Journal of Food and Drug Analysis, Vol. 16, No. 5, 2008, Pages 21-27 藥物食品分析 第十六卷 第五期

A Dose-Dependent Pharmacokinetic Study of Levodopa by Intramuscular Administration in Rabbits

LI-HSUAN WANG[1], KUANG-YANG HSU[2], FENG-LIN HSU[3] AND SHWU-JIUAN LIN[1]*

[1.] *Department of Medicinal Chemistry, College of Pharmacy, Taipei Medical University, Taipei, Taiwan (R.O.C.)*
[2.] *Department of Pharmaceutics, College of Pharmacy, Taipei Medical University, Taipei, Taiwan (R.O.C.)*
[3.] *Graduate Institute of Pharmacognosy, College of Pharmacy, Taipei Medical University, Taipei, Taiwan (R.O.C.)*

(Received: January 8, 2008; Accepted: March 20, 2008)

ABSTRACT

The dose-dependent pharmacokinetics of levodopa (L-dopa) was studied in rabbits by intramuscular administration. Three different doses of L-dopa/carbidopa (2/0.5, 5/1.25, and 10/2.5 mg/kg) were administered to six male rabbits via an intramuscular (IM) route, and one dose of L-dopa/carbidopa (2/0.5 mg/kg) was administered via an intravenous (IV) route with a washout period of 1-week between different doses. Plasma samples were collected after each treatment and the concentrations of L-dopa and 3-*O*-methyldopa (an L-dopa metabolite, 3-OMD) were measured by a sensitive high-performance liquid chromatographic (HPLC) method. Subsequently, these measurements were used to determine the pharmacokinetic behavior of L-dopa and 3-OMD. The results indicated that the absorption of L-dopa was fast with the time to the peak within 30 min, but the formation of 3-OMD was slow with the time to the peak of 120-180 min after IM administration. The IM bioavailability of L-dopa was in the range of 0.70-1.21, and the relative ratios of the formation of 3-OMD at different doses of L-dopa were in the range of 0.79-1.24. No statistically significant difference could be observed for IM bioavailability of L-dopa or for the relative ratios of the formation of 3-OMD in this dose range. The elimination half-lives of L-dopa and 3-OMD also exhibited no significant differences for each dose after IM administration. In addition, both the area under the curve (AUC) and maximum plasma concentration (C_{max}) values of L-dopa and 3-OMD increased proportionally over the dose range of 2/0.5-10/2.5 mg/kg for L-dopa/carbidopa, suggesting that L-dopa and 3-OMD obeyed dose-independent pharmacokinetics.

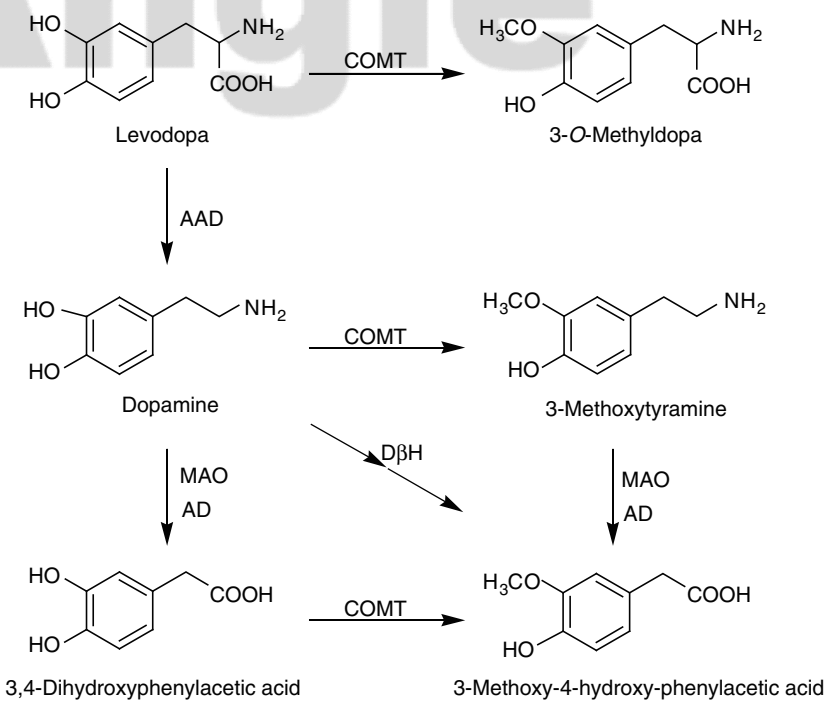
Key words: pharmacokinetics, intramuscular, bioavailability, L-dopa, rabbit.

INTRODUCTION

The administration of dopamine precursors, levodopa (L-dopa) in particular, has been the golden standard for the treatment of idiopathic Parkinson's disease (IPD) since the demonstration of dopamine deficiency in the basal ganglia of IPD patients. L-Dopa can be converted to dopamine (Figure 1). However, L-dopa is degraded to dopamine by peripheral decarboxylase, which does not pass the blood-brain barrier. Thus, a decarboxylase inhibitor needs to be coadministered. A fixed combination formulation containing L-dopa and dopa decarboxylase inhibitor in a ratio of 4 to 1 was usually used for the treatment of PD patients and also as a dopamine replacement agent. It is particularly effective for the most disabling features of the disease, namely bradykinesia and rigidity[1-2]. Unfortunately, in some patients who initially respond well to L-dopa, the control of motor symptoms gradually diminishes through the course of treatment[3].

* Author for correspondence. Tel: +886-2-27361661 ext. 6133; Fax: +886-2-28264276; E-mail: shwu-lin@tmu.edu.tw

Few study has been carried to investigate interactions of L-dopa with polyphenol compounds except for nitecapone[4], tolcapone[5-6], and entacapone[7-9], which are catechol-*O*-methyltransferase (COMT) inhibitors and may increase the systemic exposure to L-dopa. There is increasing evidence indicating the role of coffee and tea drinking modulating the risk of PD[10-12]. In particular, green tea polyphenols with many biological effects may benefit patients with PD[10,12]. This implies that the polyphenols in tea and coffee might play a role as a COMT inhibitor of L-dopa metabolism. Although we do not fully understand those specific components of tea which have benefits in treating PD, food-drug interactions possibly need to be considered. In general, pharmacokinetics plays roles in drug-drug and drug-food interactions. Therefore, it is important to understand the pharmacokinetic phenomena of L-dopa. To avoid impacts of gastrointestinal absorption on drug-drug interactions study, IV- and IM-routes of administration are better choices. However, the pharmacokinetics of L-dopa by IV or IM administration is rarely described in literatures.



AD, aldehyde dehydrogenase; COMT, catechol-*O*-methyltransferase; DβH, dopamine β-hydroxylase; AAD, aromatic L-amno acid decarboxylase; MAO, monoamine oxidase

Figure 1. Metabolism of levodopa (L-dopa).

On the other hand, the water solubility of L-dopa is poor, but can be boosted after being titrated with HCl. For the sakes of safety and tolerance, L-dopa via IM administration is better than via IV administration. Therefore, this study was aimed to investigate the pharmacokinetics of L-dopa at different doses with IM administration using rabbit as the animal model and subsequently to determine the optimal dose for advanced studies on the interactions of L-dopa with other compounds.

MATERIALS AND METHODS

I. *Chemicals and Reagents*

L-Dopa, carbidopa (an aromatic L-amino acid decarboxylase inhibitor), and 3-*O*-methyldopa (an L-dopa metabolite, 3-OMD) were obtained from Sigma Chemical (St Louis, MO, USA). HPLC grade of acetonitrile (CH_3CN), trifluoroacetic acid (TFA), and phosphoric acid (85%) were purchased from E. Merck (Darmstadt, Germany). All other chemicals were of analytical grade and used without further purification.

II. *Preparation of an L-Dopa/Carbidopa Solution*

L-Dopa/carbidopa solutions were prepared in 0.1 N HCl to obtain 10/2.5, 5/1.25, and 2/0.5 mg/kg solutions for IM administration and 2/0.5 mg/kg for IV administration.

III. *Apparatus and Chromatographic Conditions*

Plasma L-dopa and 3-OMD concentration were simultaneously determined by HPLC with fluorescence detector. Rondelli I method was employed with modifications[13]. The HPLC system was equipped with a Shimadzu LC-10AD_{VP} Pump, an SIL-HT_A/HT_C autosampler, an SPD-10A_{VP}/10AV_{VP} UV detector, and a CLASS-VP Ver. 6.1 system manager as the data processor (Shimadzu, Kyoto, Japan). Separation was effected on a Biosil ODS column (150 mm × 4.6 mm I.D., 5 μm, Biotic Chemical Co., Ltd., Taipei, Taiwan). The mobile phase consisted of 30% CH_3CN and 0.5% phosphoric acid (pH 5.0) at a flow rate of 1.2 mL/min. The fluorescence detector was set at 280 nm for excitation and 315 nm for emission. Typical chromatograms are shown in Figure 2. The standard curves show linearity over the concentration ranges of 0.025−1.5 μg/mL for L-dopa and 0.05−2.5 μg/mL for 3-OMD. The within run and between run precision were 2.5−12.6% and 5.2−9.8%, respectively, for L-dopa, and were 1.0−11.2% and 3.9−8.6%, respectively, for 3-OMD. In addition, the within run and between run accuracy were -6.2−7.2% and -2.2−3.5%, respectively, for L-dopa, and were -6.2−0.0% and -3.9−-1.9%, respectively, for

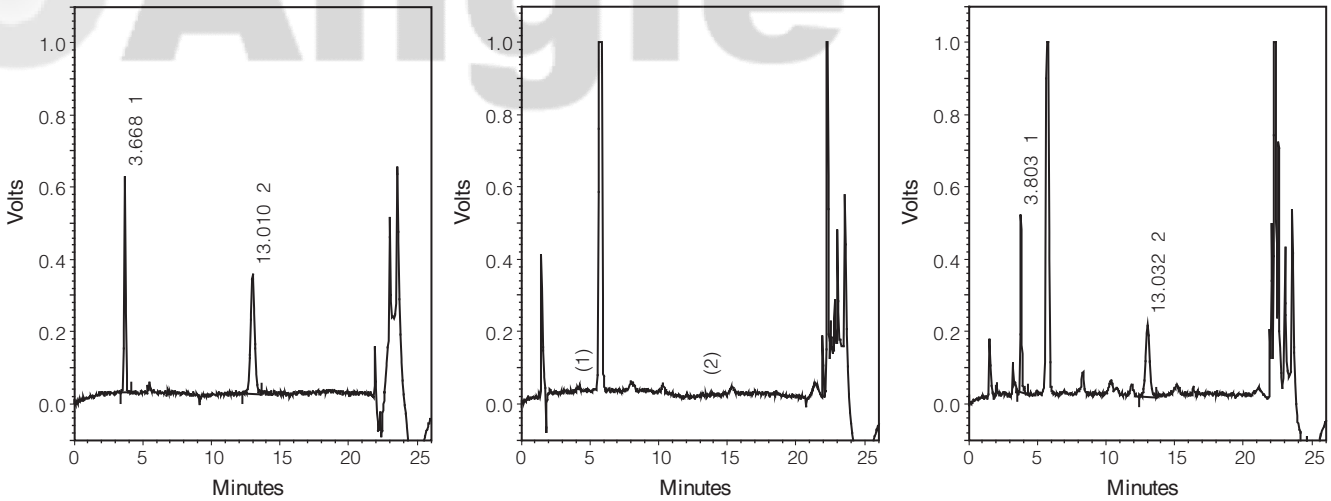


Figure 2. Typical chromatograms of (A) authentic compound, (B) drug-free plasma, (C) L-dopa and 3-OMD after 5 min by intravenous administration. 1. L-dopa; 2. 3-OMD.

3-OMD. Samples with concentration over the standard curve range were diluted before assay. The 2- and 5 times dilution integrity test showed within run and between run precision were 3.1−8.3% and 6.6−8.6%, respectively, for L-dopa, and were 2.9−7.8% and 4.4−6.9%, respectively, for 3-OMD. In addition, the 2- and 5 times dilution integrity test also showed the within run and between run accuracy were -2.4−13.2% and 2.5−7.0%, respectively, for L-dopa, and were -6.2−2.2% and -4.8−0.9%, respectively, for 3-OMD. The extraction recoveries were 94.6 and 101.0% for L-dopa and 3-OMD, respectively. The inter-assay coefficient of variation (CV) and relative error (RE) were less than 10.4 and 12.4% for L-dopa, and 7.3 and 6.9% for 3-OMD.

IV. *Sample Preparation*

Two hundred microliters of plasma in a clean culture tube was added 200 μL of 10% (v/v) TFA solution. After vortex-mixing for 1 min, samples were centrifuged for 5 min at 1,945 ×*g*. Finally, an aliquot (10 μL) of supernatant was injected into the HPLC system.

V. *Animal Study*

Six male New Zealand white rabbits weighing 2.1−3.1 kg were used in the pharmacokinetic studies. The rabbits were starved overnight before dosing. The experiments were conducted by single IV administration of 2/0.5 mg/kg of L-dopa/carbidopa. Subsequently, three different doses of L-dopa/carbidopa (2/0.5, 5/1.25, or 10/2.5 mg/kg) were administered via an IM injection in the thigh muscle. A minimum 1-week was allowed for washout between each treatment. Blood samples (1.0 mL) were collected at 0 (before drug administration), 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, 180, 240, 360, 480, 600, and 1440 min from the marginal vein of the ear after each treatment, and were immediately placed in ice bath. Plasma was separated by centrifugation at 1,945 ×*g* for 5 min, acidified by adding 20 μL of 20% v/v phosphoric acid, and stored at -80°C until analyzed.

VI. *Pharmacokinetic Analysis*

All pharmacokinetic parameters were calculated using the pharmacokinetic software WinNonlin^{TM} (version 5.2, Pharsight Corp., Mountain View, CA, USA) by the non-compartmental method. The maximum plasma concentration (C_{max}) and the time to reach C_{max} (t_{max}) were directly obtained from the plasma concentration-time curves. The elimination rate constant (K_e) was determined by simple linear regression based on the terminal log-linear part of the plasma concentration-versus-time profile. The apparent elimination half-life ($t_{1/2}$) was calculated as $0.693/K_e$. Summations of the area under the plasma concentration-time curve from 0 to the last quantifiable concentration (AUC_{0-t}) were calculated by the linear trapezoidal method. The summation of area under the plasma concentration-time curve from 0 to infinity ($AUC_{0-\infty}$) were calculated by extrapolating AUC_{0-t} to infinity using the last quantifiable concentration (C_n) divided by K_e. The IM bioavailability (BA) of L-dopa, the relative formation ratio (RFR) of 3-OMD, and the metabolic ratio (MR) were calculated as follows:

(1) BA (L-dopa) = $([AUC_{0-\infty}]_{IM,\ L\text{-}dopa}/Dose_{IM,\ L\text{-}dopa})/([AUC_{0-\infty}]_{IV,\ L\text{-}dopa}/Dose_{IV,\ L\text{-}dopa})$,

(2) RFR (3-OMD) = $([AUC_{0-\infty}]_{IM,\ 3\text{-}OMD}/Dose_{IM,\ L\text{-}dopa})/([AUC_{0-\infty}]_{IV,\ 3\text{-}OMD}/Dose_{IV,\ L\text{-}dopa})$, and

(3) MR = $([AUC_{0-\infty}]_{3\text{-}OMD})/([AUC_{0-\infty}]_{L\text{-}dopa})$, respectively.

VII. *Statistical Analysis*

Pharmacokinetic parameters are reported as the mean ± S.D.. Statistical analysis of pharmacokinetic

parameters estimated at various doses was performed by the two-way analysis of variance with $p < 0.05$ as the minimal level of significance.

RESULTS AND DISCUSSION

I. *Pharmacokinetic Parameters of L-Dopa Obtained from Rabbit Plasma after IV and IM Administration*

The plasma concentration-time profiles for L-dopa are shown in Figure 2, and pharmacokinetic parameters are summarized in Table 1. After IM administration, the absorption of L-dopa was fast with t_{max} occurring within 15-30 min. The observed C_{max} values of L-dopa were 1.32 ± 1.07, 2.81 ± 2.03, and 8.04 ± 4.84 μg/mL, respectively, after IM administration of 2/0.5, 5/1.25, and 10/2.5 mg/kg of L-dopa/carbidopa. A linear relationship existed between the dose and C_{max}. The half-lives of L-dopa for the doses of 2/0.5, 5/1.25, and 10/2.5 mg/kg of L-dopa/carbidopa by IM administration were 470.3 ± 522.1, 1016 ± 910, and 1071 ± 1492 min, respectively. After IV administration of L-dopa/carbidopa at a dose of 2/0.5 mg/kg, the half-life was 566.4 ± 265.6 min. Half-lives obtained after IM administration of L-dopa/carbidopa at doses of 5/1.25 and 10/2.5 mg/kg were longer than those obtained from doses of 2/0.5 mg/kg by IV or IM administration. However, they did not exhibit any statistically significant differences. After IV administration at a dose of 2/0.5 mg/kg, the AUC_{0-t} and $AUC_{0-\infty}$ values of L-dopa were 184.0 ± 197.8 and 249.7 ± 261.2 μg/min/mL, respectively. In addition, for IM administered doses of L-dopa/carbidopa of 2/0.5, 5/1.25, and 10/2.5 mg/kg, the AUC_{0-t} and $AUC_{0-\infty}$ values of L-dopa were 129.0 ± 159.3 and 188.9 ± 274.1; 399.9 ± 276.0 and 610.5 ± 581.2; and 748.6 ± 369.9 and 1070.0 ± 913.4 μg/min/mL, respectively. A linear relationship also existed between the dose and $AUC_{0-\infty}$ at this dose range after IM administration. The IM calculated bioavailabilities were 0.70 ± 0.40, 1.21 ± 0.67, and 1.03 ± 0.45, respectively, for doses of L-dopa/carbidopa of 2/0.5, 5/1.25, and 10/2.5 mg/kg, and no statistically significant differences were observed among the three doses after IM administration. On the other hand, as shown in Table 1, neither the dose-normal-

Table 1. Pharmacokinetic parameters for levodopa (L-dopa) and 3-*O*-methyldopa (3-OMD) after intravenous and intramuscular administration of L-dopa/carbidopa at doses of 2/0.5, 5/1.25, and 10/2.5 mg/kg, respectively, to six rabbits. Values are expressed as the mean ± S.D. ($n = 6$)

	L-dopa/carbidopa dose (mg/kg)							
	Intravenous administration		Intramuscular administration					
	2/0.5		2/0.5		5/1.25		10/2.5	
Parameter	L-dopa	3-OMD	L-dopa	3-OMD	L-dopa	3-OMD	L-dopa	3-OMD
AUC_{0-t} (μg/min/mL)	184.0 ± 197.8	382.2 ± 193.5	129.0 ± 159.3	251.5 ± 198.2	399.9 ± 276.0	1151.4 ± 807.3	748.6 ± 369.9	2157.9 ± 1071.1
$AUC_{0-\infty}$ (μg/min/mL)	249.7 ± 261.2	459.1 ± 203.4	188.9 ± 274.1	337.1 ± 200.1	610.5 ± 581.2	1383.5 ± 1008.2	1070.0 ± 913.4	2379.1 ± 1296.0
C_{max} (μg/mL)		0.85 ± 0.29	1.32 ± 1.07	0.47 ± 0.26	2.81 ± 2.03	1.61 ± 0.80	8.04 ± 4.84	3.31 ± 1.27
t_{max} (min)		45.0 ± 9.5	19.2 ± 8.6	112.5 ± 26.4	27.5 ± 11.3	175.0 ± 35.1	15.8 ± 4.9	130.0 ± 31.0
$t_{1/2}$ (min)	566.4 ± 265.6	503.7 ± 206.6	470.3 ± 522.1	466.2 ± 172.1	1016 ± 910	469.3 ± 115.5	1071 ± 1492	377.6 ± 97.4
BA			0.70 ± 0.40		1.21 ± 0.67		1.03 ± 0.45	
RFR				0.79 ± 0.33		1.24 ± 0.55		1.06 ± 0.35
MR		2.74 ± 1.59		3.18 ± 1.67		2.47 ± 0.72		2.67 ± 1.21
Normalized								
$AUC_{0-\infty}$ (μg/min/mL)	124.9 ± 130.6	229.6 ± 101.7	94.5 ± 110.3	168.6 ± 193.1	122.1 ± 116.2	276.7 ± 201.6	107.0 ± 91.4	237.9 ± 129.6
C_{max} (μg/mL)	2.50 ± 1.99	0.43 ± 0.15	0.66 ± 0.71	0.23 ± 0.26	0.56 ± 0.40	0.32 ± 0.16	0.80 ± 0.48	0.33 ± 0.13

[a] AUC_{0-t}, the area under the plasma concentration-time curve from 0 to the last quantifiable concentration; $AUC_{0-\infty}$, the plasma concentration-time curve from 0 to infinity; C_{max}, maximum plasma concentration; t_{max}, the time to reach C_{max}; $t_{1/2}$, half-life; BA, bioavailability; RFR, relative formation ratio; MR, metabolic ratio.

Journal of Food and Drug Analysis, Vol. 16, No. 5, 2008

izing $AUC_{0-\infty}$ nor C_{max} showed any statistically significant differences among these doses after IM administration. The pharmacokinetic parameters of bioavailability, elimination half-life, dose-normalized $AUC_{0-\infty}$, and C_{max} did not show statistically significant differences for each dose after IM administration, indicating that L-dopa exhibited dose-independent pharmacokinetics following IM administration over the dose range of 2/0.5−10/2.5 mg/kg of L-dopa/carbidopa.

II. *Pharmacokinetic Parameters of 3-OMD Obtained from Rabbit Plasma after IV and IM Administration*

The plasma concentration-time profiles for 3-OMD are shown in Figure 3, and the pharmacokinetic parameters are summarized in Table 1. The t_{max} values were 113−175 min after IM administration; however, t_{max} was about 45 min for IV administration. This indicated that the formation of 3-OMD was slower after IM administration of L-dopa/carbidopa, in comparison to IV administration. The observed C_{max} values of 3-OMD were 0.47 ± 0.26, 1.61 ± 0.80, and 3.31 ± 1.27 μg/mL, respectively, after IM administration of 2/0.5, 5/1.25, and 10/2.5 mg/kg of L-dopa/carbidopa. A linear relationship existed between the dose and C_{max}. After IV administration, the observed C_{max} of 3-OMD was 0.85 ± 0.29 μg/mL, which was greater than that of the same dose of 2/0.5 mg/kg after IM administration. Obviously, the observed t_{max} of 3-OMD was much shorter after IV administration, and subsequently a higher C_{max} value was obtained. The half-lives of 3-OMD at doses of L-dopa/carbidopa of 2/0.5, 5/1.25, and 10/2.5 mg/kg by IM administration were 466.2 ± 172.1, 469.3 ± 115.5, and 377.6 ± 97.4 min, respectively. After IV administration at a dose of 2/0.5 mg/kg, the half-life was 503.7 ± 206.6 min. The results indicated the half-lives of 3-OMD of no statistically significant differences among these doses after IM administration or between IV and IM administration routes. After IV administration at a dose of 2/0.5 mg/kg of L-dopa/carbidopa, the AUC_{0-t} and $AUC_{0-\infty}$ values of 3-OMD were 382.2 ± 193.5 and 459.1 ± 203.4 μg/min/mL, respectively. In addition, for doses of 2/0.5, 5/1.25, and 10/2.5 mg/kg after IM administration, the AUC_{0-t} and $AUC_{0-\infty}$ values of 3-OMD were 251.5 ± 198.2 and 337.1 ± 200.1, 1151.4 ± 807.3 and 1383.5 ± 1008.2, and 2157.9 ± 1071.1 and 2379.1 ± 1296.0 μg/min/mL, respectively. A linear relationship was found between the dose and $AUC_{0-\infty}$ in this dose range after IM administration. The relative ratios of the formation of 3-OMD were 0.79 ± 0.33, 1.24 ± 0.55, and 1.06 ± 0.35, respectively, for the IM administration of 2/0.5, 5/1.25, and 10/2.5 mg/kg of L-dopa/carbidopa, and no statistically significant differences were observed among these doses. It seems that the relative ratio for the formation of 3-OMD was similar to the bioavailability of L-dopa at each dose. Therefore, we inferred that L-dopa was metabolized to 3-OMD and a constant ratio existed at the range of 2/0.5 to 10/2.5 mg/kg. On the other hand, as shown in Table 1, neither the dose-normalizing $AUC_{0-\infty}$ nor C_{max} values exhibited statistically significant differences among these doses after IM administration. Pharmacokinetic parameters of bioavailability, elimination half-life, dose-normalized $AUC_{0-\infty}$, and C_{max} showed no statistically significant differences for each dose after IM administration, indicating that 3-OMD also exhibited dose-independent pharmacokinetics following IM administration over the dose range of 2/0.5−10/2.5 mg/kg of L-dopa/carbidopa.

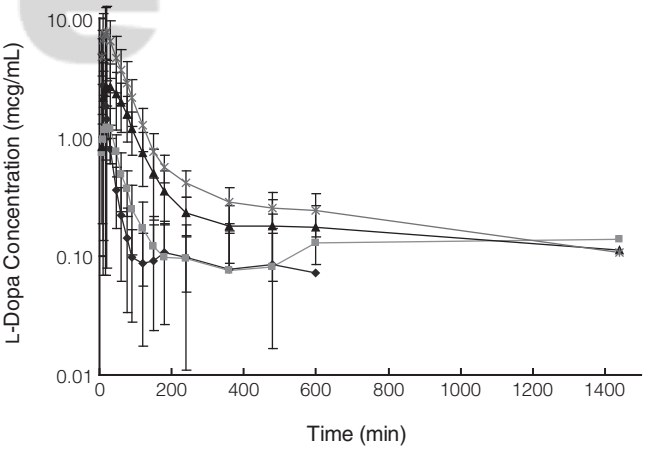


Figure 3. Plasma concentration-time profiles for levodopa after the intravenous (IV) administration of L-dopa/carbidopa at a dose of 2/0.5 mg/kg (♦) and intramuscular (IM) administration of doses of 2/0.5 (■), 5/1.25 (▲), and 10/2.5 (×) mg/kg, respectively, to six rabbits. Data are shown as the mean ± S.D.

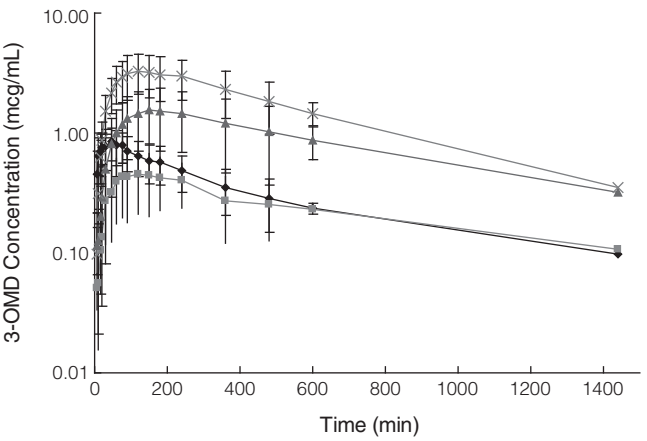


Figure 4. Plasma concentration-time profiles for 3-*O*-methyldopa (3-OMD) after the intravenous (IV) administration of L-dopa/carbidopa at a dose of 2/0.5 mg/kg (♦) and intramuscular (IM) administration of doses of 2/0.5 (■), 5/1.25 (▲), and 10/2.5 (×) mg/kg, respectively, to six rabbits. Data are shown as the mean ± S.D.

Many reports generally agreed that 3-OMD is an important negative determinant of the clinical response to L-dopa[14-18]. Increased 3-OMD concentrations in circulation or a high ratio of 3-OMD/L-dopa in plasma have been correlated with the poor response of Parkinso-

nian patients to L-dopa therapy. Furthermore, a previous report showed measurements of COMT activity in erythrocytes from several animal species and also demonstrated that the value of COMT activity in the rabbit was closest to the human value[19]. As shown in Table 1, $AUC_{0-\infty}$ values of 3-OMD were greater than those of L-dopa. The AUC ratios of 3-OMD/L-dopa were 3.18 ± 1.67, 2.47 ± 0.72, and 2.67 ± 1.21 for doses of 2/0.5, 5/1.25, and 10/2.5 mg/kg of L-dopa/carbidopa, respectively, after IM administration. Following IV administration, the AUC ratio of 3-OMD/L-dopa was 2.74 ± 1.59. As the data show, AUC ratios of 3-OMD/L-dopa were similar among these doses after IM administration and were also similar between the IM and IV administration routes, indicating that AUC ratios of 3-OMD/L-dopa were dose-independent. In addition, good linear relationships also existed for $AUC_{0-\infty}$ as well as C_{max} between L-dopa and 3-OMD over the dose range after IM administration. Therefore, at the doses of 2/0.5, 5/1.25, and 10/2.5 mg/kg of L-dopa/carbidopa, L-dopa, 3-OMD, and the AUC ratios of 3-OMD/L-dopa could be used as indicators to evaluate interactions between L-dopa and other compounds in the rabbit.

CONCLUSIONS

The present study demonstrates that the pharmacokinetic parameters of L-dopa and 3-OMD were independent of dose over the dose range of 2/0.5−10/2.5 mg/kg of L-dopa/carbidopa after IM administration in rabbits. Within this dose range, the IM bioavailability was about 0.70-1.21 and the relative formation ratio of 3-OMD was about 0.79-1.24. In addition, AUC ratios of 3-OMD/L-dopa after IM administration were 2.47-3.18. To evaluate interactions between L-dopa and other compounds in rabbits, doses of L-dopa/carbidopa of 2/0.5−10/2.5 mg/kg are suitable. L-Dopa, 3-OMD, and the AUC ratios of 3-OMD/L-dopa can be used as indicators to evaluate interactions between L-dopa and other compounds in the rabbit.

ACKNOWLEDGMENTS

The authors wish to thank Miss Su-Wen Zheng for her help in the HPLC analysis of samples. Financial support through a research grant (NSC96-2320-B038-013) from the National Science Council of Taiwan is gratefully acknowledged.

REFERENCES

1. Laar, T. V. 2003. Levodopa-induced response fluctuations in patients with Parkinson's disease. CNS Drugs 17: 475-489.
2. Deleu, D., Northway, M. G. and Hanssens, Y. 2002. Clinical pharmacokinetic and pharmacodynamic properties of drugs used in the treatment of Parkinson's disease. Clin. Pharmacokinet. 41: 261-309.
3. Marsden, C. D. and Parkes, J. D. 1977. Success and problems of long-term levodopa therapy in Parkinson's disease. Lancet 12: 345-349.
4. Kaakkola, S., Gordin, A., Järvinen, M., Wikberg, T., Schultz, E., Nissinen, E., Pentikäinen, P. J. and Rita, H. 1990. Effect of a novel catechol-*O*-methyltransferase inhibitor, nitecapone, on the metabolism of L-dopa in healthy volunteers. Clin. Neuropharmacol. 13: 436-447.
5. Sědek, G., Jorga, K. M., Schmitt, M., Burns, R. S. and Leese, P. 1997. Effect of tolcapone on plasma levodopa concentrations after coadministration with levodopa/carbidopa to healthy volunteers. Clin. Neuropharmacol. 20: 531-541.
6. Jorga, K. M. 1998. Pharmacokinetics, pharmacodynamics, and tolerability of tolcapone: a review of early studies in volunteers. Neurology 50 (Suppl 5): S31-S38.
7. Ruottinen, H. M. and Rinne, U. K. 1996. Entacapone prolongs levodopa response in a one month double blind study in parkinsonian patients with levodopa related fluctuations. J. Neurol. Neurosurg. Psychiatry 60: 36-40.
8. Keränen, T., Gordin, A., Karlsson, M., Korpela, K., Pentikäinen, P. J., Rita, H., Schultz, E., Seppälä, L. and Wikberg, T. 1994. Inhibition of soluble catechol-*O*-methyltransferase and single-dose pharmacokinetics after oral and intravenous administration of entacapone. Eur. J. Clin. Pharmacol. 46: 151-157.
9. Ahtila, S., Kaakkola, S., Gordin, A., Korpela, K., Heinävaara, S., Karlsson, M., Wikberg, T., Tuomainen, P. and Männistö, P. T. 1995. Effect of entacapone, a COMT inhibitor, on the pharmacokinetics and metabolism of levodopa after administration of controlled-release levodopa-carbidopa in volunteers. Clin. Neuropharmacol. 18: 46-57.
10. Tan, E. K., Tan, C., Fook-Chong, S. M. C., Lum, S. Y., Chai, A., Chung, H., Shen, H., Zhao, Y., Teoh, M. L., Yih, Y., Pavanni, R., Chandran, V. R. and Wong, M. C. 2003. Dose-dependent protective effect of coffee, tea, and smoking in Parkinson's disease: a study in ethnic Chinese. J. Neurol. Sci. 216: 163-167.
11. Ascherio, A., Zhang, S. M., Hernán, M. A., Kawachi, I., Colditz, G. A., Speizer, F. E. and Willett, W. C. 2001. Prospective study of caffeine consumption and risk of Parkinson's disease in men and women. Ann. Neurol. 50: 56-63.
12. Pan, T., Jankovic, J. and Le, W. 2003. Potential therapeutic properties of green tea polyphenols in Parkinson's disease. Drugs Aging 20: 711-721.
13. Rondelli, I., Acerbi, D., Mariotti, F. and Ventura, P. 1994. Simultaneous determination of levodopa methyl ester, levodopa, 3-*O*-methyldopa and dopamine in plasma by high-performance liquid chromatography with electrochemical detection. J. Chromatogr. B 653: 17-23.
14. Nutt, J. G. and Fellman, J. H. 1984. Pharmacokinetics

of levodopa. Clin. Neuropharmacol. 7: 35-49.
15. Männistö, P. T. and Kaakkola, S. 1990. Rational for selective COMT inhibitors as adjuncts in the drug treatment of Parkinson's disease. Pharmacol. Toxicol. 66: 317-323.
16. Rivera-Calimlim, L., Tandon, D., Anderson, F. and Joynt, R. 1977. The clinical picture and plasma levodopa metabolite profile of Parkinsonian nonresponders. Treatment with levodopa and decarboxylase inhibitor. Arch. Neurol. 34: 228-232.
17. Reilly, D. K., Rivera-Calimlim, L. and Dyke, D. V. 1980. Catechol-*O*-methyltransferase activity: A determinant of levodopa response. Clin. Pharmacol. Ther. 28: 278-286.
18. Muenter, M. D., Sharpless, N. S. and Tyce, G. M. 1972. Plasma 3-*O*-methyldopa in L-dopa therapy of Parkinson's disease. Mayo Clin. Proc. 47: 389-395.
19. Zürcher, G., Prada, M. D. and Dingemanse, J. 1996. Assessment of catechol-*O*-methyltransferase activity and its inhibition in erythrocytes of animals and humans. Biomed. Chromatogr. 10: 32-36.