Variable Absorption of Carbidopa Affects Both Peripheral and Central Levodopa Metabolism

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*Carbidopa (CD), a competitive inhibitor of aromatic l-amino acid decarboxylase that does not cross the blood-brain barrier, is routinely administered with levodopa (LD) to patients with Parkinson disease (PD) to reduce the peripheral decarboxylation of LD to dopamine. Using a stable isotope-labeled form of LD, the authors examined in 9 PD patients the effects of variable CD absorption on peripheral and central LD metabolism. Subjects were administered orally 50 mg of CD followed in 1 hour by a slow bolus intravenous infusion of 150 mg stable isotope-labeled LD (ring 1',2',3',4',5',6'-^{13}C). Eight patients underwent a lumbar puncture 6 hours following the infusion. Blood and cerebrospinal fluid (CSF) samples were analyzed for labeled and unlabeled metabolites using a combination of high-performance liquid chromatography and mass spectrometry. When patients were divided into "slow" and "rapid" CD absorption groups, significantly greater peripheral LD decarboxylation (as measured by area under the curve [AUC]-labeled serum HVA) was noted in the poor absorbers (*p = *0.05, Mann-Whitney U test). Elimination half-lives for serum LD did not differ between groups, suggesting a further capacity for decarboxylation inhibition in the "rapid" absorbers. A significant correlation between AUC serum CD and percent-labeled HVA in CSF was found for all patients (*R = *0.786,* p = *0.02). "Rapid" as compared to "slow" CD absorbers had significantly more percent-labeled CSF HVA (60 vs. 49,* p = *0.02, Mann-Whitney U test), indicating greater central-labeled DA production in the better CD absorbers. The data suggest that peripheral aromatic l-amino acid decarboxylase activity is not saturated at CD doses used in current practice. The authors believe that future studies to better examine a dose dependence of CD on peripheral LD decarboxylation and LD brain uptake are warranted.*

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Carbidopa (CD), a competitive inhibitor of aromatic l-amino acid decarboxylase (AAAD) that does not cross the blood-brain barrier, is routinely administered with levodopa (LD) to reduce the peripheral decarboxylation of LD to dopamine (DA). Inhibiting this peripheral metabolism minimizes systemic DA-induced side effects (nausea, vomiting, cardiac arrhythmias) and allows more LD to enter brain.[1] The latter is supported by positron emission tomography (PET) studies, which have shown that CD administration prior to intravenous fluorodopa enhances scan activity.[2,3] It is uncertain, however, if CD doses used in current practice are high enough to maximally inhibit peripheral AAAD activity. If such doses do not saturate peripheral AAAD, it would be expected that variable absorption of an oral CD dose might have a significant impact on both peripheral and central LD metabolism.

Previous attempts to study the effects of CD on peripheral and central LD decarboxylation have been severely compromised by an absence of a safe and adequate labeling technique for LD. We have developed a new labeled form of LD using stable isotopes. Stable isotopes are naturally occurring isotopes that usually differ from their parent atom (the most abundant form of the element) by the addition of one or more neutrons. These substances are not radioactive. An example of one such stable isotope is ^{13}C (as compared to the usually occurring ^{12}C). Since there is no toxicity associated with the use of stable isotopes, the margin of safety in applying ^{13}C as a pharmacologic label has been described as virtually limitless.[4] A drug labeled with such isotopes can be quantitated along with its metabolites using mass spectrometry (MS). The labeled LD form

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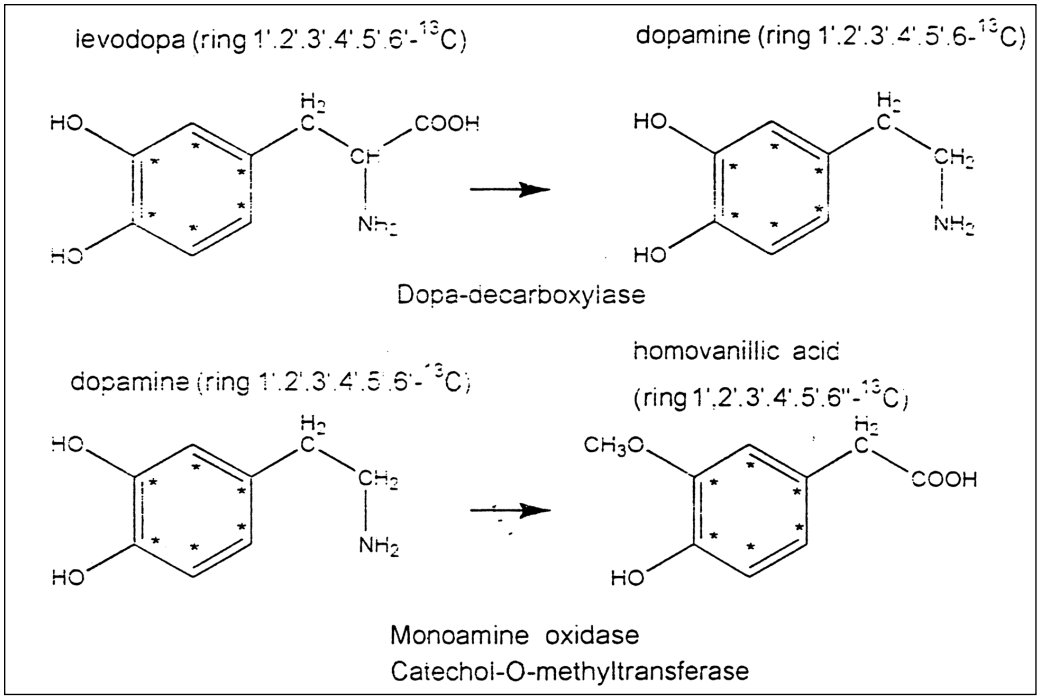

Figure 1. The represented stable isotope-labeled levodopa form has all carbons within the ring replaced with ^{13}C, making it six mass units greater than unlabeled levodopa. These "heavy" carbons are marked by an asterisk. The major catabolic route of levodopa pictured involves metabolism occurring completely outside the ring structure. Thus, all metabolites derived from the labeled form will also be six mass units greater and detectable from background metabolite concentrations via mass spectrometry.

used in this study had all carbons within the catechol ring replaced with the stable isotope ^{13}C, making the compound and all subsequent metabolites six atomic mass units greater than the unlabeled analogue (Figure 1). Since this LD form is not radioactive, we are able to administer a full pharmacologic dose that is completely labeled rather than the tiny tracer amounts used in radioactive LD studies. All quantitation of metabolites, therefore, represents actual amounts produced from an administered LD dose and is not subject to errors based on estimation with tracer doses.

MATERIAL AND METHODS

Nine patients participated in the infusion studies. Subjects were all diagnosed with idiopathic Parkinson disease (PD) by the principal author (R.D.) and at least one other neurologist. All patients had experienced previous benefit to LD except for one. This latter patient had been newly diagnosed, and his first LD exposure was the stable isotope-labeled LD given in our study. The mean (*SD*, range) age of our patient group was 61.4 years (12.3, 44-75), and they averaged 8.1 years for duration of disease (4.9, 0.5-15). None had undergone surgical therapy for PD. Patients with a prior history of cardiac disease or arrhythmias were excluded from participation. For patients taking CD/LD, their average daily dose prior to study was 102 mg (46, 37.5-175)/538 mg (240, 150-1000). Six patients were taking pergolide, 3 selegiline, and 1 bromocriptine before the stable isotope-labeled LD infusion. All medications were discontinued on the day of the infusion.

After signing informed consent, patients were admitted to the hospital on the afternoon prior to the day of study. Approval for the project had been given by the Research and Development Committee and Human Studies Subcommittee at the Boston Veterans Administration Medical Center. A Food and Drug Administration (FDA) investigational new drug approval had been obtained for the stable isotope-labeled LD form by one of us (R.D.) before use. The stable isotope-labeled LD form was more than 99% isotopically pure at each labeled site, and chemical purity was 98.1%. The source of this labeled form was Cambridge Isotopes Laboratory (Andover, MA). On the day of investigation, food and all medications were held from 12 midnight. At 7 a.m., subjects were connected to a cardiac monitor, and two heparin locks (one for blood drawing and one for the LD infusion) were placed in arm veins (one lock in each arm). All patients received 50 mg of CD orally at 8 a.m. after a blood sample (their last CD dose as part of their daily CD/LD regimen in all cases had been taken at least 12 hours earlier). At 9 a.m., subjects were examined using a modified Unified Parkinson Disease Rating Scale (UPDRS), and another blood sample was

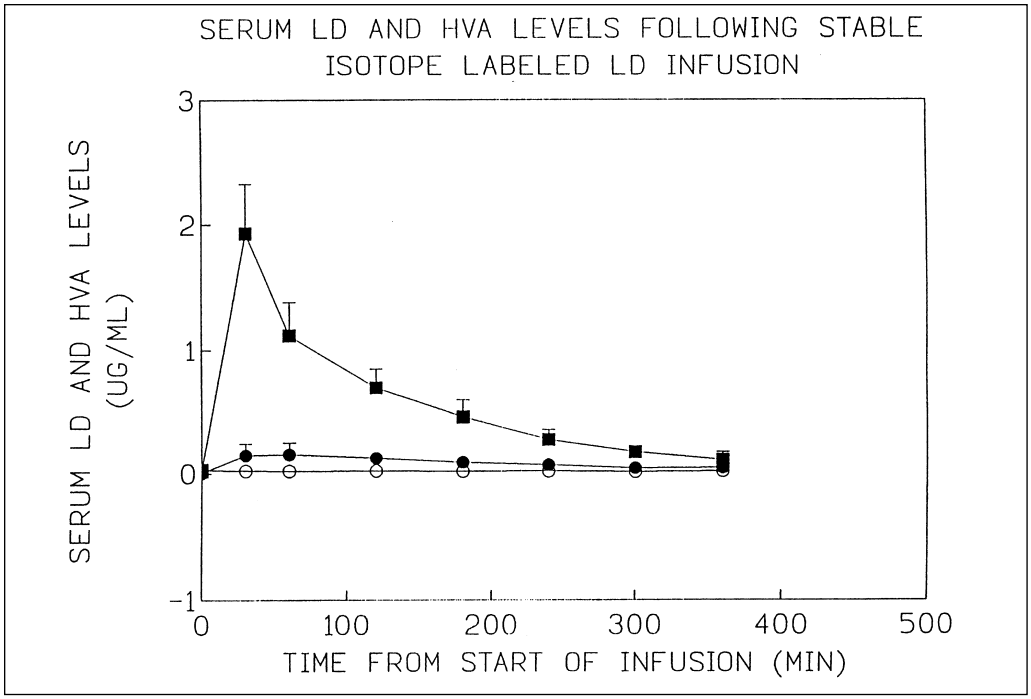

Figure 2. Mean-labeled serum levodopa levels (filled squares), mean-labeled serum HVA levels (filled circles), and mean-unlabeled serum HVA levels (open circles) obtained over a 6-hour period following an intravenous bolus infusion of 150 mg of stable isotope-labeled levodopa at time 0 are shown. Each point is the average of 9 patients. Error bars indicate standard deviation.

drawn (heparin within the lock was always first discarded). Immediately following this serum sample, a bolus infusion was started of 150 mg (1 mg/ml) over 12 to 15 minutes of stable isotope-labeled LD (ring 1',2',3',4',5',6'-^{13}C) in 5% Dextrose Injection USP solution (pH = 4.5) using Harvard infusion pumps (Harvard Apparatus Syringe Infusion Pump 22). Blood samples were drawn at 30- or 60-minute intervals throughout the duration of the study until 360 minutes post-infusion. These samples were immediately spun and resultant serum pipetted into polypropylene tubes and then frozen (–70°C). In addition, at 360 minutes, a lumbar puncture was performed in 8 patients with cerebrospinal fluid (CSF) collected in 5 ml aliquots (total: 25 ml). The last aliquot (20th-25th ml) was used for CSF HVA analysis. The severity of patients' parkinsonism and blood pressure were measured every 30 minutes during the study.

Laboratory methodologies involved the use of both high-performance liquid chromatography (HPLC) and MS. HPLC was used for quantitation of total (labeled and unlabeled) HVA, CD, and LD concentrations. MS was employed to determine the ratio of labeled to unlabeled HVA. By knowing the labeled/unlabeled ratio as well as total concentration, we were able to calculate absolute amounts for labeled and unlabeled metabolite. Our HPLC assay was derived from a previously published methodology[5] and allowed for the simultaneous analysis of LD, CD, and HVA. The ratios between labeled and unlabeled forms of HVA were measured as their O-acetyl, pentafluorobenzyl esters by negative ion chemical ionization gas chromatography/MS. The employed methodology was a modified technique of that described by De Jong et al.[6] Pharmacologic data were analyzed using WinNonlin software (Scientific Consulting, Inc., Cary, NC), and statistical analysis involved use of the Mann-Whitney U Test and Pearson correlations.

RESULTS

All patients received benefit following the labeled LD infusion. Specifically, the average maximal percentage of improvement noted was 63% (29, 13-100), with a mean duration response of 237 minutes (89, 120-360). The average reduction in systolic blood pressure was 22 mmHg (14, 10-40). No prolonged (> 5 seconds) cardiac arrhythmias were noted during the infusion or subsequent 6-hour monitoring period.

Our bolus infusion methodology produced levels of LD in blood, as seen in Figure 2. The highest level measured was at T = 30 minutes (15 minutes after cessation of the infusion). The mean concentration at this time for the 9 patients was 2.00 ug/ml (0.43, 1.3-2.6). These peak concentrations are similar to those attained after

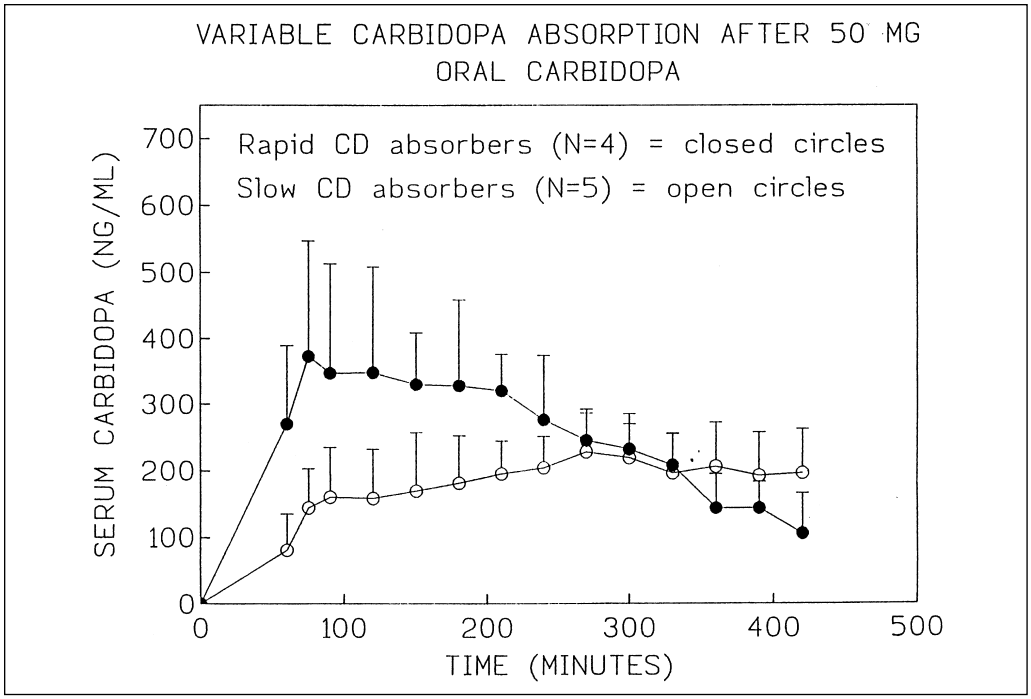

Figure 3. Serum concentrations of CD are presented for "slow" (open circles) and "rapid" (filled circles) CD absorbers. The two groups significantly differ for AUC CD (p = 0.02, Mann-Whitney U test). Error bars indicate standard deviation.

an oral 25/250 (CD/LD dose). Thereafter, serum concentrations steadily declined to approximately baseline levels at T = 300 minutes. For all subjects, mean absolute levels of serum-labeled HVA were approximately tenfold those of baseline-unlabeled HVA levels at times 30 and 60 minutes. Labeled HVA at these respective times accounted for 83% and 85% of the total serum HVA concentration. Even in the last sampling period (T = 360 minutes), labeled HVA still represented 68% of the total serum HVA concentration. For all patients, previous daily CD dose did not influence peripheral LD decarboxylation (i.e., area under the curve [AUC]–labeled serum HVA) ($R = 0.170$, $p = 0.69$).

The observed average time to maximum concentration of serum CD (t_{max}) was 205 minutes. The variation in CD t_{max} among patients ranged from 75 to 420 minutes. Patients were divided into "slow" absorbers (those unable to attain a serum CD level of at least 300 ng/ml within 3 hours after CD administration) and those with more "rapid" absorption (patients obtaining a level of 300 ng/ml or greater within the first 3 hours). AUC serum CD for "slow" absorbers averaged 69,632 ng/ml × 7 hours ($n = 5$) as compared to 101,985 ng/ml × 7 hours in those with "rapid" absorption ($n = 4$) ($p = 0.02$, Mann-Whitney U test) (Figure 3). Variability in CD absorption directly affected peripheral LD decarboxylation. Specifically, AUC-labeled serum HVA in the "slow" CD absorbers approached twice that of "rapid" absorbers (44,597 ng/ml × 6 hours vs. 25,455 ng/ml × 6 hours, $p = 0.05$, Mann-Whitney U test) (Figure 4). For all patients, AUC serum CD significantly correlated with percent-labeled CSF HVA ($R = 0.786$, $p = 0.02$). In addition, a significantly greater percent-labeled CSF HVA was present in "rapid" as compared to "slow" CD absorbers (60% vs. 49%, $p = 0.02$, Mann-Whitney U test). Other pharmacologic parameters for the two groups are given in Table I. There was no evidence of peripheral-labeled HVA contaminating CSF HVA as the correlation between AUC serum-labeled HVA and CSF-labeled HVA was not significant ($R = -0.222$, $p = 0.60$). The duration of response and maximal percentage of improvement achieved after the labeled LD infusion did not significantly differ between the two groups. Similarly, the observed reduction in blood pressure following the infusion was not statistically different for "slow" and "rapid" absorbers.

DISCUSSION

Peripheral AAAD is located in gastrointestinal mucosa,[8] kidney and liver,[9] blood, and capillary endothelium.[10] Intravenously administered LD is metabolized to some degree by all of these compartments, including gastrointestinal mucosa.[11] Several observations in our study indicate that peripheral AAAD is not saturated with doses of CD used in current practice. First, we

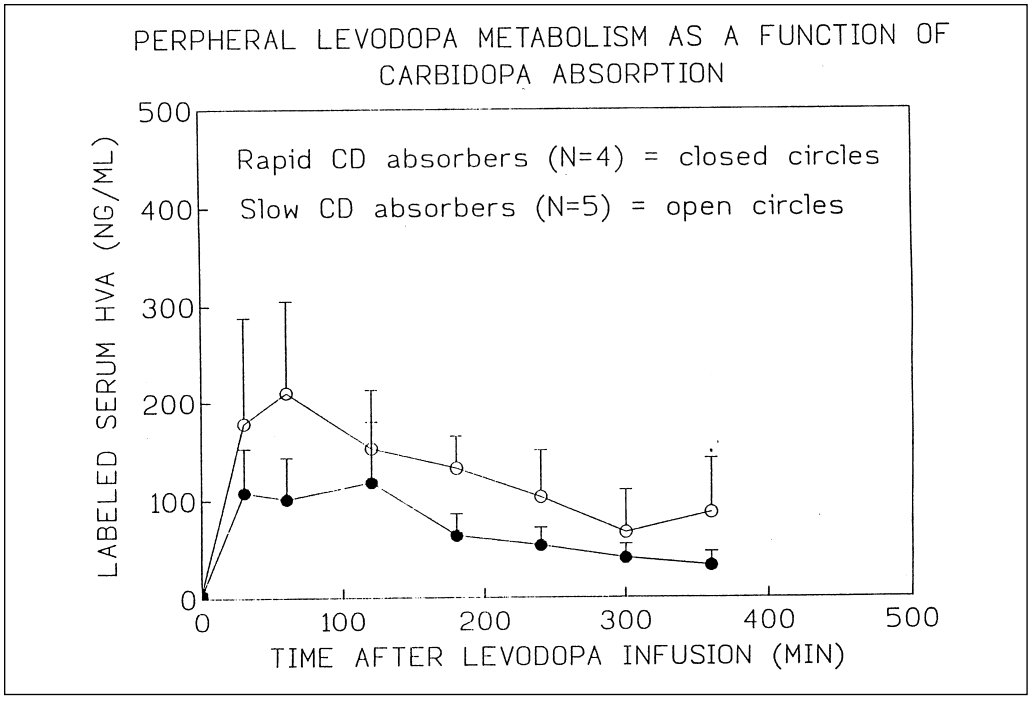

*Figure 4. Serum concentrations of labeled HVA are presented for "slow" (open circles) and "rapid" (filled circles) CD absorbers. The two groups significantly differ for AUC-labeled HVA (*p = *0.05, Mann-Whitney U test). Error bars indicate standard deviation.*

found that variable CD absorption significantly affects enzyme inhibition. Such would not have been possible if achieved levels of CD in blood were well above those required for saturation. Second, our observation that serum LD elimination half-lives did not differ between "slow" and "rapid" CD absorbers would indicate that even in the "rapid" absorption group, enzyme saturation was not approximated. Specifically, decarboxylation of LD is the major catabolic route for systemic LD.[12] Had peripheral AAAD been nearly or fully saturated, we would have expected the elimination half-life for serum LD to be longer in "rapid" CD absorbers (i.e., the major catabolic route for LD would have been significantly blocked, resulting in a diminished capacity to eliminate LD). Finally, levels of CD achieved in blood closely predicted percent-labeled CSF HVA. The latter suggests that there was no cap on peripheral AAAD inhibition (i.e., increasing serum CD levels allowed for still greater inhibition of systemic LD catabolism and subsequently more LD penetration into the brain). We note that within the clinical community, 75 to 100 mg/day of CD delivered in 25 or 50 mg doses has frequently been accepted as blocking all peripheral LD decarboxylation.[13] Evidence for the latter, however, is

Table I Pharmacologic and Clinical Parameters by CD Absorption Group

Parameter	"Slow" CD Absorption	"Rapid" CD Absorption
AUC serum CD (ng/ml × 7 hours)	69,623 (5, 12,999)	101,985 (4, 19,098)*
AUC serum-labeled LD (ug/ml × 6 hours)	225 (5, 71)	217 (4, 47)
AUC serum-labeled HVA (ng/ml × 6 hours)	44,597 (5, 16,099)*	25,455 (4, 9298)
Labeled CSF HVA (ng/ml)	43.6 (4, 25.6)	54.8 (4, 37.1)
Percent-labeled CSF HVA	49.1 (4, 8.2)	60.3 (4, 5.2)*
Percentage of improvement	57.8 (5, 21.4)	69.5 (4, 38.8)
Duration of response (min)	252 (5, 86.4)	217 (4, 102)
LD elimination half-life (min)	71 (5, 13.6)	68.8 (4, 7.0)

CD, carbidopa; AUC, area under the curve; LD, levodopa; CSF, cerebrospinal fluid.
*$p \leq 0.05$.

indirect and based primarily on clinical observations rather than direct pharmacokinetic data.[1] In fact, the few radioactive LD studies that are available appear to support our findings of nonsaturation. Specifically, with tiny radioactive LD tracer doses, Messiha et al[14] found persistent labeled HVA production in blood despite pretreatment with 100 mg CD. In addition, Ward et al[15] observed a continuing inhibition of peripheral LD decarboxylation at CD doses up to 2.5 mg/kg by measuring exhaled radioactive CO_2 after a tracer radioactive LD dose. Our study adds to this literature because we have employed pharmacologic rather than tracer-labeled doses and therefore more closely replicated the clinical setting. Our methodology is also considerably more direct since a specific metabolite indicative of decarboxylation is measured rather than a more remote product (CO_2).

Little is known regarding gastrointestinal absorption of CD. It probably does not involve the same gastrointestinal blood-brain barrier, facilitated amino acid transport system used by LD[12] since CD does not cross the blood-brain barrier. CD absorption is slower and more variable than that of LD, with a t_{max} range reported to be 30 minutes to 5 hours in PD patients.[1] This wide CD t_{max} range is consistent with our data. It is probable in this study that we achieved higher blood levels of CD at peak LD levels than are present after an oral CD/LD dose since we gave CD approximately 1 hour before LD administration. Our experimental design also differed from the normal clinical setting in two other ways. Rather than giving both CD and LD orally, we administered the stable isotope-labeled LD form intravenously. Oral absorption of LD is highly variable, with attainment of peak plasma levels ranging from 15 minutes to 2½ hours.[16] Our slow bolus LD infusion methodology allowed us to minimize this variation among patients and improve statistical power, yet simulate peak LD concentrations seen following oral 25/250 CD/LD tablets.[7] Another difference was our administration of a single CD dose as compared to the typical clinical setting in which patients take three to four doses of CD per day. Again, we believe our study paradigm is reflective of clinical treatment (a daily, multiple CD dose regimen) because the plasma half-life of CD is reported to be 2 to 3 hours.[1] Consequently, a steady-state CD level is unlikely attained during the course of a day for most PD drug regimens (i.e., CD dosing 4 hours apart or greater with no administration of drug overnight). The latter is supported by our own data showing the absence of serum CD after an overnight fast and the lack of a significant influence of previous daily CD dosage on the peripheral decarboxylation of our labeled LD dose. We would caution, however, that the small number of patients in our study might have resulted in insufficient power to detect such an effect. Longer washout periods for CD may prove more optimal for future studies if prolonged effects of CD on decarboxylation can be shown in larger patient populations.

To measure decarboxylation of LD, we employed serum HVA as a marker for DA. HVA is the final peripheral and central metabolite of DA and is routinely used as a stable marker for the neurotransmitter.[17] Experiments in rats[18] and man[14] demonstrate that serum HVA levels parallel peripheral DA concentrations when decarboxylation pathways are inhibited with CD. We note that peak levels of serum-labeled HVA in our study were attained almost immediately following the LD infusion. While we have no similar in vivo human studies to compare with, such rapid systemic conversion of LD to HVA after parenteral LD administration has been observed in animals even after pretreatment with CD.[18] While significant differences were noted in AUC serum-labeled HVA between "slow" and "rapid" CD absorption groups, similar findings were not found comparing serum LD. This lack of effect of CD on LD pharmacokinetics in blood has been noted in animals. Specifically, Deleu et al[19] reported that the preadministration of CD increased LD AUC in muscle extracellular fluid but not in plasma. We hypothesize that distribution and metabolism of LD in blood are extremely rapid when peripheral AAAD activity is not saturated. As a consequence, CD-induced effects might only be detected in more stable end-point metabolites such as HVA or in compartments in which distribution has been slowed (e.g., muscle).

There is an abundance of literature indicating that CSF HVA is derived from central dopamine production and not from blood-brain or blood-CSF transfer.[17] Consequently, the significant correlation between serum AUC CD and percent-labeled CSF HVA in all patients and the difference in percent-labeled CSF HVA between "rapid" and "slow" CD absorbers are in vivo pharmacologic support for the possibility that higher serum CD levels will promote greater entry of LD into the brain. Our data suggest that CD doses greater than those currently employed in practice might further increase central uptake of LD and promote greater DA production. Future studies to better examine the dose dependence of CD on LD peripheral metabolism and uptake will be needed to further investigate this issue.

We could not demonstrate a clinical difference in magnitude or duration of response to LD based on serum CD levels or peripheral LD decarboxylation. Similarly, we found no difference in systemic DA-related side effects (nausea, vomiting, or cardiac arrhythmias) based on these same variables. We believe that this may

relate to our small number of study subjects, which allowed for only large differences to be detected. The possibility that clinical differences might have been seen in a larger patient group is underscored by recent success in treating PD patients with peripheral catechol-O-methyltransferase (COMT) inhibitors.[20-23] These latter studies clearly demonstrate that further diminishing peripheral LD catabolism in patients taking usual therapeutic CD/LD doses beneficially affects treatment. The administration of LD in combination with CD and peripheral COMT inhibitor doses that maximally inhibit peripheral LD catabolism might ultimately prove the most rational therapy in PD. Such an approach would theoretically optimize delivery of LD into the brain and keep unwanted peripheral DA production to a minimum.

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