Murine and Human in Vivo Penclomedine Metabolism

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ABSTRACT
Penclomedine is a multichlorinated α-picoline derivative which has shown prominent activity in murine breast cancer models and is currently undergoing clinical development. Previous in vitro research has identified several penclomedine metabolites. In this study, human and murine in vivo penclomedine metabolism was examined. Upon i.v. administration to mice, no penclomedine was detectable in plasma at time points as early as 1 h postinfusion. The principle metabolite was demethyl-penclomedine [3,5-dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)pyridine]. Both penclomedine and demethyl-penclomedine could be recovered from tissues. Greater than 60% of the penclomedine dose remaining in the body at 22 h was indelibly bound to tissue and plasma proteins. Urinary metabolites of penclomedine consisted mainly of penclomic acid and additional polar metabolites. The results obtained after p.o. administration were nearly identical to i.v. administration with respect to the extent, level, and type of metabolites found in the plasma, tissues, and urine with respect to the extent of protein binding. In human subjects administered penclomedine daily for 5 consecutive days, demethyl-penclomedine could be detected in plasma and accumulated with successive doses of penclomedine, reaching peak plasma concentrations of up to 10 times that of penclomedine itself and plasma exposures of nearly 400 times that of the parent drug. It appears that patients eliminate penclomedine largely through metabolism and that this drug may be amenable to p.o. administration.

INTRODUCTION
Penclomedine (Fig. 1), a multichlorinated derivative of α-picoline, is a novel antitumor agent which is currently undergoing clinical development (1, 2). Preclinical antitumor activity was evaluated using several in vivo murine models and remarkable activity was found against several breast tumors, including both mouse tumors and human xenografts (3). Penclomedine also had activity against intracerebrally implanted MX-1 xenografts, suggesting that it penetrates the blood-brain barrier (4). This activity was up to 4-fold greater than Carmustine, the most widely used agent in the treatment of brain tumors. The compound was approximately equally effective against murine tumors whether given p.o. or i.v. (3); however, pharmacokinetic data showed that penclomedine was extensively metabolized and had an p.o. bioavailability of <2% (5). In contrast to its prominent activity against tumors in vivo, penclomedine had little antiproliferative activity in cell culture (5). These observations suggest that penclomedine may be acting as a prodrug.

Previous in vitro work in this laboratory has elucidated the structures of several metabolites of penclomedine which are formed under aerobic and anaerobic conditions (6). Although numerous metabolites could be discerned, the rate of metabolism was relatively slow, particularly considering the low p.o. bioavailability of the drug and the hypothesis that penclomedine might be acting as a prodrug. Therefore, the current investigation examines the in vivo metabolism and tissue distribution of i.v. and p.o. administered penclomedine in mice. In addition, material from Phase I clinical studies allowed initial investigation of human penclomedine metabolism. A preliminary account of this investigation has been presented (7).

MATERIALS AND METHODS
Penclomedine (dry powder and clinical formulation) and ¹⁴C-labeled penclomedine (17.6 mCi/mmol, labeled as in Fig. 1) were obtained from the Pharmaceutical Resources Branch, Division of Cancer Treatment, National Cancer Institute (Baltimore, MD). The clinical formulation consisted of a de novo emulsion containing 10 mg penclomedine, 100 mg soybean oil, 30 mg egg phospholipid, and 20 mg glycerine/ml. Demethyl-penclomedine¹ and penclomic acid were synthesized as described below; all other metabolites were synthesized and generously provided by Dr. Robert Struck (Southern Research Institute, Birmingham, AL). Additional reagents were of the highest grade commercially available.

Penclomic acid was prepared by heating a 10 mm solution of penclomedine in 50% aqueous acetonitrile at 80°C for 48 h. The acetonitrile was evaporated, the resultant solution was al-

¹ The abbreviations used are: demethyl-penclomedine, 3,5-dichloro-2-methoxy-4-hydroxy-6-(trichloromethyl)pyridine; BSTFA, N.O-bis(tri- methylsilyl)trifluoroacetamide; dechloro-penclomedine, 3,5-dichloro-2,4-dimethoxy-6-(dichloromethyl)pyridine; demethyl-dechloro-penclomedine, 3,5-dichloro-2-methoxy-4-hydroxy-6-(dichloromethyl)pyridine; demethyl-penclomedine acid, 3,5-dichloro-2-methoxy-4-hydroxy-6-carboxy pyridine; GC/EI/MS, gas chromatography/electron ionization/ mass spectrometry; HPLC, high-performance liquid chromatography; penclomic acid, 3,5-dichloro-2,4-dimethoxy-6-carboxy pyridine.
Fig. 1. Structure of penclomedine. $^{14}$C label is as marked with asterisk.

Fig. 2. HPLC chromatographic analysis of penclomedine-derived murine plasma radioactivity. A, i.v. administration; B, p.o. administration. The scales of the ordinate axes for the 2- and 22-h postadministration samples are the same. The concentrations of demethyl-penclomedine (IV) at 2 h are 53 and 63 µM for i.v. and p.o. treated mice, respectively. Metabolites are identified with Roman numerals consistent with those in Fig. 1.

Kalined with sodium bicarbonate, extracted with diethyl ether, and the aqueous phase was neutralized to crystallize the product.

Demethyl-penclomedine was prepared by heating a 200 mM solution of penclomedine in anhydrous DMSO at 150°C for 90 min. The principle products were demethyl-penclomedine and demethyl-penclomic acid. The DMSO was evaporated under a vacuum, the residue was dissolved in chloroform, and demethyl-penclomic acid was extracted into water. Demethyl-penclomedine was purified by precipitation from methanol/water.

BALB/c × DBA/2 mice were obtained from Harlan Sprague-Dawley (Frederick, MD). During the course of the experiment, mice were housed in polyethylene shoe box cages without bedding and given access to food and water. Four mice were used per time point. $^{14}$C-penclomedine (1 mCi/ml in ethanol) was added to the clinical penclomedine emulsion to give an activity of 40 μCi/ml (4% ethanol final concentration). The mice were administered the above penclomedine formulation at 40 mg/kg (120 mg/m²; ~100 µl/animal) either i.v. via tail vein or p.o. via gavage. At 1, 2, 4, and 22 h after i.v. administration and 2, 4, 6, and 22 h after p.o. administration, animals were euthanized with carbon dioxide, and blood and tissues were collected. Blood and tissues from each time point were pooled. Blood was centrifuged, and plasma and RBCs were separated; tissues were homogenized in three volumes of 100 mM ammonium formate buffer (pH 6.5). Urinary output was estimated by washing the cages with water after removing food and feces and concentrating these cage washings.

The investigation of the human metabolism of penclomedine was undertaken as part of a Phase I trial of penclomedine in patients with solid tumor malignancies performed at the Johns...
Hopkins Oncology Center. Prior to treatment, written informed consent was obtained from each patient according to federal and institutional guidelines. Penclomedine was administered as a 1-h infusion daily for 5 days every 3 weeks. Blood samples were collected during days 1 and 5 of the first cycle of treatment, at 0, 15, 45, and 59 min during infusion, and then at 2, 5, 10, 20, 30, 45, 60, 90, 120, 240, and 360 min after infusion. On days 2 through 4, blood samples were drawn immediately before the infusion and at 10 min after infusion. The blood samples were centrifuged, and the plasma was removed and stored at −70°C prior to analysis.

A 1-ml aliquot of tissue homogenate or a 200-μl aliquot plasma was acidified with 200 μl 0.7 M ammonium phosphate (pH 2.7), and then 3 ml ethyl acetate were added. This mixture was vortexed, centrifuged, and the organic layer was collected. Fifty μl DMSO were added, and the ethyl acetate was concentrated to approximately 100 μl with a stream of dry nitrogen. Fifty μl acetonitrile were added to this residue, and the resulting solution was analyzed using HPLC. Human plasma was processed in a manner identical to mouse plasma, with the exception that 500 μl plasma were extracted. Concentrated cage washings were analyzed using HPLC without further processing.

Plasma and tissue protein binding were measured from the residue of the above extractions. The aqueous phase was washed with 5 ml ethanol, centrifuged, and the ethanol-insoluble precipitate was washed again with 5 ml ethanol. After centrifugation, the pellet was resuspended in 0.5 ml 6 M guanidinium chloride, added to 10 ml 3a70B scintillation cocktail (Research Products International Corp., Mount Prospect, IL), and counted for 14C.

HPLC Assays. The HPLC system consisted of a Hewlett-Packard Series II 1090 liquid chromatograph with diode array detector (Hewlett-Packard, Palo Alto, CA). The column used was an Alltech Adsorbosphere HS C18 5 μ 250 × 4.6-mm column (Alltech Associates, Deerfield, IL). The system used a gradient elution consisting of 100% 10 mm ammonium phosphate buffer (pH 2.7) progressing to 100% acetonitrile over 25 min at 1 ml/min. Detection was by means of UV absorbance at 240 nm as well as by detection of radioactivity using a Radiomatic Flo-One/Beta A140 radioactive flow detector (Packard Instrument Co., Downers Grove, IL) equipped with a 500-μl liquid cell and Flo-Scint VI scintillation cocktail at a 2:1 ratio.

GC/EI/MS Metabolite Identification. Metabolites were identified by comparing the mass spectra of the incubation extracts with those of synthetic standards. Mass spectra were obtained on a Hewlett Packard 5890 Series II Gas Chromatograph equipped with a Model 5971 Mass Selective Detector (Hewlett-Packard Co.). Compounds were separated on a 20 m × 0.25-mm DB-5 fused silica capillary column (Alltech Associates). Helium was used as the carrier gas at a flow rate of 0.6 ml/min; the temperatures of the injector and transfer lines were 200 and 270°C, respectively. The column temperature was held at 150°C for 4 min after sample injection and then linearly increased to 290°C at a rate of 10°C/min.

Sample Preparation for GC/EI/MS. Plasma and tissue homogenates were extracted with ethyl acetate, and the organic phase was evaporated with a stream of dry nitrogen. Samples were either reconstituted in ethyl acetate and injected directly

\( \text{Fig. 3. HPLC chromatographic analysis of plasma extracts from a single patient receiving 236 mg/m}^2 \text{ of penclomedine. A, plasma sample taken before penclomedine administration; B, plasma sample taken immediately after a 60-min penclomedine i.v. infusion; C, plasma sample taken immediately before penclomedine infusion on the second day of therapy. IV designates demethyl-penclomedine.} \)

(2 μl) into the GC or reacted with BSTFA (Supelco Inc., Bellefonte, PA).

RESULTS

Plasma Penclomedine Metabolites in Mouse and Human Plasma. 14C-Penclomedine could not be detected in HPLC radiochromatograms of mouse plasma sample extracts as early as 1 h after i.v. or 2 h after p.o. drug administration, although small amounts of penclomedine (≤1.6 μM) could be detected in the RBCs. A major peak (IV) and three minor peaks (I, II, and III) were observed in the HPLC radiochromatograms as seen in the 2-h samples in Fig. 2, A and B; peaks I, II, and III and IV were still detectable in mouse plasma 22 h after drug administration. The extent of metabolism observed in these studies was considerably greater than that which was predicted from previous in vitro studies (6). The types and amounts of metabolites observed were nearly identical whether mice received i.v. or p.o. administered penclomedine. The retention times of peaks I, II, III, and IV corresponded to the retention times of the synthesized proposed metabolites and were tentatively identi-
identified as demethyl-pencloemic acid, pencloemic acid, dechloro-demethyl-pencloemic acid, and demethyl-pencloemic acid, respectively. Results similar to the murine i.v. data were also seen in studies using rats (8).

Fig. 3B shows a HPLC chromatogram of a human plasma sample taken immediately following a 1-h i.v. infusion of pencloemic acid. This revealed the presence of pencloemic acid and a peak with the same UV spectrum and retention time as peak IV observed in mouse plasma. These two peaks were not detected in pretreatment samples (Fig. 3A). Twenty-four h after pencloemic acid administration to humans, plasma levels of peak IV increased approximately 3-fold over the end of infusion concentrations, and the parent drug was undetectable (Fig. 3C). Endogenous compounds present in the human plasma extracts precluded HPLC detection of the minor peaks I, II, and III observed in mouse plasma.

**GC/EI/MS Identification of Pencloemic Acid Metabolites.** Identification of pencloemic acid metabolites in human and mouse plasma extracts was confirmed by GC/EI/MS after silylation with BSTFA and comparison to the mass spectra of silylated synthesized proposed metabolites. The same five metabolites were present in both mouse plasma and human plasma; therefore, only the mass spectra of the metabolites found in human plasma are reported. The human plasma samples analyzed for these mass spectra were isolated samples from patients receiving pencloemic acid i.v. and were drawn on the fifth day of therapy when levels were expected to be highest. Not all patients were analyzed in this manner, since the mass spectra were used only for identification, not quantification. The mass spectrum of each of the metabolites observed in the plasma samples presented in Fig. 4 was indistinguishable from the mass spectrum of synthetic me-
Fig. 5. HPLC radiochromatographic analysis of mouse urine after 14C-pencloomedine administration. A, i.v. administration; B, p.o. administration. Urinary output was estimated from cage washings; therefore, each tracing represents the total urinary output from the start of administration. Tracings have been normalized to peak II, pencloomic acid.

tabolite standards. Demethyl-pencloomedine (IV) was the major metabolite detected, and mass spectra of this monosilylated metabolite (Fig. 4A) contained a molecular ion cluster at m/z 381, consistent with a compound containing five chlorine ions. This compound eluted at approximately 27 min in the radiochromatogram traces. Mass spectral fragmentation occurred primarily through loss of a methyl or chlorine radical, giving rise to the ion clusters observed at m/z 366 and m/z 346, respectively. The compound which eluted at 19 min in the radiochromatogram plots was determined to be pencloemic acid (II). The demethyl analogue of this compound (I) eluted at 15 min. Electron impact spectra of monosilylated pencloemic acid (Fig. 4B) and disilylated demethyl-pencloemic acid (Fig. 4C) show molecular ion clusters at m/z 323 and m/z 381, respectively, and the isotopic content of these clusters confirm the presence of two chlorine atoms. The most abundant fragment ion cluster present in the spectra both for compounds at m/z 308 (Fig. 4B) and at m/z 366 (Fig. 4C) was produced by loss of a methyl radical from the molecular ion. The peak in the radiochromatograms at 30 min corresponded to loss of one chlorine at the α carbon of pencloomedine. This dechboro-pencloomedine (V) gave a molecular ion at m/z 289, with the major fragmentation being loss of a chlorine ion to form the cluster at m/z 254 (Fig. 4D). DMethyl-dechloro-pencloomedine (III) was also observed in human plasma extracts, eluting at 25 min in the radiochromatogram traces;
electron impact mass spectra of its monosilylated derivatives shown in Fig. 4E produced a molecular ion cluster at m/z 347, consistent with a compound containing four chlorine atoms. Loss of a methyl radical or a chlorine radical from the molecular ion produced the major ion fragments observed at m/z 332 and m/z 312, respectively.

Disposition of 14C-Pencloomedine in Mouse Urine and Tissues. 14C-Pencloomedine-derived radioactivity was determined in mouse urine samples collected at 1, 2, 4, and 22 h following i.v. and p.o. administration of 14C-pencloomedine, and the results of this HPLC analysis are shown in Fig. 5. No pencloomedine was observed in urine samples following i.v. administration; trace quantities of pencloomedine that were detected in mouse urine after p.o. administration were probably due to partial regurgitation of the drug. The profile of the pencloomedine-derived radioactivity found in chromatograms of urine extracts were similar following i.v. (Fig. 5A) or p.o. (Fig. 5B) administration. Peaks I and II correspond to the retention times observed for the proposed synthetic metabolites demethyl-pencloemic acid and pencloemic acid, respectively. The identity of the remaining radioactivity present has not been determined. Urine samples taken at later time points appear to have a larger proportion of more polar metabolites than earlier samples. Total recovery of pencloomedine-related radioactivity from urine was 13 and 17% of the pencloomedine dose 22 h after i.v. or p.o. drug administration, respectively.
Penclomedine and a number of metabolites could be detected in most mouse tissues, as seen in Fig. 6 and Table 1. Unlike the plasma samples, all 2-h tissue samples contained detectable amounts of penclomedine. There were considerable differences in metabolite distribution among the different tissues; e.g., liver contained the highest levels of demethyl-penclomedine and the lowest levels of penclomedine. The highest levels of penclomedine were detected in the fat and carcass. As in the previous sections, there were very few differences in the type or the amount of metabolites between p.o. and i.v. administration. Most compounds present in the 2-h samples were detectable in the 22-h samples at levels from 10 to 50% of those seen at 2 h.

**Protein Binding of Penclomedine-derived Radioactivity.** Total plasma radioactivity is displayed in Fig. 7. For both p.o. and i.v. administration, the total plasma radioactivity at 22 h is nearly the same as that at the first sampling point. It is thus apparent that there is more total radioactivity present at 22 h than can be explained by the HPLC analyses in Fig. 2, A and B, which represent extractable radioactivity and which at 22 h only account for 10 to 30% of the radioactivity at 2 h. A portion of the plasma radioactivity could not be removed by extraction with ethyl acetate or ethanol however; this fraction is also displayed in Fig. 10 and is assumed to be covalently bound to plasma proteins. At 22 h, 77 and 79% of total radioactivity measured from i.v. and p.o. administration, respectively, was associated with plasma proteins. Irreversibly bound radioactivity was present at similar levels in selected tissues as seen in Fig. 8, ranging from 40 to 60% of the total radioactivity measured 22 h after either route of administration.

**Plasma Penclomedine Disposition in Patients.** Because it was possible to quantify demethyl-penclomedine in human...
Table I  Concentrations of penclomedine and metabolites in pooled murine tissues

Tissue concentrations of penclomedine and principal metabolites are determined from radiochromatographic analysis based on the specific activity of penclomedine and assumption that the metabolites have a specific activity identical to that of penclomedine.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Demethyl-dechloro-penclomedine (III)</th>
<th>Demethyl-penclomedine (IV)</th>
<th>Dechloro-penclomedine (V)</th>
<th>Penclomedine</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.v.</td>
<td>p.o.</td>
<td>i.v.</td>
<td>p.o.</td>
</tr>
<tr>
<td>Liver</td>
<td>9.15</td>
<td>8.5</td>
<td>40.5</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>13.5</td>
<td>5.65</td>
</tr>
<tr>
<td>Kidney</td>
<td>4.02</td>
<td>6.10</td>
<td>13.7</td>
<td>13.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>4.89</td>
<td>2.08</td>
</tr>
<tr>
<td>Brain</td>
<td>0.32</td>
<td>n.d.</td>
<td>2.02</td>
<td>3.36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>0.54</td>
<td>0.54</td>
</tr>
<tr>
<td>Fat</td>
<td>n.d.</td>
<td>n.d.</td>
<td>3.43</td>
<td>4.46</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>0.40</td>
<td>n.a.</td>
</tr>
<tr>
<td>Carcass</td>
<td>9.00</td>
<td>6.25</td>
<td>11.4</td>
<td>10.3</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>22</td>
<td>2.85</td>
<td>0.85</td>
</tr>
</tbody>
</table>

* n.d., not detectable; n.a., not available.

DISCUSSION

This investigation demonstrates that penclomedine is eliminated primarily through metabolism, as has been previously suggested by Reid et al. (5). Fig. 11 shows a proposed metabolic scheme for the metabolites of penclomedine identified in this study. Note that both oxidative and reductive components are indicated, which earlier in vitro work had suggested (6). It is not necessary to invoke a metabolic process for the formation of penclomic acid and demethyl-penclomic acid, since these compounds can form spontaneously from aqueous solutions of the parent compounds. This process is several orders of magnitude faster in the presence of viable tissue, however, making it likely that enzymatic processes are involved. The site of loss of the methyl group in the demethyl-metabolites could not be determined from this mass spectral study; however, other analytical...
Fig. 8. Murine tissue-bound radioactivity. Percentage of tissue binding was determined as the ratio of protein-bound radioactivity determined from extracted and washed tissue samples over total tissue radioactivity as determined by direct scintillation counting of tissue homogenate. ○, i.v. administration; ■, p.o. administration.

Fig. 9. Human plasma levels for penclomedine and the demethylpenclomedine metabolite, as determined by HPLC chromatographic analysis with UV detection. These results are for a single patient and are typical. ○, demethyl-penclomedine; ■, penclomedine. The displayed curves were fit using a biexponential decay for penclomedine (α = 3.88 h⁻¹, β = 0.456 h⁻¹) and an exponential rise to a constant value for demethyl-penclomedine.

Fig. 10. Penclomedine and demethyl-penclomedine levels on each of 5 treatment days. Data are from a single patient receiving 315 mg/m² penclomedine and are typical of those seen in other patients. The same ordinate scale is used for both penclomedine and demethyl-penclomedine. □, penclomedine, preinfusion (present at very low levels); ■, penclomedine, end of infusion; ▪, demethyl-penclomedine, preinfusion; □, demethyl-penclomedine, end of infusion.

studies indicate that the methyl group is lost from the 4-carbon. From urinary excretion data, it appears that penclomic acid is only produced during the initial few h following drug administration. This is consistent with the proposed route of formation of penclomic acid; presumably, this metabolite is made in a direct route from penclomedine, and therefore could only be formed when circulating penclomedine is actually present. The

5 R. Struck, personal communication.
Table 2  Accumulation of demethyl-penclomedine in plasma of clinical subjects

Plasma concentrations of demethyl-penclomedine were measured at 10 min after the end of infusion for each of 5 days and are expressed as mM.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Dose (mg/m²/day)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<tr>
<td>1</td>
<td>90</td>
<td>1.1</td>
<td>5.1</td>
<td>4.0</td>
<td>8.2</td>
<td>n.a.*</td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>1.6</td>
<td>5.1</td>
<td>6.1</td>
<td>11.5</td>
<td>22.4</td>
</tr>
<tr>
<td>3</td>
<td>135</td>
<td>0.6</td>
<td>4.8</td>
<td>7.0</td>
<td>5.7</td>
<td>19.0</td>
</tr>
<tr>
<td>4</td>
<td>180</td>
<td>8.2</td>
<td>n.a.</td>
<td>17.9</td>
<td>38.1</td>
<td>51.7</td>
</tr>
<tr>
<td>5</td>
<td>180</td>
<td>2.7</td>
<td>7.7</td>
<td>16.1</td>
<td>26.7</td>
<td>42.6</td>
</tr>
<tr>
<td>6</td>
<td>236</td>
<td>1.2</td>
<td>7.9</td>
<td>19.1</td>
<td>28.7</td>
<td>35.4</td>
</tr>
<tr>
<td>7</td>
<td>236</td>
<td>0.9</td>
<td>6.7</td>
<td>17.6</td>
<td>31.3</td>
<td>32.2</td>
</tr>
<tr>
<td>8</td>
<td>315</td>
<td>1.5</td>
<td>5.9</td>
<td>14.2</td>
<td>15.6</td>
<td>25.6</td>
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<tr>
<td>9</td>
<td>315</td>
<td>3.8</td>
<td>17.9</td>
<td>29.1</td>
<td>41.3</td>
<td>50.0</td>
</tr>
</tbody>
</table>

* n.a., not available.

structures of the more polar metabolites have yet to be established, however demethyl-penclomic acid, the breakdown product and presumed metabolite of demethyl-penclomedine, is probably present based on the retention time of the main cluster of metabolites.

Significant amounts of penclomedine-derived radioactivity can be found in most murine tissues. Tissue accumulation of penclomedine itself probably accounts for a large portion of the loss of drug from the plasma. Large amounts of penclomedine metabolites could be detected in murine tissue extracts; however, these metabolites were generally less polar than those found in tissue extracts of rats receiving penclomedine (8).
Much of the penclomedine-derived radioactivity found in murine tissues could not be removed by extraction however. This irreversibly bound material accounts for approximately 60% of the total radioactivity present at 22 h and may explain the persistently high levels of plasma radioactivity noted by Reid et al. (5). The identity of the binding metabolite or metabolites, the nature of the bond, and the binding site are not currently known. Native penclomedine does not bind to macromolecules in non-metabolically active systems at 37° (data not shown). Several of the proposed intermediates to the identified metabolites shown in Fig. 11 are potential alkylators or acylators, making it likely that a metabolite of penclomedine is the actual binding species. The binding noted here is consistent with the proposed mechanism of action of penclomedine as an alkylating agent (4). This evidence per se does not prove that penclomedine or its metabolites act by alkylation, but only that it can bind to tissues.

Demethyl-penclomedine could easily be detected in clinical samples and persisted in the plasma for longer than 24 h. Indeed, after repeated penclomedine administration, plasma concentrations of this metabolite could be observed to reach greater than 10 times the peak plasma level of penclomedine and produced drug exposures of up to 400 times that of the parent compound. Considering the high plasma levels attained for demethyl-penclomedine, it is important that the toxicity of this metabolite be ascertained. Neurotoxicity has been the principle toxicity associated with penclomedine administration at the dose levels assessed in this study. This toxicity consists of light-headedness, and at higher dose levels ataxia and dysmetria (1). These symptoms occur during the period of infusion, are dose related, and are self-limiting and noncumulative. Hematological toxicity has not been observed at these dose levels. The pattern of toxicities observed in these early clinical trials indicate that demethyl-penclomedine is unlikely to be involved as the toxic agent. In this study, levels of this metabolite increased as toxicity resolved, and on repeated administration of penclomedine neurotoxicity did not increase, whereas levels of the metabolite increased to several times the plasma levels of the parent drug. Additional studies are under way to determine the clinical significance of this metabolite.

This investigation supports the concept that a clinical trial of p.o. administered penclomedine is warranted. Preclinical studies have already shown that p.o. penclomedine is as effective as i.v. penclomedine against intracerebral MX-1 xenografts (4). The present investigation shows that p.o. and i.v. administered penclomedine produces nearly identical levels of penclomedine and penclomedine metabolites in mice for all tissues studied. This finding was somewhat unexpected, because Reid et al. (5) demonstrated that penclomedine has negligible p.o. plasma bioavailability, making it unlikely that much intact penclomedine would be available for tissue distribution. The present data indicate that at least some penclomedine is absorbed intact, but is rapidly redistributed to tissue sites, and consequently significant plasma levels are not obtained. Thus, clinical studies with p.o. administered penclomedine might produce levels of the active species that are very similar to those seen with i.v. administration.

This study indicates that penclomedine is rapidly metabolized by both mice and humans and that p.o. and i.v. administration produce nearly identical results with respect to murine plasma and tissue levels of penclomedine and its metabolites and with respect to the extent of potential alkylation. It remains to be definitely shown which, if any, of these metabolites are involved in the antitumor activity ascribed to penclomedine, but the extremely high concentrations of demethyl-penclomedine suggest that this species could play a major role.

ACKNOWLEDGMENTS

We thank Dr. Robert Struck (Southern Research Institute, Birmingham, AL) for much helpful advice during the course of the study as well as the generous gift of the synthetic penclomedine metabolites. The excellent nursing assistance of Katherine Bowling, Susan Sartorius, and Barbara Lubejko is gratefully acknowledged.

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Murine and human in vivo penclom edine metabolism.
