J. Pharm. Pharmacol. 1987, 39: 421–425
Received October 13, 1986

Effect of age on gastrointestinal and hepatic first-pass effects of levodopa in rats

KIKUO IWAMOTO*, JUN WATANABE†, MIKA YAMADA†, FUMIE ATSUMI† AND TOMOKO MATSUSHITA†

Department of Hospital Pharmacy, Shimane Medical University Hospital, 89-1, Enya-cho, Izumo 693, Japan and †Department of Biopharmaceutics, Faculty of Pharmaceutical Sciences, Nagoya City University, 3-1, Tanabe-dori, Mizuho-ku, Nagoya 467, Japan

Presystemic elimination of levodopa (20 mg kg^{-1}) has been studied in 5- to 104-week-old male Wistar rats. The gastrointestinal and hepatic contribution to the overall first-pass effect was estimated separately after the drug had been administered intravenously, orally and intraportally. The contribution of the gut to the overall first-pass effect of this drug was greater than that of the liver in any age-group of the rats. Both the overall and intestinal first-pass effects of levodopa were greatest in 11-week-old rats and relatively small in both young (between weeks 5 and 7) and aged (between weeks 52 and 104) rats. In contrast, the hepatic first-pass effect did not show any significant age-dependent change. Age-related change in the jejunal blood flow could not explain the unique age-dependence in the intestinal first-pass metabolism of levodopa. However, the present age-dependence in the oral systemic availability of this drug between adult (11 weeks) and aged (52 to 104 weeks) rats was found to be similar to the tendency that has been reported between normal adult subjects and aged patients with Parkinson's disease.

Different dose requirements among elderly parkinsonian patients from those in the other age-groups (Broe & Caird 1973; Vignalou & Beck 1973; Grad et al 1974) might be indicative of either variability in the systemic availability or changes in the elimination kinetics of levodopa. A 'tailor-made' dosage regimen for each patient has therefore been characteristic of levodopa therapy (Bianchine & Sunyapridakul 1974). Evans et al (1980) reported that the area under the plasma levodopa concentration-time curve in elderly parkinsonian patients ($\bar{x}$ 77 years) was significantly larger than that in young healthy volunteers ($\bar{x}$ 26 years) despite the same elimination half-life in both groups.

Levodopa is subject to the intestinal and hepatic first-pass effect. To identify where the first-pass metabolism occurs, a drug is either directly administered into the hepatoportal vein, whereby the extraction and/or metabolism component in the gut is by-passed (Harris & Riegelman 1969; Iwamoto & Klaassen 1977a,b; Back et al 1978; Iwamoto et al 1982a, 1983), or a portal systemic shunt that by-passes the liver is created (Gugler et al 1975). The present work was designed to investigate the effects of age on the oral-systemic availability, and the relative contribution of intestinal and hepatic first-pass metabolism to the overall first-pass effect of levodopa by comparing the plasma drug levels after intravenous, oral and intraportal administration to rats aged between 5 and 104 weeks.

* Correspondence

MATERIALS AND METHODS

Materials

Levodopa (L-3,4-dihydroxyphenylalanine) was supplied by Sankyo Pharmaceutical Co. (Tokyo, Japan); L-3,4-dihydroxyphenyl [3-^{14}C]alanine ([^{14}C]levodopa, specific activity 10·9 mCi $mmol^{-1}$, radiochemical purity >97%) was from Amersham (Bucks, UK). Bio Rad ion-exchange resin (AQ 50W × 4, 200–400 mesh), from Wako Pure Chemicals (Nagoya, Japan), was used to separate plasma-unchanged levodopa from its metabolite. An RF-510 spectrofluorometer (Shimadzu Seisakusho, Kyoto, Japan) was used for analysing the unchanged levodopa. Fine SIL C_{18}T(18 μm), used in the HPLC analysis, was from Japan Spectroscopic Co. (Hachioji, Japan). The HPLC system (Japan Spectroscopic Co.) was equipped with a Trirotor-II solvent delivery system, a VL-variable loop injector and a Uvidec-100-III variable wavelength UV detector operated at 220 nm which was used for the analysis of levodopa. A hydrogen monitor (PHG-300, M.T.-Giken Co., Nagoya, Japan) was used for measuring jejunal and hepatic blood flow; it included a needle electrode (Pt-Pt black H_2-electrode) with an Ag-AgCl electrode as reference. All other chemicals were of analytical grade.

Animals

Male Wistar rats, 5(110–135 g), 7(210–230 g), 9(300–325 g), 11(355–390 g), 15(385–420 g), 26(495–520 g), 52(610–730 g) and 104(785–880 g) weeks old were used. Each was cannulated in the jugular vein one day before the experiment, fasted overnight, and used once as previously reported (Iwamoto et al 1982a,b, 1983, 1985a,b). For the measurement of the blood flow, each rat was lightly anaesthetized with urethane (800 mg kg^{-1} i.p.).

Measurement of urinary and faecal excretion of radioactivity after intravenous and oral administration of [^{14}C]levodopa

After either a bolus intravenous or oral administration of [^{14}C]levodopa [20 mg(10 μCi) kg^{-1}] to unanaesthetized rats (n = 4) in each age-group, periodical urine samples and cumulative faecal samples were collected from these rats up to 24 h in the metabolic cage. An aliquot (100 μL) of appropriately diluted urine was mixed with 10 mL of the scintillator (PPO 5 g, POPOP 300 mg, toluene 700 mL, Triton X-100 300 mL) and then its total radioactivity was counted in Mark II liquid scintillation spectrometer (Nuclear-Chicago Corp., Des Plaines, USA). Total radioactivity in the faeces was analysed as reported previously (Iwamoto et al 1982b). The counting efficiencies were determined by a ^{133}Ba external standardization method.

Measurement of plasma levodopa after intravenous, oral and intraportal administration to rats

Unanaesthetized rats (n = 4) in each age-group were given 20 mg kg^{-1} of levodopa either intravenously via the cannula or orally by gastric intubation, whereas other age-matched rats (n = 4) that were anaesthetized lightly with ether were given the same dose as above intraportally by a constant rate infusion over 5 min via the ileocolic vein in the same manner as described previously (Iwamoto et al 1982a, 1983). The abdominal midline incision was tied off before the rat recovered from anaesthesia (about 5 to 10 min after the infusion).

Blood samples (9 to 10 samples, about 0·12 mL for 5-week-old rats and 0·23 mL for all other age-groups) were withdrawn periodically from the jugular vein cannula over 180 min, placed in chilled micro-centrifuge tubes containing 1 unit of heparin, and then centrifuged at 3000 rev min^{-1} for 10 min to obtain the plasma samples. The plasma (50 or 100 μL) so obtained was used for the determination of unchanged drug either by a spectrofluorometric method after hydroxyindole derivatization (Laverty & Taylor 1968) of levodopa separated from its metabolites by means of ion-exchange chromatography using Bio Rad AQ 50W × 4 resin (Atack 1973), or by an HPLC analysis method which was in principle the same as reported recently (Nakagawa et al 1983) using *O*-methyldopa as an internal standard. These independent analytical methods were found to correlate with each other (r = 0·988).

Measurement of jejunal and hepatic blood flow

An abdominal midline incision (about 3 cm) was made in lightly anaesthetized rats which were given the same i.v. dose of levodopa as described above. After a needle H_2-electrode was placed into the upper jejunum and liver tissue, the blood flow in these organs was measured in the same manner as reported previously (Iwamoto et al 1985a,b). These measurements were done at 20 to 30 min after the i.v. administration of levodopa. Flow data representing one organ (or tissue) were obtained from the average value of five determinations (coefficients of variation 7·3 to 9·5% and 3·6 to 7·8% for the jejunum and liver, respectively).

Calculation of pharmacokinetic parameters

Plasma concentration(C)-time data after the intravenous administration were analysed according to least-squares regression analysis program MULTI (Yamaoka et al 1981) for the bi-exponential decline ($C = Ae^{-\alpha t} + Be^{-\beta t}$). Criteria for both convergence and best fit were the same as described in the original report (Yamaoka et al 1981). The best fit of the data was achieved by weighting with the reciprocal of plasma concentration (C). Significant difference was quantified by Student's *t*-test.

RESULTS

Cumulative urinary and faecal excretion of levodopa and its metabolites after intravenous and oral administration

Table 1 summarizes the cumulative percentage of dose excreted as total (unchanged levodopa and its metabolites) radioactivity in the urine and faeces over 24 h after intravenous and oral administration of [^{14}C]levodopa (20 mg kg^{-1}) to 5- to 104-week-old rats and the resultant extent of bioavailability. The extent of urinary excretion of the radioactivity was more than 90% of the i.v. dose in every rat, whereas those after oral dosing decreased with age between 15 and 104 weeks. Therefore, the extent of oral absorption of levodopa was almost complete in 5- to 15-week-old rats, whereas that in relatively aged rats (26 to 104 weeks) was reduced significantly. These

Table 1. Cumulative urinary and faecal excretion of total radioactivity in 24 h following intravenous or oral administration of [^{14}C]levodopa [20 mg (10 μCi) kg^{-1}] to rats 5 to 104 weeks old.

Route of administration		Cumulative % of dose, mean ± s.d. for rats[a] of age (weeks)							
		5	7	9	11	15	26	52	104
i.v.	Urine	96·7 ± 2·8	94·5 ± 5·1	93·4 ± 1·9	95·1 ± 4·4	93·5 ± 3·0	92·9 ± 4·9	90·5 ± 2·2	90·1 ± 6·3
	Faeces	1·5 ± 0·3	1·4 ± 0·4	1·5 ± 0·2	1·2 ± 0·3	1·0 ± 0·4	1·3 ± 0·2	1·4 ± 0·5	1·5 ± 0·4
Oral	Urine	92·9 ± 6·4	93·0 ± 4·7	91·7 ± 2·6	93·9 ± 3·1	90·7 ± 4·6	65·3 ± 5·2	59·0 ± 4·8	54·8 ± 8·9
	Faeces	2·7 ± 0·8	3·2 ± 0·9	3·4 ± 0·8	3·5 ± 1·0	3·4 ± 1·0	24·6 ± 7·2	31·7 ± 6·5	35·4 ± 7·0
$\frac{\text{Urine}_{\text{oral}}{}^{b}}{\text{Urine}_{\text{i.v.}}}$		0·961	0·984	0·982	0·987	0·972	0·703	0·652	0·608

[a] n = 4. [b] Ratio of the mean value.

mean data for the extent of oral absorption were taken into account in estimating the overall first-pass effect and relative contribution of the gut in producing the first-pass effect of this drug.

Plasma levodopa levels after intravenous, oral and intraportal administration

Fig. 1 shows typical plasma concentration-time curves for levodopa after intravenous, oral and intraportal administration (20 mg kg^{-1}) to 7-, 11- and 52-week-old rats. Similar results were obtained in other age-groups. In each age-group, the drug level after the bolus intravenous dosing was highest or followed closely that after intraportal infusion, whereas the plasma level after oral administration was always the lowest of the three. The plasma level after intravenous dosing declined bi-exponentially with time. Elimination half-life ($t_{\frac{1}{2}}$), area under the plasma concentration-time curve (AUC), systemic availability (F) and relative contribution of the gut (f_g) and the liver (f_h) in producing the first-pass effect are summarized in Table 2. In each age-group, there was no significant difference in the elimination $t_{\frac{1}{2}}$ after these routes of administration. However, the half-life tended to increase with age between weeks 7 and 104 (approximately 20 to 40 min). Oral availability was lowest in 11-week-old rats (about 0·2), whereas for relatively young (5 to 7 weeks) and aged (52 to 104 weeks) rats it tended to be higher. The relative contribution of the gut (f_g) to the overall first-pass effect was larger than that of the liver (f_h) in all age-groups and showed age-dependence, the largest value being in 11-week-old rats and with smaller values in both young (5 to 7 weeks) and aged (52 to 104 weeks) rats. In contrast, f_h did not show any distinct age-dependence.

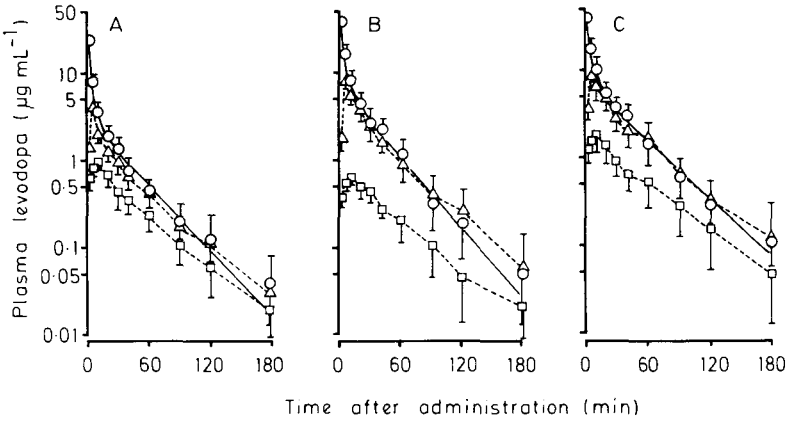

FIG. 1. Plasma levodopa concentration-time curves after intravenous (○), intraportal (△) or oral (□) administration of 20 mg kg^{-1} to four rats which were 7(A), 11(B) and 52(C) weeks old. Each point represents the mean data point with s.d. The solid line for intravenous administration represents best computer-fitted bi-exponential curve weighted with the reciprocal of the individual plasma concentration.

Table 2. Pharmacokinetic parameters for levodopa following intravenous, intraportal or oral administration (20 mg kg^{-1}) to rats 5 to 104 weeks old.

	Mean ± s.d. for rats[a] of age (weeks)							
Parameters	5	7	9	11	15	26	52	104
$t_{\frac{1}{2}}$ (min^{-1})								
i.v.	23·1 ± 3·8	21·4 ± 3·2	24·8 ± 3·1	26·5 ± 2·7	29·1 ± 3·5	32·7 ± 3·3	33·1 ± 3·6	40·1 ± 4·2
intraportal	24·1 ± 4·4	23·1 ± 3·8	23·9 ± 3·9	28·7 ± 3·6	29·8 ± 3·9	32·8 ± 4·7	34·9 ± 3·8	40·3 ± 4·9
oral	24·7 ± 4·0	23·0 ± 6·1	24·9 ± 4·7	28·4 ± 5·3	31·1 ± 5·7	34·1 ± 5·9	35·6 ± 4·9	42.1 ± 6.1
AUC (μg min mL^{-1})								
i.v.[c]	228 ± 44	226 ± 36	233 ± 40	269 ± 37	301 ± 43	340 ± 41	364 ± 48	379 ± 59
intraportal[d]	172 ± 22	169 ± 23	181 ± 26	214 ± 30	231 ± 29	270 ± 36	301 ± 39	318 ± 45
oral[d]	118 ± 28	104 ± 25	66 ± 13	55 ± 18	75 ± 21	96 ± 25	135 ± 27	116 ± 22
F								
intraportal	0·754	0·748	0·777	0·848	0·767	0·794	0·826	0·839
oral	0·518	0·461	0·285	0·204	0·250	0·282	0·371	0·305
Overall first-pass effect[e]	0·461	0·532	0·710	0·793	0·743	0·599	0·431	0·499
f_g[f]	0·286	0·375	0·626	0·756	0·665	0·495	0·312	0·400
f_h[g]	0·246	0·252	0·223	0·204	0·233	0·206	0·174	0·161

[a] n = 4.
[b] Elimination half-life.
[c] Estimated by the equation, $A/\alpha + B/\beta$.
[d] Calculated by the trapezoidal rule and extrapolation to time infinity.
[e] Estimated by the equation, $1\text{-}AUC_{oral}/AUC_{i.v.}$, after correcting AUC_{oral} according to Table 1.
[f] Estimated by the equation, $f_g = 1 -$ corrected $AUC_{oral}/AUC_{intraportal}$.
[g] Estimated by the equation, $f_h = 1 - AUC_{intraportal}/AUC_{i.v.}$.

Jejunal and hepatic blood flow

Table 3 summarizes the jejunal and hepatic blood flow per tissue weight measured by the hydrogen-gas clearance method after each rat was given the same i.v. dose (20 mg kg^{-1}) of levodopa as above. Jejunal blood flow was found to be relatively high in the younger rats between 5 and 15 weeks with a peak at 7 weeks. Liver blood flow showed a similar but less distinct age-dependence.

DISCUSSION

It has been reported that the oral absorption of [^{14}C]levodopa in 11-week-old dogs (dose 50 mg kg^{-1}) is practically complete whereas the plasma drug level after the oral dosage of 1·0 g in both adult healthy volunteers and patients without parkinsonism is variable (Abrams et al 1971). However, there have been no reports demonstrating age-related alterations in the extent of gastrointestinal absorption, systemic availability and gastrointestinal or hepatic first-pass metabolism of levodopa. The present urinary excretion data of total radioactivity following the oral administration of [^{14}C]levodopa confirmed that the gastrointestinal absorption of this drug was almost complete in rats younger than 15 weeks, whereas that in rats aged between 26 and 104 weeks was incomplete (approximately 0·6 to 0·7). These mean data for absorption (Table 1) were then used to estimate the dose which was actually available after the oral dosing in each age-group.

The AUC after the intravenous administration tended to increase with age between weeks 7 and 104 (Table 2), suggesting the age-dependent reduction of total body clearance of levodopa after maturity.

Table 3. Jejunal (Q_j) and hepatic (Q_h) blood flow in rats 5 to 104 weeks old.

	Mean ± s.d. for rats[a] of age (weeks)							
	5	7	9	11	15	26	52	104
Q_j (mL min^{-1} g^{-1})[b]	2·38 ± 0·62	2·51 ± 0·54	1·99 ± 0·43	1·84 ± 0·36	1·80 ± 0·33	1·35 ± 0·41	1·41 ± 0·29	1·22 ± 0·26
Q_h (mL min^{-1} g^{-1})	1·61 ± 0·22	1·70 ± 0·20	1·61 ± 0·19	1·52 ± 0·18	1·43 ± 0·21	1·21 ± 0·20	1·13 ± 0·16	1·06 ± 0·12

[a] n = 4.
[b] Measured by hydrogen-gas clearance method and expressed as blood flow rate g^{-1} tissue.

This type of age-dependence, probably accompanied by the gradual reduction of the elimination rate with age, is thought to be very common for the total body clearance of many drugs. Oral systemic availability showed rather complicated age-dependence, yielding the lowest value in 11 weeks and moderate to higher values in both relatively young (5 to 9 weeks) and aged (26 to 104 weeks) rats. The present results between adult (11 weeks) and aged (up to 104 weeks) rats were fairly consistent with the age-dependent change in the oral systemic availability of this drug between young healthy volunteers and elderly parkinsonian patients (Evans et al 1980).

Contribution of the gut (f_g) in producing the overall first-pass effect of levodopa was greater than that of the liver (f_h) in all age-groups. In addition, the age-dependence in f_g was very similar to that in the overall first-pass effect. These results suggest that the intestinal first-pass metabolism may be a determinant in the oral systemic availability of this drug. When administered orally, levodopa is known to be decarboxylated to dopamine to a considerable extent at the peripheral sites of localizing dopa-decarboxylase, such as stomach mucosa (Hakanson & Owman 1966), intestinal tissue and liver (Awapara et al 1962). Shindo et al (1972, 1973) have also suggested a rapid and extensive decarboxylation of levodopa in rat peripheral tissues including the gastrointestinal tract. In addition, Komai et al (1978) have reported that the small intestine contains appreciably higher dopa-decarboxylase activity (about 280 nmol dopamine formed min^{-1} (g tissue)$^{-1}$ when the supernatant of the tissue homogenate from 7- to 8-week-old male Wistar rats was incubated with 1 mM levodopa). Hence the present unique age-dependence in f_g may be largely due to the complex age-dependent changes in the intestinal function to decarboxylate this drug.

Age-dependent change in the jejunal blood flow could not explain the unique age-dependence in f_g, since the peak blood flow was observed at 7 weeks. Age-related factors other than the splanchnic blood flow (such as the intrinsic activity of the intestinal decarboxylase) should be taken into account in clarifying the age-dependent determinant in the intestinal first-pass metabolism of levodopa.

Acknowledgement

This work was supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (61571099).

REFERENCES

Abrams, W. B., Coutinho, C. B, Leon, A. S., Speigel, H. E. (1971) J. Am. Med. Assoc. 218: 1912–1914

Atack, C. V. (1973) Br. J. Pharmacol. 48: 699–714

Awapara, J., Snadman, R. P., Hanley, C. (1962) Arch. Biochem. Biophys. 98: 520–525

Back, D. J., Brekenridge, A. M., Crawford, F. E., L'E Orme, M., Rowe, P. H., Smith, E. (1978) J. Pharmacol. Exp. Ther. 207: 555–565

Bianchine, J. R., Sunyapridakul, L. (1974) Med. Clin. North Am. 58: 1071–1081

Broe, G. A., Caird, F. I. (1973) Med. J. Aust. 1: 630–635

Evans, M. A., Triggs, E. J., Broe, G. A., Saines, N. (1980) Eur. J. Clin. Pharmacol. 17: 215–221

Grad, B., Werner, J., Rosenberg, G., Werner, S. W. (1974) J. Am. Geriatr. Soc. 22: 489–494

Gugler, R., Lain, P., Azaroff, D. L. (1975) J. Pharmacol. Exp. Ther. 195: 416–423

Hakanson, R., Owman, C. (1966) Biochem. Pharmacol. 15: 489–499

Harris, P. A., Riegelman, S. (1969) J. Pharm. Sci. 58: 71–75

Iwamoto, K., Klaassen, C. D. (1977a) J. Pharmacol. Exp. Ther. 200: 236–244

Iwamato, K., Klaassen, C. D. (1977b) Ibid. 203: 365–376

Iwamoto, K., Takei, M., Watanabe, J. (1982a) J. Pharm. Pharmacol. 34: 176–180

Iwamoto, K., Watanabe, J., Imai, K., Shibata, M., Hirate, J., Ozeki, S. (1982b) Chem. Pharm. Bull. 30: 1422–1429

Iwamoto, K., Arakawa, Y., Watanabe, J. (1983) J. Pharm. Pharmacol. 35: 687–689

Iwamoto, K., Watanabe, J., Araki, K., Deguchi, N., Sugiyama, H. (1985a) Ibid. 37: 466–470

Iwamoto, K., Watanabe, J., Araki, K., Satoh, M., Deguchi, N. (1985b) J. Pharmacol. Exp. Ther. 234: 470–475

Komai, T., Kawai, K., Nambu, K., Miyakoshi, N., Shindo, H. (1978) J. Pharm. Dyn. 1: 271–280

Laverty, R., Taylor, K. M. (1968) Anal. Biochem. 22: 269–279

Nakagawa, T., Shibukawa, A., Uno, T. (1983) J. Chromatogr. 254: 27–34

Shindo, H., Miyakoshi, N., Nakajima, E. (1972) Chem. Pharm. Bull. 20: 966–973

Shindo, H., Komai, T., Tnaka, K., Nakajima, E., Miyakoshi, N. (1973) Ibid. 21: 826–836

Vignalou, J., Beck, H. (1973) Gerontol. Clin. 15: 50–64

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T. (1981) J. Pharm. Dyn. 4: 879–885