Comparative Physiological Disposition of N-(Phosphonacetyl)-L-aspartate in Several Animal Species after Intravenous and Oral Administration

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ABSTRACT

The physiological disposition of N-(phosphonacetyl)-L-aspartate (NSC 224131; PALA), a potent inhibitor of aspartate transcarbamylase, has been studied in mouse, rat, dog, and monkey after administration of [\(^{14}\)C]PALA at 120 mg/sq m i.v. or p.o. Concentrations of PALA equivalents in plasma, urine, and feces were determined radiochemically, and urine was analyzed chromatographically for PALA. The disposition of PALA equivalents in mouse tissues was determined radioautographically. After i.v. administration, PALA was rapidly (half-time, approximately 1 hr) and extensively (up to 80% of the dose) excreted in the urine of all species. Less than 5% was excreted in the feces. Only PALA was found in the urine of all four species, indicating that the metabolism of PALA, if it occurs at all, is insignificant. PALA equivalents were poorly taken up by mouse tumors and tissues, except kidney, bone, and to a lesser extent, skin and lung, and were rapidly and extensively cleared from all except bone. No differences were apparent in the uptake of PALA equivalents by Lewis lung carcinoma (sensitive to PALA treatment) and L1210 lymphocytic leukemia (insensitive). The pharmacokinetics of PALA in the plasma of rat, dog, and monkey, as well as mouse, were inconsistent with deposition of PALA in tissues and more consistent with the probable distribution of PALA into extracellular water. PALA equivalents were eliminated from all species at a rate (half-time, 1 to 1.5 hr) reflecting the rate of urinary excretion of the drug and on a secondary slower rate probably reflecting the rate of release of bound PALA from sites such as aspartate transcarbamylase. PALA was poorly absorbed into the systemic circulation when administered p.o., in that mouse, rat, and monkey excreted less than 5% of the dose in the urine after p.o. administration.

These data on the physiological disposition of PALA explain why high doses of the drug have to be administered to achieve therapeutic and toxic effects, despite the inhibitory potency of the drug on aspartate transcarbamylase. They indicate that PALA will be ineffective administered p.o. and might be contraindicated in patients with impaired renal function and that the kinetics of aspartate transcarbamylase-bound drug is probably more important in determining dose scheduling than the kinetics of free PALA.

INTRODUCTION

PALA\(^3\) (NSC 224131), an analog of the transition state for the reaction catalyzed by aspartate transcarbamylase (9), is a potent inhibitor of the enzyme (9, 17) and as such inhibits de novo pyrimidine nucleotide biosynthesis (25, 31). In comparison with other antimetabolites, the spectrum of activity of PALA against experimental tumors in mice is unique, in that L1210 and P388 leukemias and Ridgeway osteogenic sarcoma are relatively or completely insensitive to the drug, whereas Lewis lung carcinoma and B16 melanoma are sensitive (20). Since the effects of PALA on Lewis lung carcinoma are completely reversed by carbamyl-L-aspartate, its antitumor activity is probably due to its inhibition of aspartate transcarbamylase (19). PALA has been found to cause gastrointestinal toxicity in animals and at high doses central nervous system toxicity (10). PALA has undergone Phase I clinical trial and resulted in gastrointestinal and dermatological toxicity (12, 13, 15, 16, 22, 28) and is now undergoing Phase II clinical trial.

Studies on the disposition of PALA in mice after s.c. or p.o. administration showed that PALA was retained in tissues at concentrations sufficient to inhibit aspartate transcarbamylase activity and that it remained as unchanged drug (31). We have studied the physiological disposition of PALA in several species after i.v. and p.o. administration (5).

MATERIALS AND METHODS

Synthesis of [\(^{14}\)C]PALA. L-[\(^{14}\)C]Aspartate uniformly labeled (184 mCi/mmol) was purchased from New England Nuclear (Boston, Mass.). [\(^{14}\)C]PALA tetrasodium salt was prepared according to the method of Swyryd et al. (25). The overall yield of tetrasodium [\(^{14}\)C]PALA was 123 mg (20%) at 5.44 mCi/mmol. A similar large-scale synthesis was carried out to prepare nonradiolabeled drug. The radiolabeled and nonradiolabeled compounds were shown to be 95% pure by gas-liquid chromatography (4).

Animals and Dosage Schedules. [\(^{14}\)C]PALA and PALA were dissolved in 0.9% NaCl solution at various specific activities and concentrations. [\(^{14}\)C]PALA was administered to male beagles (10 to 11.5 kg) (Laboratory Research Enterprises, Inc., Kalamazoo, Mich.) at 6 mg/kg, 120 mg/sq m (10 mg/ml; 1 μCi/mg) i.v. via the brachiocephalic vein. Dogs were housed in stainless steel metabolism cages (Fenco Cage Products, Dorchester, Mass.) and allowed access to water; food was withheld for 7 hr after dosing. Blood samples were taken from the jugular vein at intervals up to 96 hr and transferred to heparinized tubes. Plasma was obtained by centrifugation and was frozen. Feces and urine were collected at 24-hr intervals up to 96 hr and were frozen. Plasma, feces, and urine in these and subsequent experiments were stored at -20º.

Female beagles (10 kg) were lightly anesthetized using sodium pentothal, catheterized for urine collection using Foley French No. 12 catheters, provided with an i.v. drip of 0.9% NaCl solution, and restrained in body slings for 8 hr when they were placed in metabolism cages. [\(^{14}\)C]PALA was administered at 6 mg/kg i.v. via the right brachiocephalic vein. Plasma and urine samples were collected at intervals up to 24 hr.

[\(^{14}\)C]PALA was administered to male rhesus monkeys (2 to 4 kg; Primate Imports Corp., Port Washington, N. Y.) at 10 mg/kg, 120 mg/
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sq m (10 mg/ml; 1 µCi/mg) i.v. via the greater saphenous vein. Treatment of monkeys and collection of biological samples were the same as for dogs, except that blood samples were taken from the femoral vein.

[123]PALA was administered to male rhesus monkeys (2 to 4 kg) at 10 mg/kg p.o. via intubation. The monkeys were handled as above except food was withheld for 16 hr prior to dosing.

Female rhesus monkeys (2 to 4 kg) were catheterized for urine collection as described for dogs above except that Foley French No. 8 catheters were used. The animals were restrained in Foringer primate chairs (Foringer Co., Rockville, Md.) for 8 hr at which time they were placed in metabolism cages. [123]PALA was administered at 6 mg/kg i.v. via the greater saphenous vein. Plasma and urine samples were collected at intervals up to 48 hr.

[123]PALA was administered to male Sprague-Dawley rats (130 to 180 g; Charles River Breeding Laboratories, Inc., Wilmington, Mass.) at 20 mg/kg, 120 mg/sq m (5 mg/ml; 1 µCi/mg) i.v. via the caudal vein. Rats were housed in conventional cages, except where stated otherwise, and were allowed water ad libitum; food was withheld for 7 hr after dosing. Blood samples were taken by decapitation of rats at intervals up to 96 hr and were collected in heparinized tubes. Plasma was obtained by centrifugation and was frozen. Feces and urine were collected at intervals up to 96 hr from rats housed in individual No. 110 metabolism chambers (Maryland Plastics, Inc., New York, N. Y.) after administration of [123]PALA at 20 mg/kg. Coprophagy was prevented by the use of tall cups (2, 30). Expired CO2 was collected in 8 M KOH over a 24-hr period from rats housed in Roth metabolism cages (Delmar Scientific, Maywood, Ill.) (24).

[123]PALA was administered to male Sprague-Dawley rats (130 to 180 g) at 20 mg/kg (5 mg/ml; 1 µCi/mg) p.o. via syringe. Rats were handled as above except that food was withheld for 16 hr prior to dosing.

[123]PALA was administered to male C57BL × DBA/2 F1 mice (20 g; Charles River Breeding Laboratories, Inc.) at 40 mg/kg, 120 mg/sq m (2 mg/ml; 1 µCi/mg) i.v. via the caudal vein. Treatment of mice and collection of biological samples were the same as for rats, except that samples from groups of mice were pooled.

[123]PALA was administered to male BD2F1 mice (20 g) at 40 mg/kg (2 mg/ml; 1 µCi/mg) p.o. via syringe. Mice were handled as above except that food was withheld for 16 hr prior to dosing.

[123]PALA was administered at 40 mg/kg (70 µCi/kg; 1.8 µCi/mg) i.v. to normal male BD2F1 mice and to those bearing 6-day solid L1210 lymphocytic leukemia or 10-day Lewis lung carcinoma, both tumors implanted s.c. in the axillary region. At various times, animals were immobilized in dry ice/hexane and were stored at −20°C. Whole-body sections were taken and radioautographed (27).

Quantitation of Total Drug Equivalents. Radioactivity in 0.1-ml samples of plasma or urine was determined in aqueous count scintillant (ACS) (Amersham/Searle Corp., Arlington Heights, Ill.) with a Searle Analytic Mark III scintillation system. Feces were mixed with distilled water until there was a consistent paste. Samples of feces were treated as described above without desalting.

Urine samples obtained at intervals after PALA administration, together with control urine samples spiked with [123]PALA, were passed through C18-Sep-Pak cartridges (Waters Associates, Inc., Milford, Mass.). Each cartridge was washed with water which was combined with the load wash, followed by 10, 20, 30, and 100% methanol solutions. Radioactivity in each was determined as described previously. Recovery of radioactivity from control and experimental car- tridges averaged 100%. The load and water washes which contained 100% of the radioactivity recovered were desalted and freeze dried as described above. In addition, since acidic conditions during desalting on AG50-X8 could destroy a potential acid-labile PALA metabolite, urine samples were passed through C18-Sep-Pak cartridges and treated as described above without desalting.

All urine residues, experiments and controls, were redissolved into water and together with a [123]PALA standard were subjected to thin-layer chromatography on precoated cellulose (0.25-mm plates (Brinkmann Instruments, Inc., Westbury, N. Y.) in solvent system: (a) ethanol:water (2:3); Rf PALA = 0.60; (b) ethanol:water:acetic acid (6:4:1); Rf PALA = 0.48; (c) 0.6 M lithium chloride:ethanol:ammonium hydroxide (5:5:1); Rf PALA = 0.33; and (d) n-butanol:acetone:acetic acid:5% ammonium hydroxide:water (18:3:4:4:8); Rf PALA = 0.15. Radioactivity was located by radioautography.

Plasma samples from all species were extracted with perchloric acid. The extracts were subjected to cation-exchange chromatography on AG50-X8 resin columns as described previously (25, 31). Radioactivity in acid-soluble and -insoluble fractions and column eluates was determined as described above.

RESULTS

PALA Concentrations in Plasma. The pharmacokinetics of PALA equivalents in the plasma of the dog and monkey and the rat and mouse are shown in Chart 1, and the pharmacokinetic parameters calculated from these data are shown in Table 1. The rates of elimination were calculated by fitting a simple exponential function to that portion of each curve which probably represents the rate of elimination of PALA from plasma. The apparent volumes of distribution (Vs) were calculated from the intercept of this slope (C0). The Honeywell time-sharing system library program LINREG was used to make the calculations. The areas under the concentration-time curves (C × ts) were calculated as described previously (7, 8).

PALA equivalents were distributed more rapidly from the plasma of rat and mouse than from that of dog and monkey into apparent volumes of distribution much greater than total body water volume (29), as compared to distribution into an apparent volume equal to total body water in dog and approximating extracellular water volume (29) in monkey. PALA equivalents were eliminated at relatively similar rates by all species; however, elimination from rat and mouse plasma was complicated by a secondary rise in concentration which indicates recycling of equivalents from another compartment back into plasma. PALA equivalents were detected in the plasma of all species at time periods later than 7 hr. Although the concentrations were low and erratic in all species, they indicate a secondary slower rate of elimination of PALA equivalents from plasma.

Urine and Fecal Excretion. The excretion patterns for total PALA equivalents were similar in all species (Chart 2). Excretion occurred primarily via the urinary route during the first 4 hr. The poorer recoveries in the urine of noncatheterized dogs and monkeys were probably due to urination occurring during...
Disposition of PALA

Chart 1. Concentrations of PALA equivalents in plasma of 4 species. [14C]PALA was administered at 120 mg/sq m i.v. in 0.9% NaCl solution. The mg/kg doses for each species are shown. Points, means for 4 dogs, monkeys, or rats or 2 groups of 5 mice; bars, S.E.

Table 1
Pharmacokinetic parameters of PALA equivalents in plasma of several species
The kinetic parameters were calculated from the data illustrated in Chart 1.

<table>
<thead>
<tr>
<th>Species</th>
<th>Dose (mg/kg)</th>
<th>V (liters/kg)</th>
<th>Rate distribution (%)</th>
<th>0- to 7-hr C x t (µg-min/ml)</th>
<th>True</th>
<th>Estimated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>120/6</td>
<td>0.6</td>
<td>65 59 (1-4)</td>
<td>1400 2300</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>120/10</td>
<td>0.35</td>
<td>53 65 (1-5)</td>
<td>4200 4200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>120/20</td>
<td>4.5</td>
<td>85 59 (1-3)</td>
<td>1300 650</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td>120/40</td>
<td>6.1</td>
<td>86 48 (1-3)</td>
<td>1800 450</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Distribution in Tissues. PALA equivalents were not taken up to any great extent by mouse tissues, except kidney and bone (Fig. 1). There was also some indication of deposition in lung and skin. No major redistribution of PALA equivalents occurred, and by 4 hr most of the equivalents had been cleared from the body, except from kidney and bone, where they were retained at 24 hr (Fig. 1). At this nontoxic dose, there was no discernible deposition of PALA equivalents in the gastrointestinal tract or the central nervous system, both sites of PALA toxicity (10).

PALA equivalents did not concentrate in tumor tissue, and by radioautographic techniques there were no detectable differences between the uptake or retention of equivalents by Lewis lung carcinoma (responder) or solid L1210 lymphocytic leukemia (nonresponder) (Fig. 2).

Metabolism of PALA. A single radioactive peak was resolved by anion-exchange chromatography on AG1-X8 columns of urine samples obtained at intervals after [14C]PALA administration to all 4 species. This peak represented nearly 100% of the radioactivity loaded on the column and had an elution volume the same as that of [14C]PALA standard (Table 2). These results indicate that PALA is not metabolized.

A single radioactive spot was resolved by thin-layer chromatography in 4 solvent systems of the radioactivity from urine of all 4 species, which had the same elution volume from AG1-X8 columns and C18-SEP-PAK cartridges as PALA standard. The best thin-layer chromatography was achieved after pretreatment on AG1-X8 columns, and 90% of the radioactivity was shown to be associated with the spot having the same Rf as the PALA standard. The remaining 10% was associated with counts which approximated background.

No evidence was found from the analysis of plasma of all 4 species for the breakdown of PALA to L-aspartate or for incorporation of radioactivity into the acid-insoluble fraction of plasma.

These data indicate strongly that PALA is not metabolized, confirming the observations of other workers (18, 31).
Bioavailability. Monkey, rat, and mouse excreted 4, 3, and 5% of the dose, respectively, via the urinary route during 96 hr after p.o. administration of \([^{14}C]\)PALA. The remainder of the dose was excreted via the fecal route. Since systemically administered PALA is excreted primarily via the urinary route, these data indicate that the drug was poorly absorbed. In support of this, the 0- to 7-hr \(C_xt\) values for PALA equivalents in plasma after p.o. administration were much lower than after an equal dose i.v.

**DISCUSSION**

PALA is an organic acid and as such might be expected to be excreted in the urine (26). Consistent with this were the excretion of the drug in the urine of all 4 species and the rapid deposition of the drug in mouse kidney.

With the exception of deposition in kidney, bone, and to a lesser extent, skin and lung, PALA was not deposited in mouse tissues. The localization of PALA equivalents in bone is probably due to PALA forming a chelate with calcium ions (1). A related compound disodium phosphonacetate has been shown to be localized and retained in bones of several species (3). The extrapolated apparent volumes of distribution for PALA ranged from a value in monkey, consistent with distribution into the extracellular water compartment and poor uptake into tissues, to values in rodents, consistent with deposition of the drug in a compartment where it is no longer in equilibrium with other species, in particular, the monkey. The drug was distributed into a volume of distribution approximating extracellular water volume and was eliminated with an initial half-time of 4 days (1). Furthermore, release from bone is much slower. Following i.p. administration of PALA to mice at 400 mg/kg the half-life in bone was approximately 23 days (1).

We concluded that PALA is a drug which is taken up poorly by the tissues of all species and by mouse tumors and is probably not metabolized. The majority of the administered dose is excreted rapidly in the urine, although a small and biologically significant fraction appears to be retained. Subsequent pharmacological studies in humans (11, 23) have shown that the disposition of PALA in humans resembled that in the other species, in particular, the monkey. The drug was distributed into a volume of distribution approximately extracellular water volume and was eliminated with an initial half-time of around 1 hr and a secondary half-time of around 5 hr and with indirect evidence for a much slower elimination rate.

These disposition studies on PALA may be correlated with experimental therapeutics and toxicological data and be applied to guiding the utilization of PALA in the clinic. Most of the data obtained in these studies were included in the Investigational New Drug Application for PALA.

The rapid elimination of PALA via the kidney, together with the poor uptake of the drug by tumors and tissues, explains why relatively high doses have to be administered to achieve antitumor effects (20) and to produce toxic effects (10), despite the inhibitory potency of the drug on aspartate transcarbamylase (9, 17).

No differences were apparent in the uptake or retention of PALA by Lewis lung carcinoma, which is sensitive to PALA treatment, and L1210 lymphocytic leukemia, which is insensitive. However, preliminary data obtained by radiochemical analysis after i.v. administration of PALA at 400 mg/kg to mice (6) indicate that PALA equivalents were retained longer in Lewis lung carcinoma than in the L1210 tumor. There is evidence that the sensitivity of murine tumors to PALA relates to low aspartate transcarbamylase levels (18, 21). However, longer retention of PALA equivalents by the sensitive tumor, especially if this reflects the slower release of PALA from aspartate transcarbamylase, could contribute to the greater sensitivity of the tumor.

Despite deposition of PALA in the kidney, toxicity was not

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**Table 2**

<table>
<thead>
<tr>
<th>Species</th>
<th>Time period (hr)</th>
<th>Radioactivity having same elution volume as ([^{14}C])PALA standard (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse</td>
<td>Control + ([^{14}C])PALA</td>
<td>100</td>
</tr>
<tr>
<td>Mouse</td>
<td>0–4</td>
<td>99</td>
</tr>
<tr>
<td>Mouse</td>
<td>4–8</td>
<td>100</td>
</tr>
<tr>
<td>Mouse</td>
<td>8–24</td>
<td>99</td>
</tr>
<tr>
<td>Rat</td>
<td>0–4</td>
<td>96</td>
</tr>
<tr>
<td>Rat</td>
<td>4–8</td>
<td>99</td>
</tr>
<tr>
<td>Rat</td>
<td>8–24</td>
<td>98</td>
</tr>
<tr>
<td>Dog</td>
<td>0–24</td>
<td>100</td>
</tr>
<tr>
<td>Monkey</td>
<td>0–24</td>
<td>99</td>
</tr>
</tbody>
</table>

\(^a\) Percentage of radioactivity recovered; this radioactivity was shown to have the same \(R_x\) as \([^{14}C]\)PALA standard in 4 thin-layer chromatography systems.
The kinetics of PALA necessitate the administration of an excess of drug in order to achieve concentrations in tumors sufficient to inhibit completely aspartate transcarbamylase. One possible way to reduce the dose necessary for therapy is to incorporate PALA into a drug delivery system, such as liposomes. Such a system has the potential for not only reducing the elimination of PALA and increasing its plasma C x t value but directly enhancing PALA uptake into tumors.

Although PALA is deposited and retained in bone, it seems unlikely that this will be therapeutically exploitable in the treatment of osteogenic sarcoma or potentially a toxicity problem in the treatment of children, since subsequent work has demonstrated that the drug appears to be located in nongrowing bone (1). It is probable that the rapid elimination kinetics of PALA will be unimportant in determining dose scheduling, since PALA did not demonstrate schedule dependency in its antitumor effects (21). This is not unexpected since the antitumor effects of PALA are due to aspartate transcarbamylase-bound drug, and the inhibitory effects of PALA on the enzyme are known to persist for days after drug administration (31). The secondary slower elimination kinetics of PALA, which may reflect the rate of release of enzyme-bound drug, will probably be more important in determining dose scheduling.

ACKNOWLEDGMENTS

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REFERENCES

Fig. 1. Radioautograms of dorsoventral (2 min) and sagittal (1, 4, and 24 hr) sections of normal mice showing distribution of PALA equivalents at intervals after i.v. administration of $[^{14}C]$PALA at 40 mg/kg (70 µCi/kg) in 0.9% NaCl solution. bo, bone; c, cerebrum; i, intestine; in, integument (skin); l, liver; lu, lung; r, renal tissue; un, urine.

Fig. 2. Radioautograms of dorsoventral (10 min) and sagittal (24 hr) sections of mice bearing 10-day Lewis lung carcinoma (LL) or 6-day solid L1210 lymphocytic leukemia at intervals after i.v. administration of $[^{14}C]$PALA at 40 mg/kg (70 µCi/kg) in 0.9% NaCl solution.
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