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ORIGINAL ARTICLE

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Bioavailablility of penclomedine and systemic exposure to 4-*O*-demethylpenclomedine in patients receiving oral and intravenous penclomedine

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Abstract *Purpose*: Oral administration of penclomedine was investigated based on preclinical studies indicating that an oral schedule of penclomedine treatment may prevent the neurotoxicity observed in phase I studies of an intravenous (i.v.) formulation, possibly by reducing maximum plasma concentrations (C_{max}) of the neurotoxic parent species. *Methods*: Penclomedine was administered i.v. (200 mg/m^2) and orally (250 mg/m^2) in alternate sequences to patients with solid tumor malignancies. Plasma concentrations of parent drug and the principal metabolite, 4-*O*-demethylpenclomedine, were determined by a reversed-phase HPLC assay. *Results*: Penclomedine was detectable in the plasma of all patients within 1 h of oral penclomedine treatment and C_{max} was reached within 1 to 4 h. Consistent with the hypothesis that an oral schedule of administration may circumvent neurotoxicity, a paired data analysis demonstrated a significant reduction in C_{max} values following oral administration ($P = 0.017$). However the magnitude of this reduction was highly variable. Similarly an extensive range in the relative exposure to both parent drug and metabolite were observed. The bioavailability of penclomedine ranged from 28% to 98% (median 73%). *Conclusions*: Oral penclomedine does produce systemic exposure, but substantial interpatient variability in absorption and systemic exposure is present which may limit the clinical role of the oral route of administration.

Keywords Penclomedine · 4-*O*-demethyl penclomedine · Pharmacokinetics · Clinical trial · Antitumor

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Introduction

The alpha picoline derivative penclomedine (NSC 338720; 3,5-dichloro-2,4-dimethoxy-6-(trichloromethyl) pyridine) was selected for clinical development by the National Cancer Institute (NCI) because of its impressive antitumor activity against $CD8F_1$ murine, MCF-7 human mammary adenocarcinoma [1], and against intracerebrally implanted MX-1 human mammary adenocarcinoma in nude mice [2]. Penclomedine is more active in vivo than in vitro [3], suggesting metabolism to an even more potent compound. Indeed, a major metabolite, 4-*O*-demethylpenclomedine, has been identified in the plasma of mice, rats, and humans [4, 5]. This compound is produced predominantly by the liver [6], and is active in vivo [7, 8]. These studies indicate that 4-*O*-demthylpenclomedine is the main, or at least a more proximal, alkylating species.

In both preclinical [9] and clinical [10, 11, 12] studies, adverse central nervous system effects and myelosuppression were the principal toxicities of intravenously (i.v.) administered penclomedine. Neurologic effects consisted of dose-related cerebellar ataxia, and were associated with Purkinje cell death in the cerebellum in preclinical correlative studies [13]. Several observations suggested that parent drug, rather than

the major metabolite 4-*O*-demethylpenclomedine, is responsible for neurotoxicity. Firstly, symptoms resolved as plasma concentrations of parent drug declined, and concentrations of the metabolite increased. Secondly, the results of pharmacodynamic modelling studies have suggested that the maximum plasma concentration (C_{max}) of penclomedine is the pharmacokinetic parameter with the highest likelihood of predicting the development of neurotoxicity [10]. Thirdly, penclomedine, but not 4-*O*-demethylpenclomedine, is detectable in brain tissue within the time frame of development of neurologic symptoms [5]. Finally, in preclinical studies Purkinje cell loss was associated with parent drug, and not with the major metabolite [13].

The development of neurotoxicity from penclomedine, which may have a potential role in the therapy of brain tumors, prompted an evaluation of alternate treatment strategies for drug administration. In preclinical studies, antitumor activity was preserved when the agent was administered orally and Purkinje cell loss was not observed in rats receiving potentially therapeutic doses of penclomedine [13]. These observations prompted an assessment of the relative exposures to penclomedine and 4-*O*-demethylpenclomedine in patients with advanced solid tumor malignancies who received oral and i.v. penclomedine.

Patients and methods

Eligibility

Patients with histologically documented solid malignancies refractory to conventional therapy, or for which no effective therapy existed, were candidates for this study. Eligibility criteria included: (1) age ≥18 years; (2) an Eastern Cooperative Oncology Group (ECOG) performance status of 2 (ambulatory and capable of self-care); (3) a life expectancy of at least 10 weeks; (4) no major surgery within 14 days, and no wide-field radiotherapy and/or chemotherapy within 28 days of entering the study; (5) adequate hematopoietic (white blood cells $>4000/\mu l$ and platelets $\geq 100{,}000/\mu l$), hepatic (total bilirubin ≤ 1.5 mg/dl), and renal (creatinine ≤ 1.5 mg/dl) functions; (6) no clinical manifestations of either primary or secondary central nervous system malignancies; (7) no history of neuropsychiatric or medical disorders which might confound toxicity assessment; (8) no history of allergies or prior hypersensitivity reactions to eggs or egg protein-containing solutions (e.g. Intralipid); and (9) no additional coexisting medical problems of sufficient severity to prevent full compliance with the study. All patients gave written informed consent according to institutional and federal guidelines before treatment.

Dosage

All patients were treated with penclomedine 200 mg/m^2 i.v. administered over 1 h. This dose was selected since it had been associated with negligible toxicity in initial clinical studies and produced high enough plasma levels to produce reliable concentration measurements [10, 11, 12]. A single oral dose of penclomedine 250 mg/m^2 was administered 1 week later. The selection of this dose was based on the results of animal studies indicating that oral doses equivalent to 130% of i.v. doses produce comparable antitumor activity. The sequence of administration of the oral and i.v. doses was alternated for patient 2 and patient 3.

Drug administration

Penclomedine for i.v. administration was supplied by the Division of Cancer Treatment and Diagnosis, NCI (Bethesda, Md.), in 10-ml vials containing 10 mg/ml penclomedine dissolved in a lipid emulsion. Each 1 ml of emulsion contained 100 mg soybean oil, 30 mg egg phospholipid, and 20 mg glycerin. The penclomedine emulsion which consisted of a 1:10 dilution of the agent in a 5% dextrose-water solution, and was infused i.v. over 1 h in the outpatient clinic.

The oral formulation of penclomedine was supplied by the Division of Cancer Treatment and Diagnosis, NCI, as 100-mg opaque soft gelatin capsules containing caprilic/capric triglycerides (Neobee-1053) in bottles containing 100 capsules. Patients were required to fast for 1 h before and after administration. The dose administered was rounded to the nearest 100 mg.

Penclomedine sampling and assay

Blood samples were obtained pretreatment, at 15, 45, and 59 min during i.v. infusions, at 2, 5, 10, 20, 30, 45, 60, and 90 min postinfusion, at 2, 4, 6, 24, 30, 48, and 72 h postinfusion, and 1 or 2 weeks postinfusion. Blood samples were also obtained pretreatment, at 15, 30, 45, 60, 75, 90, 120, 150, and 180 min after oral administration, at 4, 6, 24, 30, 48, 72 h after oral administration, and 1 or 2 weeks after oral administration. Blood samples were centrifuged at 1000 *g* for 10 min at room temperature immediately after sampling, and the plasma was removed and stored at –20°C until assayed. A 500-µl aliquot of plasma was added to a 15-ml polypropylene centrifuge tube containing 3 ml ethyl acetate, 100 µl 0.7 *M* ammonium phosphate buffer, pH 2.7, and 50 µl 50 µ*M* 4-ethylpenclomedine in acetonitrile as an internal standard. The samples were then mixed using a vortexer, centrifuged, and the organic phase was drawn off and placed in a separate 15-ml centrifuge tube. To these organic phases, 50 µl dimethylsulfoxide was added to prevent complete evaporation, and the resulting solution was concentrated to approximately 100 µl under a stream of dry nitrogen. These concentrates were diluted with 50 µl acetonitrile and 100-µl aliquots were injected into the high-performance liquid chromatography (HPLC) system for analysis.

The HPLC system consisted of a Hewlett-Packard Series II 1090 liquid chromatograph with diode array detector (Hewlett-Packard, Palo Alto, Calif.). The column used was an Alltech Adsorbosphere HS C18 5 µm 250×4.6 mm column (Alltech Associates, Deerfield, Ill.). The system used gradient elution consisting of 100% 10 m*M* ammonium phosphate buffer, pH 2.7, progressing to 100% acetonitrile over 25 min at 1 ml/min. Detection was by means of ultraviolet absorbance at 240 nm. The lower limits of detection of the assay for penclomedine and 4-*O*-demethylpenclomedine were 0.1 and 0.2 µ*M*, respectively. The coefficient of interday variability, which was calculated by comparing the slopes from seven calibration curves obtained over 4 months, was 13%.

For the purposes of the assay two metabolites of penclomedine were synthesized, 4-*O*-demethylpenclomedine from penclomedine as previously described [4], and 4-ethylpenclomedine in the following manner. Approximately 200 mg of 4-*O*-demethylpenclomedine was placed in a 5-ml reaction tube containing 1 ml iodoethane and approximately 100 mg potassium bicarbonate. This mixture was heated with stirring at 65°C for 24 h. The iodoethane was then evaporated under a stream of dry nitrogen, and the residue was dissolved in water and extracted twice with dichloromethane. The 4-*O*-ethylpenclomedine was purified by preparative HPLC. Purity was established by analytical HPLC and the structure was verified by gas chromatography-mass spectrometry

using a Hewlett-Packard Series II gas chromatograph equipped with a 5971 mass selective detector (Hewlett-Packard, Palo Alto, Calif.). Yield was not determined.

Pharmacokinetic and statistical analysis

Data on individual plasma penclomedine and 4-*O*-demethylpenclomedine concentrations were plotted separately for each patient. Plasma concentration-time curves for penclomedine and 4-*O*-demethylpencomedine plasma levels were analyzed using nonlinear regression analysis (MicroMath Scientist for Windows, Version 2.01, MicroMath). The Wilcoxon paired signed ranks test was used to assess differences in the pharmacokinetic disposition of penclomedine following oral or i.v. administration. All tests for significance were two-tailed. A *P*-value threshold of 0.05 was considered significant for all tests. Statistical analysis was performed using Statistica 5.0 for Windows (Statsoft, Tulsa, Okla.).

A mixed linear and logarithmic trapezoidal rule was then used to calculate the area under the penclomedine plasma concentration-time curve (AUC) [14, 15]. Bioavailability was calculated as follows:

$$\text{Bioavailability} = \text{AUC}_{\text{oral dose}}/(\text{AUC}_{\text{i.v.dose}} \times 1.25)$$

Penclomedine plasma clearance values were determined as the ratio of penclomedine dose to the AUC.

Results

Between January and November 1997, eight patients were enrolled in the study. One patient with metastatic pancreatic cancer was removed from study before completion due to the development of progressive metastatic disease. The relevant demographic characteristics of the remaining seven evaluable patients are displayed in Table 1. Plasma concentration-time curves of both penclomedine and 4-*O*-demethylpenclomedine were quite variable as shown in Fig. 1 for patients 1, 2, and 6. Penclomedine plasma profiles were similar for all three patients in that penclomedine C_{max} values were greater following i.v. than following oral administration. However, considerable differences were observed for the 4-*O*-demethylpenclomedine plasma concentration-time curves. Similar to penclomedine, both patients 2 and 6 exhibited greater 4-*O*-demethyl-penclomedine C_{max} values following i.v. than following oral penclomedine administration, irrespective of the dosing sequence. In contrast, the 4-*O*-demethylpenclomedine C_{max} for patient 1 was greater following oral than following i.v. administration.

After oral treatment, plasma penclomedine concentrations could be detected in the plasma within approximately 1 to 4 h of administration (median lag time 77 min, range 49 to 249 min; Table 2). The most prolonged lag time was observed in one of three patients who was being treated with narcotics for pain control. The median time to maximum plasma concentration (T_{max}) was 155 min (range 64 to 250 min). A paired analysis demonstrated a significant reduction in maximum plasma concentrations (C_{max}) when i.v. and oral schedules were compared (Fig. 2).

A tenfold difference among the seven patients was observed for total penclomedine plasma clearance (median 1.19 l/min per m^2, range 0.39 to 4.12 l/min per m^2; Table 2). Compared with i.v. administration and corrected for dose, the median bioavailability of the parent compound was 73% (range 28–89%). The penclomedine pharmacokinetic parameters for plasma disposition curves following i.v. treatment were similar to those obtained in previous studies [10, 11].

As found in previous studies [4], 4-*O*-demethylpenclomedine plasma concentrations were detectable for 2 weeks after the second dose of penclomedine in five out of seven patents who had plasma sampling performed. The plasma elimination half-lives determined for these five patients ranged from 6 to 20 days (median 14 days).

Other than mild nausea and vomiting in one patient after treatment with i.v. penclomedine, no adverse events were observed in the study.

Discussion

In the present study, the potential role of an oral schedule of administration to improve the therapeutic index of penclomedine was evaluated. The premise of the study was that increased first-pass hepatic metabolism would lower the C_{max} of the neurotoxic parent compound. While reductions in C_{max} were consistently

Table 1 Patient characteristics

Patient	Diagnosis	Age (years)	Race	Prior therapy	Concomitant medications
1	Adenocarcinoma of unknown primary	48	Caucasian	Chemotherapy, radiation therapy	Coumadin, zolipedem, acetaminophen, propoxyprophen, hydromorphone
2	Colon cancer	43	Caucasian	Chemotherapy	None
3	Non-small-cell lung cancer	69	African-American	Chemotherapy	Morphine, oxycodone, lorazepam, loratidine, nabumetone
4	Colon cancer	71	Caucasian	Chemotherapy	None
5	Non-small-cell lung cancer	59	African-American	Chemotherapy, radiation therapy	Morphine
6	Renal cancer	73	Asian	Immunotherapy	Guaifenesin, codeine
7	Renal cancer	75	Asian	Immunotherapy, chemotherapy	None

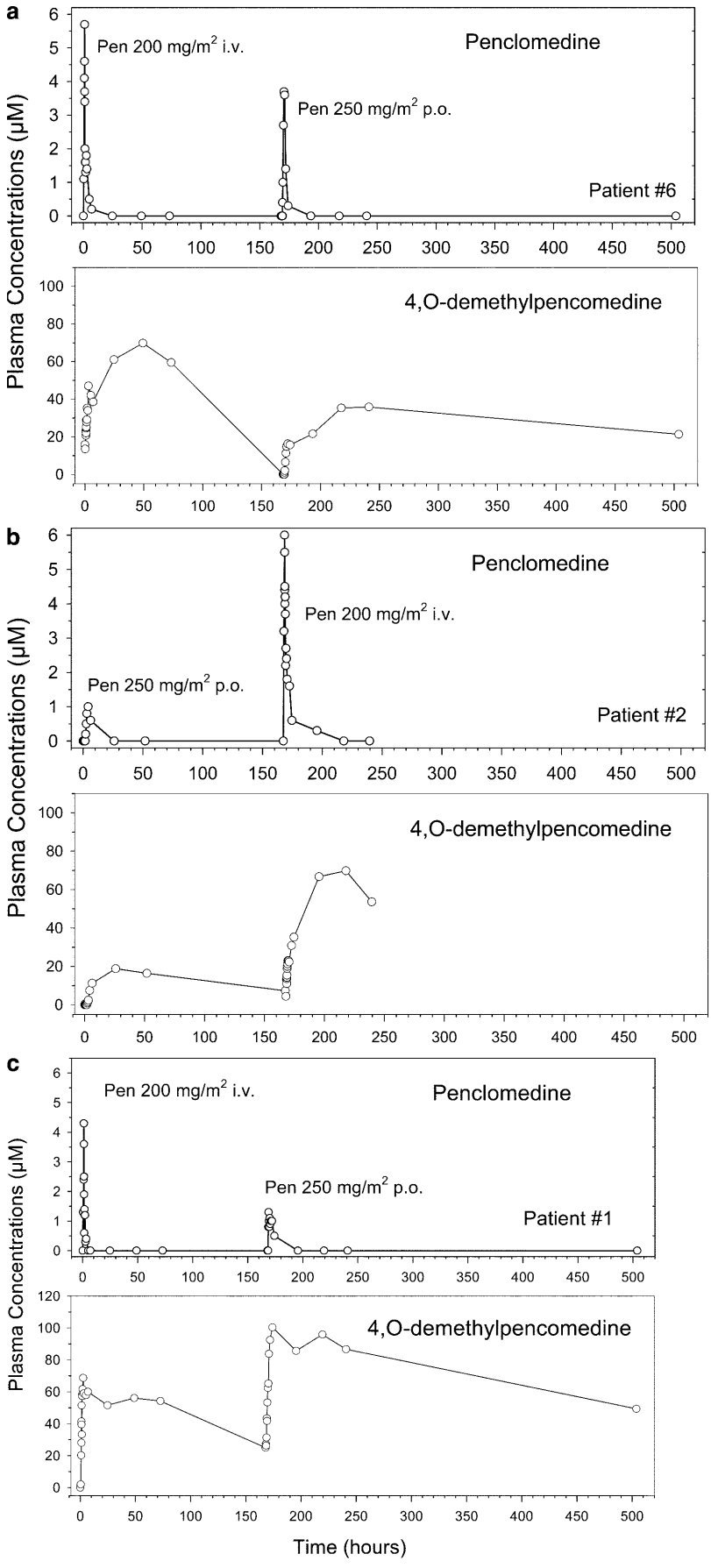

Fig. 1a–c Penclomedine and 4-*O*-demethylpenclomedine plasma concentration-time curves determined following two penclomedine doses for: (**a**) patient 6, i.v. (200 mg/m^2) on day 1 and oral (250 mg/m^2) on day 8; (**b**) patient 2, oral (250 mg/m^2) on day 1 and i.v. (200 mg/m^2) on day 8; (**c**) patient 1, i.v. (200 mg/m^2) on day 1 and oral (250 mg/m^2) on day 8

Table 2 Penclomedine pharmacokinetic parameters

	Parameter	Patient number						
		1[a]	2[b]	3[b]	4[a]	5[a]	6[a]	7[a]
Intravenous administration	AUC (μM·min)	281	1566	518	344	149	569	616
	Clearance (l/min/m^2)	2.18	0.39	1.19	1.79	4.12	1.08	1.00
	C_{max} (μM)	4.3	6.0	6.1	3.8	2.0	5.7	4.7
Oral administration	AUC (μM·min)[c]	237	432	379	218	142	560	450
	T_{lag} (min)	49	128	66	77	249	77	65
	T_{max} (min)	64	250	240	92	249	152	155
	C_{max} (μM)	1.3	1.0	1.2	0.8	0.6	3.7	3.4
Penclomedine bioavailability (%)		84	28	73	63	95	98	73

[a]Penclomedine dose week 1 (i.v.) and week 2 (oral)
[b]Penclomedine dose week 1 (oral) and week 2 (i.v.)
[c]AUC_{oral} corrected for dose

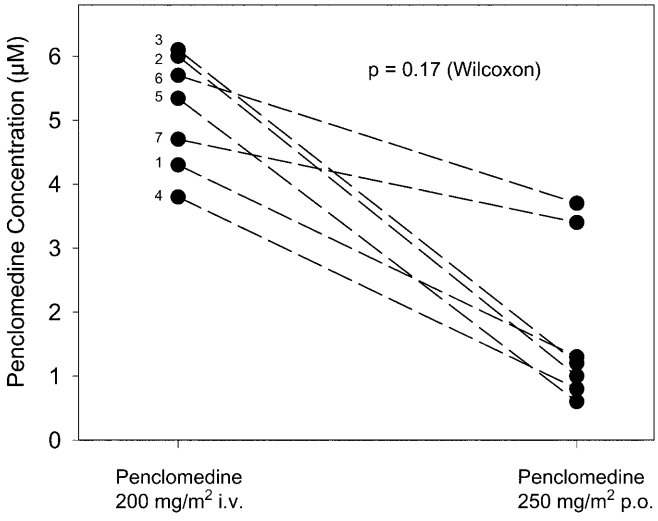

Fig. 2 Effect of route of administration on penclomedine plasma C_{max}. The *P*-value was obtained from the Wilcoxon paired signed ranks test

observed following oral administration, the magnitude of the decline varied substantially between patients, indicating that interpatient variability in neurologic toxicity in subsequently conducted phase I dose escalation studies may be substantial. In addition, while neurologic effects were observed at the end of infusion in phase I studies of the i.v. formulation, the broad range of T_{max} values in the present study suggests that the time frame for onset of symptoms may be quite variable and may occur up to 6 h after therapy. These results were based upon a minimum of 1 h fasting before and after treatment. In addition, there may be other determinants of the observed variability.

In preclinical studies of penclomedine, antitumor activity was retained at oral doses of 125% of their i.v. equivalents. In the present study, significant interpatient variability in the bioavailability of parent drug was observed. The range of interindividual variability was similar to those obtained in bioavailability studies of other antineoplastic agents such as etoposide phosphate (range 35–112%) [16] and topotecan (range 21–45%) [17]. However, the clinical significance of this broad range of penclomedine bioavailability with respect to antitumor activity is unclear. The agent is extensively metabolized, and the exact cytotoxic species has not yet been isolated.

The prolonged half life of 4-*O*-demethylpenclomedine in the present study (median 13 days) is consistent with the findings of Reid et al. who reported that plasma concentrations of radiolabel were detectable in plasma for several weeks following the administration of radiolabeled penclomedine to mice [3]. Similarly in rats, high levels of unextractable radiolabel were measured in tissue samples after treatment [5]. Whether high plasma levels represent protein turnover and recirculation of previously covalently bound drug is unknown. Of concern is the implication of this prolonged half-life for the development of penclomedine-based combination chemotherapy regimens, or the subsequent treatment of patients with other cytotoxic agents after penclomedine therapy. This prolonged half-life also indicates that the use of protracted oral administration schedules may be superior to the daily ×5 every 4-week regimen which was initially evaluated.

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