Pharmacokinetic and Pharmacodynamic Studies of L-Dopa in Rats. I. Pharmacokinetic Analysis of L-Dopa in Rat Plasma and Striatum

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The purpose of this investigation was to quantitatively describe the pharmacokinetics of exogenous and endogenous L-dopa in plasma and the striatum using a basic physiological model, and to determine the apparent metabolism clearance from L-dopa to dopamine in the striatum. Male Wistar rats were used in this study. The time courses of L-dopa concentrations in plasma and the striatum were determined before and after the rapid i.v. injection of 10, 50 and 100 mg/kg. Plasma and striatum samples were obtained over 480 min (17 time points) from different group of animals and then assayed by HPLC-ECD. The endogenous L-dopa concentration in plasma before drug administration was 2.1 ± 0.6 mg/l. The exogenous L-dopa concentration declined biexponentially with time after drug injection. The total clearance of endogenous L-dopa in plasma was 3.13 (l/h)/kg. The production rate constant of endogenous L-dopa in plasma was 6.59 (mg/h)/kg. The value of the production rate constant of endogenous L-dopa in plasma could be calculated by the multiplication of the total clearance of L-dopa and the endogenous L-dopa concentration in plasma before drug injection. The pharmacokinetics of endogenous and exogenous L-dopa in plasma could be described quantitatively by a two compartment model which included the production rate constant of endogenous L-dopa. The time course of L-dopa concentrations in the striatum was analyzed on a hybrid model in which the striatum compartment is independently connected with the plasma compartment by the apparent diffusion clearance. The striatum compartment has two apparent first-order clearance terms, one from the plasma to the striatum, the other from the striatum to the outside of the striatum, including the metabolism clearance from L-dopa to dopamine in the striatum. The time course of L-dopa concentration in the striatum could be described by the basic physiological model, and the apparent metabolism clearance from L-dopa to dopamine in the striatum could be determined by the basic physiological model.

Keywords L-dopa; plasma; striatum; pharmacokinetics

L-Dopa is one of the most widely used drugs for the treatment of Parkinsonism. The drug has a very complex mode of action in pharmacokinetics, pharmacodynamics and clinical responses. The relationship among individual doses of L-dopa, plasma concentration of L-dopa and clinical response is still unclear. Algeri et al. reported little or no correlation among dose, plasma concentration and clinical response. 1) Although several types of clinical fluctuations related to drug treatment have been identified, the most common cause of therapy related fluctuations is an end-of-dose phenomenon, often called “wearing-off” or “on-off”. 2) It has been suggested that the possible cause of these phenomena are caused by both an impaired conversion of L-dopa to dopamine and to a reduced capacity for the storage of newly synthesized dopamine in dopaminergic neuronal terminals in Parkinson's disease striatum. As the disease of Parkinsonism progresses, there is a progressive reduction of dopa decarboxylase activity in the striatum. 3) Spencer and Wooten found that the administration of L-dopa resulted in a time-dependent elevation of striatal dopamine content that was of a smaller magnitude and briefer in dopaminergic de-nervated striatum than in the control, and they suggested that “wearing-off” is a consequence of a reduction in the rate of conversion of L-dopa to dopamine and perhaps of a diminished capacity for the storage of dopamine. 4) Nutt and Woodward have demonstrated that the duration, but not the magnitude, of clinical response was linearly related to plasma concentrations during the infusion of L-dopa in Parkinsonian patients. 5) However, pharmacokinetic studies on the penetration of L-dopa into the striatum and the metabolism from L-dopa to dopamine in the striatum have not been comprehensively performed. The time course of L-dopa concentrations in the striatum was analyzed on a hybrid model in which the striatum compartment is independently connected with the plasma compartment by an apparent diffusion clearance. The hybrid model provides an advantage in that much less data is required and fewer equations need to be solved for a single compartment. 6) The purpose of this investigation was to develop a physiologically based pharmacokinetic model for L-dopa in plasma and striatum and to determine the apparent metabolism clearance from L-dopa to dopamine in the striatum.

MATERIALS AND METHODS

Chemicals L-Dopa (Wako Pure Chemical Industries, Ltd., Osaka, Japan) was purchased commercially and used without further purification. This drug was dissolved in isotonic sodium chloride solution (dissolved with the aid of 0.1 N HCl) and was administered intravenously. All other chemicals were of reagent grade and were obtained commercially (Wako Pure Chemical Industries, Osaka, Japan).

Animal Experiments Inbred male Wistar rats (Sankyo Lab Service Corporation, Inc., Shizuoka, Japan) were used in this investigation. Before the investigation, the rats were housed individually in metal cages in an environment of controlled temperature (22–24°C) and alternating 12 h light (7 a.m.—7 p.m.) and dark cycles. Food and water were withdrawn in the morning on the day of the exper-
iment and these rats were placed in individual plastic metabolic cages before the experiment. The rats used for the pharmacokinetics of L-dopa in plasma and striatum had an indwelling cannula implanted in the right jugular vein 1 d before the experiment. To characterize the L-dopa disposition in plasma and the striatum using a basic physiological model, L-dopa 10, 50 and 100 mg/kg was injected rapidly into the right jugular vein. The blood samples (0.2 ml) were collected prior to the drug administration (for endogenous L-dopa concentrations) and at 1, 2, 3, 5, 7, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, 360 and 480 min after the dosing. Immediately after blood collection, each rat was sacrificed by 2 mmol/ml KCl (1 ml) injection, and their whole brains were quickly excised, rinsed with cold physiological saline and dissected into corpus striatum according to a modification of the method of Glowinski and Iversen. The plasma sample was obtained after blood collection by centrifuge at 10000 rpm for 2 min. The plasma and striatum samples were stored at −80°C until analysis.

**Assay Methods for L-Dopa**

The plasma and striatum concentrations of L-dopa were determined by a modification of the high-performance liquid chromatographic assay of Murali et al. with isoproterenol (Wako Pure Chemical Industries, Ltd., Osaka, Japan) as the internal standard. The plasma samples (20 µl) were placed in 1.5 ml microtubes to which were added 180 µl of 0.1 mh perchloric acid containing 10 µmol EDTA 2 Na and isoproterenol for the precipitation of protein. After centrifugation at 4000 rpm for 10 min at 4°C, the clear supernatants were filtered through a 0.45 µm filter (disposable syringe filter unit, dismic-3cp cellulose acetate, Advantec, Tokyo, Japan), and 10 µl of the filtrates were injected onto the HPLC system. The striatum samples (0.05 g) were put onto a glass test tube and homogenized with a polytron homogenizer (PT 10-35, Kinematica, Switzerland) at 15000 rpm for 10 s in 500 µl of 0.1m perchloric acid containing 10 µmol EDTA 2 Na and isoproterenol. The supernatants after centrifugation were prepared by the same procedure as described for the plasma. The resultant filtrate was loaded onto a reversed-phase HPLC column (Supelcosil LC-18-DB, Supelco, Inc., PA, U.S.A.). The solvent delivery system (L-5000 LC controller and 655A-11 pump, Hitachi, Ltd., Tokyo, Japan) was equipped with an electrochemical detector (ECD-100, EICOM, Co., Kyoto, Japan) at +0.7 V vs. an Ag-AgCl reference electrode and with an auto sampler (AS-8010, Tosoh, Co., Tokyo, Japan) and with a chromatograph-integrator (D-2500, Hitachi, Ltd., Tokyo, Japan). A guard column (Supelcosil LC-18-DB, Supelco, Inc., PA, U.S.A.) was placed between the auto sampler and the analytical column. The mobile phase was 0.01 M citrate buffer (pH 4.4)–MeOH (90:10, v/v) containing 10 µmol EDTA 2Na and 0.5 mm sodium 1-octanesulfonate, and the flow rate was 1.0 ml/min. Retention times were 4 and 45 min for L-dopa and the internal standard, respectively.

**Pharmacokinetic Analysis**

Assuming that the pharmacokinetic behavior of endogenous and exogenous L-dopa are the same, the pharmacokinetic model for L-dopa was constructed and is shown in Fig. 1. The time courses of L-dopa concentration in the striatum were analyzed on a hybrid model in which the striatum compartment is independently connected with the plasma compartment by apparent diffusion clearance. It was assumed that the pharmacokinetics of L-dopa in plasma can be described by linear elimination kinetics. The exogenous L-dopa concentrations in plasma fitted to a biexponential equation as follows.

\[ C_{m(\text{exo})} = A \exp(-\alpha t) + B \exp(-\beta t) \]  

Where \( C_m \) is the concentration (mg/l) at time \( t \) (h), and \( A, B, \alpha \) and \( \beta \) are constants (mg/l and h⁻¹). The computer program FKDM² was used for the nonlinear least squares analysis using a digital computer (PC-9821 Ac, NEC Corp., Tokyo, Japan). The total clearance of L-dopa was calculated from the parameters of the biexponential Eq. 1 in the usual manner. The endogenous L-dopa in plasma and striatum are assumed as a zero-order infusion rate, the endogenous L-dopa in plasma can be expressed using the following equation:

\[ C_{s(\text{end})} = k_o \frac{A + B}{\alpha + \beta} \left( 1 - \frac{k_o}{\alpha} \exp(-\alpha t) - \frac{k_o}{\beta} \exp(-\beta t) \right) \]  

where \( k_o \) is the zero-order production rate of endogenous L-dopa in plasma (mg/h/kg), \( A/\alpha + B/\beta \) is the area under the curve (AUC) of the plasma L-dopa concentration ((mg h)/l). AUC/dose is 1/CL. Therefore, at steady state (time is infinity), the value of the production rate constant of endogenous L-dopa in plasma can be calculated by the following equation, the multiplication of the total clearance of L-dopa and the endogenous L-dopa concentrations in plasma before drug injection.

\[ k_o = C_{\text{plasma}} CL \]  

Both endogenous and exogenous L-dopa concentrations in plasma before and after i.v. administration of L-dopa can be expressed using the following equation and were simulated using the parameters of \( A, B, \alpha, \beta \) and \( k_o \) which were estimated by Eqs. 1 and 3.
\[ C_{\text{striata}} = \frac{k_0}{\text{dose}} \left( A + \frac{B}{\alpha} \right) + \frac{A}{\alpha} \left( x - \frac{k_0}{\text{dose}} \right) \exp(-\alpha t) + \frac{B}{\beta} \left( \frac{k_0}{\text{dose}} \right) \exp(-\beta t) \] 

(4)

The time course of L-dopa concentration in the striatum was analyzed using the hybrid model in which the striatum compartment is independently connected with the plasma compartment by apparent diffusion clearance.\textsuperscript{5} The striatum compartment has two apparent first-order clearance terms, one from the plasma to the striatum, the other from the striatum to outside the striatum, including the apparent metabolism clearance from L-dopa to dopamine in the striatum. The changes in the concentration of L-dopa in the striatum could be expressed as follows:

\[ V_a \frac{dC_a}{dt} = PA C_{st} - PA C_a - CL_m C_a \] 

(5)

where \( V_a \) is the striatum weight (g), and \( C_{st} \) and \( C_a \) represent the concentrations of L-dopa in plasma and striatum (mg/l and \( \mu g/g \)), respectively. \( PA \) is the apparent diffusion clearance between plasma and the striatum (ml/h). \( CL_m \) represents the apparent metabolism clearance from L-dopa to dopamine in the striatum (ml/h). The binding of L-dopa in rat plasma was determined by equilibrium dialysis. The free fraction of L-dopa in plasma was almost 1 (data not shown). Substitution of Eq. 4 into 5 and integration gives the following equation:

\[ C_{\text{max}} = \frac{k_0}{\text{dose}} \left( \frac{k_{\text{in}}}{k_{\text{out}}} \right) \left( \frac{A}{\alpha} \frac{1}{\text{dose}} \right) \exp(-\alpha t) + \frac{k_{\text{in}} A}{\alpha - k_{\text{out}}} \left( \frac{1}{\alpha} \frac{1}{\text{dose}} \right) \exp(-\alpha t) \] 

(6)

where \( k_{\text{in}} \) and \( k_{\text{out}} \) represent the apparent first-order constant from the plasma to the striatum and from the striatum to outside the striatum, including the apparent metabolism constant from L-dopa to dopamine in striatum (h\(^{-1}\)); \( k_{\text{in}} = PA/V_a \) and \( k_{\text{out}} = (PA + CL_m)/V_a \) respectively. In order to estimate the kinetic parameters of \( k_{\text{in}} \) and \( k_{\text{out}} \), the data on the concentration of L-dopa in the striatum was fitted to Eq. 6 by a nonlinear least squares regression program, FKDM.\textsuperscript{9} The value of the endogenous L-dopa concentration before L-dopa administration was calculated by the following equation:

\[ C_{\text{max}} = \frac{k_0}{\text{dose}} \frac{k_{\text{in}}}{k_{\text{out}}} \frac{1}{CL_a} \] 

(7)

RESULTS

L-Dopa Concentrations in Plasma

The time courses of L-dopa concentrations in plasma before and after the i.v.

![Fig. 2. Time Course of L-Dopa Concentrations in Plasma before and after the Intravenous Administration of L-Dopa](image-url)

(a) 100 mg/kg (n=3–6), (b) 50 mg/kg (n=3–6), (c) 10 mg/kg (n=3–6). The upper panel represents the exogenous l-dopa concentrations. The lower panel represents both exogenous and endogenous l-dopa concentrations. The plotted points represent the observed data and the solid lines represent the calculated values using Eq. 1 (upper panel) and Eq. 4 (lower panel) in the text. L-Dopa was administered at time zero, and the l-dopa values at time zero represent the endogenous l-dopa concentrations. Each experimental point is shown as the mean ± S.D.
administration of L-dopa 100, 50 and 10 mg/kg in the rats are shown in Fig. 2. The data are plotted semi-logarithmically as a function of time. The control level of plasma L-dopa concentration (endogenous L-dopa concentrations) before drug administration was 2.1 ± 0.6 mg/l. The disappearance of L-dopa concentration in plasma followed two-exponential kinetics. It was assumed that the pharmacokinetics of L-dopa in plasma could be described by linear elimination kinetics. The pharmacokinetic parameters \( A, B, x, z, \beta \) were computed by the nonlinear least squares method, and the total clearance (\( CL_t \)) for L-dopa was calculated by the common method and is listed in Table I. The values of the parameters of \( A \) and \( B \) in 10 and 50 mg/kg dosing were 0.1 \( \times A \), 0.1 \( \times B \), 0.5 \( \times A \) and 0.5 \( \times B \), respectively. Other parameters were same as at 100 mg/kg dosing. The value of the production rate constant \( k_p \) of endogenous L-dopa in plasma was calculated by multiplying the total clearance of L-dopa and the endogenous L-dopa concentration in plasma before drug injection, Eq. 3, and is listed in Table I. The pharmacokinetics of endogenous and exogenous L-dopa in plasma could be described quantitatively by a two compartment model including the production rate constant of endogenous L-dopa, Eq. 4.

**Table I. Values of Pharmacokinetic Parameters of L-Dopa in Rats Obtained from Computer Fitting of Plasma and the Striatum Concentration Data after Intravenous Administration of L-Dopa 100, 50 and 10 mg/kg**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>100 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A ) (mg/l)</td>
<td>445.9 ± 57.06</td>
</tr>
<tr>
<td>( B ) (mg/l)</td>
<td>33.11 ± 6.210</td>
</tr>
<tr>
<td>( z ) (h (^{-1}))</td>
<td>34.16 ± 4.347</td>
</tr>
<tr>
<td>( \beta ) (h (^{-1}))</td>
<td>1.750 ± 0.367</td>
</tr>
<tr>
<td>( CL_t ) (l/h)</td>
<td>3.128</td>
</tr>
<tr>
<td>( V_m ) (l)</td>
<td>2.1 ± 0.6 (15)</td>
</tr>
<tr>
<td>( k_p ) (mg/h/kg)</td>
<td>6.587</td>
</tr>
<tr>
<td>( k_p ) (h (^{-1}))</td>
<td>0.243 ± 0.034</td>
</tr>
<tr>
<td>( k_{int} ) (h (^{-1}))</td>
<td>13.18 ± 2.277</td>
</tr>
<tr>
<td>( k_m ) (h (^{-1}))</td>
<td>12.93</td>
</tr>
<tr>
<td>( V_{air} ) (g/kg)</td>
<td>0.082 ± 0.021 (114)</td>
</tr>
<tr>
<td>( P_{in} ) (ml/h)</td>
<td>19.93 ( \times 10^3 )</td>
</tr>
<tr>
<td>( CL_{int} ) (ml/h)</td>
<td>1.060</td>
</tr>
<tr>
<td>( V_{air} ) (ng/g)</td>
<td>38.8</td>
</tr>
</tbody>
</table>

\( a \) The values of the parameters of \( A \) and \( B \) following 10 and 50 mg/kg dosing were 0.1 \( \times A \), 0.1 \( \times B \), 0.5 \( \times A \) and 0.5 \( \times B \), respectively. Other parameters were the same as with 100 mg/kg dosing. \( b \) The pharmacokinetic parameters in plasma \((A, B, x, \beta)\) and in the striatum \((k_m, k_{int})\) were estimated by fitting the data to Eqs. 1 and 6, respectively, using the computer program PKDM. All values are expressed as the mean ± S.D. of the estimated parameter. \( c \) The value of this parameter was calculated by the following equation: \( CL_t = \frac{dose}{(A/z + B/\beta)} \). \( d \) The value of this parameter was measured in this study and the number of animals used is indicated in parentheses. \( e \) The value of this parameter was calculated by Eq. 3. \( f \) The value of this parameter was calculated by the following equation: \( k_{int} = k_{p} - k_m \). \( g \) The values of these parameters were measured in this study, and the number of animals used is indicated in parentheses. Rat body weight was 263.2 ± 24.6 g. \( h \) The values of these parameters were calculated by the following equation: \( P_{in} = k_m V_{air}, CL_{int} = k_m V_{air} \). \( i \) The value of this parameter was calculated by Eq. 7.

![Fig. 3. Time Course of L-Dopa Concentrations in the Striatum before and after the Intravenous Administration of L-Dopa](image)

(a) 100 mg/kg \((n=3)\), (b) 50 mg/kg \((n=3)\), (c) 10 mg/kg \((n=3)\). The upper and lower panels represent the same data in the striatum but on a different time scale. The plotted points represent the observed data, and the solid lines represent the calculated values using Eq. 6 in the text. L-Dopa was administered at time zero and the L-dopa values at time zero represent the endogenous L-dopa concentration. Although the endogenous L-dopa concentrations in the striatum before L-dopa administration could not be determined in this study, the calculated value of the endogenous L-dopa concentration in the striatum was 38.8 ng/g, and this value is consistent with the report of Ehrenstrom and Johansson. Each experimental point is shown as the mean ± S.D.
using the hybrid model. The pharmacokinetic parameters ($k_{in}$ and $k_{um}$) were computed by the nonlinear least squares method, and the other parameters ($PA$ and $CL_{m}$) were calculated and are listed in Table I. Although the endogenous L-dopa concentrations in the stratum before drug administration could not be determined in this study, the value of the endogenous L-dopa concentrations in the stratum could be calculated using Eq. 7 and are listed in Table I.

**DISCUSSION**

L-Dopa is still the most effective and widely used drug in treating Parkinson's disease. Clinical experience has revealed that chronic L-dopa treatment is often associated with various side effects, including a gradual decline in efficacy. However, pharmacokinetic studies on the penetration of L-dopa into the stratum and on the metabolism from L-dopa to dopamine in the stratum have not been comprehensively performed. The purpose of this investigation was to develop a physiologically based pharmacokinetic model for L-dopa in plasma and the stratum and to determine the apparent metabolism clearance from L-dopa to dopamine in the stratum.

L-Dopa is an endogenous compound. The control concentration of L-dopa in plasma was $2.1 \pm 0.6$ mg/l, and this value is consistent with the reports of Rose et al. and Mearrick et al. In order to describe the disposition of the endogenous L-dopa in plasma, it was assumed that the production rate of the endogenous L-dopa in plasma is the zero-order infusion rate in this study. In the case of the two compartment model, drug concentrations in plasma during the zero-order intravenous infusion may be written using the following equation:

$$C = \frac{k_0}{V_d k_{10}} \left( 1 + \frac{\beta - k_{10}}{\alpha} \exp(-\alpha t) + \frac{k_{10} - \alpha}{\alpha - \beta} \exp(-\beta t) \right)$$

where $V_d$ is the apparent volume of distribution, $k_{10}$ is the apparent first-order elimination rate constant. The apparent volume of distribution ($V_d$) and the apparent first-order elimination constant ($k_{10}$) can be expressed in the following equations, respectively:

$$V_d = \frac{dose}{A + B}$$

$$k_{10} = \frac{(A + B)\beta}{A \beta + B}$$

The Eq. 2 was obtained by the substitution of Eqs. 9 and 10 into Eq. 8, in order to express Eq. 8 using the parameters of $A$, $B$, $x$, $\beta$ and doses which were obtained by the biexponential equation. The values of the total clearance and $\beta$ of L-dopa in plasma were $13.7$ ml/min and 0.029 min$^{-1}$, respectively, and these values are consistent with the report of Mearrick et al. ($CL_m = 24.6$ ml/min, $\beta = 0.029$ min$^{-1}$).

Although the endogenous L-dopa concentrations in the stratum before drug administration could not be determined in this study, the calculated value of the endogenous L-dopa concentrations in the stratum before drug administration was 38.8 ng/g, and this value is consistent with the report of Ehrenstrom and Johansson. It has been demonstrated that the L-dopa is transported into the brain through a carrier-mediated system, and the transport of L-dopa into the brain is limited because of the competition with an endogenous large neutral amino acid in plasma. The estimated value of the apparent diffusion clearance from plasma to the stratum ($PA$) and the apparent metabolism clearance from L-dopa to dopamine in the stratum ($CL_m$) were $19.93 \times 10^{-3}$ and 1.06 ml/h, respectively. The value of $PA$ was much smaller than the plasma flow rate ($Q$) into the rat brain (64.73 ml/h, rat body weight 0.25 kg). This result suggests that the pharmacokinetics of L-dopa into the rat stratum can be explained by the diffusion-limited model. Moreover, the values of $PA$ and $CL_m$ were much smaller than the total clearance of L-dopa in plasma. This result suggests that the elimination of L-dopa from the stratum is negligible compared to the systemic elimination. The time courses of the stratum L-dopa concentrations were reasonably described by the hybrid model.

The value of $PA$ contains the values of both the passive diffusion clearance and the carrier-mediated diffusion clearance from plasma to the stratum. However, in this analysis, it was very difficult to estimate the values of the passive and active diffusion clearance from plasma to the stratum separately. Since it was assumed that the value of the returned diffusion clearance from the stratum to plasma is the same as the value of the diffusion clearance from plasma to the stratum, there is little possibility of an overestimation or underestimation of the value of $CL_m$. In the results of this analysis, it was recognized that the value of $CL_m$ is about 50 times the value of $PA$. Therefore, it can be considered that there is no effect of the underestimation of the value of $CL_m$ on the analysis of the metabolism from L-dopa to dopamine. Moreover, in the case of the overestimation of the value of $CL_m$, it can be considered that the contribution of $CL_m$ on the enlargement of dopamine concentrations in the stratum after the L-dopa administration might be diminished. The obtained value of $CL_m$ will be able to be used for the analysis of the metabolism from L-dopa to dopamine, and the contribution of $CL_m$ on the enlargement of dopamine concentration in the stratum after L-dopa administration will be examined in the subsequent paper.

In conclusion, the calculation of L-dopa concentrations in plasma and the stratum after i.v. administration of L-dopa were performed using the two compartment model, including the production rate of endogenous L-dopa in plasma and the physiologically based pharmacokinetic model. The time courses of the L-dopa concentrations in plasma and the stratum were well explained by these models, and the metabolism clearance from L-dopa to dopamine in stratum was estimated. In the subsequent paper, the time courses of the dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) concentrations in the stratum after the i.v. administration of L-dopa will be elucidated using the estimated value of the metabolism clearance from L-dopa to dopamine.
REFERENCES