Pharmacokinetics of Positron-labeled 1,3-Bis(2-chloroethyl)nitrosourea in Human Brain Tumors Using Positron Emission Tomography

Mirko Diksic, Kazuhiro Sako, William Feindel, Amami Kato, Y. Lucas Yamamoto, Simin Farrokhzad, and Christopher Thompson

Montreal Neurological Institute and Department of Neurology and Neurosurgery, McGill University, Montreal, Quebec H3A 2B4, Canada

ABSTRACT

The nitrosoureas are widely used in the chemotherapy of brain tumors, two of the most common being 1,3-bis(2-chloroethyl)nitrosourea and 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea. However, we do not understand how these compounds work, nor do we know which part of the molecule has antitumor activity.

In six patients with brain tumor, we measured the kinetic behavior of positron-labeled 1,3-bis(2-chloroethyl)nitrosourea in both the tumor and the normal brain with the aid of positron emission tomography; we also analyzed the distribution of radioactivity in plasma. We found the clearance of total radioactivity from the tumor to be significantly slower than from the contralateral brain and plasma, indicating a different rate of 1,3-bis(2-chloroethyl)nitrosourea decomposition in the tumor than in normal brain.

INTRODUCTION

Over the past decade, BCNU has been used increasingly to treat certain neoplastic diseases (22, 25, 26). Although widely applied for brain gliomas (12, 23, 26), its in vivo pharmacokinetics has not yet been established in humans.

Although the in vitro decomposition of BCNU in various environments has been well-documented (1, 2, 15, 19), some of the conditions under which decomposition was studied were not physiological. Levin et al. (16) studied BCNU pharmacokinetics in humans and observed that the degradation rate of BCNU in the sera of normal volunteers was greater after a meal with high lipid content than before the meal. They also measured the half-life of the BCNU clearance in patients after administration of therapeutic doses of BCNU. The behavior of this chemotherapeutic agent in the human body may well differ from that observed in vitro (22, 27). Since the antitumor action of this class of chemotherapeutic agents is related to their mode of decomposition, the identification of such differences is important.

We report now on the in vivo pharmacokinetics of 11C-labeled BCNU in the carbonyl position, and of 14N-labeled BCNU in the nitroso nitrogen atom. The biological behavior of these 2 types of BCNU was mapped by positron emission tomography to provide 3-dimensional in vivo images of uptake, distribution, and clearance of the drug in gliomas and in distant normal brain.

MATERIALS AND METHODS

Tomograph. A 2-ring positron emission tomograph, each ring containing 64 bismuth germanate crystals (24), provided full width at half-maximum resolution in the dynamic mode of about 1.7 cm. Three "slices" were obtained simultaneously. Scans were made at 30-sec intervals for the first 10 min, 2-min intervals for the next 20 min, 5-min intervals for the following 15 to 20 min, and 10-min intervals for the final 20 to 30 min.

BCNU Labeling. The 11C-labeled synthesis of BCNU is based on the reaction of 11C-phosgene with ethylenimine in ether at 5° to 7°. This synthesis yields 11C-BCU, which by nitrosation yields BCNU. Since storing and handling of ethylenimine is difficult because of its exothermic and self-sustaining polymerization reaction, we developed a new synthesis based on the reaction of 2-chloroethylamine hydrochloride with 11C-phosgene in ether or dioxane at room temperature (4). The nitrosation of 11C-BCU with NaNO2 in formic acid gives 11C-BCNU in a quantitative yield (6). Both syntheses were done at the carrier-free level ("no carrier added") to yield a product of high specific activity, about 30 mCi/mmol (140 mCi/mg), at the time of administration. The dilution of specific activity resulted from impurities in the chloride. A dose of 10 to 15 mCi of 11C-BCNU was used in the patient studies.

11N-Nitroso-labeled BCNU was synthesized by adapting the Sloan-Kettering procedure (21). The specific activity of 11N-BCNU was 10 mCi/mmol at injection time.

Handling of Blood Samples. During the scan, venous blood samples were taken every min for the first 4 min, every 2 min for the next 8 min, and then every 5 min to the end of the scan. The activity of whole blood, RBC, and plasma was measured with a well-type NaI(T1) detector connected to a single or multichannel analyzer. RBC were separated from plasma by spinning the whole blood for 1 min at 12,500 x g. Chart 1 shows a flow diagram for handling blood samples. Plasma was extracted with CHCl3, and both fractions were assayed for radioactivity. The chloroform extract was evaporated to dryness, and the residue dissolved in 20 μl of ether. About 1 μg of BCNU and BCU was added to serve as an internal standard and the mixture spotted on silica gel, thin-layer chromatograph plates. The plates were developed in a saturated solution of iodine in ethanol or ether. After development, the plates were scanned on a thin-layer radiochromatograph (a typical thin-layer radiochromatograph is shown in Chart 2) and examined under UV lamps at 254 and 366 nm. To examine the binding of BCNU or its decomposition fragments to the RBC, RBC ghosts were washed with 0.9% NaCl solution (saline) after hemolysis, and the radioactivity of the supernatant fluid was measured after 6 consecutive washings. This decreased according to a simple exponential law, proving that the drug and its products are not bound to the RBC membrane.

Patients. The BCNU studies were carried out on patients diagnosed by biopsy or surgery as having gliomas. Ten to 15 mCi of 11C-labeled BCNU in 2 ml of saline solution were injected i.v. as a bolus. Scanning started with injection of the tracer and followed the schedule mentioned earlier.

After reconstruction, the scans were analyzed by selecting a region of interest in the tumor and one in the area of normal brain tissue on the opposite side. The regional blood flow was determined by a 14CO2 scan (11, 29, 31). Two regions of interest were used in the analyses of scans. Their radii were 2 and 3 pixels, with areas of 1.3 and 3.1 sq cm, respectively.
RESULTS AND DISCUSSION

Generally, there were 2 types of tumor, one well-perfused and the other poorly perfused, as compared to the cortical gray matter measured with \([^{15}O]CO_2\) scans. The tumors always had higher blood flow than the contralateral white matter. Preliminary results of our studies on pharmacokinetics and metabolism in malignant gliomas have been reported earlier (5, 8–10, 29).

Kinetic analysis of data from the normal brain reveals that \([^{11}C]BCNU\) at first diffuses throughout the brain. Between 1 and 3 min, the distribution of \([^{11}C]\)-radioactivity correlates with blood flow (Fig. 1). (This has also been confirmed in autoradiography experiments when \([^{11}C]BCNU\) and \([^{14}C]\)-labeled 4-iodoantipyrine were injected simultaneously into a rat and the animal was sacrificed 60 sec after the start of the injection.

Table 1

<table>
<thead>
<tr>
<th>Component</th>
<th>Plasma</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ([^{11}C])-radioactivity</td>
<td>1.5 ± 0.2 (a,c)</td>
<td>4 ± 1.6 (c)</td>
</tr>
<tr>
<td>([^{11}C]BCNU)</td>
<td>1.0 ± 0.2 (b)</td>
<td>4.1 ± 2.1 (c)</td>
</tr>
<tr>
<td>Tumor</td>
<td>80 ± 15</td>
<td>266 ± 22 (d)</td>
</tr>
<tr>
<td>Contralateral side</td>
<td>120 ± 30</td>
<td></td>
</tr>
</tbody>
</table>

\(a\) Mean ± S.D.
\(b\) Significantly different—paired statistics, \(p < 0.05\).
\(c\) Statistically the same—paired statistics.
\(d\) Clearance of tumor and plasma by paired statistics differs at 0.001 level of significance.
\(e\) Difference significant at \(p < 0.001\), paired statistics.

BCNU Pharmacokinetics with Positron Emission Tomography

Scans of a malignant glioma taken 30 and 55 min after injection (Fig. 1) show radioactivity accumulating in the tumor. The lack of blood flow in the necrotic area results in the radioactivity entering only by diffusion. The clearance half-life of the necrotic and cystic area is relatively long (~500 min) and cannot be properly evaluated in the time scale used in our experiments. Because they are dead tissue, cystic and necrotic areas were excluded in calculations of the average half-life of the clearance given in Table 1. The edematous zone around the tumor exhibits a half-life clearance between that of normal brain and tumor (100 to 160 min). (This zone was not included in Table 1 under tumor clearance half-life, because the tissue volume analyzed is a variable mixture of solid tumor, peritumoral tissue, and relatively "normal" brain.)

BCNU is unstable and decomposes both in vitro and in vivo (1, 2, 15, 17, 19, 28) at a rate depending on the solution used. Its decomposition fragments have antineoplastic properties (1, 2, 15, 18, 20, 28). BCNU may break down as follows (Chart 4):
M. Diksic et al.

Chart 4. Schema for decomposition of $^{11}$C- or $^{13}$N-labeled BCNU.

isocyanate (Compound 3), suggested as a product (1, 13, 18, 23, 26), can form carbamate (Compound 4), which in turn could decompose to 2-chloroethylamine (Compound 5). In this decomposition process, the $^{11}$C label in the carbonyl carbon atom would be lost from the molecule as $^{11}$CO$_2$, giving 2-chloroethylamine. Since isocyanate (Compound 3) is chemically very reactive, reaction with an amino group would occur in a short time ($t_{1/2} = 17$ sec) (14). The half-life of 2-chloroethylisocyanate has been estimated as 17 sec (14) and that of 2-chloroethylcarbocation, formed during transformation of Compound 2 into Compound 6 (Chart 4), as 2 to 3 sec (27). In this reaction, the radioactivity would become bound to a relatively large molecule and would stay in the supernatant after chloroform extraction.

The origin of BCU in the plasma is more difficult to explain. Theoretically, Compound 5 can react with Compound 3 to yield BCU 7 (Chart 4). Since the absolute concentration of BCNU injected is only about 100 $\mu$g ($\sim 0.5$ µmol), however, the likelihood of this reaction is small. A more probable explanation is that BCU derives from BCNU after oxidation in the liver. [In vitro experiments show that BCNU is oxidized by liver microsomes (13).] While this oxidation could explain BCU in plasma, it does not explain results with animal tumors, where BCU was found in the brain as well as in the tumor.5 (Recent analysis of brain tumor tissue taken during surgery after injection of [$^{13}$C]BCNU revealed [$^{13}$C]BCU in the chloroform extract of the tissue homogenate.)

Thin-layer chromatographic analysis of the plasma chloroform extract revealed that the ratio between BCNU and BCU reaches a constant value of $0.9 \pm 0.2$ (S.D.) after 25 min.

The half-life for the clearance of radioactivity from the plasma has both a fast and a slow component (Table 1), the latter being related to the absorption of lipid-soluble, $^{11}$C-labeled BCNU in the whole body. Levin et al. (16) also observed 2 components in the plasma clearance curve after chemotherapeutic doses of BCNU were administered over 32 to 45 min. Since administration in their experiments was prolonged, the comparison with short-lived components is probably not valid. However, the long-lived components observed in their experiments [$t_{1/2} \approx 80$ to 160 min (16)] are comparable to those obtained in our work (Table 1).

Brain and tumor also have fast components, which are not significantly different one from the other but are significantly slower than in plasma. This disparity can be partially explained by the difference in blood flow through brain and tumor. It is also evident that the half-life of the fast component is shorter in blood than in brain. This is not unexpected, since BCNU is metabolized in the liver (13), which should contribute to faster clearance from the blood and therefore a shorter apparent biological half-life in the blood.

Analysis of whole blood revealed that 60 to 70% of the $^{11}$C-radioactivity is in the plasma between 10 and 60 min after injection. The remainder, inside the RBC, is released after hemolysis, indicating that radioactivity is not bound to the RBC membrane. Thin-layer chromatography revealed that radioactivity inside the RBC derives from BCNU and BCU, as well as from radioactivity bound to the proteins or nucleic acids. Both the amount and the ratio between these chemical forms change with time. The BCNU:BCU ratio, as well as the radioactivity bound to the protein, do not differ significantly in RBC or in blood plasma. The ratio between the $^{11}$C-radioactivity remaining in the plasma and that extracted with chloroform increases for the first 40 to 50 min, at which point it reaches a plateau (Table 2), indicating that radioactivity bound to amino groups in plasma proteins or nucleic acids, product(s) of decomposition of [$^{11}$C]BCNU, is increasing. Similar distribution of total radioactivity was noted with implanted tumors in animals. Radioactivity bound to protein results from the reaction of 2-chloroethylisocyanate with the amino groups of a protein or nucleic acid. This can be concluded from the high chemical reactivity between isocyanate and amines.

These results suggest that both chemical and metabolic BCNU "decomposition" are different in normal brain than in the tumor. Since the blood-brain barrier is defective with these tumors, one can speculate that radioactivity bound to the protein [isocyanate (Compound 3)] will bind to amino groups of protein in the plasma. This accumulation may be due to extravasation of protein-bound radioactivity. However, this process is much slower taking 1 to 2 hr before significant amounts of labeled macromolecules are extravasated (30). If this is indeed the mechanism, the half-life for the clearance of $^{11}$C-radioactivity from the tumor should be similar to that in the blood. Our results, however, show that the clearance (half-life) times for these 2 compartments are significantly different (Table 1). Since our studies included well-perfused tumors, blood flow is obviously not the only factor responsible for accumulation in the tumor. A difference in the decomposition rate in the tumor and normal brain could explain the accumulation of $^{11}$C-radioactivity in the brain tumor observed during our in vivo measurements (Fig. 1; Chart 3; Table 1). With radioactivity cleared more slowly from the tumor than from the normal brain, decomposition of BCNU most likely occurs at a higher rate in the tumor itself. [The products of BCNU decomposition have been themselves suggested as antineoplastic agents (1, 2, 15, 18).] Since these intermediaries have short

<table>
<thead>
<tr>
<th>Time after injection (min)</th>
<th>Ratio$^a$ of [11C]BCNU</th>
<th>Ratio$^a$ of [13N]BCNU</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>1.1</td>
<td>0.29</td>
</tr>
<tr>
<td>10</td>
<td>1.20</td>
<td>0.49</td>
</tr>
<tr>
<td>15</td>
<td>1.46</td>
<td>0.92</td>
</tr>
<tr>
<td>20</td>
<td>1.77</td>
<td>1.04</td>
</tr>
<tr>
<td>40</td>
<td>2.26</td>
<td>1.36</td>
</tr>
<tr>
<td>50</td>
<td>2.56</td>
<td>1.58</td>
</tr>
<tr>
<td>60</td>
<td>2.65</td>
<td>1.65</td>
</tr>
</tbody>
</table>

$^a$ Ratio is expressed as plasma radioactivity after extraction: chloroform radioactivity. Ratios are mean values for 6 patients; S.D., 15%.
half-lives (14, 27), they would be expected to react further before diffusing out from the area of decomposition. Since our results show accumulation of radioactivity in the brain tumor and our work on animal tumors showed significant increase in the radioactivity that could not be extracted with chloroform from the (tumor) tissue homogenate, we would hypothesize that BCNU decomposes more rapidly in the tumor tissue.

$^{13}$N-labeled BCNU clears from the blood at a different rate than $^{14}$C-labeled BCNU. If BCNU decomposed only via compounds (3) and (2), the ratio between the radioactivity in plasma after extraction and that extracted into chloroform would be small. $^{[2}$-Cloroethylidiazonium hydroxide (Compound 2) loses the $^{14}$N label by decomposing to 2-chloromethanol (Compound 6) and evolving nitrogen. Furthermore, the ratio would not change with time, because gaseous nitrogen would not remain in plasma. However, radioactivity increases with time (Table 2), indicating that BCNU partially decomposes into BCU by denitroization (oxidation). A product of this oxidative decomposition would be NO$_2^-$ or NO$_3^-$, both of which would remain in plasma, increasing its radioactivity (Table 2). Digenis et al. (3) also observed 2-component clearance in experiments on $^{13}$N-labeled BCNU in rats.

Our findings through positron emission tomography imaging indicate that BCNU decomposes faster in brain tumor tissue than in normal brain. The presence of the $^{13}$N-label in the plasma after its extraction with chloroform indicates denitroization (oxidative metabolism), which could account, in part, for faster clearance, as compared to $^{14}$C-labeled BCNU, from this compound from the brain and tumor. The clearance of radioactivity from the brain gloma is slower than from the normal brain, irrespective of the blood flow through the brain tumor. In vivo behavior of $^{13}$N-labeled BCNU differs markedly from that of $^{14}$C-labeled BCNU.

Kinetic analysis of $^{13}$N-labeled BCNU positron emission tomography scans provides a means to determine the level of BCNU retention in gliomas. This information can then be used to evaluate and predict results of BCNU chemotherapy in brain tumors.

ACKNOWLEDGMENTS

We would like to thank Dr. Rhoda Blostein, University Clinic, Royal Victoria Hospital; Dr. Lawrence Panasci, Department of Medicine, McGill University; and Dr. Roger Hand, McGill Cancer Center, for valuable discussions. Our thanks to Dr. Victoria Lese for her editorial assistance, and to the staff of the Medical Cyclotron Unit and Neuroisotope Laboratory for their help.

REFERENCES

Fig. 1. Scans taken at 1 (A), 3 (B), 30 (C), and 55 (D) min after injection of $^{11}$BCNU. Arrow, tumor. N-arrow, necrotic area of tumor. T-arrow, solid part of the tumor. This necrosis as well as clinical diagnoses were positively established with computer tomographic scans, and pathological findings were established during surgery.
Pharmacokinetics of Positron-labeled 1,3-Bis(2-chloroethyl)nitrosourea in Human Brain Tumors Using Positron Emission Tomography

Mirko Diksic, Kazuhiro Sako, William Feindel, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/44/7/3120

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.