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Pharmacokinetics of Nanosomal Form of Levodopa in Intranasal Administration

Andrey Anatolievich Nedorubov*, Alexey Nikitich Pavlov, Natalia Valeryevna Pyatigorskaya, Galina Eduardovna Brkich, Marina Maksimovna Shabalina

Institute of Pharmacy and Translational Medicine, Sechenov First Moscow State Medical University, Trubetskaya Street, 8, Moscow, Russian Federation

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***Correspondence:** Andrey Anatolievich Nedorubov. Institute of Pharmacy and Translational Medicine, Sechenov First Moscow State Medical University, Trubetskaya Street, 8, Moscow, Russian Federation. E-mail: a.a.nedorubov@mail.ru

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Abstract

BACKGROUND: Parkinson's disease is one of the most common neurological diseases. Pathogenesis of the disease is associated with destruction and death of neurons that produce the neurotransmitter dopamine. The precursor to dopamine, which crosses the protective blood-brain barrier, is the amino acid 3, 4-dihydroxy-L-phenylalanine – levodopa, L-DOPA. The investigational drug is a pharmaceutical composition, containing L-DOPA as an active substance, which is distributed in a polymer matrix based on a biodegradable copolymer of lactic/glycolic acids.

AIM: This work aimed to study the main pharmacokinetic parameters for the drug "L-DOPA – PC, nasal drops" and comparator drugs "L-DOPA in oil", "L-DOPA – PC in purified water", reference product – tablets "Madopar 125".

METHODS: To increase the bioavailability of the active substance L-DOPA, a new route of administration was used for the first time – nasal administration. Pharmacokinetics of the innovative drug with the intranasal route of administration was investigated in rabbits. The L-DOPA concentration in blood plasma was determined by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS).

RESULTS: Bioavailability of the drug – nasal drops were 244.4% compared with the drug "Madopar 125".

CONCLUSION: Assay procedure for the determination of L-DOPA in animal blood plasma using liquid chromatography with tandem mass-selective detection (HPLC-MS/MS) was developed and validated.

Introduction

Parkinson's disease is a slowly progressing socially significant chronic neurological disease, characteristic of the older age group [1], refers to the degenerative diseases of the extrapyramidal motor system. The cause of the disease is progressive destruction and death of neurons that produce neurotransmitter dopamine [2], primarily in the black substance, as well as in other parts of the central nervous system. Insufficient production of dopamine leads to the activating influence of basal ganglia on the cerebral cortex. Leading symptoms are muscle rigidity, hypokinesia, tremor, postural instability.

The innovative drug L-DOPA-PC, nasal drops, is an original pharmaceutical composition containing L-DOPA (3, 4-dihydroxy-L-phenylalanine, levodopa) as an active ingredient, distributed in the polymer matrix based on a biodegradable copolymer of lactic/glycolic acids (polylactide glycolide, PLGA 50/50) [3].

L-DOPA's mechanism of action is well studied and described in the literature [4], [5], [6], [7], [8]. L-DOPA eliminates hypokinesia, rigidity, tremor, dysphagia, salivation. L-DOPA is an amino acid, an

immediate metabolic predecessor of dopamine, which unlike dopamine is able to cross the blood-brain barrier and compensate dopamine deficiency in the brain that underlies many clinical manifestations of Parkinson's disease. L-Dopa is captured by the endings of remaining dopaminergic nigrostriatal neurons, undergoes decarboxylation in them, turns into dopamine, which is released into the synaptic cleft, thus maintaining an adequate functional state of the neurons of the striatum and other basal ganglia.

Modern scientists' efforts are concentrated on medical drugs application frequency decreasing, preserving their efficiency [9], [10]. Nasal delivery method is one of the most promising. The most important feature of the medical products intranasal delivery is the opportunity to penetrate them directly into the central nervous system without entering the blood circulatory system. The medical products transportation from the nasal cavity to the central nervous system is implemented without the mucous participation. It is done using an extracellular tract through the epithelial barrier in the course trigeminal and olfactory nerves. It was earlier believed that all L-dopa is entirely utilised in the sympathetic nerves ends and does not get out in the extracellular space. Nevertheless, rather, an important part of L-dopa leaves sympathetic nerves and arrives in the arterial and venous system, which supplies extremities, head, heart, adrenal glands and intestines with the blood [11].

To increase the bioavailability of the active substance L-DOPA, we used the nasal route of administration of the drug [4]. This work aimed to study the main pharmacokinetic parameters for the drug "L-DOPA – PC, nasal drops" and comparator drugs "L-DOPA in oil", "L-DOPA – PC in purified water", reference product – tablets "Madopar 125".

Material and Methods

"L-DOPA-PC nasal drops" was compared with:

1. L-DOPA (3.75% of levodopa substance) in oil.

2. L-DOPA – PC (3.75% of levodopa substance) in purified water.

3. "Madopar 125", dispersible tablets, (levodopa content-100 mg). Composition: levodopa 100 mg, benserazide hydrochloride 28.5 mg. *Excipients:* anhydrous citric acid; corn starch pre-gelatinized; microcrystalline cellulose; magnesium stearate. The tablet was dispersed in 10 ml of purified water and administered orally as a suspension through a feeding tube.

The study was conducted on 24 male rabbits weighing 2900-3200 g. The animals were divided into 4 groups of 6 rabbits each. Groups 1-3 administered the drug intra-nasally; the volume of administration was not more than 200 µl. Group 4 received the drug through oesophagal atraumatic bougie; the volume of administration was not more than 0,5 ml. The dose of administration for L-DOPA was 2.5 mg/kg. Blood samples were taken at the following time points: 0, 0.08, 0,16, 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, 48 h. Five ml of blood was taken from the ear vein. The design of the study is given in Table 1.

Table 1: Scheme of intranasal and oral administration of drugs based on L-DOPA

Name of the administered sample	Group of animals			
	Group 1	Group 2	Group 3	Group 4
	Nasal oil drops based on L-DOPA-PC	Levodopa suspension in olive oil	L-DOPA-PC suspension in purified water	Tablets "Madopar 125"
Content of Levodopa (L-DOPA)	5.0 %	3.75 %	3.75 %	100 mg
Route of administration		nasally		orally
Rate of application	once	once	once	once
L-DOPA dose	2.5 mg/kg	2.5 mg/kg	2.5 mg/kg	2.5 mg/kg

There are many L-DOPA research methods in various assay samples (HPLC-UV, HPLC-MS/MS and others). Method HPLC-UV is suitable for the determination of L-DOPA in dosage form [12]. HPLC-MS/MS is used for pharmacokinetic studies owing to increased selectivity for the substance [11]. To improve the quality of the study, the method HPLC-MS/MS was chosen.

Determination of L-DOPA concentration in blood plasma was performed by high-performance liquid chromatography with tandem mass spectrometry (HPLC-MS/MS). Equipment used for the study – tandem quadrupole mass spectrometric detector, Shimadzu, manufactured in Japan; liquid chromatograph LC-30 Nexera, Shimadzu, Japan.

Statistical data processing was carried out using Statistica 12.0 software.

Results

Four preparations of different compositions were obtained for the study; they contained the amino acid (3,4-dihydroxy-L-phenylalanine, levodopa, L-DOPA) as an active ingredient.

All the blood plasma samples obtained were analysed by HPLC-MS/MS. During the development of the assay procedure of the analyte understudy, a mass spectrum of L-DOPA was obtained. The mass spectrum was obtained in the full scan mode of fragment ions in the range of mass-to-charge ratios from 100 m/z to 500 m/z. Based on the data obtained,

characteristic ion transitions for the analyte under study were determined. The mass spectrum of L-DOPA is shown in Figure 1.

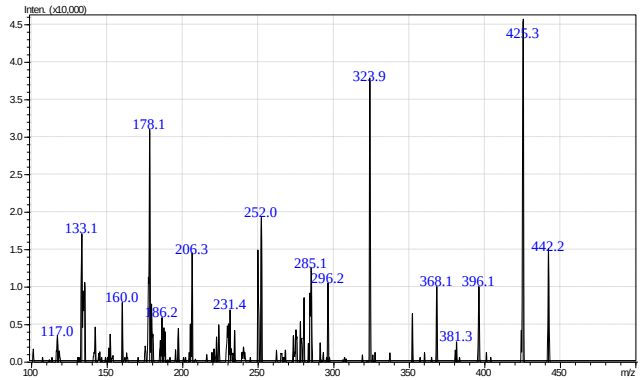
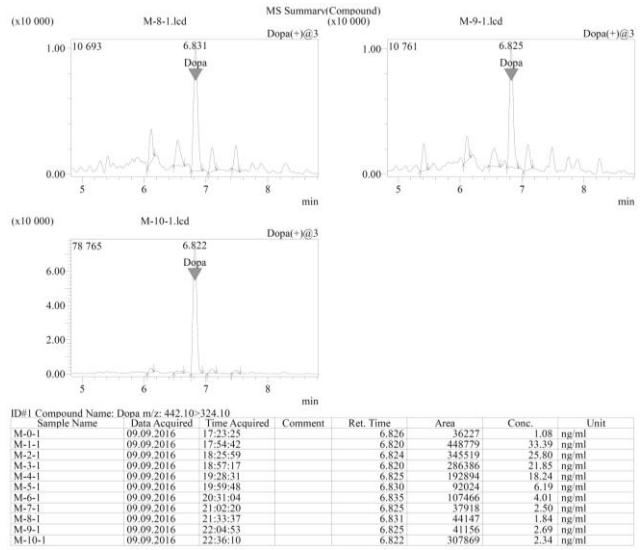


Figure 1: Mass spectrum of L-DOPA

Typical results of L-DOPA concentration determination are shown in Figure 2.



Sample Name	Data Acquired	Time Acquired	Comment	Ret. Time	Area	Conc.	Unit
M-0-1	09.09.2016	17:23:25		6.826	36227	1.08	ng/ml
M-1-1	09.09.2016	17:54:42		6.820	448779	33.39	ng/ml
M-2-1	09.09.2016	18:25:59		6.824	345519	25.80	ng/ml
M-3-1	09.09.2016	18:57:17		6.820	286386	21.85	ng/ml
M-4-1	09.09.2016	19:28:31		6.825	192894	18.24	ng/ml
M-5-1	09.09.2016	19:59:48		6.830	92024	6.19	ng/ml
M-6-1	09.09.2016	20:31:04		6.835	107466	4.01	ng/ml
M-7-1	09.09.2016	21:02:20		6.825	37918	2.50	ng/ml
M-8-1	09.09.2016	21:33:37		6.831	44147	1.84	ng/ml
M-9-1	09.09.2016	22:04:53		6.825	41156	2.69	ng/ml
M-10-1	09.09.2016	22:36:10		6.822	307869	2.34	ng/ml

Figure 2: Mass chromatograms of L-DOPA in the blood plasma of rabbits after a single intranasal administration of levodopa suspension in olive oil (2), sample №1

Figure 3 shows pharmacokinetic profiles of L-Dopa in the blood of rabbits, in linear coordinates, after a single administration of the drugs "L-Dopa-PC nasal drops", "L-DOPA in oil", "L-DOPA-PC in purified water" and "Madopar 125" capsules.

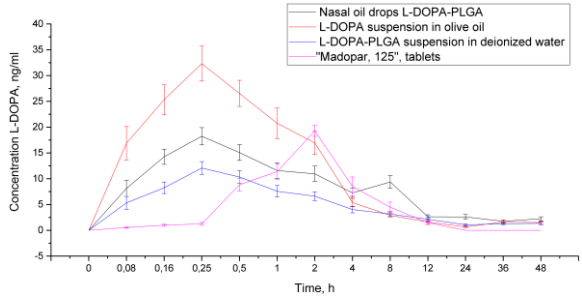


Figure 3: Graph of averaged pharmacokinetic profiles (in linear coordinates) for L-DOPA (observation from 0 to 48 hours)

The main pharmacokinetic parameters are presented in Table 2.

Table 2: Pharmacokinetic parameters calculated from averaged data

Group	Parameter	$T_{½}$	T_{max}, h	C_{max}, ng/ml	$AUC_{0→48}$, ng × h/ml	$AUC_{0→\infty}$, ng × h/ml	MRT, h	$C_{max}/AUC_{0→t}$	$C_{max}/AUC_{0→\infty}$	K_{el} (h^{-1})
(1)	Mean	15.39	0.25	18.2	181.88	231.64	27.3	0.1002	0.0787	0.0452
	Gmean	15.38	0.25	18.1	180.75	230.91	27.3	0.1005	0.0787	0.0451
	Median	15.06	0.25	18.0	175.27	229.30	26.3	0.1026	0.0794	0.0460
	Min	14.53	0.25	15.5	167.81	214.83	25.6	0.0891	0.0721	0.0395
	Max	17.54	0.25	20.3	203.94	254.75	34.5	0.1073	0.0848	0.0477
	SD	1.09	0.00	1.7	13.76	16.52	3.5	0.0068	0.0051	0.0029
(2)	Mean	10.92	0.25	32.31	135.53	159.32	20.19	0.2383	0.2027	0.0636
	Gmean	10.91	0.25	32.16	135.24	159.09	20.12	0.2378	0.2022	0.0635
	Median	11.06	0.25	31.54	134.51	158.05	20.89	0.2426	0.2088	0.0627
	Min	10.12	0.25	28.21	124.73	149.54	17.23	0.2167	0.1788	0.0614
	Max	11.29	0.25	37.02	152.75	171.45	21.70	0.2613	0.2172	0.0685
	SD	0.44	0.00	3.38	9.82	9.46	1.82	0.0169	0.0161	0.0027
(3)	Mean	15.99	0.25	12.08	100.36	131.22	29.00	0.1209	0.0927	0.0437
	Gmean	15.92	0.25	12.03	99.62	129.97	28.96	0.1208	0.0926	0.0435
	Median	15.24	0.25	12.12	98.19	129.16	28.41	0.1215	0.0935	0.0455
	Min	14.73	0.25	10.54	86.43	109.17	26.96	0.1104	0.0825	0.0357
	Max	19.39	0.25	14.01	126.96	169.87	31.76	0.1275	0.0983	0.0471
	SD	1.73	0.00	1.23	14.02	20.73	1.75	0.0058	0.0055	0.0041
(4)	Mean	3.25	2.00	19.28	87.52	94.68	5.00	0.2220	0.2048	0.2265
	Gmean	3.14	2.00	19.26	87.14	94.43	4.95	0.2210	0.2040	0.2204
	Median	2.96	2.00	19.07	89.33	93.46	4.80	0.2210	0.2082	0.2343
	Min	2.50	2.00	17.95	72.91	86.44	4.29	0.1937	0.1769	0.1323
	Max	5.24	2.00	20.73	95.70	104.80	6.47	0.2487	0.2338	0.2777
	SD	1.01	0.00	1.06	8.71	7.56	0.82	0.0225	0.0201	0.0520

Discussion

Comparative analysis of main pharmacokinetic parameters for the investigational drug "L-Dopa-PC nasal drops"(1) and "L-Dopa in oil"(2), "L-Dopa-PC in purified water"(3), "Madopar 125" capsules (4) (table 3) showed that the studied drugs quickly enter systemic circulation, however, at different rates.

Table 3: Relative bioavailability of L-Dopa-PC-based nasal oil drops [1]

Name of the administered sample	Group of animals		
	Group 2	Group 3	Group 4
	L-Dopa suspension in olive oil	L-Dopa-PC suspension in purified water	"Madopar 125", capsules
Dosage	2.5 mg/kg	2.5 mg/kg	2.5 mg/kg
RELATIVE BIOAVAILABILITY	145.2 %	176.3 %	244.4 %
Group 1 L-DOPA – PC based nasal oil drops Dosage - 2.5 mg/kg			

Difference between L-DOPA absorption rate values ($Cmax/AUC_{0→t}$) for investigational drugs (1), (2), (3) and (4) is statistically-valid and for (1) was (0.1002 ± 0.0068) h^{-1}, for (2) – (0.2383 ± 0.0169) h^{-1}, for (3) – (0.1209 ± 0.0058) h^{-1} , for (4) – (0.2220 ± 0.0225) h^{-1}, and the individual variance of values is insignificant - CV was 22-23%. Time to reach maximum L-DOPA concentration (Tmax) for (1), (2), (3) was 0.25 h on average, which indicates that there were no significant differences in the time of reaching maximum concentration (Tmax) between the studied drugs. For the drug (4) time to reach maximum L-DOPA concentration was 2 hours due to the oral route of administration, which shows a significant difference in the time to reach the maximum concentration (Tmax) between the studied drugs (1) and (4).

The average maximum concentration of L-DOPA, determined in the blood plasma of rabbits (Cmax), was for the drug (1) – (18.23 ± 1.68) ng/ml, for (3) – (12.08 ± 1.23) ng/ml, for (4) – (19.28 ± 1.06) ng/ml and (2) – (32.31 ± 3.38) ng/ml. L-Dopa is then slowly excreted from the body and after 48 hours it is still found in the blood plasma of rabbits after administration of drugs (1), (2) and (3), but after oral administration of the drug (4) after 12 hours L-Dopa is not detected. The results of the Student's test for Ln (Cmax) for the values between and within the groups (1-2) ($t = -16.8105$ at $p < 0.05000$), (1-3) ($t = 10.80150$ for $p < 0.05000$), showed a statistically significant difference between groups (1) and (2), (1) and (3). The results of the Student's test for Ln (Cmax) for the values between and within the groups (1-4) ($t = 1.74050$ at $p < 0.05000$) showed that there was no statistically significant difference between the groups.

Analysis of the main parameter characterising the degree and rate of bioavailability of the active substance (L-DOPA) from the dosage form – the area under the pharmacokinetic curve ($AUC_{0\rightarrow t}$) indicates a significant variability of this value. The average value of $AUC_{0\rightarrow t}$ for the drug (1) was (181.88 ± 13.76) ng/ml×h, for (2) – (135.53 ± 9.82) ng/ml×h, for (3) – (100.36 ± 14.02) ng/ml × h, for (4) – (87.52 ± 8.71) ng/ml × h. The average value of $AUC_{0\rightarrow\infty}$ for the drug (1) was (231.39 ± 16.52) ng/ml × h, for (3) – (131.22 ± 20.73) ng/ml × h, for (2) – (159.32 ± 9.46) ng/ml × h, for (4) – (94.68 ± 7.56) ng/ml × h. The results of the Student's test for Ln ($AUC_{0\rightarrow t}$) for the values between and within the groups: (1-2) ($t = 17.83326$ at $p < 0.05000$), (1-3) ($t = 9.041106$ at $p < 0.05000$) (1-4) ($t = 18.05994$ for $p < 0.05000$) showed that there is a statistically significant difference between groups.

As can be seen from Table 3, the relative bioavailability of the drug "L-DOPA–PC nasal drops " (1) relative to the drug "L-DOPA in oil" (2) was 145.2%. The relative bioavailability of the drug "L-DOPA-PC nasal drops"(1) relative to the drug "L-DOPA-PC in purified water" (3) was 176.3%. The relative bioavailability of the drug "L-DOPA-PC nasal drops"(1) relative to the drug "Madopar 125" (4) was 244.4%.

Parkinson's disease is the most frequent neurodegenerative disease after Alzheimer's disease [13], [14]. The disease is common in occurrence. Disease prevalence varies from 120 to 180 cases per 100 thousand of the population; the number of patients increases significantly among the older age group. The prevalence per 100,000 population for the different age groups: 41 for the 40-49 age group; 107 for the 50-59 age group; 173 for the 55-64 age group; 428 for the 60-66 age group; 425 for the 65-74 age group; 1087 for the 70-79 age group; and 1903 for the age group over 80 years [15]. The average age of disease onset is 65.3 ± 2.6 years. The incidence in men is higher than in women [16]. No significant racial differences in the morbidity patterns were found [2].

A new method of administering nanoparticles, containing levodopa, allows rapid delivery of the dopamine neurotransmitter precursor to the brain cells. The most important feature of intranasal administration of drugs is the possibility of their penetration directly into the central nervous system. Transport of drugs from the nasal cavity to the central nervous system is performed without the involvement of the mucosa, extracellularly along the trigeminal and olfactory nerves. Within 10-15 minutes chemical agents administered intranasally are found in the brain. Drugs penetrate the brain only from the olfactory region, where there is a possibility of extra - and intracellular penetration of drugs through the epithelial barrier and getting not into the bloodstream, but directly to meninges.

The study showed that the use of nasal delivery method improves pharmacokinetic parameters of the drug in comparison with oral administration. All the above mentioned allowed to increase the time of drug presence in the blood and, potentially, to use the drug in much smaller doses with a reduction in risk of toxic complications.

In conclusion, a new form of the drug (oil-based drops), containing the precursor of dopamine - L-DOPA, was developed for intranasal administration. Pharmacokinetic parameters of "L-DOPA – PC nasal drops" were studied in comparison with three other potentially active dosage forms, containing L-DOPA in the same concentrations as the study drug: "L-DOPA in oil", "L-DOPA-PC in purified water", "Madopar 125". Assay procedure for the determination of L-DOPA in animal blood plasma using liquid chromatography with tandem mass-selective detection (HPLC-MS/MS) was developed and validated.

The relative bioavailability of the drug "L-DOPA–PC nasal drops" relative to the drug "L-DOPA in oil" was 145.2%; relative to the drug "L-DOPA-PC in purified water" – 176.3%; relative to the drug "Madopar 125" – 244.4%.

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