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Research Article

The Effects of Carbidopa Dose and Time and Route of Administration on Systemic L-Dopa Levels in Rats

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The effects of carbidopa dose and time and route of administration on systemic plasma levels of parenterally and nonparenterally administered L-dopa were examined in rats. Intravenous coadministration of L-dopa + carbidopa resulted in significant ($P < 0.05$) carbidopa-dependent increases in both the area under the plasma L-dopa concentration versus time profile (AUC; +27%) and the plasma L-dopa half-life ($t_{1/2}$; +35%). Simultaneous duodenal or rectal carbidopa administration did not alter the L-dopa i.v. pharmacokinetic profile. Carbidopa pretreatment significantly increased the i.v. L-dopa AUC (+38 and +82% for i.v. and duodenal pretreatments, respectively) compared to simultaneous administration. Both i.v. and duodenal carbidopa increased duodenal L-dopa AUC to a similar extent (+282 and +239% for i.v. and duodenal administration, respectively). Rectal studies indicated poor absorption of both L-dopa and carbidopa, with no demonstrable effect on plasma L-dopa. The results indicate that the timing and route of carbidopa and L-dopa administration are important in determining the extent of i.v. or duodenal L-dopa systemic availability. The rat model affords results similar to those reported in human studies and may be useful for more extensive evaluation of L-dopa and carbidopa interactions.

KEY WORDS: L-dopa; carbidopa; rats; i.v.; duodenal; rectal.

INTRODUCTION

Aromatic amino acid decarboxylase (AAAD), or dopa decarboxylase, is found at low concentrations in most body tissues and high concentrations in liver, kidney, intestinal mucosa, and plasma (1–3). In the peripheral circulation, L-dopa is rapidly decarboxylated to dopamine by AAAD, resulting in unpleasant side effects (4) and decreased L-dopa efficacy (5,6). The AAAD inhibitor, carbidopa (L-α-methyldopahydrazine), is routinely coadministered with L-dopa in the form of Sinemet (Merck Sharp & Dohme) for the treatment of Parkinson's disease. Carbidopa competitively inhibits the extracerebral decarboxylation of L-dopa to dopamine (4,7–10), thus reducing the unpleasant side effects (5,11) and improving L-dopa efficacy (4,11–13) by increasing the proportion of the L-dopa dose available for transport across the blood–brain barrier. Two formulations of Sinemet, based on the L-dopa dose and providing different ratios of L-dopa:carbidopa (100:25 and 250:25 mg), are used clinically. The dose of carbidopa required to inhibit adequately peripheral decarboxylase activity in humans was determined empirically in human clinical observations (12). However, studies in healthy volunteers (14) and Parkinson's disease patients (12,15) have indicated significant enhancement of L-dopa efficacy with carbidopa doses exceeding those currently prescribed without inhibition of L-dopa transport across the blood–brain barrier (4,9,13).

In the studies described here, the effect of carbidopa administration on systemic L-dopa levels was examined in rats. The dependence on routes of administration, timing relative to L-dopa administration, and dose of carbidopa were examined. The studies were directed toward examining the influence of carbidopa on systemic L-dopa levels and assessing the utility of the rat as an animal model for this interaction.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats, 250–300 g, were fasted 20–24 hr prior to use. Sodium pentobarbital (Nembutal, Abbott Laboratories; 50 mg/kg i.p.) was used for initial and maintenance anesthesia. Whole blood samples (0.5 ml) were withdrawn from the external jugular vein, added to 10 μl 0.1 *M* EDTA anticoagulant, and processed immediately for 100-μl plasma samples. Plasma samples were quickly frozen and stored at −80°C until assayed for L-dopa by high-pressure liquid chromatography (HPLC).

All drug solutions were prepared daily. L-Dopa and/or carbidopa were dissolved in a small amount of 0.2 *N* HCl and 0.2% (w/v) ascorbic acid (final ascorbic acid concentration, 0.02%). Solutions were titrated to pH 5.0–5.5 with 0.5 *N* NaOH, and water was added q.s. to the appropriate final concentration of L-dopa and/or carbidopa. Compounds were routinely (co)administered in a volume of 250 μl. In the case of the highest dose of carbidopa (1.0 mg) coadministered intravenously with L-dopa, a total volume of 500 μl was used due to the solubility limits of the two compounds.

Analytical Procedure. A stock solution of L-dopa, 100

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μg/ml, was prepared daily in 0.1% H_3PO_4 and diluted to 10.0 μg/ml. Serial dilutions (1:1) with 0.1% H_3PO_4 yield the following series of standard concentrations: 5.00, 2.50, 1.25, 0.625, 0.312, 0.156, 0.078, 0.039, and 0.0195 μg/ml. To a 500-μl Eppendorf centrifuge tube, 100 μl of the aqueous standard was added to 100 μl of blank rat plasma and immediately deproteinized with 10 μl of perchloric acid. These standards were vortexed 1 min and centrifuged 3 min in an Eppendorf 5414 centrifuge. The supernatant was analyzed by HPLC. Unknown plasma samples were treated similarly, except 100 μl of 0.1% H_3PO_4 was substituted for the aqueous standard.

Chromatographic Conditions. The HPLC system consisted of a primary or analytical subsystem and a secondary or cleanup subsystem. The sample (10 μl) was injected from the autosampler (Perkin–Elmer ISS100) onto the secondary precolumn loop (Brownlee, RP8, 2.1 × 3 cm) at 1.0 ml/min (Scientific Systems pump, Model 300). The secondary mobile phase consisted of 10 m*M* sodium perchlorate, 0.05% perchloric acid, and 0.01% Na-EDTA. The catecholamines were retained on the reverse-phase column with the aqueous mobile phase, while more polar contaminants were flushed to waste. Using timed control valves, the secondary loop was switched in line with the primary column (Brownlee, RP8, 2.1 × 3 cm, and Analytichem, SCX, 4.6 × 10 cm). The primary mobile phase consisted of 10 m*M* sodium perchlorate, 0.05% perchloric acid, 0.01% EDTA, and 20% methanol. The primary flow rate was 1.0 ml/min (Environmental Science Assoc., Model 5700). The methanol in the primary mobile phase quickly eluted the catecholamines and the secondary column was then switched out of line. It was flushed with 50% acetonitrile and then equilibrated with the secondary mobile phase. An electrochemical detector (Environmental Science Assoc., Model 5100A) containing two porous graphite electrodes in series was used in a screening mode. The first electrode, set at −0.05 V, oxidized some contaminants but not the compounds of interest. The second electrode, set at +0.35 V, maximally oxidized L-dopa and carbidopa. A rise time of 2 sec and gain of 5 × 10 were used. A Hewlett–Packard 9816 computer with Nelson Analytical XTRACHROM software (Ver 6.3) and A/D interface (Model 761) were used to collect and integrate data.

Unknown sample concentrations were calculated by external standard from the equation ($y = mx + b$) as determined by weighted (weight α $1/y$) linear regression. Standards were linear over the range 0.02–5.00 mg/ml, where $m = 9 \times 10^{-6}$ and $b = -4 \times 10^{-3}$, with a correlation coefficient of 0.9999. Nominal standard values agreed with those calculated from this line within 3% at concentrations greater than 0.04 mg/ml and within 10% at lower concentrations. Replicate standards ($N = 6$) were assayed to assess intraday precision. Coefficients of variation were less than 5% at concentrations greater than 0.04 mg/ml, 10% for 0.039 mg/ml, and 24% for 0.020 mg/ml. The limit of detection for L-dopa was 0.01 mg/ml.

Data Analysis. The area under the plasma L-dopa versus time curve (AUC) was calculated by the trapezoid summation method. The plasma half-life ($t_{1/2}$) was calculated by linear regression analysis of the terminal portion of the i.v. plasma L-dopa concentration versus time profile. Statistical significance was determined by Student's *t*-test (16).

RESULTS AND DISCUSSION

Intravenous Coadministration of L-Dopa and Carbidopa

Increasing amounts of carbidopa were intravenously administered to rats along with a fixed dose of L-dopa in order to examine the effect of the total carbidopa dose and ratio of carbidopa to L-dopa on the pharmacokinetics of L-dopa. Increased carbidopa concentrations resulted in significant increases in the L-dopa AUC ($P < 0.05$) and the plasma half-life ($t_{1/2}$; $P < 0.025$) between 0.5 and 1.0 mg carbidopa. Results are shown in Fig. 1. At the higher ratio of L-dopa: carbidopa prescribed clinically (4:1; 2.0 mg L-dopa:0.5 mg carbidopa), inhibition of peripheral aromatic amino acid decarboxylase (AAAD) activity was substantial but incomplete, as a twofold increase in carbidopa dose resulted in significant increases in both L-dopa AUC (+16%; $P < 0.05$) and $t_{1/2}$ (+17%; $P < 0.025$) relative to the lower dose of carbidopa. These results indicate that the effect of carbidopa is dependent on the total dose of carbidopa rather than the ratio of carbidopa to L-dopa. Such observations are consistent with those reported in humans (12,14). Kaakkola *et al.* (14) demonstrated in healthy human subjects that increasing the ratio of carbidopa:L-dopa from 1:10 to 1:4 resulted in significant increases in the apparent $t_{1/2}$ and AUC of L-dopa. Similarly, in studies with Parkinson's disease patients, Cedarbaum *et al.* (12) observed significant increases in L-dopa peak plasma levels and AUC from a single dose of L-dopa when the carbidopa dose was doubled.

Simultaneous Administration of i.v. L-Dopa with Nonparenteral Carbidopa

Carbidopa was administered duodenally and rectally to determine whether nonparenteral administration produced similar effects on the plasma L-dopa AUC and $t_{1/2}$ as found for i.v. carbidopa. Results are shown in Fig. 2. Although intravenous coadministration of carbidopa and L-dopa resulted in significant increases in the L-dopa AUC and $t_{1/2}$ (Figs. 1 and 2), carbidopa administration via the duodenal or

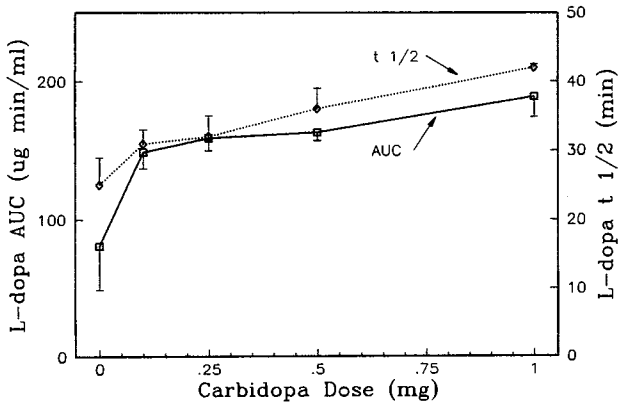

Fig. 1. Effect of carbidopa dose on L-dopa plasma AUC and half-life ($t_{1/2}$) following intravenous coadministration. Carbidopa + 2.0 mg L-dopa was adminstered in a 250-μl bolus injection in all cases except the highest carbidopa dose (1.0 mg), which was administered in 500 μl due to the solubilities of the two compounds. Each point represents the mean ± standard deviation of three determinations.

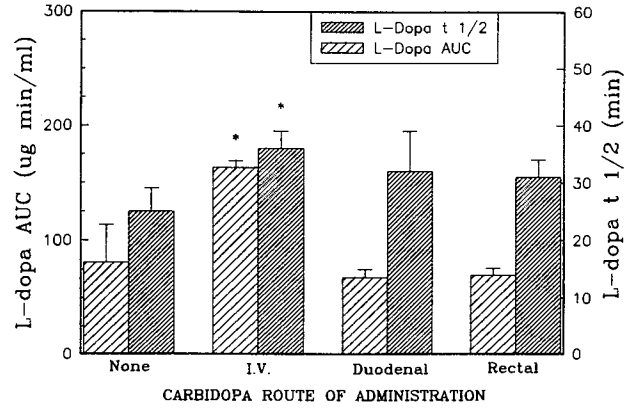

Fig. 2. Effect of the route of carbidopa administration on L-dopa plasma AUC and half-life ($t_{1/2}$) following simultaneous intravenous administration of L-dopa. L-Dopa (2.0 mg) was intravenously administered simultaneous with the administration of carbidopa (0.5 mg) via the following routes: intravenous (coadministered with L-dopa), duodenal (intralumenal injection through the pylorus), and rectal (microenema at an intrarectal depth of 2.5 cm). Each bar represents the mean ± standard deviation of the determinations. Asterisks indicate values significantly different from the value for no carbidopa (none) at the $P < 0.05$ level.

rectal route simultaneous with i.v. L-dopa administration afforded no increase in the L-dopa AUC or $t_{1/2}$ (Fig. 2). The inability of nonparenterally administered carbidopa to affect the AUC and $t_{1/2}$ might be accounted for by the fact that the L-dopa dose, administered i.v., was "instantaneously" susceptible to high concentrations of systemic or postabsorptive (plasma, liver, kidney) decarboxylase activity. Lovenberg *et al.* (7) observed *in vitro* that dopa was decarboxylated by AAAD isolated from guinea pig kidney more rapidly than any other aromatic amino acid substrate. Therefore, a large proportion of an L-dopa dose, administered intraveously without a decarboxylase inhibitor, might be rapidly decarboxylated during distribution into tissues containing high concentrations of AAAD (e.g., liver, kidney, intestine). Although carbidopa was simultaneously administered either duodenally or rectally, the rate or extent of carbidopa absorption might be insufficient to prevent decarboxylation of L-dopa by postabsorptive AAAD. Little is known about the pharmacokinetics of carbidopa in rats, including intestinal absorption. The lack of effect of duodenal carbidopa on i.v. L-dopa plasma AUC and $t_{1/2}$ may be due to poor absorption of carbidopa from the rat gastrointestinal tract. Evidence in the literature (17) suggests that this is the case at the blood–brain barrier. If carbidopa behaves similarly at the mucosal-cell barrier, this could account for the absence of an effect. Additionally, previous studies (8) with an enzyme preparation from guiena pig kidney indicated an extremely rapid interaction between AAAD and carbidopa *in vitro*. Carbidopa complexed with the enzyme *in vitro* within 2 min, even in the presence of L-dopa, with a dissociation constant so low as to be practically irreversible. Since the concentration of AAAD in the small bowel is high, this could result in a decreased extent of carbidopa absorption due to pseudoirreversible complexing with the enzyme in the lumen of the gastrointestinal tract in the current study.

Pretreatment with Carbidopa 30 min Prior to i.v. L-Dopa

In order to examine whether a slow rate of absorption of nonparenteral carbidopa might account for the inability of the dose to alter the AUC or $t_{1/2}$ of i.v. L-dopa, carbidopa was administered intravenously, duodenally, or rectally 30 min prior to intravenous administration of L-dopa. Preadministration via the intravenous and duodenal routes resulted in significant increases ($P < 0.01$ and $P < 0.005$, respectively) in L-dopa plasma AUCs (Fig. 3) over those obtained with simultaneous administration via the same routes (Fig. 2). The $t_{1/2}$ values were similar for both patterns of administration (Figs. 2 and 3). These results were consistent with results reported in Parkinson's disease patients receiving i.v. infusions of L-dopa with and without concurrent oral carbipoda administration (17,18). Nutt *et al.* (18) observed that oral pretreatment and concurrent treatment with carbidopa reduced by 50% the L-dopa infusion rate required to obtain therapeutically effective plasma L-dopa i.v. levels. However, contrary to the results of the current study, concurrent oral carbidopa administration also modestly extended the plasma half-life of L-dopa. This inconsistency may be due to repeated administration of carbidopa to the Parkinsonian patients during the course of the infusion, while in the present case only a single intraduodenal bolus injection was administered to rats 30 min prior to i.v. L-dopa. Also, higher extravascular AAAD levels in rats may account for the difference.

Rectal pretreatment with carbidopa afforded no increase in i.v. L-dopa AUC or $t_{1/2}$. This result suggests that carbidopa is much more readily and/or extensively absorbed by the duodenal than the rectal route in the rat. The rate of duodenal carbidopa absorption, however, does not appear to be sufficient to prevent significant plasma decarboxylation of intravenously administered L-dopa unless the carbidopa is administered prior to the i.v. L-dopa administration (e.g., 30 min pretreatment).

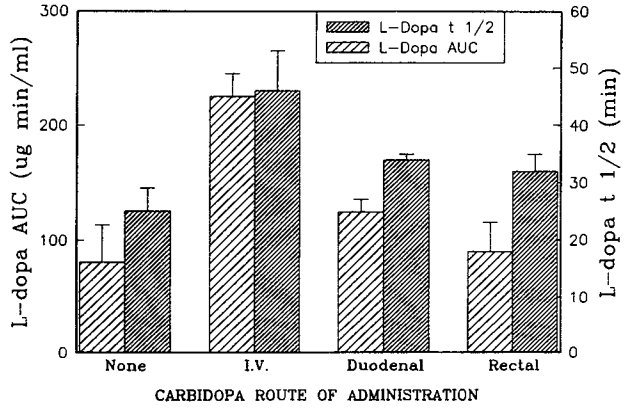

Fig. 3. Effect of carbidopa pretreatment on plasma AUC and half-life ($t_{1/2}$) of intravenously administered L-dopa. Carbidopa (0.5 mg) was administered intravenously (bolus injection), duodenally (intralumenal injection), or rectally (microenema) 30 min prior to intravenous administration of L-dopa (2.0 mg). Each bar represents the mean ± standard deviation of three determinations. Asterisks indicate values significantly different from the value for no carbidopa (none) at the $P < 0.05$ level.

Nonparenteral L-Dopa Simultaneously with i.v., Duodenal, and Rectal Carbidopa

The effect of the carbidopa administration route on systemic levels of nonparenterally administered L-dopa was also examined to compare the relative degrees of pre- and postabsorptive decarboxylation (Table I). When L-dopa was administered duodenally, carbidopa administered simultaneously by i.v. or duodenal routes resulted in an approximate fourfold increase ($P < 0.05$) in the L-dopa plasma AUC over that obtained when no carbidopa was given. Although rectal carbidopa administration appeared to increase the plasma AUC of duodenally administered L-dopa approximately 1.6-fold over that seen in the absence of carbidopa, the increase was not statistically significant. It is worth noting that, when L-dopa was administered duodenally, intravenous (postabsorptive) and duodenal (preabsorptive) administration of carbipoda resulted in similar L-dopa plasma AUC values. This observation may be due to multiple decarboxylation sites and the relative influences of i.v. and duodenal carbidopa on these sites. Intravenous carbidopa might be expected to inhibit effectively the postabsorptive (liver, kidney, plasma) AAAD activity but not significantly affect the preabsorption activity in the mucosal tissue, allowing decarboxylation of a portion of the duodenally administered L-dopa prior to gaining access to the systemic circulation. In contrast, duodenally administered carbidopa could result in inhibition of both presystemic (intraluminal and mucosal) and systemic (liver, kidney, plasma) AAAD. The inhibition of systemic AAAD by duodenally administered carbidopa, obviously, would be dependent on the rate and extent of carbidopa absorption. It was shown previously (Figs. 2 and 3) that there was a lag time between duodenal administration of carbidopa and expression of systemic AAAD inhibition. Therefore, under the experimental conditions employed in the current study, the L-dopa plasma AUC observed following duodenal carbidopa administration is probably due to a combined, although incomplete, effect of carbidopa on pre- and postabsorptive AAAD activity. This relative accessibility of carbidopa to the two sites of AAAD activity may account for the observed similarity of response seen with duodenal L-dopa administration in the presence of i.v. or duodenal carbidopa.

Table I. Effect of Simultaneous Administration of L-Dopa and Carbidopa via Various Routes on L-Dopa Plasma AUC[a]

	L-Dopa plasma AUC (μg · min/ml)[b]	
	Duodenal L-Dopa	Rectal L-Dopa
No carbidopa	28 ± 13	ND[c]
i.v. carbidopa	107 ± 11	4 ± 1
Duodenal carbidopa	85 ± 7[d]	2 ± 1
Rectal carbidopa	45 ± 15	8 ± 5

[a] L-Dopa (2.0 mg) was administered duodenally (intraluminal injection) or rectally (microenema) simultaneous with the administration of carbidopa via the intravenous, duodenal, or rectal route. In the cases where L-dopa and carbidopa were administered via the same route, both compounds were administered in a single bolus.
[b] Values represent $\bar{X}$ ± SD of three determinations.
[c] No L-dopa detected in plasma.
[d] Average ± range of two determinations.

Table II. Comparison of the Effect of Carbidopa Administration Concomitantly with, vs 30 minutes Prior to, L-Dopa Administration via the Same Route on L-Dopa Plasma AUC[a]

	L-Dopa plasma AUC (μg · min/ml)[b]	
Administration route	Coadministration	Pretreatment
Duodenal	85 ± 7	109 ± 20
Rectal	8 ± 5	10 ± 1

[a] Carbidopa (0.5 mg) was administered duodenally (intraluminal injection) or rectally (microenema) concomitantly with, or 30 min prior to, administration of L-dopa (2.0 mg) via the same route.
[b] Values represent $\bar{X}$ ± SD of three determinations.

When rectally administered without carbidopa, L-dopa was not detected in the plasma (Table I). However, simultaneous administration of carbidopa by any of the routes resulted in detectable but very low plasma L-dopa levels. These results indicate that the administration of carbidopa by any route increases the AUC of rectally administered L-dopa. However, rectal absorption of L-dopa compared to duodenal absorption is extremely low.

Nonparenteral Pretreatment with Carbidopa 30 min Prior to L-Dopa Administration by the Same Route

As shown in Figs. 2 and 3, the rate of duodenal or rectal absorption of carbipoda is apparently not sufficient to inhibit systemic (postabsorptive) decarboxylation of concurrently administered i.v. L-dopa, while 30-min duodenal pretreatment significantly decreased postabsorptive decarboxylation. In order to determine if such pretreatment exhibited a similar effect on decarboxylation of nonparenterally administered L-dopa, rats were duodenally or rectally pretreated with carbidopa 30 min prior to L-dopa administration via the same route (i.e., duodenal–duodenal, rectal–rectal). Resulting L-dopa plasma AUCs are shown in Table II. Luminal pretreatment with carbidopa 30 min prior to L-dopa administration afforded no statistically significant increase in plasma AUC (Table II) over coadministration of the two compounds by the duodenal or rectal routes.

The failure of luminal pretreatment with carbidopa to provide an increase in the L-dopa AUC over coadministration of L-dopa and carbidopa might be attributable to the rate at which carbidopa binds pseudoirreversibly to AAAD. It was reported (8) that carbidopa complexed with AAAD *in vitro*, even in the presence of L-dopa, within 2 min. In the current study, carbidopa coadministered with L-dopa may bind with the luminal decarboxylase at such a rate as to make pretreatment unnecessary for full inhibition of the preabsorptive enzyme.

These results indicate that the rat model responds to variations in L-dopa and carbidopa dosing regimens in a manner similar to that reported for humans. In this model, the L-dopa plasma AUC resulting from duodenal coadministration of the 4:1 L-dopa:carbidopa ratio prescribed clinically for oral administration in humans is not significantly improved by changes in the route or timing of carbidopa administration.

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