Tissue and Tumor Distribution of $^{14}$C-Penclomedine in Rats


ABSTRACT

Penclomedine, a lipophilic α-picoline derivative, is undergoing clinical development presently because of its pronounced antitumor activity against intracerebral (i.c.) tumor xenografts. Penclomedine may be metabolized in vivo to a more potent compound. Although it may be useful in the treatment of brain tumors, the drug has caused significant neurotoxicity in early clinical trials. The possibility that antitumor activity and neurotoxicity may be mediated by different mechanisms prompted a study assessing the differential distribution of penclomedine and penclomedine metabolites to brain and i.c.-implanted tumors in rats. In the present study, quantitative autoradiographic analysis demonstrated a homogenous distribution of $^{14}$C-penclomedine in all organs within 1 h of administration. Levels of $^{14}$C-penclomedine in both i.c. and s.c. tumors were three times higher than in normal brain tissue. High-performance liquid chromatography combined with gas chromatography and mass spectrophotometry demonstrated that two metabolites, O-demethyl penclomedine and penclomic acid, were responsible for most of the plasma radioactivity. Penclomic acid was also the most common urinary metabolite of penclomedine. In liver samples, although a large number of metabolite peaks were detected, no parent compound could be identified. However, in tumors and all other tissues, penclomedine was the main compound detected. The finding of penclomedine in normal brain tissue indicates not only that this drug may be useful in tumors with normal blood-brain barrier function, but also that it may be directly neurotoxic.

INTRODUCTION

Penclomedine [3,5-dichloro-2,4-dimethoxy-6-(trichloromethyl)pyridine; National Service Center number 338720], an α-picoline derivative, was selected for clinical development by the National Cancer Institute because of its pronounced preclinical antitumor activity against CD8F1 murine and MCF-7 human mammary adenocarcinomas (1) and against i.c.-implanted MX-1 xenografts (2). The antitumor activity of penclomedine against i.c.3 MX-1 xenografts was up to four times greater than that of carmustine, the most commonly used agent for the treatment of primary brain tumors. Although the mechanism of the antitumor activity of penclomedine activity is unclear, studies in leukemia cell lines that are resistant to alkylating agents indicate that penclomedine itself may be an alkylating agent (2). Penclomedine is more active in vivo than in vitro (3), suggesting that it may be metabolized to a more potent compound. Indeed, NADPH-dependent oxidative and reductive metabolites of penclomedine have been detected when penclomedine is incubated with mouse microsomal liver preparations (3); at least five metabolites are produced following the incubation of penclomedine in human liver microsomes and liver slices (4).

Neurological and hematological toxicities were the main toxicities of penclomedine in preclinical (5) and early clinical studies (6, 7). Dose-related neurotoxicity in rats was indicated by muscle tremors, incoordination, convulsions, and reduced activity. Apparently, neurotoxicity correlated with peak plasma drug concentrations, because it developed during or immediately after infusion and could be abrogated by decreasing the rate of drug administration. Similarly, in dogs, severe emesis and seizures were associated with plasma penclomedine levels above 30 μM. When penclomedine was administered as a 1-h infusion on each of 5 consecutive days to patients with advanced solid tumors, neurotoxicity, as indicated by dysmetria, ataxia, and vertigo, was also the principal dose-limiting factor (6, 7). The finding of severe toxicities at much lower peak plasma concentrations than had been reported in preclinical studies may preclude administration of the higher doses of penclomedine needed to achieve concentrations associated with optimal antitumor activity.

The mechanism of neurotoxicity of penclomedine is unknown, as is the question of whether penclomedine itself or its putative metabolites are involved. The principal objective of this study was to determine the distribution of both penclomedine and its metabolites in both control and tumor-bearing rats by using a combination of quantitative autoradiography and a sensitive HPLC assay with radiochemical detection.
MATERIALS AND METHODS

i.c. and s.c. Tumor Implantation. All animal care and experiments were performed in accordance with the guidelines set forth by the Animal Care and Use Committee of The Johns Hopkins Medical Institutions. The Walker 256 tumor used in this study was harvested from malignant ascites in Sprague-Dawley carrier rats. Two to 4 days after i.p. injection of tumor cells, the rats were anesthetized with Metafane (Pitman Moore, Washington Crossing, NJ); 20 ml heparinized PBS were injected i.p. into each animal; and 15–20 ml ascites were removed. The ascites was placed in a sterile 50-ml tube and centrifuged at 1000 rpm. This procedure was repeated at least three times, to remove RBC from the ascites. The pellet was resuspended in 20 ml of PBS; a viable cell count was performed; and the suspension was diluted to achieve a concentration of 10^7 cells/ml.

Following anesthetization with pentobarbital (50 mg/kg i.p.), the male Sprague-Dawley rats used in the study had their heads and left flanks shaved and scrubbed with Betadine (Purdue Fredrick Co., Norwalk, CT) and 70% ethanol solution. Under aseptic conditions, a 2-cm midline incision was made on the scalp to expose the sagittal and coronal sutures, and a 2-mm hole was drilled through the skull 4 mm lateral and just posterior to the coronal suture. The dura matter was pierced, and 10 μl Walker 256 cell suspension (10^7 cells/μl) were injected slowly at a depth of 3 mm. The skull was flushed with saline, and the scalp incision was closed with wound clips. The flanks of two animals were also injected with 100 μl cell suspension.

Preparation of 14C-Pencloomedine for Injection. 14C-Pencloomedine (specific activity, 17.6 and 18.9 mCi/mM; Fig. 1), which was obtained from Dr. G. Taylor (Research Triangle Institute, Research Triangle Park, NC), was stored at −20°C. The site of the radiolabel has been conserved in all pencloomedine metabolites identified to date. HPLC analysis with radiochemical detection determined that the composition of the radioactive material was 99% 14C-pencloomedine. In preparation for i.v. administration, the 14C-pencloomedine was warmed to room temperature and suspended in 100% ethanol. Liposyn III solution (Abbott Laboratories, North Chicago, IL) was then added, and the final injectable solution containing 10% ethanol was stored at −20°C.

Administration of i.v. 14C-Pencloomedine. Thirteen male Sprague-Dawley rats weighing from 360 to 530 g were anesthetized with inhaled Metafane, and 1.8 mg/kg (30 μCi/rat) 14C-pencloomedine were injected i.v. into the tail vein of each animal. Following drug administration, 250 μl of Liposyn vehicle were also injected. Animals were sacrificed by intracardiac injections of pentobarbital at either 1 or 24 h after drug administration. Immediately after death, the heart, lungs, liver, spleen, kidney, testis, intestine, brain, cervical spinal cord, and a wedge of hind limb skeletal muscle were removed and snap frozen in hexane at −40°C. All tissues were stored at −20°C prior to sectioning. A single lobe of liver, one kidney, and a section of forebrain were placed on ice for metabolite studies.

A portion of each i.c. and s.c. tumor was dissected from the surrounding brain and muscle, respectively, and placed on ice for subsequent metabolite studies. The remainder of each tumor was snap frozen in hexane at −40°C for subsequent autoradiographic analysis.

Blood, urine, and cerebrospinal fluid were collected from the animals without implanted tumors at the time of sacrifice; however, only blood and urine were obtained from tumor-bearing animals. Whole blood samples were placed in polypropylene tubes containing lithium heparin and were centrifuged at 2500 rpm for 5 min. Plasma was removed, and both plasma and packed RBC fractions were stored in polypropylene tubes at −20°C. Urine and cerebrospinal fluid were centrifuged in polypropylene microcentrifuge tubes for 5 min, and the supernatants were stored at −20°C.

Preparation of Tissues for Quantitative Autoradiography. Frozen organs and tissue samples were mounted on cryostat chucks with embedding matrix (Miles, Inc., Elkhart, IN) and cut into 20-μm sections in a cryostat (Hacker Instruments, Research Triangle Park, NC).
Table 1  14C-penclomedine concentrations in tissues and body fluids of rats without implanted tumors  

14C-penclomedine concentrations, as determined by quantitative autoradiography, 1 and 24 h after i.v. administration of 14C-penclomedine (1.8 mg/kg) to rats.

<table>
<thead>
<tr>
<th>Fluid/tissue</th>
<th>Mean (tissue) 14C-penclomedine concentration (µM)*</th>
<th>Mean tissue:plasma ratio</th>
<th>Mean (tissue) 14C-penclomedine concentration (µM)*</th>
<th>Mean tissue:plasma ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>13.8 (8.8–24.1)</td>
<td>1.0</td>
<td>4.96 (4.87–5.00)</td>
<td>1.0</td>
</tr>
<tr>
<td>Urine</td>
<td>129.7 (22–238.5)</td>
<td>9.4</td>
<td>17.36 (13.5–32.75)</td>
<td>3.5</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>0.41 (0.14–0.62)</td>
<td>0.03</td>
<td>0.15 (0.2–0.09)</td>
<td>0.03</td>
</tr>
<tr>
<td>Packed RBC</td>
<td>6.62 (2.57–7.63)</td>
<td>0.48</td>
<td>1.29 (0.73–2.55)</td>
<td>0.26</td>
</tr>
<tr>
<td>Forebrain</td>
<td>0.23 (0.15–0.3)</td>
<td>0.02</td>
<td>0.24 (0.16–0.32)</td>
<td>0.05</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.28 (0.26–0.32)</td>
<td>0.02</td>
<td>0.37 (0.22–0.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>0.27 (0.22–0.32)</td>
<td>0.02</td>
<td>0.37 (0.18–0.62)</td>
<td>0.08</td>
</tr>
<tr>
<td>Brain stem</td>
<td>0.22 (0.14–0.29)</td>
<td>0.02</td>
<td>0.36 (0.10–0.87)</td>
<td>0.07</td>
</tr>
<tr>
<td>Liver</td>
<td>12.53 (10.5–13.6)</td>
<td>0.9</td>
<td>4.79 (3.88–7.05)</td>
<td>0.97</td>
</tr>
<tr>
<td>Kidney</td>
<td>6.48 (3.9–8.9)</td>
<td>0.47</td>
<td>3.15 (2.94–3.6)</td>
<td>0.64</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.43 (0.14–58)</td>
<td>0.03</td>
<td>0.52 (0.27–0.81)</td>
<td>0.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.74 (2.3–3.09)</td>
<td>0.2</td>
<td>1.29 (0.4–2.66)</td>
<td>0.26</td>
</tr>
<tr>
<td>Heart</td>
<td>2.59 (2.1–2.99)</td>
<td>0.19</td>
<td>1.63 (0.81–2.49)</td>
<td>0.33</td>
</tr>
<tr>
<td>Lung</td>
<td>2.74 (0.83–4.42)</td>
<td>0.2</td>
<td>2.11 (0.8–2.95)</td>
<td>0.4</td>
</tr>
<tr>
<td>Testis</td>
<td>0.87 (0.8–0.97)</td>
<td>0.06</td>
<td>0.9 (0.47–1.37)</td>
<td>0.18</td>
</tr>
<tr>
<td>Intestine</td>
<td>3.55 (2.0–5.09)</td>
<td>0.26</td>
<td>1.27 (1.1–1.37)</td>
<td>0.26</td>
</tr>
</tbody>
</table>

* Values were derived from four animals in each group.

Table 2  14C-penclomedine concentrations in tissues and body fluids of rats with implanted tumors  

14C-penclomedine concentrations, as determined by quantitative autoradiography, 1 and 24 h after i.v. administration of 14C-penclomedine (1.8 mg/kg) to tumor-bearing rats.

<table>
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<tr>
<th>Fluid/tissue</th>
<th>Mean (tissue) 14C-penclomedine concentration (µM)*</th>
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<th>Mean tissue:plasma ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>6.78 (4.8–9.8)</td>
<td>1.0</td>
<td>2.13</td>
<td>1.0</td>
</tr>
<tr>
<td>Urine</td>
<td>219.3 (145.0–263.2)</td>
<td>38.7</td>
<td>8.52</td>
<td>4.0</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>3.8 (3.25–5.0)</td>
<td>0.56</td>
<td>0.47</td>
<td>0.22</td>
</tr>
<tr>
<td>Packed RBC</td>
<td>0.40 (0.3–0.49)</td>
<td>0.06</td>
<td>0.20</td>
<td>0.1</td>
</tr>
<tr>
<td>Forebrain</td>
<td>0.38 (0.3–0.49)</td>
<td>0.06</td>
<td>0.15</td>
<td>0.07</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.33 (0.22–0.42)</td>
<td>0.05</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>0.3 (0.22–0.35)</td>
<td>0.05</td>
<td>0.17</td>
<td>0.08</td>
</tr>
<tr>
<td>Brain stem</td>
<td>1.25 (0.95–1.49)</td>
<td>0.18</td>
<td>0.53</td>
<td>0.25</td>
</tr>
<tr>
<td>i.c. tumor</td>
<td>1.3 (1.4–1.23)</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>s.c. tumor</td>
<td>12.4 (10.1–14.8)</td>
<td>1.83</td>
<td>2.91</td>
<td>1.36</td>
</tr>
<tr>
<td>Liver</td>
<td>10.8 (6.3–20.0)</td>
<td>1.59</td>
<td>2.31</td>
<td>1.08</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.7 (0.3–1.2)</td>
<td>0.1</td>
<td>0.36</td>
<td>0.17</td>
</tr>
<tr>
<td>Muscle</td>
<td>2.72 (2.27–3.88)</td>
<td>0.4</td>
<td>0.89</td>
<td>0.42</td>
</tr>
<tr>
<td>Spleen</td>
<td>2.23 (0.7–4.95)</td>
<td>0.33</td>
<td>0.82</td>
<td>0.38</td>
</tr>
<tr>
<td>Heart</td>
<td>4.44 (2.43–6.22)</td>
<td>0.66</td>
<td>1.73</td>
<td>0.81</td>
</tr>
<tr>
<td>Lung</td>
<td>0.60 (0.32–0.8)</td>
<td>0.09</td>
<td>0.31</td>
<td>0.14</td>
</tr>
<tr>
<td>Testis</td>
<td>8.15 (3.25–20.5)</td>
<td>1.2</td>
<td>3.4</td>
<td>1.6</td>
</tr>
</tbody>
</table>

* Values were derived from four animals in 1-h group and one animal in 24-h group.

Inc., Fairfield, NJ) at -17°C. Six pairs of consecutive sections were selected randomly from each organ or tissue sample. One section from each pair was mounted on a gelatin-coated slide and stained with hematoxylin and eosin for histological review; the other section was mounted on a gelatin-coated glass slide, warmed to 60°C, and used for quantitative autoradiography. Slides for autoradiography were heat fixed on a slide warmer for 15 min after preparation.

14C standards (Amersham, Arlington Heights, IL) with eight levels of activity (40–1069 nCi/g tissue) and the slides containing the tissue sections for quantitative autoradiography were placed against radiation-sensitive SB5 film (Eastman Kodak Co., Rochester, NY) in an X-ray cassette and exposed for 5 days at room temperature. The films were developed with a Kodak RP X-OMAT processor.

Quantitative Autoradiography Analysis. The autoradiographs and 14C standards were digitized on a RAS-R1000 receptor autoradiography analysis system (Loats Associates, Inc., Westminster, MD). The absorbances of the 14C standards were corrected for background radioactivity and were then used...
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Fig. 2  Hematoxylin and eosin-stained sagittal section of rat forebrain (A) and the corresponding autoradiograph (B) obtained 1 h after i.v. injection of $^{14}$C-penclomedine into rats with i.c. Walker 256 carcinoma implants. The tumor areas in both sections are indicated by arrows. Similar magnifications were used for both sections.

Fig. 3  Hematoxylin and eosin-stained sagittal section of s.c. tumor (A) and the corresponding autoradiograph (B) obtained 1 h after i.v. injection of $^{14}$C-penclomedine into rats with s.c. Walker 256 carcinoma implants. The tumor areas in both sections are indicated by arrows. Similar magnifications were used for both sections.

to generate a standard curve, which converted absorbance data into dpm/mg tissue. A power function using least square regression of the absorbances of the $^{14}$C standards provided the highest coefficient of determination ($r^2 > 0.97$). The specific activity of $^{14}$C-penclomedine is known; therefore, dpm/mg tissue could be converted into units of concentration ($\mu$M).

At least three autoradiographic images from different regions of each organ sample were digitized from each animal. The absorbances of the images were used to calculate the mean and regional $^{14}$C-penclomedine concentrations for each organ. The absorbance of the entire specimen was averaged in organs with homogeneous drug distribution, as determined by minimal (<20%) variability in the absorbance of the autoradiographic image. For tissues in which there were substantial regional differences in $^{14}$C-penclomedine distribution, the average absorbance of each histological region of interest was calculated separately.

The absorbance of the least active $^{14}$C standard was equivalent to a $^{14}$C-penclomedine concentration of 2.5 $\mu$M; however, tissues with $^{14}$C-penclomedine concentrations of at least 0.2 $\mu$M could be visualized reproducibly and measured by the digitizing software. Despite the use of a 0 $\mu$M standard (background), tissue concentrations of $^{14}$C-penclomedine between 0.2 and 2.5 $\mu$M were at the lower end of the standard curve and, therefore, were subject to greater measurement error.

Metabolite Assessment. Samples of kidney, liver, forebrain, and i.c.- and s.c.-implanted tumors were weighed and homogenized in 100% methanol. Methanol was chosen to max-
imize the extraction of penclomedine and both polar and nonpolar metabolites from the tissue specimens. The tissue homogenates were centrifuged at 9000 \( \times g \) for 20 min. Following centrifugation, the supernatants were removed and stored in polypropylene tubes at \(-20^\circ C\). Prior to HPLC analysis, a 500-\( \mu l \) aliquot of each sample was concentrated to 125 \( \mu l \) on a Savant Speedvac (Savant Instruments, Inc., Farmingdale, NY).

Two hundred-\( \mu l \) aliquots of plasma and packed RBC were acidified with 100 \( \mu l \) 0.7 M ammonium phosphate (pH 2.7) and extracted with 3 ml ethyl acetate. Next, the organic phase was collected, and 50 \( \mu l \) DMSO were added. Following concentration of the extract under a nitrogen stream to \( \sim 100 \mu l \), 50 \( \mu l \) acetonitrile were added, and the resulting solutions were analyzed by HPLC. No processing of the urine and cerebrospinal fluid samples was necessary.

Samples were injected onto a Hewlett-Packard 1090 Series II HPLC system with a PV5 solvent delivery system and a diode array detector (Hewlett-Packard, Palo Alto, CA). This system was equipped with a 250-mm x 4.6-mm x 5-\( \mu m \) Alltech Adsorbosphere HS C\(_{18}\) column (Alltech Associates, Deerfield, IL). The elution of penclomedine and its metabolites was initially performed by using 100% 10 mM ammonium phosphate (pH 2.7) followed by a linear gradient elution to 100% acetonitrile in 25 min. The column effluent was monitored by both UV absorption at 240 nm and by a Radiomatic Flo-One Beta radioactive flow detector (Packard Instrument Co., Downers Grove, IL) equipped with a 500-\( \mu l \) flow cell, which used Flo-Scint IV scintillation mixture (Packard) at a 2:1 ratio.

Fifteen ml of aqueous counting solution scintillation mixture (Amersham, Arlington Heights, IL) were added to 10–20-\( \mu l \) samples of body fluids. Samples were counted in a Beckman LS8000 liquid scintillation counter (Beckman Instruments, Inc., Columbia, MD) for 1 min. The concentration of \( ^{14}C \)-penclomedine in each sample of fluid was then calculated by using the known specific activity of \( ^{14}C \)-penclomedine. The methods used for metabolite identification are described in another article.\(^4\)

RESULTS

Quantitative Autoradiographic Studies

Rats without Implanted Tumors. The tissue concentrations of \( ^{14}C \)-penclomedine at both 1 and 24 h after treatment and the ratios of tissue concentrations:plasma concentrations in animals without implanted tumors are displayed in Table 1. By 1 h after treatment, the highest concentrations of radiolabel were found in liver tissues. High concentrations were also detected in samples of kidney, spleen, heart, lung, and intestine. In these tissues, the tissue:plasma ratios of \( ^{14}C \)-penclomedine concentrations ranged from 0.19 to 0.9. In contrast, low concentrations of \( ^{14}C \)-penclomedine were found in testes, muscle, and brain. Intraregional differences in the concentrations of radiolabel in the central nervous system were not detected; however, the search for these differences was hampered by the overall low levels of radioactivity found in the brain, which approached the assay’s lower limit of sensitivity (0.2 \( \mu M \)). By 24 h after treatment, the absolute concentrations of radiolabel in the liver, spleen, and intestine had decreased, whereas \( ^{14}C \)-penclomedine levels in the brain remained constant. Tissue:plasma concentration ratios in the brain, kidney, lung, heart, and testis were 1.5–3-fold higher than ratios measured 1 h after treatment.

Tumor-bearing Rats. Following the administration of \( ^{14}C \)-penclomedine, drug distribution in tumor-bearing rats was qualitatively similar to that in rats without implanted tumors; however, drug concentrations were 2–3-fold higher in most tissues than those observed in rats without tumor implants (Table 2). This may be due, in part, to the fact that tumor-

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Metabolite Assessment

Blood. Only trace amounts of penclomedine were detectable in plasma samples 1 h after $^{14}$C-penclomedine administration. HPLC analysis demonstrated that most of the radioactivity was accounted for by two principal peaks (Fig. 4), which were identified as penclomic acid and O-demethyl penclomedine (Fig. 1). Twenty-four h after treatment, the plasma concentration of radiolabel had decreased by about 60% and consisted of only the O-demethyl penclomedine metabolite. However, most of the radiolabel detected in RBC was accounted for by the parent compound (Fig. 4). Several additional smaller peaks, including penclomic acid, were also found in RBC. Twenty-four h after treatment, the levels of radioactivity detected in RBC had declined by 80%, with the remaining radioactive species being mainly penclomedine and an as-yet unidentified metabolite that eluted at 29.8 min.

Urine. Penclomic acid was the predominant chemical species identified in urine collected 1 and 24 h after treatment (Fig. 4); penclomedine was not detected. Several additional peaks with earlier elution times were also present, particularly in those samples collected at 24 h after treatment, at which time these peaks accounted for 20–50% of the radioactivity of the urine. Neither parent drug nor metabolite peaks were detected in cerebrospinal fluid.

Liver. High concentrations of penclomic acid were detected at both 1 and 24 h after treatment (Fig. 5). Several unidentified metabolites were also detected that had elution times similar to those of the metabolites that were prominent in 24-h urine samples. One h after treatment, penclomedine was not detected in the liver tissues of 10 of 13 animals; in the remaining 3, only trace amounts of penclomedine were detected. The concentration of O-demethyl penclomedine detected also varied between animals; in some samples, its concentration was about that of penclomic acid, in others, only trace amounts were detected. Twenty-four h after treatment, penclomic acid and O-demethyl penclomedine were the only metabolites detected definitely in liver samples. Other metabolite peaks were much less prominent and were only present in trace amounts.

Kidney. The pattern of metabolites in kidney extracts was qualitatively similar to that seen in liver samples. Quantitatively, however, penclomic acid was the predominant metabolite; additional metabolite peaks with earlier and later elution times were only present in trace amounts (Fig. 5). Trace amounts of penclomedine were observed in most of the kidney extracts. The 24-h kidney samples had much less radioactivity than the 1-h samples and showed persistent trace levels of penclomedine.

Brain. In brain extracts, penclomedine was detected 1 h after treatment and was the predominant radioactive species; penclomic acid accounted for a minute portion of radioactivity (Fig. 6). HPLC analysis of extracts from 24-h samples did not reveal any penclomedine or metabolite peaks.
Tumors. By HPLC analysis, radiolabeling of extracts from i.c. tumors and normal brain tissue were comparable at 1 h after treatment and remained so at 24 h (Fig. 6). Penclo-
medine was the predominant species observed in s.c.-implanted tumors (Fig. 6). Metabolite assessment of plasma, RBC, and urine and liver and kidney extracts in tumor-bearing rats gave results similar to those observed in rats without implanted tumors.

DISCUSSION

The results of the studies described in this report indicate that 14C-penclo-
medine is metabolized extensively after i.v. ad-
ministration to rats. Two major metabolites were identified in plasma and tissue samples. The largest number of metabolite peaks was found in liver samples, which is consistent with the results of hepatic microsomal studies (3, 4). The amount of O-demethyl penclo-
medine that was detected in these samples varied substantially between animals, suggesting that this compound is an intermediate metabolite, which is converted subsequently to a more polar compound. Penclo-
ic acid and several additional metabolites detected in the liver were also found in urine and indicate that penclo-
medine is metabolized by the liver to more polar compounds, which are excreted subsequently in the urine.

The distribution of 14C-penclo-
medine to all tissues and the persistence of unmetabolized drug at these sites, despite tracer levels of penclo-
medine in plasma, are consistent with the lip-
ophilic nature of this drug. Twenty-four h after administration, autoradiography demonstrated that tissue levels of radioactivity remained elevated compared with levels recorded at earlier times. However, HPLC analysis of methanol extracts of these tissues revealed low levels of both penclo-
medine and metabolites. Further analysis demonstrated that kidney and liver samples contained high levels of unextractable radioactivity (data not shown), indicating avid binding of penclo-
medine and/or metabolites to tissue. The discrepancy between the levels of radioactivity detected by autoradiography and by HPLC is consistent with the hypothesis that either penclo-
medine or a major metabolite is an alkylating agent (2).

Penclo-
medine was the predominant species identified in brain tissue, indicating that the parent compound is most likely responsible for the central nervous system toxicity observed in animal and human studies. This hypothesis is also consistent with the observation that peak plasma concentrations of penclo-
medine correlate with neurotoxicity (5, 7). The noncumulative, reversible nature of this toxicity suggests that the nonextractable metabolites, which were observed in tissue and plasma samples, are less likely to be responsible for these central nervous system effects. Therefore, therapeutic strategies to circumvent neurotoxicity might be directed initially at the penclo-
medine itself rather than at putative metabolites. The detection of penclo-
medine in brain extracts, despite only low levels in plasma or undetectable levels in cerebrospinal fluid, indicates that drug levels in these fluids may not reflect penclo-
medine concentrations in the brain. Pharmacokinetic evaluation of penclo-
medine levels in RBC may be a better marker and warrants investigation. Regional differences in the central nervous system distribution of the radiolabel were not observed in this study; therefore, the basis for the selective vestibulocerebellar toxicity of penclo-
medine that has been observed in clinical trials is unclear. The levels of radioactivity in brain samples, however, approached the lower limits of sensitivity of the autoradiographic assay; it is possible that, with the use of therapeutic (30–70 mg/kg) rather than tracer (1.8 mg/kg) doses of penclo-
medine, intraregional differences might have been detected.

Pharmacokinetic studies of penclo-
medine in mice have demonstrated that penclo-
medine is cleared from the circulation rapidly (plasma clearance, 114 ml/min/m2) and has a large steady-state volume of distribution (4.8 liters/m2; 3). Furthermore, plasma levels of radioactivity remained elevated for up to 48 h following the administration of 14C-penclo-
medine; however, penclo-
medine was not detected in plasma for most of this time. Thirty and 14% of the total radioactivity were eliminated in urine and feces, respectively. The high level of radioactivity...
in the urine may be attributed to the urinary excretion and presence of penclomic acid and/or polar penclomedine metabolites, which were observed in rats in the present study. In addition, the persistently high levels of radioactivity in plasma reported in murine studies may be due to extensive drug metabolism similar to that in rats. The fecal excretion of the radiolabel in mice and the high levels of radioactivity demonstrated by autoradiography are consistent with a biliary route of elimination for penclomedine and its metabolites.

One h after the administration of ^14^C-pencloomedine, higher levels of radiolabel were observed in the brain, kidney, liver, and muscle of tumor-bearing rats compared with animals without implanted tumors. There are several possible explanations for these results. First, tumor-bearing animals showed evidence of focal neurological deficits and dehydration at the time of drug administration; consequently, hypovolemia may have caused selective perfusion of vital organs, such as the central nervous system. Second, the metabolism of pencloomedine might be substantially different in tumor-bearing rats (8–10). Higher rates of metabolism of fostemustine and mercaptopurine and lower rates of metabolism of cyclophosphamide and pentobarbital have been reported in tumor-bearing animals compared with controls (8, 10). Third, the tumor-bearing rats in this study were exposed to anesthetic agents 1 week prior to receiving ^14^C-pencloomedine. It is possible that these anesthetics, which are known to be modulators of cytochrome P450 microsomal metabolism, may have altered pencloomedine metabolism in the liver. Last, pencloomedine is a highly lipophilic agent; thus, adipose tissue depletion in tumor-bearing animals may have reduced the volume of distribution of pencloomedine and may have increased plasma drug concentrations.

At present, many of the available chemotherapeutic agents have a limited role in the treatment of patients with brain tumors, in part, because most of these compounds are not able to cross the blood-brain barrier. Previous studies have demonstrated that pencloomedine is effective against i.c.-implanted tumors in which blood-brain barrier disruption is likely to have occurred (2). The studies detailed here indicate that the distribution of pencloomedine to normal brain tissue is such that it also may be useful in the treatment of tumors that do not disrupt blood-brain barrier integrity and in regions of tumors in which blood-brain barrier disruption is relatively insignificant. These findings provide a strong rationale for the evaluation of this agent in patients with brain tumors. The extensive hepatic metabolism of pencloomedine observed in this study may have important implications for clinical trials, particularly regarding patients with altered liver function and patients concurrently receiving medications that may either induce or inhibit cytochrome P450-mediated drug metabolism, such as anticonvulsant agents and corticosteroids. These agents are administered routinely to patients with brain tumors and have been shown to alter the pharmacological and metabolic profiles of other antineoplastic drugs (11). Therefore, pharmacological monitoring should be continued in disease-directed clinical evaluations involving such patients.

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