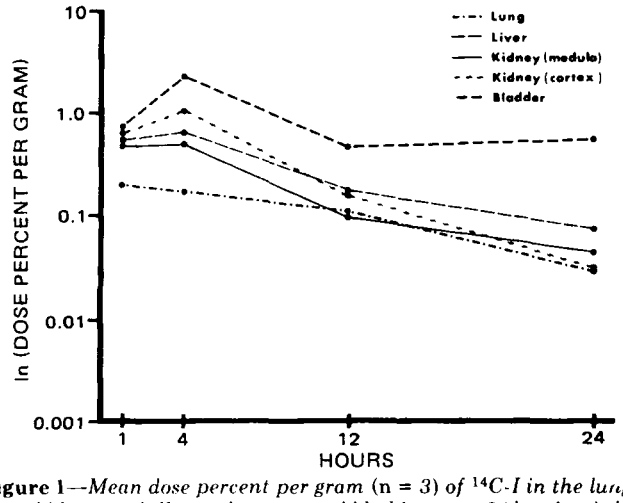

Figure 1—*Mean dose percent per gram* (n = 3) *of* ^{14}*C-I in the lungs, liver, kidney medullar and cortex, and bladder up to 24 hr after injection.*

For the tumor, a consistent ^{14}C-I uptake was observed at all times in the order: fluid > capsule > necrotic portion (Table II). The dose percentage per gram ratio of the tumor capsule to the brain rose rapidly to a maximum of 8.31 after 12 hr and decreased thereafter (Table III). Tumor to muscle and tumor to blood ratios were maximum after 4 hr and also decreased rapidly thereafter.

DISCUSSION

The localization and sizing of neoplasms were demonstrated with various tumor-specific radiopharmaceuticals (11). However, current radiodiagnostic agents are limited in their ability to be specific markers for tumors. Better definition not only could help in the identification of the histology but also could more precisely delineate and assist with effective treatment. Tumors may be defined better if radiopharmaceuticals are developed whose structures are based on chemotherapeutic agents.

The *N*-substituted nitrosoureas, a class of synthetic drugs currently studied for their anticancer properties (12), were selected for study. Their structural diversity (Table I) and limited chemical stability *in vivo* led investigators to hypothesize that their mechanism of action is alkylation (13–15). The 1-alkyl-1-nitrosoureas are believed to decompose under physiological conditions to yield alkyl diazohydroxides, which are progenitors of carbonium ions, and isocyanic acid or alkyl isocyanates, which undergo further reaction (13).

Compound I initially metabolizes to an alkyl diazohydroxide and an alkyl isocyanate. The former structure undergoes further decomposition to yield a vinyl carbonium ion, which eventually breaks down to acetaldehyde. The alkyl isocyanate reacts with water to form the corresponding carbamic acids, which decompose to yield cyclohexylamine. If the concentration of the latter compound is high, as is the case when therapeutic amounts are administered, a symmetrical urea is formed. Under the conditions of this investigation, nontherapeutic amounts of I were administered (0.385 mg of I/0.3-kg rat or 1.28 mg/kg), thus reducing the probability of a symmetrical urea. The production of alkylating agents during the *in vivo* decomposition of I results in the attack of nucleophilic

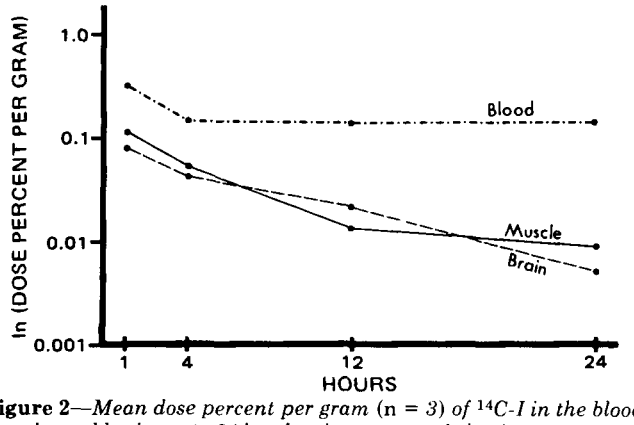

Figure 2—*Mean dose percent per gram* (n = 3) *of* ^{14}*C-I in the blood, muscle, and brain up to 24 hr after intravenous injection.*

Table III—Dose Percent Mean ^{14}C-I per Gram Ratios of Tumor to Various Organs (n = 3)

Hours	Tumor / Brain	Tumor / Muscle	Tumor / Blood
1	0.765	0.566	0.153
4	5.94	4.58	1.66
12	8.31	1.36	1.25
24	6.41	3.77	0.238

centers of biologically important molecules by these products. Reactions with amino, sulfhydryl, or hydroxyl groups yield alkylated materials, which alter the functional ability of biological materials. Such alkylation is thought to produce the mutagenic, teratogenic, carcinogenic, and cytotoxic activity of this class of chemotherapeutic agents (16).

Previous work with ^{14}C-chloroethyl-labeled I showed the label to be bound to nucleic acids and proteins both *in vitro* and *in vivo* (17). Specifically, the carbon-14 attached to nucleic acid bases and tRNA. Several investigators studied cyclohexyl derivatives of I in aninals, where extensive fixation of the label to the plasma proteins was found (18, 19). The tissue biodistribution of ^{14}C-cyclohexyl-I was studied in rabbits, where most of the label was found in the plasma, liver, kidneys, and lungs; after 12 hr, almost all of the carbon-14 label was eliminated *via* the kidneys and bile (5). The preparation and biodistribution of ^{13}N-bis(2-chloroethyl)nitrosourea, labeled primarily to the nitroso group, was reported recently (20). This positron-labeled agent was detected in a human neuroblastoma (mouse model) shortly after administration.

The results with ^{14}C-I correlate well with the literature relative to rapid blood disappearance and uptake in the liver and intestine (5, 18). The maximum tumor to organ ratios (Table III) suggest that γ-emitting radionuclides such as ^{99m}Tc ($t_{1/2p}$ = 6 hr) and ^{111}In ($t_{1/2p}$ = 2.6 days) labeled to I have potential as brain tumor scanning agents.

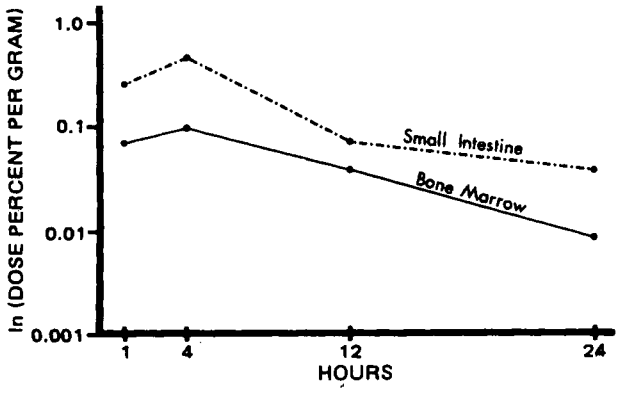

Figure 3—*Mean dose percent per gram* (n = 3) *of* ^{14}*C-I in the small intestine and bone marrow up to 24 hr after intravenous injection.*

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ACKNOWLEDGMENTS

Supported in part by U.S. Public Health Service Grant RR-05486-12.