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Study of adhesive properties of new dosage forms for Nano-L-DOPA nasal delivery system based on PLGA nanoparticles

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Abstract

In the present study, a modern approach to the pharmaceutical development of new L-dopa medicinal preparations has been implemented for the treatment of the Parkinson's disease as a result of the creation of the Nano-L-Dopa nasal delivery system on the basis of polymer PLGA nanoparticles and rational dosage forms. Twenty compounds have been proposed, and experimental batches of nasal drops, gels and ointments containing 5% of Nano-L-Dopa have been developed. The experimental studies of the adhesive properties of nasal dosage forms have been carried out on the in vitro model of the nasal-cavity lining mucosa. Appropriate adhesive properties of dosage forms account for their uniform distribution in the lining mucosa and the necessary rate of flowing down from the nasal cavity. The optimum Nano-L-Dopa dosage forms have been selected: oil and combined nasal drops, as well as nasal gels.

Keywords: Nano-L-DOPA, PLGA Nanoparticles, Parkinson's disease, nasal drug delivery system, adhesive properties, dosage forms of drugs, drops, gels, ointments.

INTRODUCTION

More than 4 million people in the world suffer from the Parkinson's disease (PD), and the annual growth trend of the number of cases has been observed [1]. Levodopa (L-dopa) is a metabolic predecessor of dopamine. Its introduction into the medical practice embodies a substitutional neurotransmitter therapeutic approach and the "gold standard" of the PD treatment [2, 3]. One of the major drawbacks of the traditional L-DOPA oral dosage forms is their low bioavailability [3].

The promising trends of pharmaceutical technology include the creation of the nasal delivery systems for medicinal preparations on nanocarriers [4-8]. For example, creation of the nanosomal L-DOPA (Nano-L-Dopa) through the inclusion of the micronised L-DOPA in polymeric nanoparticles based on biodegradable copolymer of the poly(lactic-co-glycolic acid) (PLGA) enables adjustable release, increased bioavailability and, respectively, the therapeutic efficacy of the medicinal preparation in case of the nasal route of administration in PD patients [9, 10].

The pharmaceutical development of optimal formulations of the Nano-L-Dopa nasal forms is of great interest. An important factor in providing the nasal dosage forms with the adequate characteristics, such as uniform distribution in the nasal-cavity lining mucosa and the optimum rate of flowing down from the nasal cavity, is the presence of sufficient adhesive properties**.**

The aim of this study is to investigate the adhesive properties of various forms (drops, gels and ointments) of the Nano-L-Dopa nasal delivery system.

MATERIALS AND METHODS

Nano-L-Dopa was created on the basis of biodegradable polymer nanoparticles as per the improved technologies of Zhou Y. Z et al (2013) and Barsegyian G.G. et. al, (2014) [11, 12]. The composition of Nano-L-Dopa per 100 g,% wt.:

- L-Dopa (micronized) –9.5-10.0;
- PLGA polymer 50/50 - 79.0 –80.0;
- D-mannitol - 8.0 - 8.3;
- Polyvinyl alcohol -1.7 –3.5 (the rest, below 100.0).

Nano-L-Dopa is an amorphous lyophilized, white or light white, hygroscopic, photosensitive, odorless powder. It is soluble in dimethylsulfoxide and dimethylformamide, and poorly soluble in ether, hexane, 96% alcohol; it forms a suspension with water. The size of PLGA nanoparticles in the main fraction of the aqueous suspension of Nano-L-Dopa is not more than 500 nm. The pH value of 1% aqueous suspension of Nano-L-Dopa is in the range of 4.5 to 7.0. The water content is below 1.0%. The quantitative content of L-Dopa in Nano-L-Dopa is 9.10-9.54%.

The following was used in the preparation of Nano-L-Dopa: micronised L-Dopa (3,4-dihydroxy-L-phenylalanine, L-Dopa> 98%, USP36, "Sigma-Aldrich", USA); Poly(lactic-co-glycolic acid) - PLGA 50/50 (Poly (DL-lactide-co-glycolide) nominal, Ester Terminated, Inherrent Viscosity 0.37 dL/g, USP36; LACTEL® Absorbable Polymers International, USA); polyvinyl alcohol (87-90% hydrol., average mol. wt 30,000-70,000, "Sigma-Aldrich", USA); and D-Mannit (D-Mannitol> 98%, "Fluka", USA).

To study the adhesive properties of new dosage forms of the nano-L-Dopa nasal delivery system, an in vitro model system simulating nasal-cavity lining mucosa was used. The model was developed on the basis of the modified technique of D.L. Shobolov et al. [13]. The assessment was made based on the parameters of the distribution area and the degree of adhesion of the samples on the model surface. The methylene blue or Sudan III dyes, respectively, were preliminarily injected in the samples, depending on the predominant content of hydrophilic or hydrophobic phase. To determine the distribution area, the test sample was placed on a model lining mucosa surface located in a Petrie dish and preheated to 37 °C, covered with a lid, thermostated at 37 °C for 15 minutes. The distribution area S (cm^2) of each sample was determined using graph paper.

Adhesive properties of various dosage forms of the Nano-L-Dopa nasal delivery system were assessed by the rate of sample flowing down a sloping surface of the model underlayer, moistened with the model medium. The test sample was applied to the start line of a substrate preheated to 37 ° C (the surface in a Petrie dish). The substrate was raised at angles of 10° and 70°, and after 30 seconds the path traversed by the front boundary of the sample was measured. The rate of flowing down V (mm/s) was calculated by the following formula:

$$v = \frac{L}{\tau},$$

where L wasthe path traversed by the front boundary of the sample, mm;

τ was the flowing down time, sec.

RESULTS

Experimental batches of the following dosage forms for the L-Dopa nasal delivery system have been developed in the laboratory: nasal drops, ointments and gels containing 5% of Nano-L-Dopa. The compositions of the dosage forms are presented in Table 1.

To study the adhesive properties, the samples of Nano-L-Dopa nasal dosage forms were taken in an amount of 5.0 ml (5.0 g), which corresponded to the content of 0.25 g in terms of Nano-L-Dopa, or 0.025 g in terms of L-Dopa.

Table 1. Composition of Nano-L-Dopa nasal dosage forms

Component	Composition, per 10.0																			
	Nasal drops														Nasal gel			Nasal ointment		
	aqueous				oil				Combined											
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Hypromellose			0.1												0.3	0.4	0.5			
Methylhydroxypropylcellulose				0.1																
Vaselene																		5.0	3.0	3.0
Vaselene oil										0.1	0.2	0.3	0.4	0.5					1.5	1.5
Tween 80							0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.01	0.01	0.01			
Benzyl alcohol			0.10	0.10																
Cetyl alcohol										0.01	0.01	0.01	0.01	0.01						
Sorbitol 70%		0.020	0.02	0.02					0.02						0.1	0.1	0.1			
Propylene glycol									0.1											
Softisan 649																				0.5
Lanolin																			0.5	
Sodium chloride		0.004																		
Sodium dihydrogen phosphate dihydrate		0.004																		
Disodium hydrogen phosphate dihydrate		0.003																		
Edetate disodium		0.001		0.001																
Anhydrous sodium phosphate															0.01	0.01	0.01			
Sodium citrate				0.004																
Chlorohydric acid				0.003																
Citric acid monohydrate															0.02	0.02	0.02			
Olive oil					below 10.0		below 10.0		2.0	2.0	0.5	1.0	1.5	2.0	2.0	2.0	2.0			
Sunflower oil						below 10.0		below 10.0												
Purified water	below 10.0	below 10.0	below 10.0	below 10.0					below 10.0	below 10.0	below 10.0	below 10.0	below 10.0	below 10.0	2.56	2.46	2.36			

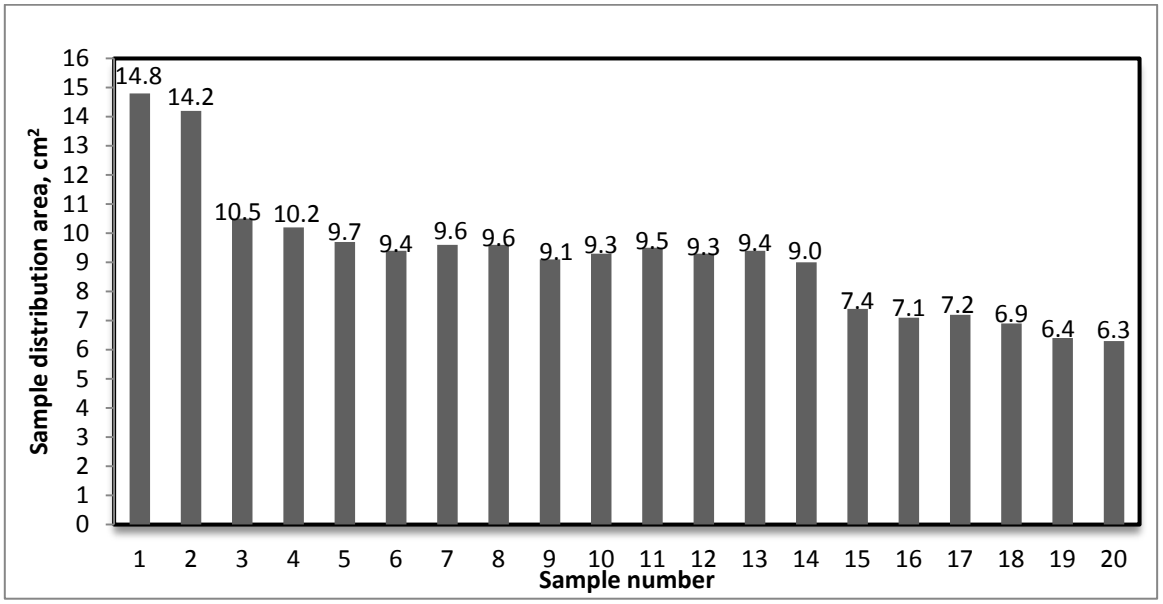

Figure 1. Samples distribution areas of Nano-L-Dopa nasal dosage forms on the model surface of the nasal-cavity lining mucosa in vitro. The abscissa indicates the sample number (the composition is indicated in the table), the ordinate - the sample distribution area, cm^2.

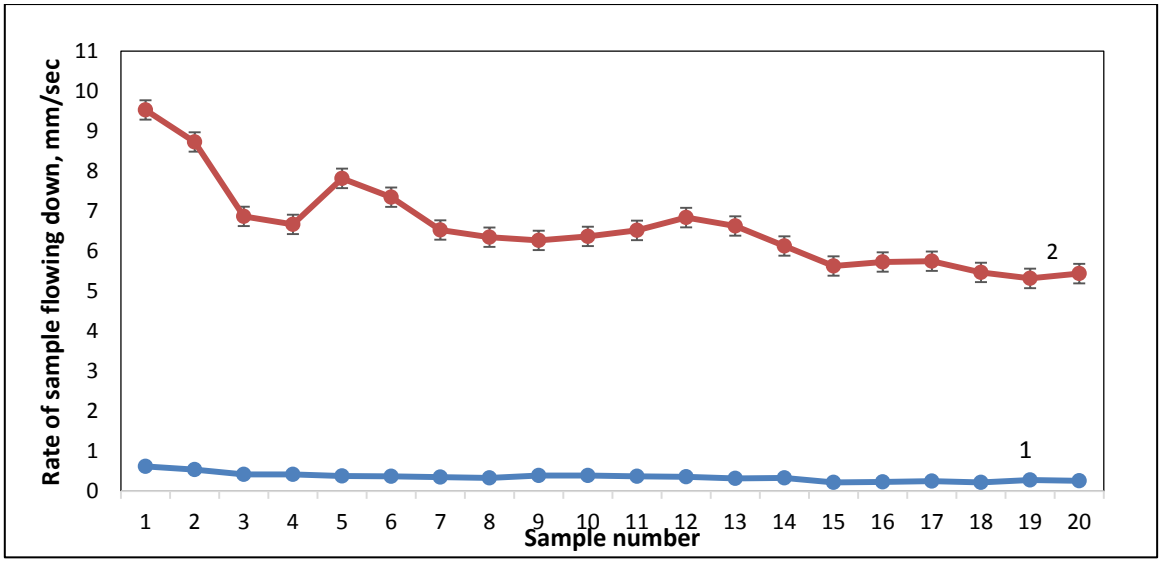

Figure 2. The rate of flowing down of the Nano-L-Dopa nasal dosage form samples along the model surface of the in vitro nasal-cavity lining mucosa at slope angles of the surface of 10 °(1) and 70 °(2). The abscissa indicates the sample number (the composition is indicated in the table), the ordinate - the sample flowing down rate, mm/sec.

The distribution area of Nano-L-Dopa nasal dosage form samples on the model surface was 6.3 to 14.8 cm^2, the results are shown in Figure 1. The maximum distribution area was found for water-based nasal drops (14.2-14.8 cm^2), which allowed to predict their rapid flowing down from the nasal cavity and, therefore, the impossibility of accurate dosing. When 1% of hypromelose and 1% of methylhydroxypropylcellulose have been added to water drops, the distribution area was reduced to 10.2-10.5 cm^2. The distribution area of samples of combined and oil drops was in the range from 9.0 to 9.7 cm^2. A significant decrease in the distribution area (by 1.6-3.4 cm^2) was recorded in the study of samples of soft dosage forms - gels and ointments. The ointments were characterized by the minimum distribution area (6.3-6.9 cm^2). The optimum distribution of nasal dosage forms on the model surface was 7-10 cm^2. The following dosage forms correspond to the specified criterion: oil and combined drops, as well as gels.

The rate of the samples' flowing down the substrate, wetted with a model medium, was determined at slope angles of 10 ° and 70 °, taking into account the localization of the simulated surface of nasal-cavity lining mucosa. In the physiologically normal state in humans, slope angle of the surface of nasal-cavity lining mucosa along the bottom wall is about 10-20° to the horizontal surface, and along the upper wall of the nasal cavity it reaches 70°. The results of the study of adhesive properties of Nano-L-Dopa nasal dosage forms are presented in Figure 2.

The rate of the samples' flowing down an underlayer, wetted with a model medium, was 0.26-0.62 mm/s at the surface slope angle of 10 ,°and 5.44-9.53 mm/s at the surface slope angle of 70°. It should be noted that the difference among the samples' flowing down rate at a slope angle of 10 °was insignificant (0.40 mm/s), and this criteria was not suitable to be used for comparing the adhesive properties of Nano-L-Dopa nasal dosage forms.

On the contrary, the rate of the samples' flowing down an underlayer, wetted with a model medium, at the surface slope angle of 70 °can be used as an informative indicator for comparing adhesive properties. As known, the physiological rate of the nasal mucociliary transport in adults over 50 years old is in the range from 3.1 to 7.0 mm/s [14]. If we accept these values as normal range for establishing a comparison criterion of the sample adhesive properties, then the sample advance speed along the mucosa within the indicated range (3.1-7.0 mm/s) was demonstrated by: water drops with the inclusion of 1% hypromelose (formulation 3) and 1% methylhydroxypropylcellulose (formulation 4), oil drops with the inclusion of 0.1% Tween 80 (formulations 7 and 8), combined drops (formulations 9-14), gels (formulations 15-17) and ointments (formulations 18-20).

Thus, based on the results of the study of adhesive properties of various dosage forms of the Nano-L-Dopa nasal delivery system in the in vitro experiment on the model of the nasal-cavity lining mucosa, the oil drops with 0.1% Tween-80 (formulations 7 and 8), combined drops (formulations 9-14) and gels (formulations 15-17) should be considered the most promising for further study.

DISCUSSION

One way to achieve the goal is to develop nanosomal drug products and to use the nasal administration route for the treatment of neurodegenerative brain diseases [6, 7, 15].

Nasal drug delivery provides local and systemic effects on the human body, creates contemporary alternative to oral and injectible prescriptions. Nasal route of administration is noninvasive, with low probability of injury or transmission of infection, simple and convenient for the patient, cost effective, and provides high bioavailability and therapeutic efficacy of drug products. Pharmaceutical substances are absorbed quickly through the amply vascularized and well-permeable nasal cavity, enter the bloodstream without the primary passage through the liver and start acting in a few minutes, which is important in emergency care. The rate of the therapeutic action development commensurates with the injectable administration route, and the systemic effect in the nasal application develops in 5-10 min [16, 17].

The nose-brain route leads to the rapid delivery of certain drugs directly to the central nervous system through the blood-brain barrier. Thus, the nasal drug delivery system opens the revolutionary possibilities of treating neurodegenerative brain diseases [7, 15, 17].

However, the nasal drug delivery has a number of restrictions associated with a low bioavailability of "large" molecules (e.g., of a peptide nature), the presence of a mucociliary clearance system, and enzymatic degradation. The use of new

forms of the nasal delivery system based on nanocarriers allows to minimize these disadvantages [18].

The developed technology for obtaining a nasal delivery system for nanosomal L-Dopa (Nano-L-Dopa) based on polymeric biodegradable PLGA nanoparticles by modifying the technology previously proposed by Zhou Y. et al. (2013) and BarsegYan G.G. et al. is of particular interest (2014) [11, 12]. The antiparkinsonian activity of the Nano-L-Dopa based on PLGA nanoparticles in the nasal administration has been experimentally confirmed in vivo in animals. The incorporation of L-Dopa into polymeric PLGA nanoparticles significantly increases specific activity and provides a prolonged effect of the pharmaceutical substance. Nasal administration of Nano-L-Dopa significantly reduces the daily dose of L-Dopa [9, 10].

The present experimental study aims at the development of optimal pharmaceutical dosage form of the Nano-L-Dopa nasal delivery system. Given the high dispersity of pharmaceutical substance (micronized L-Dopa in PLGA nanoparticles), the need for the systemic and/or central action in the PD treatment, the need for a long stay on the nasal mucosa to ensure the completeness of absorption and prolonged therapeutic effect, the regularity of applying the formulation on the nasal-cavity lining mucosa, as well as its use by older persons, the drops, gels, ointments were selected as tested dosage forms of the Nano-L-Dopa nasal delivery system.

The choice of excipients during pharmaceutical development of the Nano-L-Dopa nasal dosage form was due to the following factors: the physico-chemical properties of the pharmaceutical substance, the physicochemical parameters of the nasal-cavity lining mucosa and nasal secretions; and the physicochemical and technological properties of excipients.

Having analyzed various factors of the nasal mucosa and nasal secretions' operation, a simple and accessible in vitro model was used allowing to investigate the behavior of various nasal dosage forms of Nano-L-Dopa in the nasal cavity. Based on the properties of "nasal mucosa/nasal secret" system, the behavior of nasal dosage forms in such a system is characterized by the following parameters: distribution area over the surface and the ability to be held on a sloped surface; it is also dependent on the physical and chemical characteristics of two basic functional-morphological units - mucosa and nasal secretions of the nasal cavity. As a result of the in vitro experimental study of the adhesive properties on the model of the nasal-cavity lining mucosa, the following promising for further investigation Nano-L-Dopa dosage forms have been selected: nasal drops and gels of formulations No. 7, 8, 9-14, 15-17.

CONCLUSION

Twenty compounds have been proposed, and experimental batches of nasal drops, gels and ointments containing 5% of Nano-L-Dopa have been developed. The experimental studies of the adhesive properties of nasal dosage forms have been carried out on the in vitro model of the nasal-cavity lining mucosa. The following Nano-L-Dopa dosage forms have been selected: nasal oil and combined drops, as well as nasal gels with required characteristics, capable of ensuring an even distribution of the dosage form on nasal-cavity lining mucosa and the optimum rate of flowing down from the nasal cavity when used for PD treatment through the Nano-L-Dopa nasal delivery system. It is advisable to recommend the measurement of the distribution area on the model surface and the rate of sample flowing down a sloping surface of the model underlayer, wetted by the model medium, at an angle of 70°, for the study of adhesive properties of nasal dosage forms in the in vitro experiments and for selection of the optimal formulations.

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