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Importance of Within Subject Variation in Levodopa Pharmacokinetics: A 4 Year Cohort Study in Parkinson's Disease

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The purpose of the study was to describe the population pharmacokinetics of levodopa in patients with Parkinson's disease studied in 5 trials (10 occasions) over 4 years. Twenty previously untreated Parkinsonian patients were investigated. Each trial consisted of a 2-hr IV infusion of levodopa (1 mg/kg/h) with concomitant oral carbidopa given on two occasions separated by 72 hr with no levodopa in between. This trial design was repeated at 6, 12, 24 and 48 months. A two-compartment pharmacokinetic model with central volume (V_1), peripheral volume (V_2), clearance (CL) and inter-compartmental clearance (CL_{ic}) was used to fit plasma levodopa concentrations. The model accounted for levodopa dosing prior to each trial and endogenous levodopa synthesis. Population parameter estimates (geometric mean) and population parameter variability (PPV; SD of normal distribution) were V_1 11.4 l/70 kg (0.44), CL 30.9 l/h/70 kg (0.25), V_2 27.3 l/70 kg (0.27), and CL_{ic} 34.6 l/h/70 kg (0.48). PPV was partitioned into between subject variability (BSV) which was 0.12 V_1, 0.13 CL, 0.15 V_2, 0.28 CL_{ic}, within trial variability (WTV) which was 0.16 V_1, 0.13 CL, 0.08 V_2, 0.18 CL_{ic} and between trial variability (BTV) which was 0.40 V_1, 0.17 CL, 0.21 V_2, 0.34 CL_{ic}. Neither structural nor random levodopa pharmacokinetic parameters were associated with the time course of development of fluctuation in motor response. Variability in levodopa pharmacokinetic parameters (particularly V_1) may result in variability in plasma levodopa concentrations that could contribute to fluctuations in motor response.

KEY WORDS: levodopa; within subject variability; pharmacokinetics; Parkinson's disease; population approach.

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INTRODUCTION

Long-term therapy with levodopa is commonly associated with variation in motor function from hour to hour. Development of motor fluctuations typically develops after 2–4 years of levodopa treatment (1–3). The incidence of motor fluctuations was 58% after a mean of 35 months treatment from the time of levodopa treatment initiation. Development of motor fluctuations may be related to changes in peripheral and/or central (brain) levodopa pharmacokinetics (4–6). Variability in plasma levodopa concentration could contribute to fluctuations in motor response. It has been shown that continuous intravenous infusion of levodopa reduces the frequency of motor fluctuations and the duration of immobile periods (7–9). In Parkinson's disease, the pharmacokinetics of levodopa has been found to be highly variable between patients (10,11). Nevertheless, no trend for a systematic change in plasma levodopa pharmacokinetics was observed in 28 levodopa treated patients who were followed up for 4 years (12), and no difference in pharmacokinetics of levodopa was found in patients with stable or fluctuating motor response (13–15). Within subject variability in pharmacokinetics defines the limit of predictability of concentrations (16). This information can be used to decide if measurement of concentration is a practical means of dose individualization. It may also provide insight into within subject variability in response e.g. motor fluctuations observed in patients treated with levodopa.

In general variability can be considered as a predictable effect (e.g. using some characteristic such as weight) or a random effect i.e. unpredictable based on current knowledge. We have examined the predictable and random components of the pharmacokinetic model parameters for levodopa.

Population parameter variability (*PPV*) is composed of between subject variability (*BSV*) and within subject variability (*WSV*). *BSV* can be further divided into predictable (*BSVP*) and random (*BSVR*) components. BSVP denotes the variability that can be explained by covariates while *BSVR* is random and unpredictable. Covariates are individual characteristics (e.g. sex, weight), which may be used to identify predictable between subject differences in pharmacokinetic parameters.

The identifiable component of *WSV* may be modeled using an occasion as a covariate to identify between occasion variability (*BOV*, also known as interoccasion variability (17)). For the purposes of this study, we further divide *WSV* into components based upon repeated trials and repeated occasions as covariates. We define a trial to mean a period involving two infusions of levodopa separated by 3 days and an occasion to mean the period from the start of the first levodopa infusion until the next

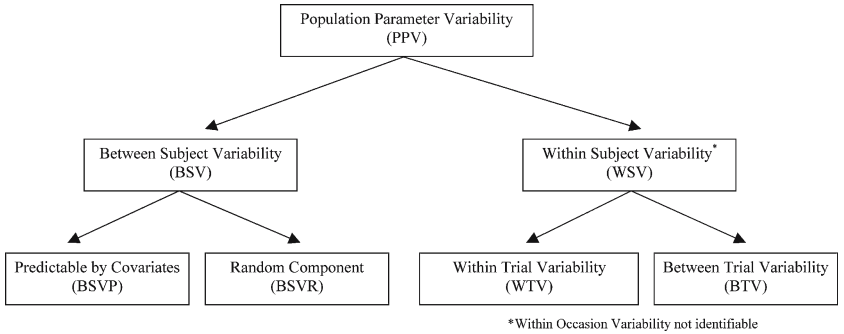

Fig. 1. Components of population parameter variability.

infusion starts or from the start of the second infusion until 5 hr later. If we consider the average value of a parameter estimated on separate occasions within a trial we can determine the within trial variability (*WTV*) around this occasion averaged value. *WTV* is the same as *BOV*. Further, if we consider the average value of a parameter estimated in all trials within a study then we can determine the between trial variability (*BTV*) around this trial averaged value. Figure 1 shows the individual components of *PPV*.

There is another quite different kind of random variability. Random unidentified variability (*RUV*), also known as residual error, accounts for errors arising from all other sources, including experimental errors such as dosing size and times, measurement error, and model misspecification. Unlike *PPV* which measures the parameter variability, *RUV* is the residual variability in the observations.

The objective of this study was to investigate the population pharmacokinetics of levodopa and its variability in patients with Parkinson's disease followed from the start of treatment with levodopa and continued for up to 4 years. Of particular interest we wished to determine if there was a systematic change in levodopa pharmacokinetic parameters or in the variability of the parameters during long-term therapy which might contribute to the development of motor fluctuations. The current analysis uses data from a previously published clinical study (3).

METHODS

Data

The details of experimental design have been reported previously (3). In brief, twenty subjects (12 men, 8 women) with idiopathic Parkinson's

Table I. Patient Demographics of the Study Population

Patients (12 M/8 F)	Mean	SD	Range
Weight (kg)	78.7	12.4	60–100
Age at entrance of study (Years)	59.8	10.7	40–75
Age of onset of disease (Years)	56.1	10.9	39–73
UPDRS at baseline (units)[a]	31.5	13.8	10.5–61

[a] Unified Parkinson's disease rating scale was obtained at baseline in 11 out of 20 patients.

disease and with no prior levodopa treatment (who were judged to require initiation of chronic levodopa therapy) were followed for 4 years (Table I). Parkinson's disease was defined by history, symptoms and signs (18,19). All subjects participating in this study gave informed consent to a protocol approved by the Oregon Health Sciences University (OHSU) Institutional Review Board.

At each individual trial (0, 6, 12, 24, and 48 months measured from the time of starting long-term levodopa), patients were hospitalized in the OHSU clinical research center for 5 days and oral levodopa withheld the night before and through each trial. Day 0 was admission to the center. A 2-hour (9:00 AM to 11:00 AM) constant rate levodopa IV infusion (1 mg/kg/hr) with concomitant oral carbidopa (25 mg administered at 8:00 AM, 10:00 AM and 12:00 PM) was given on two occasions separated by 72 hr (20). Patients received no levodopa treatment in between the two infusions. Following the first trial period and prior to all subsequent trial visits, patients took oral levodopa (with carbidopa), adjusted as needed for optimum symptomatic benefit. No explicit record of individual dose compliance was kept but the typical daily dose immediately prior to each trial was noted (427 ± 163 mg for 6 months, 489 ± 196 mg for 12 months and 579 ± 332 mg for 48 months) (3,21). Other anti-parkinsonian medications were taken as necessary. Plasma levodopa concentrations (μmol/l) were collected at 0, 0.25, 0.5, 1, 1.5, 2, 2.25, 2.5, 3, 4 and 5 hr after the infusion start. Levodopa concentration was assayed by high-performance liquid chromatography with electrochemical detection (22). The within-day assay precision was 3.5% and the between assay precision was 7.9%. The lower limit of quantitation was 0.1 μmol/l.

Pharmacokinetic Model Development

Structural Model

Zero-order input with one- and two-compartment pharmacokinetic models using the subroutines from the NONMEM library (ADVAN1

TRAN2 and ADVAN3 TRAN4) were fitted to the data. For the two-compartment pharmacokinetic model, the estimated parameters were central (V_1) and peripheral (V_2) compartment volumes, total body clearance (CL) and inter-compartmental clearance (CL_{ic}). Duration of infusion was fixed to the nominal protocol duration of 2 hr. Estimation of the individual input durations did not improve the model fit.

Rate of Endogenous Levodopa Synthesis and Prior Trial Dosing

Two additional parameters were used to extend the base pharmacokinetic model to account for endogenous levodopa synthesis (Rsyn) and concentrations arising from exogenous levodopa prior to each trial (Css_{pre}) (Fig. 2).

Exogenous oral levodopa was modeled by a steady state infusion with constant but unknown rate, which ended at the start of the first levodopa infusion of each trial. The rate of steady state infusion ($R1$) was calculated from:

$$R1 = CL \bullet Css_{pre}$$

For the first occasion of the 0 month trial and for the second occasion at each subsequent trial, Css_{pre} was assumed to be zero as no levodopa treatment was received prior to the start of the first trial or during the drug holiday between trial occasions.

The synthesis rate of endogenous levodopa was assumed to be constant (Rsyn). Concentrations arising from endogenous synthesis were added to the predicted levodopa concentration induced by external input (Cex). CP denotes the sum of the predicted levodopa concentrations for both endogenous and external inputs.

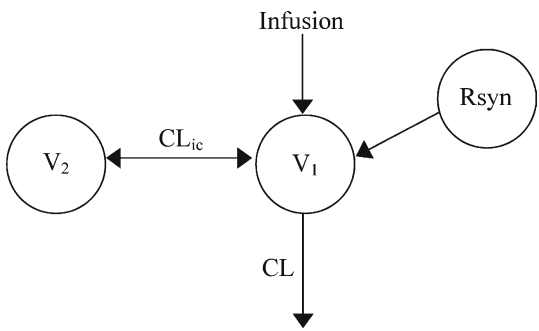

Fig. 2. Pharmacokinetic model for levodopa. Parameters are central (V_1) and peripheral (V_2) compartment volumes, total body clearance (CL), intercompartmental clearance (CL_{ic}), rate of endogenous levodopa synthesis (Rsyn). Infusion represents the constant rate (1 mg/kg/h) IV levodopa infusion. A concentration arising from exogenous levodopa prior to each trial (Css_{pre}) was assumed at time zero in both compartments.

$$CP = C\text{ex} + \frac{R\text{syn}}{CL}$$

Modeling BSV, WTV and BTV

$\eta BSV, \eta WTV$ and ηBTV were assumed to be random variables with mean zero and standard deviation of *BSV, WTV* and *BTV*. Variability in parameters was assumed to arise from a lognormal distribution. Random effects (η) are assumed to arise from a normal distribution with mean 0 and standard deviation reflecting each component of variability e.g.

$$\eta PPV_{CLijk} = \eta BSV_{CLi} + \eta WTV_{CLij} + \eta BTV_{CLik}$$

$$CL_{ijk} = CL_{GRP} \bullet e^{\eta PPV_{CLijk}}$$

ηPPV_{CLijk} is the random effect for CL for the ith patient at the jth occasion of the kth trial. It is the sum of the random effects due to between subject (ηBSV), and between trial (ηBTV) and within trial (ηBTV) differences. CL_{GRP} is the group value (23) of CL based on fixed effects such as weight. CL_{ijk} is the predicted individual CL for the ith patient at the jth occasion of the kth trial.

Residual Error Model

RUV was described using an additive, a proportional or a combined additive and proportional error model.

$$Y = CP + \varepsilon ERR_{SD}$$

$$Y = CP \bullet e^{\varepsilon ERR_{CV}}$$

$$Y = CP \bullet e^{\varepsilon ERR_{CV}} + \varepsilon ERR_{SD}$$

Y is the observed value whereas $\varepsilon\text{ERR}_{\text{CV}}$ and $\varepsilon\text{ERR}_{\text{SD}}$ are random variables with mean zero and standard deviation ERR_{CV} and ERR_{SD}.

Covariate Model

The influence of covariates was modeled as effects on each of the pharmacokinetic parameters (V_1, CL, V_2 and CL_{ic}) in the two-compartment model. An exponential model (except for weight) was used to restrict pharmacokinetic parameters to be positive.

Weight

An allometric model was applied to standardize the pharmacokinetic parameters with an assumption of a standard body weight (WT) of 70 kg (24). For CL and CL_{ic}, the 3/4 power model was used. CL_{GRP} and V_{1GRP} are the group parameter values for CL and V_1

$$CL = CL_{GRP} \bullet \left(\frac{WT}{70\,kg}\right)^{3/4}$$

$$V_1 = V_{1GRP} \bullet \frac{WT}{70\,kg}$$

Sex

$$V_1 = V_{1GRP} \bullet e^{K_{SEX\_V_1}}$$

$K_{SEX\_V_1}$ is a parameter describing the change in V_1 in males in relation to females. $K_{SEX\_V_1}$ has a value of zero for females.

Age and Age of Onset

$$V_1 = V_{1GRP} \bullet e^{(K_{AGE\_V_1} \bullet (AGE-60))}$$

$K_{AGE\_V_1}$ is a parameter describing the change in V_1 in relation to a standard age of 60 years. The age of onset (AON) was modeled in the same way with a standard AON of 56 years (mean of the 20 patients).

Disease Duration

$$V_1 = V_{1GRP} \bullet e^{(K_{DD\_V_1} \bullet DD)}$$

K_{DD_V1} is a parameter describing the change in V_1 in relation to the duration of disease (DD).

Computation

Parameter estimation was performed using mixed effects non-linear regression (NONMEM version V release 1.1) (25) under MS-DOS on a Pentium III 600 under NT4.0 in conjunction with the Compaq Visual Fortran 77 compiler (version 6.6A). All covariate model building and final

parameter estimation was performed using the first-order conditional estimation method with interaction.

Models were compared using the objective function value (*OBJ*) and the model fits were also judged by eye. The null hypothesis that a full model was equivalent to a simpler model was tested by referencing the difference to a chi-square distribution with $\alpha = 0.05$ and degrees of freedom equal to the difference in the number of estimated parameters between the two models (26,27).

Population Parameter Variability Components

The individual components of *PPV* can be computed from the variance estimates from four models: (i) base model (random effect model); (ii) base model with WT as covariate (fixed effect for BSV model); (iii) base model using occasion and trial to identify WTV and BTV (fixed effect for WSV model) and; (iv) base model with WT, occasion and trial as covariates (final or true mixed effect model).

In the base model, all of the components of *PPV* (*BSVP, BSVR, WTV* and *BTV*) are embedded within a single random parameter variability (*RPV*) value. In the fixed effect for BSV model, the predictable component of BSV (*BSVP*) is explained by the covariate WT, and the RPV estimate consists of the random component of *BSV* (BSVR), plus *WTV* and *BTV*. The *WTV* and *BTV* components are identified using occasion and trial as covariates in the fixed effect for *WSV* model with the associated RPV estimate consisting of the sum of *BSVP* and *BSVR*. In the final model, BSVP is explained by *WT* while *WTV* and *BTV* are estimated using occasion and trial as covariates, thus only *BSVR* is included in *RPV*. Table II summarizes the *PPV* components embedded in each of the four models. *BSVR* was obtained from the final model (estimate of

Table II. Method for Determining the Components of Population Parameter Variability (PPV); BSVP and BSVR represent the Predictable and Random Components of Between Subject Variability (BSV); WTV and BTV represent Within Trial and Between Trial Variability; RPV describes the random parameter variability estimated with each model; See text for the details of different models

Model	Identified Components	RPV
Base (Random effect)	–	PPV
Fixed effect for BSV	BSVP	BSVR+WTV+BTV
Fixed effect for WSV	WTV+BTV	BSVP+BSVR
Final (True mixed effect)	BSVP+ WTV+BTV	BSVR

RPV); *BSVP* was computed by subtracting *BSVR* from the fixed effect for *WSV* model estimate of *RPV*; *WTV* and *BTV* estimated directly from the *WTV* and *BTV* components of the fixed effect for *WSV* model. Each of the components was then expressed as a percentage of the total *PPV* of the fixed effect for *WSV* model. The fixed effect for *WSV* model was considered to give a better estimate of *PPV* than the base model. The estimate of *PPV* in the base model was underestimated because some of the variability is accounted for by *RUV*.

Model Validation Using Prediction Intervals

We performed a predictive check to determine if the final model and its parameter estimates adequately describe the observed data. Data was simulated with the final model and its parameter estimates under the same experimental design that the original data. Fifty instances of the original data were concatenated to create a single dataset of 1000 subjects. The similarity between the actual observed data and simulated data was examined by comparing the 95% prediction intervals of the simulated data with the original observed data.

RESULTS

The Patients

A total of 164 time-concentration profiles with 1477 concentration observations were collected on up to 10 occasions over 4 years (5 trials). Eighteen out of twenty patients were followed for 4 years. One subject was lost to follow up after the first year and another subject had not completed the 48 months trial at the time of performing this analysis. Data from a minimum of 3 and maximum of 5 trials (6–10 occasions) were available on each subject.

Model Building

Base Model Development

A two-compartment pharmacokinetic model described the data better than a one-compartment model. The OBJ fell by 1270 units with the addition of 4 parameters (2 structural and 2 RPV, Table III). Introduction of *R*syn and Css_{pre} separately reduced the OBJ by 98 and 70 units respectively, and 259 units collectively. This showed that both *R*syn and Css_{pre} were important in describing the baseline levodopa concentrations. The residual error (RUV) was described by a model combining both additive

Table III. Model Building – Forward Addition

Model	Model description	*N*	ΔOBJ	Conclusion
1	One-compartment	8	–	
2	Two-compartment	12	1270[a]	Two-compartment model better
3	Two-compartment model with introduction of Rsyn	14	98.1[b]	Rsyn important
4	Two-compartment model with introduction of Css_{pre}	14	70.2[b]	Css_{pre} important
5	Two-compartment model with introduction of Rsyn and Css_{pre}	16	259[b]	Rsyn and Css_{pre} important (base model)
6	Base model with introduction of WTV	20	872[c]	WTV important
7	Base model with introduction of BTV	20	673[c]	BTV important
8	Base model with introduction of WTV and BTV	24	981[c]	WTV and BTV important
9	Fixed effect for WSV model with WT on all PK parameters	24	11.8	(fixed effect for WSV model) WT prediction better
10	Fixed effect for WSV model with DD on all PK parameters	26	17.2	DD prediction better
11	Fixed effect for WSV model with covariance of BSV parameters	30	35.5	Covariance of BSV important
12	Fixed effect for WSV model with covariance of WTV parameters	30	122	Covariance of WTV important
13	Fixed effect for WSV model with covariance of BTV parameters	30	152	Covariance of BTV important
14	Fixed effect for WSV model with covariance of BSV, WTV and BTV parameters	42	196	Covariance of BSV, WTV and BTV important
15	Fixed effect for WSV model with covariance of BSV, WTV and BTV parameters and WT on all PK parameters	42	28.2[d]	WT prediction better (final model)
16	Fixed effect for WSV model with covariance of BSV and WTV parameters and DD on all PK parameters	44	19.2[d]	DD possibly predictive

N, number of structural and error model parameters; ΔOBJ, difference in objective function value between the current and the fixed effect for WSV models except.
[a]compared with Model 1.
[b]compared with Model 2.
[c]compared with base Model 5.
[d]compared with Model 14.

and proportional error functions. Inclusion of Rsyn and Css_{pre} with a zero-order input two-compartment model and a combined additive and proportional error functions was chosen for the base model.

Full Model Development

Introduction of *WTV* and *BTV* reduced the *OBJ* by 981 units with an addition of 8 parameters. Inclusion of covariance of *BSV*, *WTV* and *BTV* parameters further reduced the *OBJ* by 196 units. Weight and disease duration were showed to be possible predictors of parameter variability.

Final Model Development

Once the full model had been developed, model elements were removed in order to confirm if a covariate or a component was vital to the final model. The *OBJ* increased by 890 and 359 units, respectively, when only an additive or a proportional error model was used (Table IV) confirming the choice of a combined residual error model. Weight was a significant covariate because exclusion of weight resulted in an increase of OBJ by 28.2 units. The influence of disease duration disappeared when weight was included as a covariate (OBJ reduced by 2.1 units). There was no apparent influence of sex, age or age at onset in explaining the variability of the pharmacokinetic parameters.

The final model (Model 15), was a zero-order input two-compartment pharmacokinetic model with *R*syn and Css_{pre} components, weight, occasion and trial as covariates, covariance of *BSV*, *WTV* and *BTV* and a combined additive and proportional residual error model.

Population Pharmacokinetics of Levodopa

The use of *WT* as a predictor of pharmacokinetic parameter variability only influenced *BSV* and not *WTV* or *BTV*. This was expected because most subjects had a stable body weight throughout the 4 years and weight was assumed to be unchanged within a trial. Only 3 subjects had a more than 10 kg (11,13 and 22 kg) change in weight. Inclusion of weight as a covariate resulted in a decrease in *BSV* for V_1 (15.0%) and *CL* (10.5%) and an increase for V_2 (7.4%) and CL_{ic} (9.5%). *RUV* slightly decreased from 19.18 to 19.16%.

Introduction of WTV and BTV substantially reduced residual error from 25.9% to 19.2% with an increase in *PPV* (e.g. V_1 33 to 46%). *PPV* of individual parameters was partitioned into *BSV, WTV* and *BTV* in the final model (Table V). Correlations of individual parameters for *BSV, WTV* and *BTV* are shown in Table VI.

Table IV. Model Building – Backwards Elimination

Model	Model description	*N*	ΔOBJ	Conclusion
17	No influence of prior dosing (Css_{pre})	40	−719	Css_{pre} important
18	No influence of endogenous levodopa synthesis (Rsyn)	40	−394	Rsyn important
19	No influence of Css_{pre} and Rsyn	38	−125	Both Css_{pre} and Rsyn important
20	No influence of weight (WT) on all PK parameters	42	−28.2	WT prediction better
21	No WTV	32	−391	WTV important
22	No BTV	32	−69.2	BTV important
23	No WTV and BTV	22	−271	Both WTV and BTV important
24	Additive population error	41	−890	Combined error model better
25	Exponential population error	41	−359	Combined error model better
26	No covariance of BSV parameters	36	−18.3	Covariance of BSV important
27	No covariance of WTV parameters	36	−48.7	Covariance of WTV important
28	No covariance of BTV parameters	36	−57.1	Covariance of BTV important
29	No covariance of BSV and WTV parameters	30	−65.8	Covariance of BSV and WTV important
30	No covariance of BSV and BTV parameters	30	−81.5	Covariance of BSV and BTV important
31	No covariance of WTV and BTV parameters	30	−175	Covariance of WTV and BTV important
32	No covariance of BSV, WTV and BTV parameters	24	−212	Covariance of BSV, WTV and BTV important
15	WTV and BTV; WT on all PK parameters; covariance of BSV, WTV and BTV; Combined RUV	42	0	Final model
33	Influence of sex on all PK parameters	46	2.72	No influence of sex
34	Influence of age on all PK parameters	46	0.24	No influence of age
35	Influence of age of onset (AON) on all PK parameters	46	−0.29	No influence of AON
36	Influence of disease duration (DD) on all PK parameters	46	2.13	No influence of DD

N, number of structural and error model parameters; ΔOBJ, difference in objective function value compared to Model 15.

Table V. Population Parameter Estimates for the Final Model (Model 15)[a]

	Parameter Estimate	BSV[b]	WTV	BTV
V_1 (l/70 kg)	11.4	0.12	0.16	0.40
CL (l/h/70 kg)	30.9	0.13	0.13	0.17
V_2 (l/70 kg)	27.3	0.15	0.08	0.21
CL_{ic} (l/h/70 kg)	34.6	0.28	0.18	0.34
Rsyn (μmol/h/70 kg)	3.1	0.74		
Css_{pre} (μmol/l)	0.075	1.24		
Residual error[c] (%)	19.16	–		

[a] Because of the complexity of the model, the NONMEM covariance step was not successful. Therefore, precision of parameter estimates was not available. The run time of the model (40 h) made it impractical to use a non-parametric bootstrap to obtain parameter confidence intervals.
[b] BSV, WTV and BTV are standard deviations obtained from the square root of variance estimates.
[c] $ERR_{CV} = 0.109$; $ERR_{SD} = 0.158$.

Table VI. Pharmacokinetic Parameter Variability Correlations for BSV, WTV and BTV

		V_1	CL	V_2
BSV	CL	0.245	–	–
	V_2	0.868	0.695	–
	CL_{ic}	0.786	0.791	0.989
WTV	CL	0.754	–	–
	V_2	0.845	0.987	–
	CL_{ic}	0.975	0.612	0.727
BTV	CL	0.603	–	–
	V_2	0.22	0.77	–
	CL_{ic}	−0.44	0.35	0.775

Figure 3 shows an example of an individual fit generated by the final model for the 5 trials. This plot illustrates the within subject variability in the plasma levodopa concentration profile on different occasions.

Population Parameter Variability

Components of the population parameter variability of the pharmacokinetic parameters computed from different models are shown in Table VII. The *BSV* component of *PPV* on V_1 (17.6%) was substantially smaller than in *CL* (36%). Nevertheless, *BTV* accounts for the majority of the total *PPV* in all parameters (range 43–70%). In V_1 and *CL,* about 11% (60%

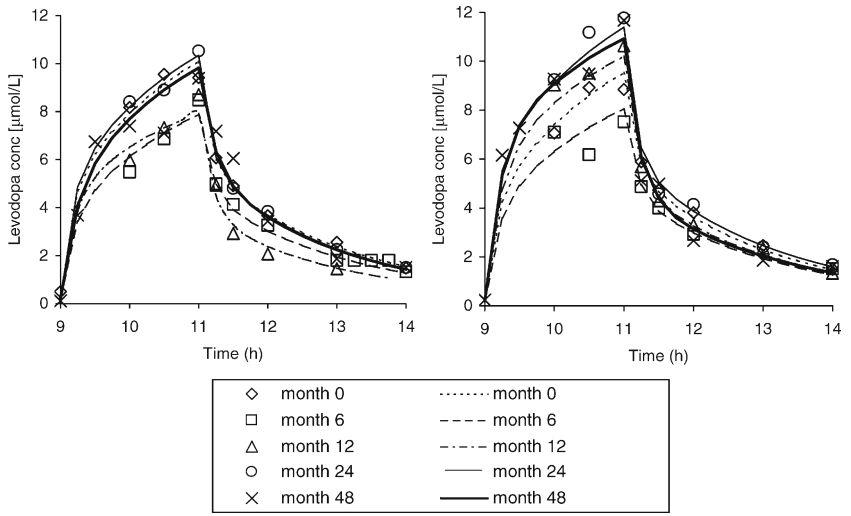

Fig. 3. Time course of plasma levodopa concentration showing within subject variability over 4 years. First infusion is on the left and second infusion (after 3 days levodopa withdrawal) is on the right. Infusion starts at 9 and stops at 11 hr.

of BSV) and 15% (41% of BSV) of the total *PPV* was predicted by weight. A negative *BSVP* in V_2 and CL_{ic} indicated difficulties in obtaining consistent estimates of parameter variability with the different models. Figure 4 shows the variability of the maximum *a posteriori* Bayesian estimates of the pharmacokinetic parameter for the 20 patients on all occasions. For V_1,

Table VII. Population Parameter Variability (PPV) of Levodopa Pharmacokinetics; Values are Obtained from the Square Root of the Variance Estimates[a], Percentages of Total PPV are Shown in Parentheses; Total PPV was Calculated from the Square Root of the Sum of the Variances of the Component Values

	V_1	CL	V_2	CL_{ic}
Total *PPV*	0.46	0.27	0.27	0.45
BSVP (%)	0.15 (10.6)	0.11 (14.9)	_[b]	_[b]
BSVR (%)	0.12 (7.0)	0.13 (21.1)	0.15 (30.3)	0.28 (39.2)
WTV (%)	0.16 (12.0)	0.13 (21.1)	0.08 (9.7)	0.18 (15.3)
BTV (%)	0.39 (70.4)	0.18 (42.8)	0.22 (67.4)	0.34 (55.0)

[a] Values were obtained from different models. See text for detailed description on how individual population parameter variability components were computed.
[b] Numerical inconsistencies in the variance parameter estimates led to negative predicted variances for BSVP.

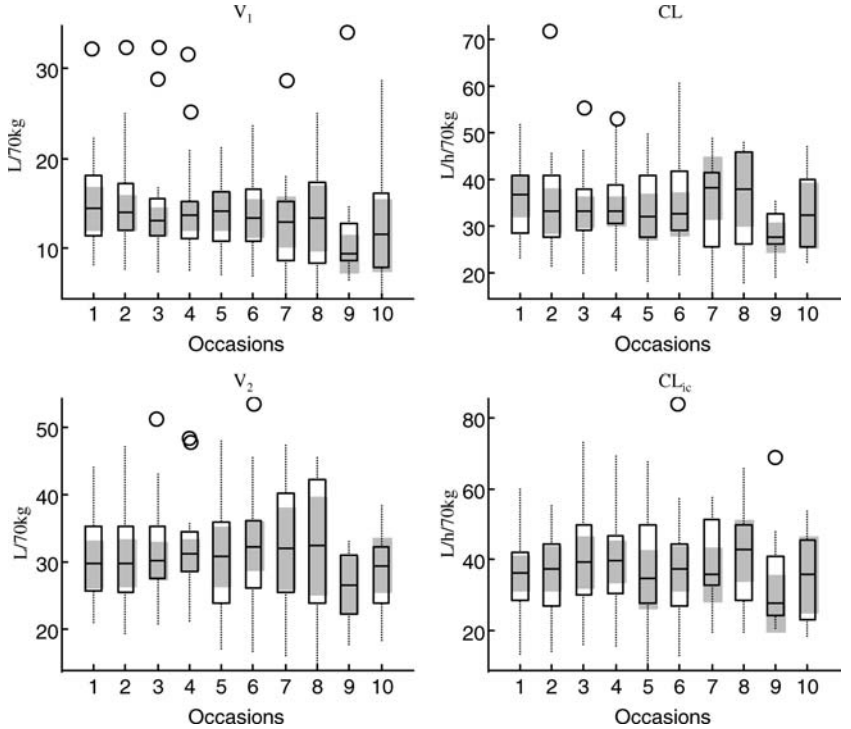

Fig. 4. Pharmacokinetic parameter distributions on different occasions in 20 subjects. All parameters based on WT standard of 70 kg. Boxes indicate the interquartile range with the median of the *post hoc* individual estimates shown by a horizontal line. Dotted lines represent the whisker lines (1.5 times of the interquartile range below the 1st quartile or above the 3rd quartile) and circles represent outliers. Shaded boxes show the 95% confidence intervals of the *post hoc* individual estimates.

there is a tendency for a decrease in the median, no statistical significance was reached ($p = 0.3869$) determined by the Kruskal–Wallis rank sum test on the maximum *a posteriori* Bayesian estimates with occasion as a factor (S-PLUS 6.2 for Windows, Professional edition).

Predictive Performance

A good fit was shown between the observed and the individual predicted plasma levodopa concentrations (Fig. 5 top panel) and no systematic relationship is seen in the weighted residual plot for the final model (Fig. 5 bottom panel).

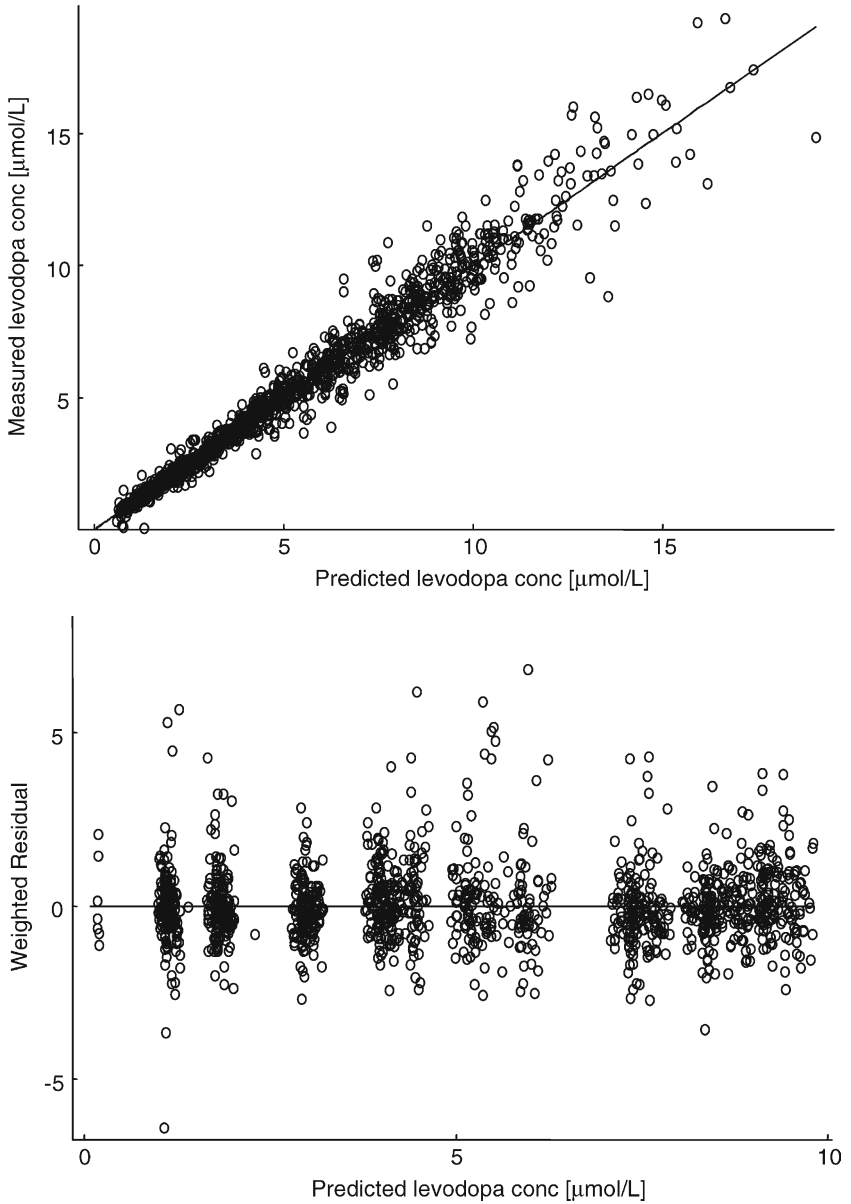

Fig. 5. Goodness of fit plots for the final population model. (Top panel) Observed versus predicted plasma levodopa concentrations. The solid line represents the line of identity. (Bottom panel) Weighted residual versus predicted plasma levodopa concentrations.

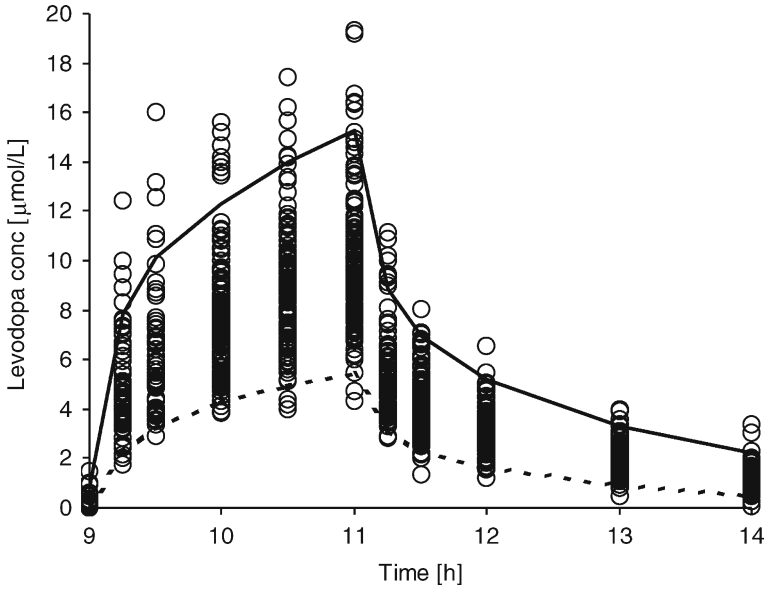

Fig. 6. Validation of the final model by assessing the similarity of the 95% prediction intervals (2.5% percentile dotted line and 97.5% percentile solid line) of the simulated plasma levodopa concentration (obtained from simulation using the final model and its parameter estimates) and the original observed concentration (open circles).

A comparison of the predicted concentrations generated from simulation using the final model and its parameter estimates against the observed concentrations is presented in Fig. 6. The overall 95% prediction interval was constructed using all predicted concentrations at particular time point irrespective of trial or occasion. Table VIII shows the percentage of observations outside the 95% prediction intervals. In general, a biased pattern was shown with a tendency for underestimation of levodopa concentration from the start of infusion until 0.25 hr after its end. Observed concentrations were described well from half an hour after the end of the infusion.

DISCUSSION

Structural Model

We have developed a model for the time course of plasma levodopa concentrations in patients with Parkinson's disease receiving multiple IV levodopa infusions over 4 years. The model takes into the account the endogenous levodopa synthesis as well as levodopa dosing prior to

Table VIII. Percentage of Observations Outside the 95% Predictive Interval (PI)

Clock time	% below 2.5% PI	% above 97.5% PI
9:00 (Start of infusion)	0	**5.13**
9:15	**4.69**	**7.81**
9:30	1.52	**7.58**
10:00	1.88	**4.95**
10:30	1.88	**4.41**
11:00 (End of infusion)	1.19	**3.57**
11:15	1.85	**8.20**
11:30	2.48	1.87
12:00	1.22	1.22
13:00	1.22	2.50
14:00	1.83	1.83

the observation periods. This allows more flexibility in describing the variability of baseline observations between individuals and with different trials. Our model indicates that 60% of the baseline concentration (after overnight withdrawal of oral levodopa) is attributable to endogenous levodopa synthesis. At the start of the study (occasion 1 of trial 1) and after 3 days levodopa withdrawal in all trials, the baseline observations were explained solely by the endogenous levodopa synthesis component. On the other hand, the baseline observations at the first occasion of the subsequent trials were explained by both endogenous levodopa synthesis and prior levodopa dosing.

Unlike other population analysis studies of oral levodopa pharmacokinetics (28,29), a two-compartment pharmacokinetic model described the current intravenous data set much better than a one-compartment model.

With an expectation of 95% of observations within the 95% prediction intervals, the predictive check shows that the final model tends to underpredict concentrations during the infusion period. The underprediction of levodopa concentration at the time of infusion start indicates difficulty estimating endogenous levodopa synthesis and/or concentrations arising from prior exogenous levodopa dosing. The underprediction of levodopa concentration during the infusion period may reflect pharmacokinetic model misspecification, biased estimates of the pharmacokinetic parameters V_1 and CL_{ic} and/or model misspecification for the distribution of random errors. It should be recognized that the use of a 95% prediction interval is a high standard for prediction and the error in the prediction is expected to be of little consequence for pharmacodynamic simulations. The model described the observations well from half an hour after the end of infusion.

Covariate Model

In the final model, a large proportion of BSV was explained by weight (60% in V_1 and 41% in CL). This suggests that weight is a strong predictor of these pharmacokinetic parameters. The effect of weight as a predictor of between subject variability is a fundamental biological expectation. Because models with and without weight are not nested the usual criterion for assessing the change in objective function is not appropriate for evaluating the improvement in model fit. We prefer to use more meaningful criteria based on the magnitude of variability explained by weight. In the population levodopa pharmacokinetic study done by Jorga *et al.* (28), weight was only found to be a determinant of volume of distribution in patients with motor fluctuation, whereas total body clearance was not significantly associated with weight in patients with and without motor fluctuation. Jorga *et al.* (28) also found sex was a factor in determining volume of distribution in patients without motor fluctuations and total body clearance in patients with motor fluctuations, but these were not supported by the current study. The failure to find weight as a covariate on CL is not related to a narrower overall range of weights in Jorga's study (36–153 kg) compared with our study (60–100 kg) (28) but may be due to the inappropriate use of an objective function criterion for rejecting the null hypothesis.

Because the pharmacokinetics of levodopa have been shown to be highly variable between individuals (10,11), it was expected that demographic factors might have some degree of influence on pharmacokinetic parameters. During the model building process, disease duration showed the possibility of predicting pharmacokinetic parameters, but the influence disappeared by including weight as a covariate. No other study has reported that disease duration is a factor determining levodopa pharmacokinetics. Contin *et al.* (12) found no evidence for a change in levodopa pharmacokinetics in a cohort of patients with established disease studied for 4 years. This was supported by the current study though our patients were less severe than Contin et al and had not been treated with levodopa prior to the study.

Comparison with Previous Studies

In general, the estimates of the pharmacokinetic parameters CL and steady state volume ($V\text{ss} = V_1 + V_2$) were found to be consistent with other four studies using standard two-stage approaches (11,14,30,31). The ranges for CL are 9.1–31.1 l/h for levodopa naive and 7.7–38.5 l/h for levodopa treated patients. Similarly, the ranges for $V\text{ss}$ are 18.2–58.8 l and

15.4–83.1 l for levodopa naive and treated patients, respectively. The results of the current study can only be cautiously compared with other population studies (28,29) as levodopa was administered by the oral route in these studies and a different disposition (one-compartment) model was used. With a first-order one-compartment model, Triggs *et al.* (29) found that the population mean of V/F was 35.4 l with BSV of 30.4% in patients receiving oral levodopa with benserazide. The population mean of V/F was similar to the estimates generated by our final model (Vss = 38.7 l) which is compatible with an oral bioavailability of over 80% for levodopa with a decarboxylase inhibitor (32,33). With a zero-order one-compartment model, Jorga *et al.* (28) found that the population mean of V was 99.2 l for fluctuators and 124 l for nonfluctuators in patients receiving oral levodopa with carbidopa or benserazide. BSV were reported to be 42% for fluctuators and 80% for nonfluctuators (BOV, equivalent to WSV in the current study was not estimated). The mean values of V/F were higher, as expected, than our findings (Vss 38.5–43.3 l). CL/F ranged from 18.1 l/h to 28.5 l/h for fluctuators and 17.0–24.9 l/h for nonfluctuators. BSV and BOV (equivalent to WSV in the current study) were reported to be 26% and 29% (CV%) for fluctuators and 33% and 16% for nonfluctuators. We have calculated the corresponding PPV values to be 0.39 and 0.37. The mean values of CL/F were smaller than our findings for CL (30.7–33.5 l/h) while the PPVs were higher than in our analysis (0.25) which is expected given additional variability due to oral bioavailability.

Components of PPV

A key objective of this study was to describe the population pharmacokinetic parameter variability of levodopa. To our knowledge, this is the first study which has attempted to dissect the components of population parameter variability. With the design of the current study, we were able to further define the components of within subject variability (WSV) to differentiate the within trial variability (WTV) from the between trial variability (BTV). In comparison with BSV and BTV, WTV only contributes a relatively small proportion of PPV. Our results show that most of the overall variability in parameters (PPV) is due to within subject variation (estimated by BTV). In line with the study by Karlsson and Sheiner (17), the results showed that ignoring WSV (WTV+BTV) inflates both BSV and residual error (RUV). Our results also showed that ignoring WSV may cause imprecision in parameter estimation and an underestimate of total PPV. In the present study, the differences in CL, V_1, CL_{ic}, Rsyn and Css_{pre} between the fixed effect for BSV (without WSV) and final (with WSV) models were found to be 0.65, 0.42, 1.42, 1.16 and 17.4%, respectively.

The major drawback of the current method of determining the components of PPV was the numerical errors arising from subtracting estimates obtained from different models. If the shape of the minimization surface is relatively flat then different models may converge with estimates of parameter variability that are not reflective of a global minimum. Changes in the final parameter estimates may have little impact on the objective function but can give rise to inconsistent values as shown by the negative estimate for the component of V_2 and CL_{ic} variability predicted by weight. We do not consider this a serious criticism of the method because estimation of these two parameters is typically harder than V_1 and CL and it may therefore be more difficult to attribute the influence of weight as an explanatory covariate.

In comparison with other population pharmacokinetic studies, the estimated WSV of CL (0.22) agrees with other studies while V_1 (0.42) was much higher. The ranges of published WSV (CV%) were 10%–34% and 11%–35% for CL and Vss, respectively (17,34–36). No information about WSV for CL_{ic} could be found in the literature.

To our knowledge, this is the first population study to attempt to quantify within subject variability from as many as 10 occasions from an individual. We found no evidence for systematic changes in levodopa pharmacokinetics in previously untreated parkinsonian patients over the first 4 years of levodopa treatment. However, due to large within subject variability in pharmacokinetic parameters it is impractical to predict the future concentrations in an individual using prior measurements in that individual. There is therefore no merit in attempting target concentration intervention (16) for patients taking levodopa.

Levodopa Pharmacokinetics and Motor Fluctuations

Distribution of levodopa out of the central compartment (e.g. crossing the blood brain barrier) may be influenced by the presence of other amino acids, e.g. 3-O-methyldopa, a long-lived metabolite of levodopa ($t_{1/2} \sim 15$ h) (37,38). The degree of build up of levodopa and 3-O-methyldopa in long-term levodopa treatment and their loss during the drug withdrawal period may contribute to the variation in V_1 and CL. Changes in the activity of levodopa metabolic enzymes and reversal of the O-methylation reaction (37,39) might also explain some of the variability in CL.

The current study indicated that the variability in predicted peak plasma levodopa concentration on different occasions in an individual subject could be largely explained by variation in V_1. Because we found no systematic time related changes in either the pharmacokinetic parameters or their variability (predictable and random) we have no evidence that

changes in levodopa disposition contribute to the development of fluctuations in motor response. However, pharmacodynamic changes associated with chronic treatment, especially the shortening of the time for equilibration between plasma and effect site (12,40) would be expected to expose increased sensitivity to variability in volume of distribution because swings in plasma concentration are less effectively buffered at the effect site. Further attempts to produce slower and less variable levodopa input after oral doses would be expected to reduce motor fluctuations due to the short-duration response because concentration variability would be influenced more by CL than V_1 and within subject variability in CL is substantially lower than V_1.

GLOSSARY

AON	Age of onset
BOV	Between occasion variability
BSV	Between subject variability
BTV	Between trial variability
$BSVP$	Predictable component of BSV
$BSVR$	Random component of BSV
Cex	Exogenous input of levodopa
CL	Total body clearance
CL_{GRP}	Group parameter value for CL
CL_{ic}	Intercompartmental clearance
CP	Sum of predicted levodopa concentrations for endogenous and exogenous inputs
Css_{pre}	Parameter accounting for levodopa concentrations arising from exogenous levodopa dosing prior to each trial
DD	Duration of disease
ERR_{CV}	Residual variability of levodopa concentration for the proportional error model
ERR_{SD}	Residual variability of levodopa concentration for the additive error model
K_{AGE_V1}	Parameter describes the change in V_1 in relation to a standard age of 60
K_{DD_V1}	Parameter describes the change in V_1 in relation to DD
K_{SEX_V1}	Parameter describes the change in V_1 in males in relation to females
OBJ	Objective function value
PPV	Population parameter variability
RPV	Random parameter variability

$R1$	Rate of steady state infusion
Rsyn	Parameter account for endogenous levodopa synthesis
RUV	Random unidentified variability
V_1	Volume of central compartment
V_{1GRP}	Group parameter value for V_1
V_2	Volume of peripheral compartment
WSV	Within subject variability
WT	Body weight
WTV	Within trial variability

All variability components are quantified as the standard deviation of a normal distribution with mean 0.

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