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Evolution of the Bioavailability and other Pharmacokinetic Parameters of Levodopa (with Carbidopa) in Rabbits

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SUMMARY

Levodopa pharmacokinetics show important inter- and intraindividual differences when it is administered by the oral route. As a result of fluctuating drug plasma concentrations, patients may develop motor fluctuations and dyskinesias. Therefore, it is important to perform studies on levodopa pharmacokinetics in the same individual. The aim of this study was to contribute to a better knowledge of the evolution of the pharmacokinetics of levodopa administered with carbidopa. The study involved the oral administration of 20/5 mg/kg levodopa/carbidopa to rabbits for two different time periods (7 or 14 days), due to the fact that inhibition of aromatic L-amino-acid decarboxylase by carbidopa is not immediate. After 7 days of treatment, the levodopa AUC increased by 12.6% from day 1 (range: 114.2–150.7 μg.min/ml) to day 7 (range: 131.1–166.0 μg.min/ml) and C_{max} increased by 9.6% (range: 1.90–2.86 μg/ml on day 1 and 2.12–3.13 μg/ml on day 7). After 14 days of treatment, the increase in AUC was 17.0% (range: 119.6–160.1 μg.min/ml on day 1 and 142.9–172.7 μg.min/ml on day 14) and C_{max} increased by 6.5% (range: 2.29–2.96 μg/ml on day 1 and 2.41–3.07 μg/ml on day 14). The values obtained for C_{min} (sample obtained immediately before levodopa/carbidopa administration) in both groups increased progressively with the duration of the treatment. C_{max} and AUC values were very similar after 7 or 14 days of treatment. The time needed for C_{min} stabilization was slightly higher, because we found significant differences until day 11 of treatment.

Key words: Carbidopa - LAAD inhibitors - Levodopa - Pharmacokinetics - Rabbits

INTRODUCTION

Levodopa remains the most effective drug in the management of Parkinson's disease, despite the fact that it was introduced into therapy more than 40 years ago. After oral administration, levodopa undergoes marked presystemic and systemic decarboxylation, and it is almost always administered in combination with a peripherally acting inhibitor of aromatic L-amino-acid decarboxylase (LAAD), *i.e.*, carbidopa or benserazide. With coadministration of a peripheral decarboxylase inhibitor, levodopa bioavailability markedly increases and its side effects decrease. However, the rate and extent of levodopa absorption show important inter- and intraindividual differences, mainly related to the progression of the disease. Woitalla *et al.* (1) found that levodopa availability improved with disease progression due to the deterioration of the peripheral activity of metabolizing enzymes or to increasing enteric dysfunction with subsequently improved duodenal levodopa absorption, or both.

There are numerous factors that can modify levodopa pharmacokinetics, such as gastric emptying, the pH of gastric juice, the presence of food, different individual requirements of the LAAD inhibitor and active transport from the intestine to the blood, which can be affected by large neutral amino acids. On the other hand, the impact of body weight on pharmacokinetics is well known (2–4), and in clinical practice, drugs are often administered according to the patient's body weight. Nevertheless, patients with Parkinson's disease are usually treated with standard doses of levodopa/carbidopa without any adjustment of the dose according to body weight. Arabia *et al.* (4) found that body weight and AUC and $t_{1/2}$ were significantly and inversely correlated. Consequently, the mean pharmacokinetic data reported in different research papers are very different (*e.g.*, t_{max} of 0.5–2 h and plasma $t_{1/2}$ of 1–3 h) and show high coefficients of variation.

After several years of levodopa treatment, patients may develop involuntary movements (levodopa-induced dyskinesias) as a result of fluctuating drug plasma concentrations. Koller *et al.* (5) reported that the motor fluctuations and dyskinesias could be minimized by using the lowest possible levodopa dose throughout the years of treatment; thus, to establish the most effective treatment

regimen for levodopa, it would be advisable to monitor the patient.

The aim of this study was to contribute to a better knowledge of the evolution of the pharmacokinetics of levodopa administered with carbidopa. Because the inhibition of LAAD is not immediate (6), levodopa/carbidopa was administered for two different time periods (7 and 14 days), with the aim of establishing the stabilization period.

MATERIALS AND METHODS

Animals and experimental procedures

Twelve healthy New Zealand white rabbits weighing 2.73–3.24 kg were used. The animals were housed in individual metal cages, which allowed the isolation of feces in a lower container to avoid coprophagia. The environmental conditions were humidity 55 ± 10%, temperature 19 ± 2 °C and a 12-h light–dark cycle. Rabbits were maintained under these conditions at least 1 week before the assay, with free access to water and standard laboratory chow.

The rabbits were randomly divided into two groups (groups A and B) of 6 rabbits each. All the animals of the first group received 20 mg/kg oral levodopa and 5 mg/kg oral carbidopa (Sinemet®) dispersed in water for 7 days. The drugs were administered by gastric intubation every morning at the same time. A total of 50 ml water was used for administration and cannula cleaning. The rabbits of the second group received the same treatment but for 14 days. The first (day 1) and the last day (day 7 or 14) of treatment, levodopa concentrations were determined at different sampling times. To obtain the blood samples, rabbits were anesthetized with sodium pentobarbital (30 mg/kg i.v.) and the left carotid artery was cannulated with a silicone catheter (Silastic Medical-grade tubing, 1.02-mm inner diameter x 2.16-mm outer diameter). Drug administration was carried out after total recovery from anesthesia. Blood samples (3 ml) were obtained from the left carotid artery through the cannula into heparinized containers before and at 5, 10, 20, 30, 60, 90, 120, 180, 240 and 300 min after levodopa/carbidopa administration. In the animals of the first group, two blood samples were obtained before (C_{min}) and at 20 min after drug administration (C_{max}) on days 3 and 5 from the marginal ear vein. In the second group, these samples were obtained on days 3, 6, 9 and 11. Immediately after the collection of blood samples, plasma was separated by centrifugation and stored at –20 °C until analyzed.

Levodopa extraction from plasma samples was carried out by using a catecholamine kit (Chromsystems®) and quantitated by high-performance liquid chromatography (HPLC) with electrochemical detection. Neither heparin nor pentobarbital interfered in the assay.

Pharmacokinetic studies

Pharmacokinetic analysis was performed based on a noncompartmental description of the data observed. Maximum plasma levodopa concentration (C_{max}) and the time to reach maximum concentration (t_{max}) were read directly from the individual plasma concentration–time curves. The WinNonlin computer program and a formula described by Gibaldi and Perrier (7) were used to calculate the model-independent pharmacokinetic parameters. These parameters were the elimination rate constant (λ), area under the plasma concentration–time curve (AUC), clearance (Cl/F), volume of area (V_a/F), volume of distribution at steady state (V_{ss}/F), half-life associated with the λ phase ($t_{1/2\lambda}$), area under the first moment curve (AUMC) and mean residence time (MRT). The fraction of levodopa absorbed (F%) was calculated by dividing the mean AUC by the value of the mean i.v. AUC obtained in a previous study (4) after the administration of levodopa alone.

Statistical analysis

All pharmacokinetic parameters were calculated for each animal, and the data were presented as arithmetic mean ± standard deviation (mean ± SD). Data were analyzed by the Skewness test (to determine normality) and the Cochran test (to determine uniformity in variance). When the data were normal and there was uniformity in variance, the *t* test was used to evaluate differences between days 1 and 7 and between days 1 and 14. When the data were not normal and/or there was not uniformity in variance, the Wilcoxon test was used. To evaluate the differences in C_{min} and C_{max}, a two-way analysis of variance (ANOVA) was carried out, and Duncan's test was used to determine differences between data sets. When the data were not normal or there was no uniformity in variance, the Kruskal–Wallis test was used, and when the results were significant, the Wilcoxon test with Bonferroni correction was used to assess differences between data sets. $P \leq 0.05$ was used as the level of significance for all analyses.

RESULTS

The plots of the values obtained in each animal for plasma levodopa concentration as a function of time obtained on days 1 and 7 (group A) after oral administration of 20/5 mg/kg levodopa/carbidopa are shown in Figure 1, and the values determined on days 1 and 14 (group B) appear in Figure 2. Figure 3 includes the mean values of plasma levodopa AUC obtained in the two groups studied. The noncompartmental pharmacokinetic parameters obtained after the administration of 20/5 mg/kg levodopa/carbidopa to animals of groups A and B are summarized in Tables 1 and 2, respectively. Tables 3 and 4 include, respectively, the values for C_{min} and C_{max} obtained in group A (7 days of treatment) and group B (14 days of treatment). The determinations were carried

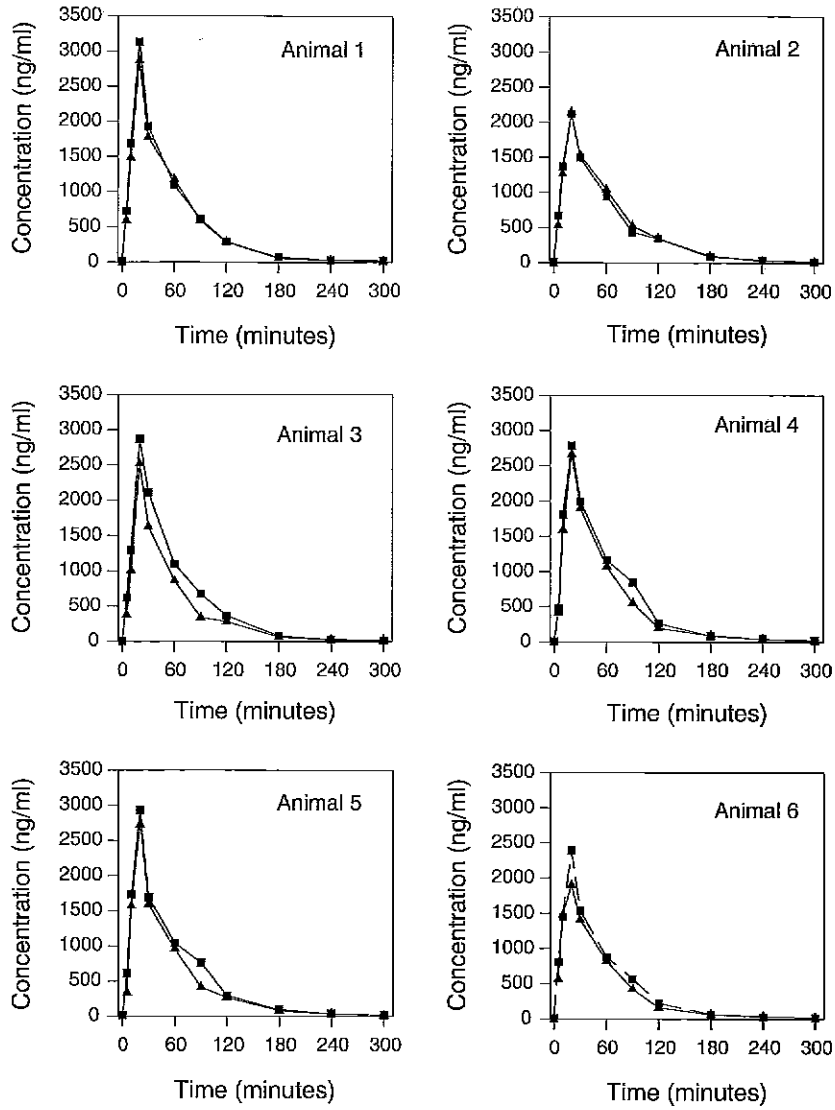

FIG. 1. Individual plasma concentrations of levodopa in rabbits after oral administration of 20/5 mg/kg levodopa/carbidopa for 7 days (group A).

out in blood samples obtained before the administration of levodopa/carbidopa and 20 min after administration. The t_{max} was determined from the AUC obtained on day 1 of treatment and was the same as that found in a previous study (8).

When levodopa and carbidopa were administered for 7 days, the AUC obtained for levodopa increased 12.6% from day 1 to day 7 (significant difference) and C_{max} increased 9.6% (significant difference). When these drugs were administered for 14 days, the increase in AUC was slightly greater (17.0%; significant difference) and the increase in C_{max} slightly lower (6.5%; significant difference). The concentrations of levodopa were higher after 14 days of treatment than after 7 days, except at three sampling times: 5 min (650 ng/ml *vs.* 648 ng/ml), 90 min (649 ng/ml *vs.* 526 ng/ml) and 120 min (296 ng/ml *vs.* 288 ng/ml). For the C_{max} obtained in the intermediate days, in group A, there were significant differences between day 1 and the other sampling days (3, 5 and 7), and between days 3 and 7. In group B, these differences were found between day 1 and the other sampling days (3, 6, 9, 11 and 14), between day 3 and the other sampling days (6, 9, 11 and 14), and between days 6 and 9. The values obtained for C_{min} in both groups increased progressively with the duration of the treatment. Thus, C_{min} increased by 10.9% from days 3 to 7 and 25.7% from days 3 to 14. In group A, there were significant differences for this parameter between days 3 and 5 and between days 3 and 7. After comparing the data obtained in group B, we found significant differences for all data except between values for days 11 and 14.

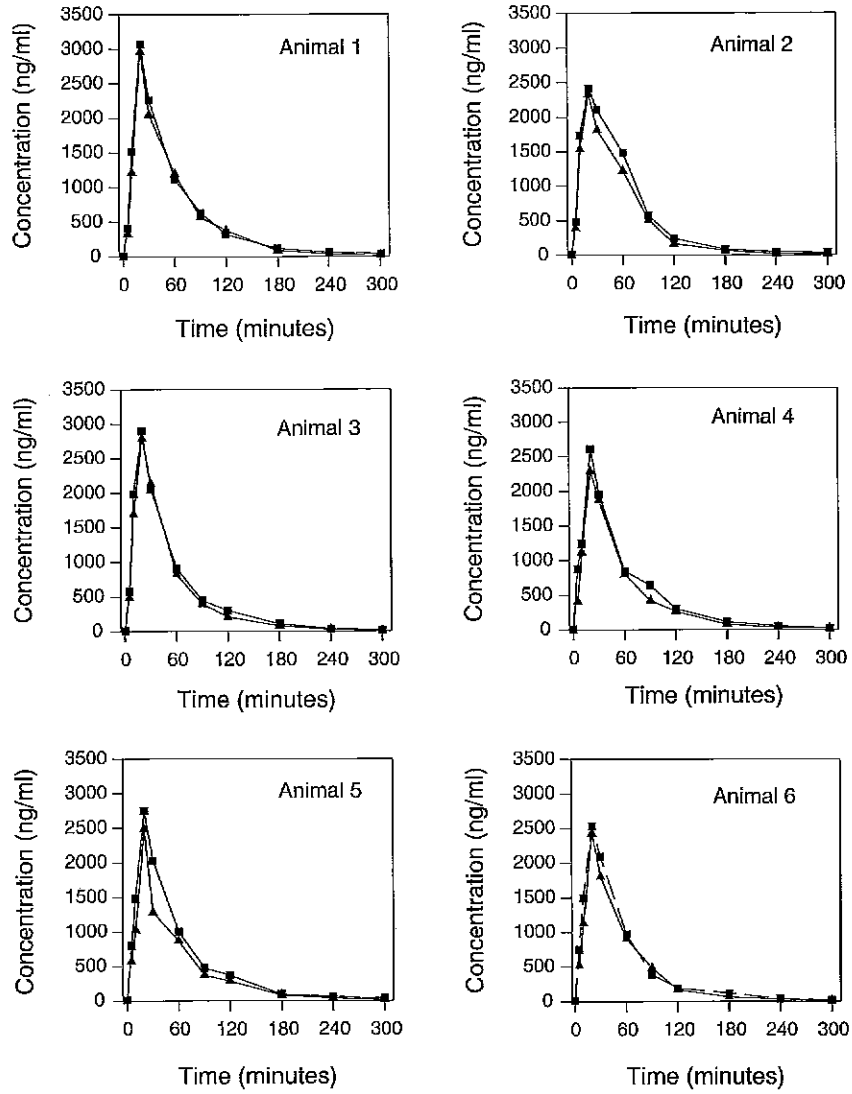

FIG. 2. Individual plasma concentrations of levodopa in rabbits after oral administration of 20/5 mg/kg levodopa/carbidopa for 14 days (group B).

The mean clearance obtained in both groups was very similar on day 1 and slightly lower on days 7 and 14: 0.149 ± 0.015 l/kg/min on day 1 in group A; 0.151 ± 0.016 l/kg/min on day 1 in group B; 0.134 ± 0.014 l/kg/min on day 7 and 0.128 ± 0.009 l/kg/min on day 14 (significant differences). Other parameters (λ, $t_{1/2\lambda}$, V_{ss}, V_a and t_{max}) did not vary significantly between day 1 and days 7 or 14.

Regarding MRT, this parameter showed no significant differences between days 1 and 7, but increased significantly between days 1 and 14.

DISCUSSION

When levodopa is orally administered, the pharmacokinetics, as determined by us and other authors (8–14), are generally noncompartmental, perhaps due to the marked fluctuations in plasma concentrations caused by the peculiar characteristics of levodopa. In this study, we carried out a compartmental analysis of the results obtained, but the plasma concentration–time data were not adequately fitted to the classic open compartmental models (one-, two- and three-compartment models) tested.

The understanding of levodopa pharmacokinetics has special importance because of its extensive presystemic metabolism, its rapid absorption in the proximal small intestine and its very short plasma half-life (14). One of the important factors affecting the rate of absorption of levodopa from the gastrointestinal tract is the stomach emptying rate. A slow emptying rate may delay and lower the peak plasma concentration and reduce the clinical efficacy of levodopa treatment (15). On the other

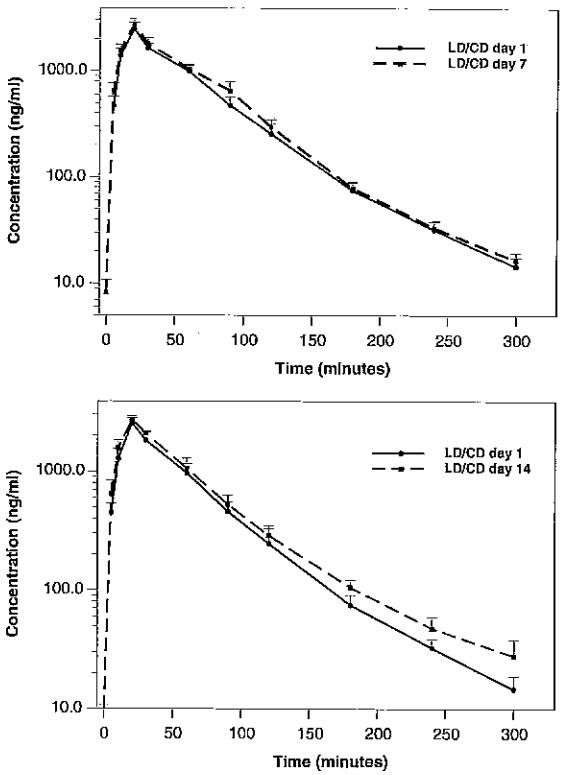

FIG. 3. Mean plasma concentrations of levodopa in rabbits after oral administration of 20/5 mg/kg levodopa/carbidopa (LD/CD) for 7 days (group A) and for 14 days (group B).

hand, the evaluation of the pharmacokinetics of levodopa is complicated by the presence of carbidopa, because carbidopa influences both the absorption and the systemic elimination of levodopa (16–18).

The values determined in both groups (7 and 14 days) for C_{max} on day 1 (2.47 and 2.55 μg/ml, respectively) are very similar to the values reported by Fernández *et al.* (8) after the oral administration of the same dose of levodopa or carbidopa to rabbits (2.74 μg/ml). After 7 or 14 days of treatment, C_{max} increased significantly, the value being very similar in both groups (2.70 and 2.71 μg/ml, respectively).

The AUC obtained on day 1 of both groups (134 μg.min/ml in group A and 136 μg.min/ml in group B) was slightly lower than the values calculated by Fernández *et al.* (8) (155 μg.min/ml). These small variations may be due to the lack of stabilization of carbidopa action. After 7 days of treatment, this parameter increased to 150 μg.min/ml, and after 14 days it was very similar (158 μg.min/ml).

Regarding t_{max}, this parameter was similar in both groups (20 min) to that reported in dogs (0.16 h) (19), the same as that reported by Fernandez *et al.* (8) in rabbits and higher than that determined when levodopa was administered alone (10 min) (9). The values found in men are higher, ranging from 30 min to 2 h (20–22). The differences in humans can be due to species-specific characteristics and also to the fact that studies in humans are carried out in patients with Parkinson's disease and the disease stage can influence levodopa pharmacokinetics (12).

The levodopa half-life is short even with the concomitant intake of LAAD inhibitors, ranging from 0.7 to 1.4 h in Parkinson's patients undergoing long-term treatment (20, 23, 24). The values obtained for the plasma elimination half-lives of levodopa (56.4 min in group A and 53.7 min in group B) fall within this range and are similar to the values reported by Fernández *et al.* (8) when orally administered with carbidopa (51.4 min) and to those reported by García *et al.* (9) when administered alone (68.4 min). These values are longer than those reported by Sasahara *et al.* (25) after i.v. bolus injections (0.65 h) and by Grange *et al.* (26) in rats after oral administration of levodopa alone (0.403 h). When these authors used levodopa combined with benserazide, the value obtained (0.834 h) was similar to ours.

TABLE 1. Pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after oral administration of 20/5 mg/kg levodopa/carbidopa for 7 days.

	Day 1			Day 7		
Parameters	Mean ± SD	Range	CV (%)	Mean ± SD	Range	CV (%)
λ (min^{-1})	0.0134 ± 0.0041	0.0082–0.0181	30.73	0.0123 ± 0.0020	0.0093–0.0153	16.34
AUC (μg.min/ml)*	133.6 ± 13.46	114.2–150.7	10.08	150.4 ± 15.33	131.1–166.0	10.19
C_{max} (μg/ml)*	2.47 ± 0.37	1.90–2.86	15.03	2.70 ± 0.37	2.12–3.13	13.84
t_{max} (min)	20.0	–	20.0	–	–	
Cl/F (l/kg/min)*	0.151 ± 0.016	0.133–0.175	10.36	0.134 ± 0.014	0.120–0.153	10.75
V_d/F (l/kg)	12.5 ± 4.95	7.73–19.79	39.61	11.22 ± 2.80	8.42–16.34	24.92
V_{ss}/F (l/kg)	8.76 ± 1.10	7.63–10.68	12.55	8.06 ± 1.09	7.17–9.69	13.55
$t_{1/2\lambda}$ (min)	56.4 ± 18.16	38.3–84.5	32.18	57.6 ± 9.89	45.3–74.5	17.18
AUMC (μg.min^2/ml)*	7910 ± 692	6961–8670	8.75	8994 ± 794	7890–10,112	8.83
MRT (min)	57.9 ± 2.56	55.1–61.2	4.42	59.9 ± 2.30	56.7–63.6	3.84
F (%)	114.25	–	–	128.61	–	–

*Significant differences between day 1 and day 7 (*t* test, $P \leq 0.05$). CV, coefficient of variation; SD, standard deviation.

TABLE 2. Pharmacokinetic parameters obtained by noncompartmental analysis in rabbits after oral administration of 20/5 mg/kg levodopa/carbidopa for 14 days.

	Day 1			Day 7		
Parameters	Mean ± SD	Range	CV (%)	Mean ± SD	Range	CV (%)
λ (min^{-1})	0.0135 ± 0.0027	0.0088–0.0161	19.83	0.0111 ± 0.0026	0.0078–0.0139	23.61
AUC (μg.min/ml)*	135.8 ± 14.15	119.6–160.1	10.42	157.5 ± 11.59	142.9–172.7	7.36
C_{max} (μg/ml)*	2.55 ± 0.27	2.29–2.96	10.42	2.71 ± 0.24	2.41–3.07	8.96
t_{max} (min)	20.0	–	–	20.0	–	–
Cl/F (l/kg/min)*	0.149 ± 0.015	0.125–0.167	9.89	0.128 ± 0.009	0.116–0.140	6.99
V_z/F (l/kg)	11.5 ± 2.80	8.93–16.32	24.44	12.03 ± 2.49	9.74–16.17	20.74
V_{ss}/F (l/kg)	8.63 ± 1.11	7.48–10.32	12.89	8.11 ± 0.48	7.53–8.80	5.92
$t_{1/2\beta}$ (min)	53.7 ± 13.27	43.0–78.8	24.72	65.9 ± 16.5	49.9–88.9	25.12
AUMC (μg.min^2/ml)*	7878 ± 963	7147–9719	12.23	10255 ± 1681	8197–12071	16.39
MRT (min)	58.1 ± 3.74	52.0–61.7	6.44	63.6 ± 5.64	57.3–70.0	8.86
F (%)	116.09		–	134.67		–

*Significant differences between days 1 and 14 (*t* test, $P \leq 0.05$). CV, coefficient of variation; SD, standard deviation.

TABLE 3. Values of C_{min} and C_{max} (mg/ml) obtained after oral administration of 20/5 mg/kg levodopa/carbidopa for 7 days to rabbits.

		Animal							
		1	2	3	4	5	6	Mean ± SD	CV (%)
C_{min} (0 min)	Day 3	0.0060	0.0059	0.0067	0.0069	0.0093	0.0098	0.0074 ± 0.0017	22.74
	Day 5[a]	0.0063	0.0061	0.0070	0.0077	0.0101	0.0110	0.0081 ± 0.0021	25.66
	Day 7[a]	0.0062	0.0057	0.0074	0.0090	0.0105	0.0106	0.0082 ± 0.0021	25.92
C_{max} (20 min)	Day 1	2.86	2.14	2.52	2.66	2.72	1.90	2.47 ± 0.37	15.03
	Day 3[b]	3.09	2.02	2.71	2.72	2.80	2.16	2.58 ± 0.41	15.83
	Day 5[b]	3.08	2.12	2.80	2.75	2.86	2.31	2.65 ± 0.36	13.64
	Day 7[a, b]	3.13	2.12	2.87	2.79	2.93	2.40	2.70 ± 0.37	13.84

Significant differences: [a]with day 3; [b]with day 1 (Duncan test, $P \leq 0.05$). CV, coefficient of variation; SD, standard deviation.

TABLE 4. Values of C_{min} and C_{max} (mg/ml) obtained after oral administration of 20/5 mg/kg levodopa/carbidopa for 14 days to rabbits.

		Animal							
		1	2	3	4	5	6	Mean ± SD	CV (%)
C_{min} (0 min)	Day 3	0.0060	0.0078	0.0054	0.0063	0.0103	0.0056	0.0069 ± 60.0019	27.15
	Day 6[a]	0.0065	0.0087	0.0064	0.0066	0.0104	0.0068	0.0076 ± 60.0016	21.49
	Day 9[a,b]	0.0073	0.0088	0.0069	0.0074	0.0110	0.0068	0.0080 ± 60.0016	20.08
	Day 11[a, b, c]	0.0076	0.0090	0.0074	0.0079	0.0113	0.0080	0.0085 ± 60.0015	17.26
	Day 14[a, b, c]	0.0077	0.0090	0.0078	0.0082	0.0118	0.0076	0.0087 ± 60.0016	18.32
C_{max} (20 min)	Day 1	2.96	2.33	2.78	2.29	2.49	2.43	2.55 ± 60.27	10.42
	Day 3[d]	2.99	2.33	2.71	2.37	2.57	2.47	2.57 ± 60.25	9.70
	Day 6[a, d]	3.05	2.39	2.83	2.48	2.58	2.48	2.63 ± 60.25	9.60
	Day 9[a,b,d]	3.11	2.41	2.81	2.50	2.65	2.50	2.66 ± 60.26	9.74
	Day 11[a,b,d]	3.04	2.39	2.86	2.54	2.69	2.49	2.67 ± 60.25	9.21
	Day 14[a,b,d]	3.07	2.41	2.90	2.61	2.75	2.53	2.71 ± 60.24	8.96

Significant differences: [a]with day 3; [b]with day 6; [c]with day 9; [d]with day 1 (Duncan test, $P \leq 0.05$). CV, coefficient of variation; SD, standard deviation.

The results obtained in this study indicate that the inhibitory effect of carbidopa on LAAD appears rapidly. Thus, C_{max} and AUC values were very similar after 7 or 14 days of treatment. The time needed for C_{min} stabilization was slightly greater, because we found significant differences until day 11 of treatment.

There is growing recognition that gastrointestinal dysfunction is common in Parkinson's disease. Virtually all parts of the gastrointestinal tract can be affected, in some cases early in the disease course. Bowel dysfunction can consist of both slowed colonic transit with consequently reduced bowel movement frequency, and difficulty in the act of defecation itself with excessive straining and incomplete emptying.

According to several authors (27), the "delayed-on" (prolonged latencies to onset) phenomenon and "non-on"

(treatment failure) phenomenon are related to alterations in the gastrointestinal transit time and absorption of levodopa. Several authors have indicated that the induction of on/off phenomena and dyskinesias may be the result of an active process of adaptation to variations in brain and plasma levodopa levels. In this way, when levodopa concentrations are maintained at a constant level by i.v. infusion, dyskinesias and fluctuations are greatly reduced (28, 29). In conclusion, further studies should be carried out to evaluate the pharmacokinetics of levodopa administered with carbidopa when gastrointestinal function is altered to simulate a situation similar to that found in clinical practice.

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