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Original Article

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Increasing the Efficiency of Parkinson's Disease Treatment Using a poly(lactic-co-glycolic acid) (PLGA) Based L-DOPA Delivery System

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To compare the efficacy of L-DOPA administered intranasally in the form of nanoparticles (nano-DOPA) and in standard drug forms using a rat Parkinson's Disease (PD) model. L-DOPA-containing nanoparticles (250±50 nm) were synthesized using the double emulsion method. The efficacy of nano-DOPA therapy was studied in Wistar rats with 6-OHDA-induced PD. Drugs were administered daily, 0.35 mg/kg (by L-DOPA). Animals' motor coordination and behavior were analyzed using the forelimb placing task and several other tests. Thirty minutes after the first administration, animals treated with L-DOPA, L-DOPA+benserazide, and nano-DOPA showed equally significant ($p<0.05$) improvements in coordination performance in comparison to the non-treated group. After 4 weeks of treatment, coordination performance in the nano-DOPA group (89±13% of the intact control level) was twice as high as in the L-DOPA and L-DOPA+benserazide groups, which did not differ from non-treated animals. The effect of nano-DOPA was significantly higher and more long-lasting (90±13% at 24 h after administration); moreover, it was still significant one week after the treatment was discontinued. Intranasal nano-DOPA was found to provide a lasting motor function recovery in the 6-OHDA-induced rat PD model with the effect sustained for one week after discontinuation, while the same doses of standard drugs provided significant effect only after the first administration. L-DOPA administered in the form of PLGA-based nanoparticles had a higher effective half-life, bioavailability, and efficacy; it was also efficiently delivered to the brain by intranasal administration.

Key words: animal models, L-DOPA, Parkinson's disease, nano-DOPA, PLGA, nasal administration

INTRODUCTION

L-DOPA (L-3,4-Dihydroxyphenylalanine) has long been the principal drug used for the therapy of Parkinson's disease (PD); each patient is ultimately bound to undergo treatment with L-DOPA. Its medical effect is based on the enhancement of dopamine production in the central nervous system.

Standard drug use disadvantages

L-DOPA has certain disadvantages, such as its low, the gradual decrease in the therapeutic effect developing in the course of treatment and the consequent necessity to increase the drug dose to maintain a stable effect, fluctuations in the gastrointestinal drug absorption rate, and the short half-life period in the blood. In addition, oral drug intake can be encumbered by dysphagia, one of PD symptoms [1].

Considerable fluctuations of L-DOPA blood levels associated with disease progression result in pulsatile stimulation of

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dopamine receptors and may produce the biphasic effect with "On" and "Off" periods. Consequently, multiple L-DOPA administration (2-3 times a day) is required to maintain the therapeutic drug level in the brain.

The disadvantages of oral L-DOPA administration, including the liver first-pass effect, which causes L-DOPA degradation and diminishes its therapeutic activity [2], can be overcome by using alternative drug delivery routes. For example, intravenous injections are not associated with gastrointestinal absorption problems [3] but are inconvenient for everyday use.

Nasal drug administration

This method is rated as an up-to-date alternative to the oral route, because nasal cavity is readily accessible, has a large surface area, porous endothelial membrane, and high total blood flow; in addition, the first-pass effect can be eliminated [4]. High efficacy of nasal drug administration was proved by a number of experiments: for instance, nasal insulin administration enhanced the memory characteristics of Alzheimer patients [5], and nasal oxytocin decreased stress level in monkeys [6]. In animals with 6-OHDA-induced Parkinson's disease, intranasal dopamine administration improved motor functions [7], and L-DOPA applied nasally improved motor functions and performance in the rotation test [8]. It was shown that absolute bioavailability of carbidopa-containing L-DOPA was 3 times higher for nasal delivery than for the oral route: 45.4% in the 2.5 mg/kg dose vs. 17.7% in the 80 mg/kg dose, respectively. At the same time, the half-life period of intranasally administered L-DOPA in the blood and in the brain was less than 30 minutes. The maximum plasma concentrations (C_{max}) of L-DOPA were nearly equal for both oral and nasal administration, although the drug dose applied intranasally was 32 times lower. The total amounts of L-DOPA in the plasma and the brain were 2.1 to 2.5 times higher for intranasal administration than for the oral route [9].

To date, the dominating approach to early PD treatment implies constant dopaminergic stimulation. In agreement with this concept, another possibility to reduce side effects and enhance drug efficacy is to develop a drug form with prolonged, controlled release. Thus, the effective drug dose could be lowered owing to controlled release and optimized biodistribution.

Poly (lactic-co-glycolic acid nanoparticles

The possibility to construct particles with controlled degradation times inspired the development of multiple drug delivery methods, including the use of biodegradable copolymers of glycolic and lactic acids (PLGA) [10]. Medical and pharmacological application of polylactides has been approved by FDA and WHO, and several recent studies described their use for nasal drug administration, including nose-to-brain delivery [11-15]. The properties of polymeric nanoparticles enable controlled, prolonged drug release, which may substantially neutralize the pulsatile effect on the blood and brain drug levels.

In our previous studies, we get some data showing dopamine levels in blood, brain and other organs after different drug (including L-DOPA and nano-DOPA administration). Measured by ELISE after a single drug administration, the brain levels of DA (AUC1-4) were double higher after nano-DOPA than L-DOPA administration, blood levels did not differ [16, 17].

The purpose of our work was to investigate the possibility of nasal administration of PLGA-based submicron L-DOPA-containing particles (nano-DOPA) for Parkinson's disease treatment, using the 6-OHDA-induced rat model of PD. Another goal was to test the efficacy of nano-DOPA in a set of behavioral tests.

MATERIALS AND METHODS

Male Wistar rats weighing 335±28 g were housed under a 12 h light/dark cycle with free access to food and water. The animals were surged to initiate 6-OHDA-induced Parkinson's disease model. All procedures were approved by the Institutional Animal Care and Ethical Committee of the Research Center for Molecular Diagnostics and Therapy in accordance with the last GLP Principles [ECD Principles on GLP. C (97)186 Final] and EU Directive 2010/63/EU principles of laboratory animal care guidelines.

Dopamine denervating lesions

All rats received unilateral dopamine (DA)-denervating lesions by injection of 6-hydroxydopamine (6-OHDA-HCl, Sigma) into the right medial forebrain bundle (mfb) [18, 19].

The rats were anesthetized with 400 mg/kg body weight chloral hydrate (Aldrich) in sterile saline. 6-OHDA was dissolved in 0.02% ascorbic acid/saline to the concentration of 4 mg/ml, and kept on ice in the dark. The toxin solution (2.5 µl) was injected at the following coordinates (in mm, relative to the bregma and the dural surface): AP=-4.4, L=-1.2, DV=-7.8 (tooth bar=-2.3), at the rate of 1 µl/min and a 5 min lag was allowed before needle extraction. Three weeks post-surgery, the extent of DA denervation was evaluated by testing the rats for apomorphine-induced rotation. Turning behavior was recorded during a 30 min period after an injection of 1.6 mg/kg Apomorphine hydrochloride (R-(-)-Apomorphine hydrochloride hemihydrate, Sigma). Only the rats that made more than five full turns/min in the direction contralateral to the lesion

were selected for the study as meeting the requirement for more than 90% striatal dopamine depletion [20].

Experimental design and drug preparation

In the experiment, a total of 48 rats with unilateral 6-OHDA lesions and 12 intact control rats were divided into five different treatment groups. Animals of the intact control and the non-treated control groups received vehicle solution containing blank PLGA particles. Different drug doses were tested in our previous experiments and nano-DOPA effects at physical power was detected in dose 1mg/kg [16], To compare nano-DOPA and standard drugs we have to use same drug doses (by L-DOPA), so the highest dose that can be used considering L-DOPA low solubility and maximum single nasal administration volume was taken. The L-DOPA treated group received 0.35 mg/kg of L-DOPA; the L-DOPA+decarboxylation inhibitor (L-DOPA+inh) treated group received 0.35 mg/kg of L-DOPA with 0.08 mg/kg of benserazide (according to the standard L-DOPA/ benserazide proportion in clinical drugs [21]). The last group received nano-DOPA suspension in the vehicle in the dose of 0.35 mg/kg (by L-DOPA). All drugs were administered daily, once a day, via the nasal delivery route. In order to analyze long-term effects of drug administration, the treatment was performed for 13 weeks, and delayed effect tests were conducted 1 and 4 weeks after the treatment was discontinued.

The nano-DOPA test drug was synthesized on the basis of L-DOPA (Sigma) and PLGA 50/50 (Plurac Biochem). The particle size was 250±50 nm, the level of L-DOPA inclusion was 10±2%. L-DOPA (Sigma) solution was used as the reference drug. For the preparation, L-DOPA was dissolved in 1% ascorbic acid, pH 2.0, to the concentration of 3.2 mg/ml; next, pH was raised to 7.0 with 5M NaOH. The vehicle solution was 1% ascorbic acid, pH 7.0. For the preparation of standard drug with inhibitor, benserazide (Sigma) was dissolved in L-DOPA standard drug solution to the concentration of 0.08 mg/ml. Drug solutions were prepared weekly and stored in the dark at 4°C. Immediately before administration, nano-DOPA was suspended in the vehicle to the concentration of 3.2 mg/ml (by L-DOPA), and blank particles were added to all other drug solutions.

Behavioral testing

Several tests were used:

Placing task

This test was used to estimate the animals' coordination performance after one-sided substantia nigra lesion. The placing task requires rats to make a directed forelimb movement in response to sensory stimuli. A rat was held so that its limbs were hanging unsupported. It was then raised to the side of a table so that its body was parallel to the edge of the table. Each rat received 10 consecutive trials with each forelimb and the total number of times the rat placed its forelimb on the top of the table was recorded [22].

In the course the treatment period, animals performed the placing task every week, twice a day: 30 min and 24 h after drug administration.

Open field test

The standard open field test was performed to check potential long-term drug effects on cognitive functions and locomotor activity [23, 24].

Vertical grid holding test

This test was used to check the animals' physical power and endurance. A rat was placed onto a vertical grid of 40x150 cm with wooden boards and allowed to hold on it. The rat was five times returned to the grid after falling. The time before the fifth fall was registered [25, 26].

Footfault asymmetry test

A rat was placed on an elevated grid with openings of 2.5 cm^2 for 2 min. As the animal was crossing the grid, a foot fault was scored each time its paw slipped through an opening in the grid. The total number of steps was also counted. The footfault index was calculated using the following formula:

$$K(faults) = \frac{n(fault\,paw\,steps)}{n(total\,paw\,steps)} * 100\%$$

The footfault asymmetry was counted as K(faults contralateral)-K(faults ipsilateral).

The score of 0 corresponded to lack of asymmetry, a positive score corresponded to a contralateral deficit, and a negative score, to an ipsilateral deficit [27, 28].

This test was performed one time, in treatment week 4.

Statistical analysis

All data were presented as group means±S.E.M. All statistics on behavioral data were performed using repeated-measures ANOVA, where treatment type and time (day of treatment) were entered as independent factors. Relevant differences were analyzed pairwise by post hoc comparisons using the Tukey's honestly significant difference (HSD) test. The threshold for statistical significance was set at 0.05.

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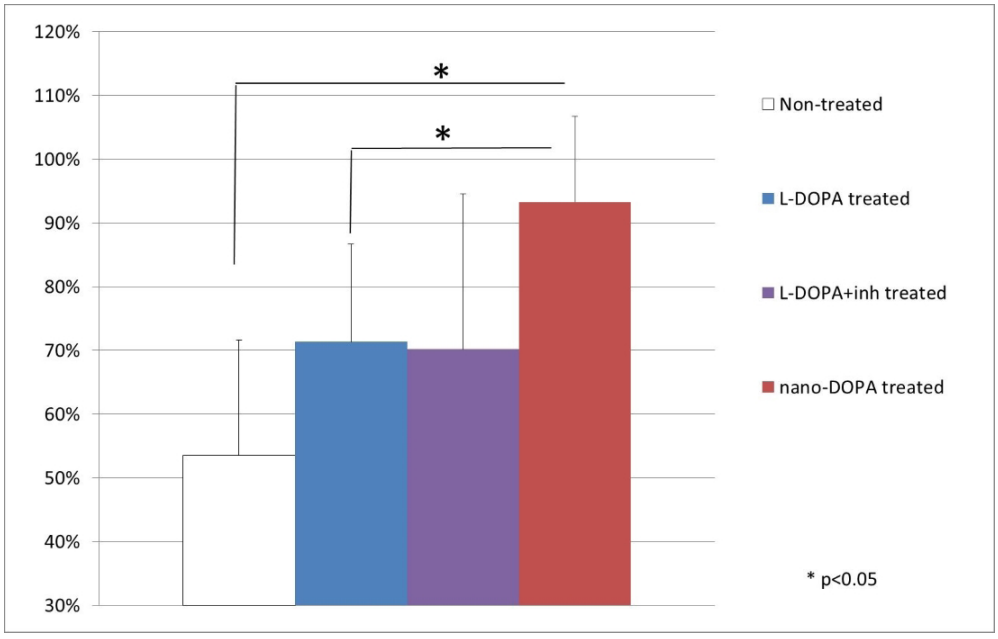

Fig. 1. Placing task scores obtained in 30 min after drug administration, 1 week after the beginning of treatment. Nano-DOPA-treated group's results are significantly higher than not only non-treated, but also L-DOPA-treated group's results ($p<0.05$). The results are shown in % of the intact control group score; values are mean±standard deviation of 12 rats in each group.

RESULTS AND DISCUSSION

The placing task was the most informative test used.

The test was first performed five weeks after surgery, before the beginning of treatment, and the results were identical in all PD-positive groups and differed significantly from the results of the intact control group ($p<0.05$).

The effect of a single drug administration was analyzed 30 min after the first nasal application. The results observed in this test were as follows: intact control group, 100±9%; no treatment group, 69±10%; L-DOPA group, 80±9%; L-DOPA+inh group, 80±3%, and nano-DOPA group, 79±10%. This means that all drug forms, including nano-DOPA, caused a significant improvement in coordination performance in comparison to non-treated animals ($P<0.05$) in 30 min after a single nasal administration, although the results of all PD-positive animal groups were significantly lower than the intact control score ($p<0.05$). Thus, a single nasal administration of both standard drugs and nano-DOPA in the dose of 0.35 mg/kg improved locomotor functions in rats.

However, after one week of treatment, we observed a decrease in the effects of the standard drugs, while the effect of nano-DOPA, on the contrary, increased. The placing task scores observed in 30 min after nasal drug administration were as follows: intact control group, 100±9%; non-treated group, 59±12%; L-DOPA group, 68±13%; L-DOPA+inh group, 73±20%, and nano-DOPA group, 87±16% (Fig. 2). Thus, nano-DOPA caused a significant improvement in coordination performance in comparison to non-treated animals and to those treated with the L-DOPA standard ($P<0.05$). The scores of all PD-positive animal groups differed significantly from the intact control ($p<0.05$).

Presumably, with the low standard drug doses used, these results illustrate the expected dose exhaustion effect, which is a well-known phenomenon in the standard drug therapy and represents one of its major disadvantages. On the other hand, no such effect was observed for nano-DOPA treatment, at least for the period of 13 weeks (Figs. 1~3).

Coordination performance was significantly higher in animals treated with nano-DOPA in comparison to all other PD-positive animal groups since the second week of treatment and throughout the whole treatment period ($P<0.05$). Moreover, starting from the third week of treatment, the scores of the nano-DOPA group did not differ from those of intact controls, indicating that this drug form was highly efficient for restoring the motor function (Fig. 2).

In the course the treatment period, animals performed the placing task twice a day: 30 min and 24 h after drug administration. The scores obtained in 24 h post-administration showed the prolonged drug effect on the motor function (Fig. 3). In animals treated with nano-DOPA, improved coordination performance was observed both 30 min after drug administration and in 24 h, immediately before the next application.

The effect of nano-DOPA was still significant 1 week after the treatment was discontinued and vanished only 4 weeks later.

The vertical grid holding test was performed in treatment week 4 and did not detect any significant differences between the experimental groups. Apparently, this means that neither the PD model, nor any type of drug treatment affected the animals'

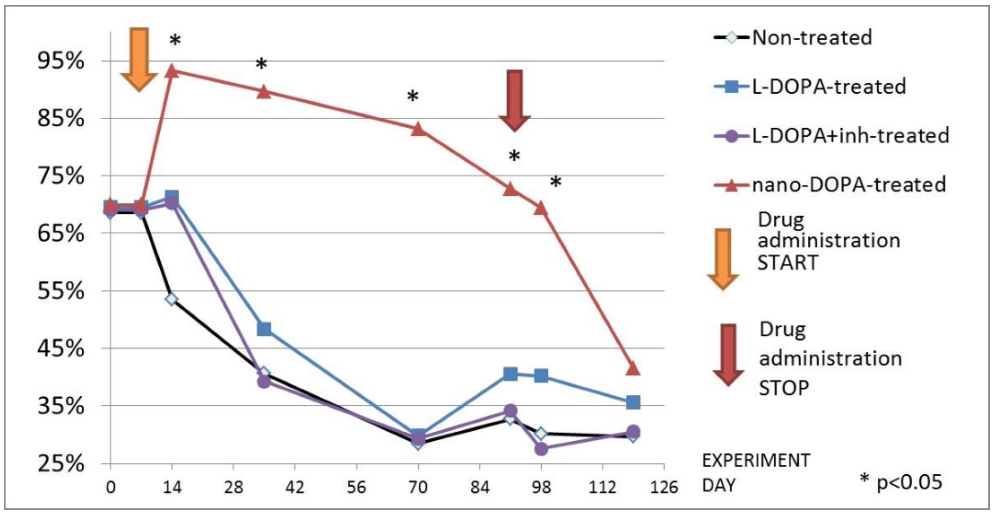

Fig. 2. Placing task scores obtained in 30 min after drug administration, score dynamics observed during of the treatment period and after discontinuation of the drug. Nano-DOPA-treated group's results were significantly higher than all other PD positive groups' results during the whole treatment period and were still significantly higher one week after the drug cancellation ($p<0.05$). The results are shown in % of the intact control group score. Number of animals in each group is 12.

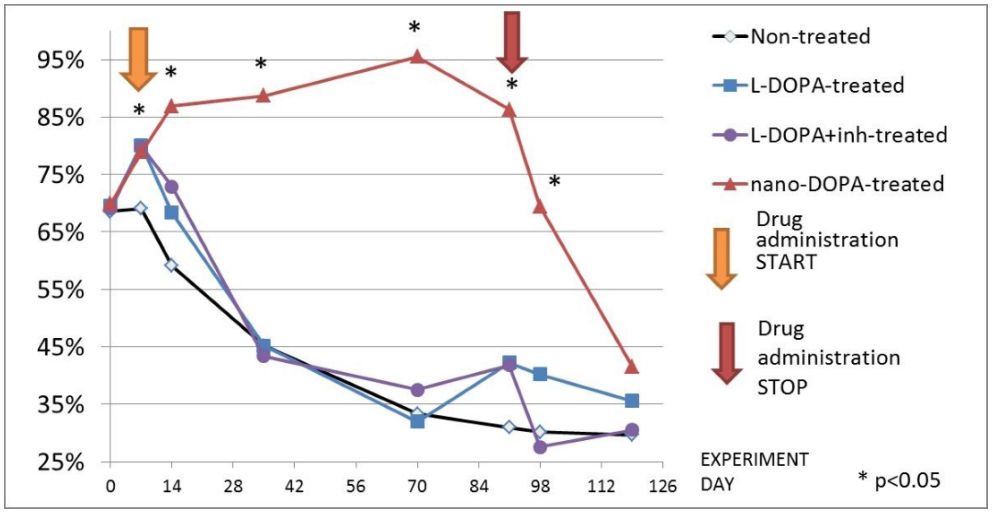

Fig. 3. Placing task scores obtained in 24 hours after drug administration; score dynamics observed during the treatment period and after discontinuation of the drug. Nano-DOPA-treated group's results were significantly higher than all other PD positive groups' results during the whole treatment period even 24 hours after the regular drug administration ($p<0.05$). The results are shown in % of the intact control group score. Number of animals in each group is 12.

endurance as measured in this test.

The open field test was carried out in treatment week 8 and did not detect any significant differences between the experimental groups. Thus, neither the PD model, nor the drug treatment affected the animals' cognition and locomotor activity as assessed by this test.

The footfault asymmetry test was performed in treatment week 4 and showed that all PD-positive groups differed significantly from the intact control group, except for the nano-DOPA treated group, where the scores were similar to those of intact control animals.

The index of step faults for the contralateral (left) paw was significantly higher in the groups that received no treatment or were treated with L-DOPA or L-DOPA+inh than in the intact control group; there were no significant differences for the ipsilateral (right) paw. The asymmetry factor for the left paw placement was as follows: intact control, -0.2±1.8%; non-treated, 5.5±4.2%; L-DOPA, 6.8±5.0%; L-DOPA+inh, 3.9±3.1%, and nano-DOPA, -0.7±2.9. The results of animals treated with nano-DOPA did not differ from those of the intact control group ones in both for the right and left paw (Fig. 4).

The asymmetry of paw placing was observed in all PD-positive rats except the group treated with nano-DOPA. This confirms that nano-DOPA therapy restored the motor function in PD-positive rats, as also suggested by the results of the placing task test. The results of both tests agreed completely and demonstrated the efficacy of nano-DOPA in the symptomatic treatment of PD.

SUMMARY

1) Standard L-DOPA-based drugs (L-DOPA and L-DOPA+benserazide) applied intranasally in the dose of 0.35 mg/kg were effective in therapeutic PD treatment only in the single-use format.

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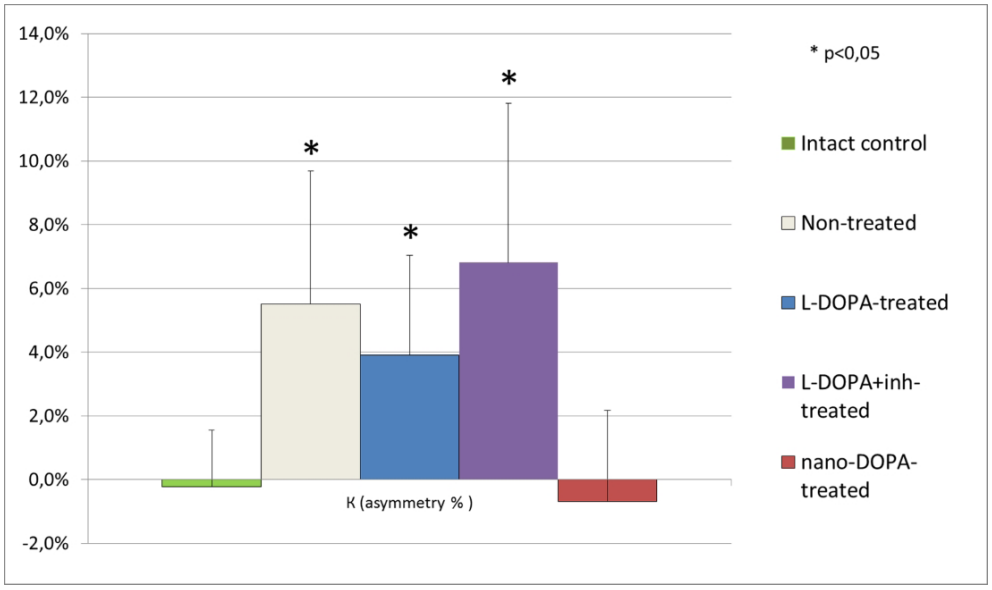

Fig. 4. The asymmetry factor (%) in the footfault asymmetry test performed 30 minutes after drug administration. Nano-DOPA-treated group's result does not differ from the intact control group's one, while all other PD positive animal groups show significantly higher footfault asymmetry score ($p<0.05$). The results are shown in % of the intact control group score; values are mean±standard deviation of 12 rats in each group.

Chronic treatment led to fast dose exhaustion.

2) Nano-DOPA preparation administered intranasally in the dose of 0.35 mg/kg (by L-DOPA) significantly improved the motor function in rats with 6-OHDA PD model throughout the whole treatment period.

3) Importantly, nano-DOPA preparation was demonstrated to have a prolonged effect: it was still effective in restoring the motor function 24 hours after previous administration and in one week after discontinuation of the drug.

CONCLUSION

Thus, the results of our study suggest that nano-DOPA administered intranasally exerts a long-lasting effect restoring the motor function in a rat PD model and may allow a considerable reduction of the effective drug dose and administration frequency in human patients. Nano-DOPA showed high efficacy while the same doses of standard drugs provided no significant effect starting from the second week of treatment and represents a promising agent for potential chronic administration in the clinical practice of the Parkinson's disease therapy.

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