A. A. Nedorubov et al /J. Pharm. Sci. & Res. Vol. 10(10), 2018, 2489-2492

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

HPLC-MS/MS Method Application for the Determination of Pharmacokinetic Parameters of Intranasal Delivered L-DOPA in Rats

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Abstract

Parkinson's disease treatment is based on the dopamine replacing therapy. Levodopa is the most successfully used drug. It is an amino acid (dopamine predecessor), which is capable to pass through the hematoencephalic barrier and to be deposited by the neurons. Peroral drug abuse results in a series of complications connected with the dopamine hormone system impact in patients' organism. Levodopa drug application with nasal forms of delivery allows to increase their bioavailability and to diminish collateral reactions. In given article we examined levodopa and dopamine pharmacokinetic profiles in rats. The values of the areas under the pharmacokinetic curves AUC0→t and AUC0→∞, received for L-dopa after intranasal injection in doses 3,4 mg/kg, 1,4 mg/kg and 0,7 mg/kg were 213,38 (± 20,86) and 252,34 (± 19,91), 117,12 (±7,86) and 136,13 (±10,59), 48,61 (±6,27) and 55,54(±6,35). As well dopamine's (levodopa metabolite) accumulation in fabrics of the brain of experimental animals in more significant concentrations in comparison with its predecessor was found out.

Keywords: levodopa, dopamine, rats, intranasal delivery, Parkinson's disease, pharmacokinetics, HPLC MS/MS.

INTRODUCTION

Parkinson's disease is a disorder connected with the gradual death of the motor nervous cells (neurons), which produce a mediator dopamine. Disease therapy is based on the dopamine deficiency compensation. Levodopa is one of the most effective drug for Parkinson disease [1, 2]. It represents a levorotary dioxyphenylalanine isomer, which transforms in dopamine under the influence of Dopa-decarboxylase enzyme. Levodopa (L-Dopa) supplies the most guaranteed effect for Parkinson's disease: it has a medical effect on patients in more than 95% of cases [3-6].

Dopamine is not only a neurotransmitter produced in the brain but is also a hormone produced with adrenal glands' medullary substance. L-dopa pharmacokinetic features are connected with the statement mentioned above: levodopa is 80-90% metabolized in gastroenteric path and vessels' endothelium under the action of peripheral Dopa-decarboxylase. This is the reason of occurrence of by-effects, such as nausea, vomiting, orthostatic hypotension. Just 10% of "pure" levodopa penetrate through brain-blood barrier transforming then in dopamine.

Modern scientists' efforts are concentrated on medical drugs application frequency decreasing, preserving their efficiency. 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 nose 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 [7, 8]. It was earlier believed that all L-dopa is entirely utilized in the sympathetic nerves ends (it quickly transforms into the dopamine) and does not get out in the extracellular space. Nevertheless, rather 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 [9].

One of the most widely examined animal models of Parkinson's disease are rats [10]. Rats with an unilateral nigrostriatal dopaminergic pathway affection by 6-hydroxy-dopamine (6-OHDA) are used frequently in preclinical evaluation of new symptomatic methods of the Parkinson's disease treatment [11] and also for a L-dopa effect improvement and new delivery method studying. The purpose of given research was an experimental studying of L-dopa pharmacokinetics after intranasal delivery L-Dopa-PC in various doses in rats [12].

MATERIALS AND METHODS

The examined pharmaceutical dosage form is "Nasal drops L-dopa-PC". The L-dopa content was 5%, there was an oil solution, levodopa is contained in PLGA- nanoparticles.

The research was carried out on rats. The males of the line Wistar with the body mass about 220-260 grams were used. At L-dopa-PC pharmacokinetics research animals were divided into 3 groups on 66 rats in each (after medical drug unitary introduction). The L-dopa introduction doses accounted for 0,7 mg/kg, 1,4 mg/kg and 3,4 mg/kg. The experimental animals' blood was selected through following time intervals after the introduction: 0 mins (control, without the drug), 0.08, 0.16, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 12.0, 24.0, 36.0 and 48.0 hours.

The L-dopa concentration in the blood plasma determination was done using the high-performance liquid chromatography tandem-mass-spectrometry method. The statistical data processing was led with the program called Statistica 12.0.

RESULTS

All received blood plasma samples were analyzed by the high-performance liquid chromatography tandem-mass-spectrometry method. Masses-spectrum of L-dopa and dopamine were received during the quantitative definition of explored analytes performance development. The masses-spectrum generation was implemented in the total scanning mode of fragmentary ions with mass to charge ratio in the range from 100 m/z to 500 m/z. Characteristic ion transitions of explored analyte were obtained based on received data. Dopamine mass-spectrum is shown in Figure 1.

L-dopa pharmacokinetics after intranasal delivery of L-dopa-PC was experimentally investigated during given research. L-dopa in rats blood plasma pharmacokinetic curves received after L-dopa-PC oil solution intranasal delivery in doses 3.4 mg/kg, 1.4 mg/kg and 0.7 mg/kg are presented in Figure 2.

Received information was used for key parameters calculation. Calculated pharmacokinetic parameters are shown in Table 1.

The L-dopa and dopamine (L-dopa main metabolite) distribution was studied in organs and fabrics with various degree of blood supply – strongly and moderately vascularized fables: heart, muscles; poorly vascularized fabrics: omentum; in organs supplying elimination - kidneys, liver; in pharmacological action zone - brain. Pharmacokinetic profiles of L-dopa (A) and

dopamine (B) in rats' brains after unitary intranasal delivery in dose 3,4 mg/kg are shown in Figure 3.

DISCUSSION

Earlier we showed that the bioavailability of L-dopa – medical drug "Nasal drops L-dopa-PC" accounted for 145.2% in comparison with "Levodopa in oil solution"; 176.3% in comparison with "L-dopa-PC in purified water"; 244.4% in comparison with medical drug "Madopar 125". Therefore for pharmacokinetics linearity research in rats we have chosen doses 3.4 mg/kg, 1.4 mg/kg and 0.7 mg/kg, which are slightly lower than doses used for L-dopa influence on rats with induced Parkinson disease research – 10 mg/kg [13]. It was earlier shown that Cmaks was equal in 2 cases: when L-dopa was intranasally delivered in rats in dose 2.5 mkg/kg and when the drug was perorally introduced in dose 80 mkg/kg. Areas under the curves AUC differed in 13.7 times [14].

After intranasal delivery of L-dopa it is quickly soaked up in the bloodstream with the maximum concentration achievement at Tmax = 0.25hr. The values of Cmax we received – 192.60 (± 10.99), 101.80 (±2.19) and 38.33 (±2.19) - changed linearly according to the entered doses. The period of semiejection after introduction in rats the largest dose of L-dopa-PC (15.72 (±0.48) hr) was more extended than the same parameter designed for smaller doses (8,10 (± 0.25) hr and 2.94 (±0.2) hr for 1.4 mg/kg and 0.7 mg/kg respectively). The values of the areas under the pharmacokinetic curves AUC0-t and AUC0-∞ received for L-dopa after intranasal delivery of levodopa as a part of polylactide particles in doses 3.4 mg/kg, 1.4 mg/kg and 0.7 mg/kg, accounted for 213.38 (± 20.86) and 252.34 (± 19.91), 117.12 (±7.86) and 136.13 (±10.59), 48.61 (±6.27) and 55.54 (±6.35) respectively. Whereas parameter AUC0-∞ changed proportionally in specified range of doses. Statistically-reliable distinctions between normalized to dose parameters AUC0-∞, AUC0-t and Cmax received from three experiments were not observed (Table 1).

In such a manner, experimental pharmacokinetics of L-dopa after L-dopa-PC intranasal delivery in rats is linear within the range of doses from 0.7 mg/kg to 3.4 mg/kg.

Dopamine (the L-dopa metabolite) was detected in rats' blood plasma in all analyzed samples of experimental animals. L-dopa and dopamine are endogenic connections and endogenic level of given connections was subtracted in each measured point during the calculation. The L-dopa and dopamine endogenic level in the rats' blood plasma, organs and fabrics concentration is presented in Table 2. Measured dopamine concentration level in all samples of rats' blood plasma ranged from 0.2 up to 2.3 ng/ml.

L-dopa and dopamine (metabolite of L-dopa) were registered in all investigated organs and fabrics: blood plasma, kidney, brain, liver, heart, muscles, except the omentum; moreover significant heterogeneity is traced in the drug distribution.

In Figure 3 L-dopa (A) and dopamine (B) pharmacokinetic profiles in the rats' brains after unitary intranasal delivery in dose 3.4 mg/kg are presented. After intranasal delivery of L-dopa as a part of polylactide particles reference level increase both in unchanged L-dopa and dopamine (the product of L-dopa's bioconversion) is observed. L-dopa concentration level change in rats' brain has a similar nature with the blood plasma. Maximum content of unchanged compound in fabric is observed in 0.25hr.

Dopamine is found out in fabrics of brains of experimental animals in more significant concentration, than its predecessor after intranasal delivery of L-dopa containing "Nasal drops L-dopa-PC". Dopamine level quickly increases in regard to the baseline and reaches the maximum value in 1 hr after L-dopa-PC introduction (Figure 3, Table 3).

Nasal delivery way using supplies rapid access to the circulation system, the drug can be delivered into the central nervous system directly through the olfactory bulb. Dihydroxyphenylalanine reaches target organ (excepting "first pass" metabolism) where decarboxylates with dopamine formation.

Table 1. Pharmacokinetic parameters of L-dopa received after intranasal delivery of oil solution L-dopa-PC in rats (n=6) in different doses.

	L-dopa-PC, dose		
Parameter, dimension	3.4 mg/kg	1.4 mg/kg	0.7 mg/kg
Lambda_z, 1/hr	0.04	0.02	0.01
t1/2, hr	15.72 (±0.48)	8.10 (± 0.25)	2.94 (±0.2)
Tmax,	0.25	0.25	0.25
Cmax, ng/ml	192.60 (± 10.99)	101.80 (±2.19)	38.33(±2.19)
AUC 0-t, ng/ml*hr	213.38 (± 20.86)	117.12 (±7.86)	48.61 (±6.27)
AUC 0-∞, ng/ml*hr	252.34 (± 19.91)	136.13 (±10.59)	55.54(±6.35)
Normalized parameter, dimension			
Cmax, ng/ml	62.96 (± 3.59)	54.49 (±1.59)	62.41 (±3.20)
AUC 0-t, ng/ml*hr	69.36 (± 6.9)	53.59 (±5.76)	63.99 (±9.20)
AUC 0-∞, ng/ml*hr	80.2 (± 8.35)	62.5(±7.77)	64.31 (±9.30)

Table 2. L-dopa and dopamine metabolite endogenic level in the rats' blood plasma, organs and fabrics (n=6).

	Concentration (±SD)						
	Plasma ng/ml	Kidneys ng/g	Brain ng/g	Liver ng/g	Heart ng/g	Muscles ng/g	Omentumng/g
L-dopa	2.65±1.55	10.67±2.6	3.40±0.9	36.98±11.4	4.94±1.2	38.89±13.1	0.00
Dopamine	1.71±0.63	8.00±2.9	99.18±20.5	52.54±15.3	0.81±0.3	35.19±11.0	0.00

Table 3. Pharmacokinetic curves of L-dopa and dopamine in rats' brains after L-dopa-PC unitary intranasal delivery in dose 3,4 mg/kg (n=6; Xcp±SD).

	Parameter, dimension					
	Kel, hr^{-1}	t1/2, hr	Tmax, hr	Cmax, ng/g	AUC 0-∞, ng/g*hr	MRT, hr
L-dopa	0.0134 (±0.0007)	51.94 (±2.48)	0.25	16.94 (±1.52)	224.11 (±53.17)	72.49 (± 3.98)
Dopamine	0.0085 (±0.0005)	81.40 (±4.78)	1.00	376.33 (3.95)	22314.46 (±2464.96)	118.23 (±7.38)

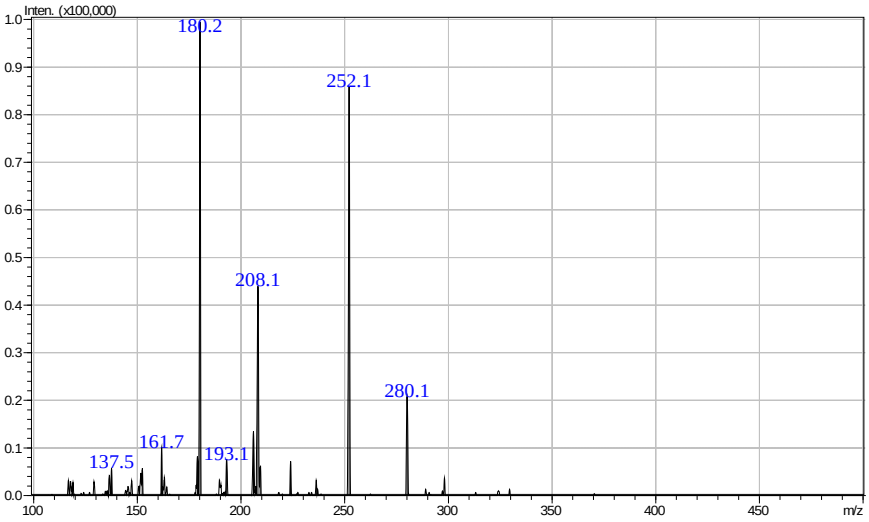

Figure 1. DA mass-spectrum.

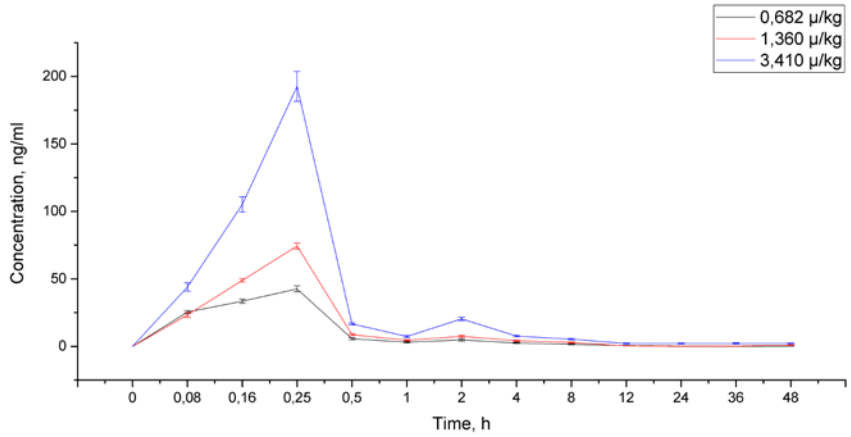

Figure 2. L-dopa in rats blood plasma pharmacokinetic curves received after intranasal delivery of "Nasal oil drops based on L-dopa-PC" in three different doses: 0.7 mg/kg, 1.4 mg/kg and 3.4 mg/kg.

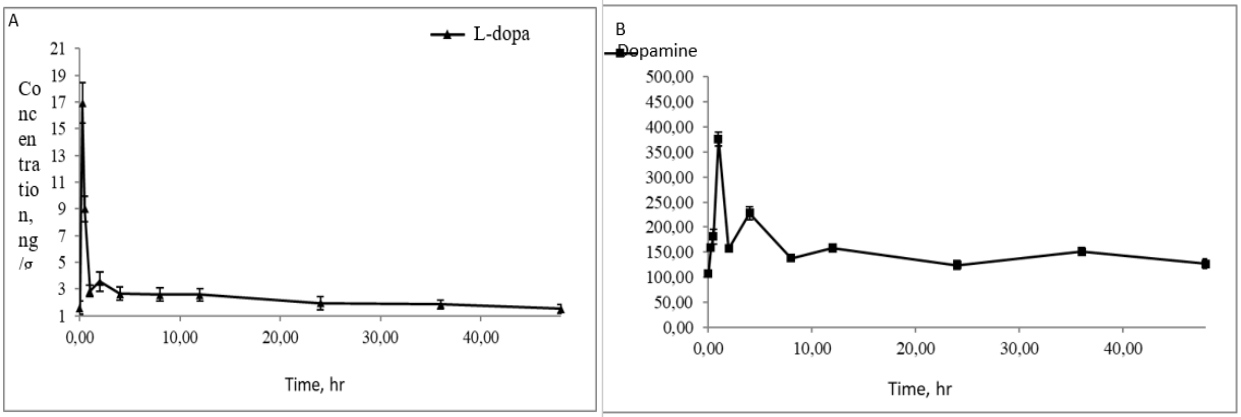

Figure 3. Pharmacokinetic curves of L-dopa (A) and dopamine (B) in rats' brains after L-dopa-PC unitary intranasal delivery in dose 3.4 mg/kg (n=6; Xcp±SD).

CONCLUSIONS

The values of the areas under the pharmacokinetic curves AUC0→t and AUC0→∞, received for L-dopa after levodopa intranasal injection as a part of medical drug "Nasal drops L-dopa-PC" in doses 3.4 mg/kg, 1.4 mg/kg and 0.7 mg/kg were 213.38 (± 20.86) and 252.34 (± 19.91), 117.12 (±7.86) and 136.13 (±10.59), 48.61 (±6.27) and 55.54(±6.35). At the same time in mentioned range of doses parameter AUC0→∞ changed proportionally. Statistically-reliable distinctions between normalized to dose parameters AUC0-∞, AUC0-t and Cmax received from three experiments were not observed. In such a manner, experimental pharmacokinetics of L-dopa after "Nasal oil drops based on L-dopa-PC" intranasal delivery in rats is linear within the range of doses from 0.7 mg/kg to 3.4 mg/kg.

ACKNOWLEDGEMENTS

Supported by the "Russian Academic Excellence Project 5-100".

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