DRUG DEVELOPMENT IN PARKINSON'S DISEASE: FROM EMERGING MOLECULES TO INNOVATIVE DRUG DELIVERY SYSTEMS

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ABSTRACT

Current treatments for Parkinson's disease (PD) are aimed at addressing motor symptoms but there is no therapy focused on modifying the course of the disease. Successful treatment strategies have been so far limited and brain drug delivery remains a major challenge that restricts its treatment. This review provides an overview of the most promising emerging agents in the field of PD drug discovery, discussing improvements that have been made in brain drug delivery for PD. It will be shown that new approaches able to extend the length of the treatment, to release the drug in a continuous manner or to cross the blood brain barrier and target a specific region are still needed.

Overall, the results reviewed here show that there is an urgent need to develop both symptomatic and disease-modifying treatments, giving priority to neuroprotective treatments. Promising perspectives are being provided in this field by rasagiline and by neurotrophic factors like glial cell line-derived neurotrophic factor. The identification of disease-relevant genes has also encouraged the search for disease-modifying therapies that function by identifying molecularly-targeted drugs. The advent of new molecular and cellular targets like α-synuclein, leucine-rich repeat serine/threonine protein kinase 2 or parkin, among others, will require innovative delivery therapies. In this regard, drug delivery systems (DDS) have shown great potential for improving the efficacy of conventional and new PD therapy and reducing its side effects. The new DDS discussed here, which include microparticles, nanoparticles and hydrogels among others, will probably open up possibilities that extend beyond symptomatic relief. However, further work needs to be done before DDS become a therapeutic option for PD patients.

Keywords: Drug delivery systems, Parkinson's disease, Symptomatic and Disease modifying treatments

Abbreviations: Parkinson's disease (PD), drug delivery systems (DDS), dopamine (DA), levodopa (L-DOPA), blood brain barrier (BBB), central nervous system (CNS), neurotrophic factors (NF), glial cell line-derived neurotrophic factor (GDNF), microparticles (MP), nanoparticles (NP), poly(lactic-co-glycolic acid) (PLGA), polyethylene glycol (PEG), 6-hydroxydopamine, (6-OHDA), 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

1 INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting 1.5% of the global population over 65 years of age. Pathologically, PD is characterized by the progressive degeneration of the nigrostriatal dopaminergic system, which causes dopamine (DA) loss. Symptomatically, PD is marked by the difficulty to perform coordinated movements. The core features are bradykinesia, resting tremor and rigidity. PD patients suffer from non-motor symptoms such as sleep disorders, neuropsychiatric issues and cognitive dysfunction [1, 2]. The current treatment, based on a DA replacement strategy, consists of the oral administration of the DA precursor levodopa (L-DOPA). However, its long-term administration produces severe detrimental L-DOPA-associated motor complications [3]. There is compelling evidence that L-DOPA-derived issues are associated with short-acting dopaminergic agents that induce pulsatile stimulation of the receptors [4, 5]. A continuous stimulation may result in better tolerability and fewer side effects [3, 5]. Thus, new approaches that can extend the length of the treatment or release the drug in a continuous manner are needed. Moreover, while motor effects of the disorder are, at least partially, being addressed with L-DOPA, alleviating non-motor symptoms is actually an unmet need. However, the development of a disease-modifying strategy able to slow or stop disease progression has become a priority.

Crossing the blood brain barrier (BBB) remains a key obstacle in the development of effective PD treatments, as only small molecules with appropriate lipophilicity, molecular weight and charge are able to diffuse from blood into the central nervous system (CNS). Approaches to transport therapeutic molecules across the BBB have recently been reviewed by Gabathuler [6]. The most accepted method used to increase the transport of therapeutics from blood into the brain is the physiological approach which takes advantage of the receptor transcytosis capacity. Other strategies include drug manipulation, BBB disruption and finding alternatives routes for drug delivery [7]. In order to solve brain delivery issues, there has been growing interest in the development of micro and nanosystems for the administration of drugs into the brain tissue.

This review provides a concise overview of the most promising emerging agents in the field of drug discovery for PD. The advances made in the last 5 years in brain

drug delivery for PD treatment are also discussed with the aim of improving both conventional and new PD therapies.

2 SYMPTOMATIC AND DISEASE MODIFYING TREATMENTS FOR PD UNDER INVESTIGATION

Drug development in PD is currently focused on: (i) reformulation of existing drugs (e.g. novel approaches for DA drug delivery including novel extended-release oral formulations), (ii) reposition of compounds approved for other indications such as the antiepileptic topiramate and (iii) development of novel small molecules and gene and cell-based approaches [8].

Most of these strategies are aimed at addressing motor symptom control and far fewer are focused on alleviating non-motor symptoms or on modifying the course of the disease [8]. For instance, at present there are 32 and 30 clinical trials open in phase III and IV respectively and none of these is evaluating neuroprotective strategies (source ClinicalTrials.gov). Regarding symptomatic therapy, rasagiline deserves special attention, as 3 of the 32 phase III trials and 7 of the 30 phase IV trials are evaluating its efficacy on different aspects of the disease such as apathy, depression, hyposmia, sleep disturbances, emotion or mood among other. It has recently been described that rasagiline may delay disease progression [9]. However, the clinical significance of these results is controversial. In this sense, there is one on-going Phase III clinical trial to evaluate rasagiline efficacy as a monoamine oxidase B inhibitor, and it is a candidate for phase II or III neuroprotective studies due to its anti-oxidant and anti-apoptotic properties (source ClinicalTrials.gov).

Concerning neuroprotective treatments, the efficacy of neurotrophic factors (NF) such as glial cell line-derived neurotrophic factor (GDNF) or neurturine have been assessed in phase I and II clinical trials (revised in [10-12]. Their delivery to the brain remains a key issue and multiple approaches are being explored to overcome this problem (see the section 3.2.2). On the other hand, the identification of disease-relevant genes has encouraged the search for disease-modifying therapies by identifying molecularly-targeted drugs. New agents targeting α-synuclein, leucine-rich repeat serine/threonine protein kinase 2 and parkin appear to be the most promising

approaches. However, these discoveries are just the first step toward new treatments [13].

It is expected that the advent of new molecular and cellular targets, together with advances in genomic and proteomic technologies, will increase the discovery of novel pharmaceutical compounds for PD treatment. However, many of the new developments will be biological therapies like proteins, cells or genes that will require innovative delivery methods.

3 DRUG DELIVERY APPROACHES TO IMPROVE PD TREATMENT

As described above, a list of PD therapies has been identified. However, drug delivery to the CNS remains a challenge in the development of effective treatments for PD. In order to solve brain delivery issues, there has been growing interest in the development of micro and nanosystems able to enhance brain delivery of drugs. DDS are capable of improving the pharmacological and therapeutic properties of conventional and new drugs, reducing their side effects. In the context of PD, DDS can be used either for local treatment administered directly into the brain or for systemic delivery for targeted action in the CNS.

DDS can be biodegradable or non-biodegradable depending upon the nature of the polymer or the material used for its preparation. Among DDS, hydrogels and polymeric or lipid microparticles (MP) and nanoparticles (NP) seem to be the most effective in providing neuroprotection and facilitating the delivery of drugs and small molecules to the brain (Figure 1).

In this section, the studies published in the last 5 years using DDS for PD treatment are reviewed (Table 1 and 2).

3.1 SYSTEMIC DDS IN PD

As far as systemic administration is concerned, it is important to consider not only the net delivery of the agent to the CNS, but also the drug ability to target the specific brain region (Table 1 and Figure 2).

3.1.1 DSS FOR DOPAMINE DELIVERY

DA, due to its hydrophilic feature and its high hydrogen bonding potential, does not cross the BBB and its brain administration constitutes a challenge.

De Giglio *et al.*, [14] published DA-loaded chitosan NP (DA-Cs-NP) formulation and characterization. Chitosan was chosen due to its high loading and good delivery ability for hydrophilic molecules. *In vitro* studies confirmed that DA-Cs-NP were less cytotoxic than the free-drug. An improvement in DA transport across the cells and oxygen reactive species reduction after 3 hours was observed. *In vivo* microdialysis studies administrating intraperitoneal DA-Cs-NP demonstrated a dose- and time-dependent striatal DA level increase [15].

An innovative tendency in drug development is the combination of a fluorescent compound with a therapeutic drug for simultaneous diagnosis and treatment. In this sense, Malvindi *et al.,* [16] combined fluorescence quantum rods with an ester bond with succinyl DA which can be hydrolyzed by cellular enzymes to release the prodrug inside the cells. This DDS was covered with a galactose shell which can be recognized by the GLUT-1 transporters to mediate its diffusion across the BBB. *In vitro* studies confirmed a high NP internalization due to the interaction between the sugar and the GLUT-1 transporters. MTT viability tests confirmed that the carbohydrate shell significantly improved NP biocompatibility compared to the non-coated nanocrystals. The ability to preserve all these properties *in vivo* is currently being tested in murine models.

DA have recently been conjugated with different aminoacid derivatives, used as prodrug molecular carriers to target the brain by using their ability to interact with the large aminoacid transporter I (LAT I) [17]. DA combined with phenylalanine derivative presented the highest affinity for LAT I receptor and the best brain uptake after intravenous administration. However, its intraperitoneal injection did not significantly increase DA striatal levels compared with L-DOPA treatment.

3.1.2 DDS FOR LEVODOPA DELIVERY

L-DOPA, a prodrug which is converted into DA in the brain, is the gold standard option in PD treatment. However, the oral bioavailability of this compound is low and has to be combined with carbidopa to avoid its peripheral degradation.

D´Aurizio *et al.* [18] synthesized the prodrug l-dopa-α-lipoic acid (LD-DA), a compound with longer plasma half-life than L-DOPA due to its lower susceptibility toward enzymatic conversion and higher lipophilicity that facilitates its delivery in the CNS. LD-DA was encapsulated in poly(lactic-co-glycolic acid) (PLGA) MP (LD-DA-MP) obtaining successful prodrug enzymatic and chemical hydrolysis prevention, and presenting sustained release via Fickian diffusion for up to more than one week. *In vivo* LD-DA-MP subcutaneous administration induced high levels of striatal DA during 4 days.

Ren *et al*., [19] encapsulated L-DOPA methyl ester, a highly soluble prodrug hydrolysable by plasma esterases, together with benserazide, a peripheral decarboxylase inhibitor in PLGA MP (LP-be-MP). MP subcutaneously administered improved motor function and stepping of the lesioned forepaw in the 6-hydroxydopamine (6-OHDA) PD rat model. LP-be-NP were also able to significantly reduce the axial, limb, orolingual and locomotive L-DOPA-induced abnormal involuntary movements or dyskinesia in the same PD model, compared to the free drug [20]. L-DOPA-induced dyskinesia molecular markers were significantly reduced in the rats presenting involuntary movements and treated with LP-be-NP.

Sharma *et al.* [21] developed a system with L-DOPA encapsulated in chitosan NP (LP-Cs-NP) and incorporated in a thermo-reversible Pluronic F127 gel for intranasal delivery. Polycation was used due to its mucoadhesive properties in addition to its ability to improve drug absorption on nasal mucosa by opening transiently tight junctions between epithelial cells and delaying mucociliary clearance. *In vivo* studies demonstrated that intranasally administered LP-Cs-NP significantly increased the drug brain content. LP-Cs-NP incorporation into the gel facilitated administration of the formulation and increased NP residence time in the nasal cavity. However, gel viscosity reduced NP migration, reducing its uptake, as the NP dispersed in saline solution intranasally administered elicited higher drug brain levels compared to the gel-embedded NP.

3.1.3 DDS FOR AGONIST DELIVERY

The use of DA structural analogs is another therapeutic strategy to provide continuous DA receptor stimulation. Most agonists present low bioavailability when they are orally administered as free drugs and they have to be administered daily in

several doses, since their effect lasts only a short time. They are thus good candidates for being formulated in DDS. With this aim, Wang *et al.*, [22] developed rotigotine loaded PLGA MP (Ro-MP) for the continuous release of this D1/D2/D3 DA agonist. The pharmacokinetic study revealed high and stable plasma and striatal drug levels for up to 14 days, as determined by microdialysis in 6-OHDA-lesioned rats. Ro-MP intramuscularly injected combined with pulsatile L-DOPA treatment induced significantly less dyskinesias in rats than the pulsatile L-DOPA monotherapy, although both treatments exerted a similar therapeutic motor effect. The toxicity of MP loaded with different drug concentrations was further evaluated in a 3-month study in monkeys. The toxicological effects were associated with rotigotine pharmacodynamic properties and with the foreign body reaction against PLGA and carboxymethylcellulose sodium, used as MP vehicle. The authors concluded that, at the end of the study, MP exhibited high safety in the monkey model. Toxicological findings recovered to a normal level except for adrenal gland vacuolar degeneration [23].

Regnier-Deplace *et al.,* [24] encapsulated the D1/D2 DA receptor agonist apomorphine in PLGA MP. The labile drug was effectively protected against degradation during MP preparation. The formulation showed a constant drug release rate for about 10 days.

Tsai *et al.* [25] encapsulated apomorphine in solid lipid NP of tripalmitin and hydrogenated soybean phosphatidylcholine for oral administration. After NP characterization, pharmacokinetic studies were carried out in rats comparing the oral formulation administration with the intravenous drug injection. Drug bioavailability was increased with the NP at all times. Apomorphine concentrations in plasma, cerebellum, brainstem and striatum were higher in NP-treated rats. The formulation efficacy was evaluated *in vivo* according to its ability to improve parkinsonian rat rotational behavior. Animals treated with orally administered NP presented significant differences in the total rotation number compared to those treated with drug solution.

Azeem *et al*. [26] developed a nanoemulsion gel for ropinirole transdermal delivery. Pharmacokinetic studies using different nanoemulsions revealed significantly greater drug release, absorption and bioavailability from the DDS compared to the conventional gel and to the orally administered marketed drug tablet suspension. These effects were attributed to the hepatic first-pass metabolism bypass. The lack of

histological injuries on the rat skin after the nanoemulsion application supports the view that the formulation is safe. The efficacy of the formulations was tested by analyzing three biochemical markers of oxidative stress in the 6-OHDA-lesioned rat striatum. 6-OHDA induced a significant increase in thiobarbituric acid reactive substances and a significant decrease in reduced glutation levels and catalase activity. Biochemistry parameters were significantly reversed and improved after the transdermal administration of the nanoemulsion, which also presented significantly better results compared to oral administration of the drug in tablets.

Chitosan NP were developed for intranasal delivery of bromocriptine [27]. NP were characterized, presenting a size of 160 nm which is compatible with transcellular transport through olfactory neurons to the brain. The mucoadhesive strength was demonstrated *in vitro* by studying mucin binding efficiency to the NP. Intranasal administration of radiolabelled drug formulations compared to free solution allowed biodistribution and pharmacokinetic studies in mice, confirming a significant increase in drug brain uptake when the drug was encapsulated in the mucoadhesive NP, and direct transport to the brain. The efficacy of the DA agonist was tested in a haloperidol-induced PD animal model. Bromocriptine in solution or nanoencapsulated was administered through the nostrils 15 minutes after the oral administration of haloperidol. The agonist was able significantly to reverse catalepsy and akinesia induced by haloperidol, producing better results with the nanoencapsulated drug.

3.1.4 DDS FOR ANTIOXIDANT DELIVERY

The antioxidant drug tempol was encapsulated in PLGA NP. OX-26 antibody was conjugated to the particles (Te-OX-NP) to enhance NP CNS delivery. NP have a size of 80–110 nm, suitable for BBB permeation. *In vitro* studies confirmed that antibody addition increased NP incorporation by the cells. Cell viability studies established that Te-OX-NP were more effective in preventing cell death induced by toxics substances than Te-NP or than the free drug in solution [28].

3.1.5 DDS FOR PROTEIN INHIBITORS, PEPTIDES AND NEUROTROPHIC FACTOR DELIVERY

The second generation monoamine oxidase MAO-B inhibitor rasagiline was encapsulated in PLGA MP. *In vitro* release studies demonstrated constant rasagiline

release for two weeks and a neuroprotective effect in neuronal-like cells treated with hydrogen peroxide. MP efficacy was tested in the rotenone-induced PD model. Although non-statistically significant differences were found between the free drug and MP regarding behavioral parameters, the controlled rasagiline release allowed a better administration regimen, which is in itself one of the goals of encapsulation [29].

Hu *et al.* [30] conjugated biodegradable PEG-PLGA NP with lactoferrin (Lf-NP), a glycoprotein whose receptor transports Lf across the BBB in different species, obtaining a DDS with a particle size around 90 nm suitable for brain capillary cell endocytosis. *In vitro* studies encapsulating a fluorescence probe demonstrated that NP incorporation into the cells was mediated by clathrin-related endocytosis. After the intravenous administration through the mouse caudal vein of the probe encapsulated in Lf-NP or in normal NP, three times more fluorescent probe was present in the substantia nigra and striatum of Lf-NP injected animals. However, Lf-NP administration caused ?mild transient acute? inflammatory reactions in liver, spleen and kidney. Lf-NP were further used for encapsulation of urocortin, which is a drug able to provide long-term nigrostriatal function restoration. Ur-Lf-NP intravenously administered significantly attenuated 6-OHDA-induced lesion evaluated using motor test and histological studies. This group also explored the intranasal drug delivery. For this purpose, PEG-PLGA NP were conjugated to odorranalectin (Ol-NP) to improve nose-to-brain drug delivery [31]. *In vitro* hemagglutination test studies using red blood cells confirmed that the lectin maintained its bioactivity after NP formulation. The biodistribution of Ol-NP compared to normal NP was determined by encapsulating a fluorescent dye after intranasal administration in mice. Ol-NP fluorescence intensity in the brain was statistically stronger than the non-coated NP in the first 4 to 8 hours after administration. Urocortin was also used as drug model to test this system's efficacy. Urocortin encapsulation in Ol-NP and its intranasal administration enhanced the drug's neuroprotective effects as confirmed in hemiparkinsonian 6-OHDA rats.

In contrast to conventional drugs focused on relieving PD symptoms, neurotrophic factors have been proposed as an alternative therapy to prevent or slow down disease progression. For this purpose, nerve growth factor was adsorbed on poly(butylcyanocrylate) NP coated with polysorbate-80 to facilitate the brain carrier transport. NP intravenously administered significantly increased GF brain concentration

[32] and induced a significant PD symptom reduction in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model.

Polymeric NP are being investigated as potential non-viral vectors for efficient brain-targeting gene delivery. With this aim, Huang *et al.,* used PANAM/PEG NP coated with Lf and encapsulating GDNF gene. Multiple NP intravenous administration presented neuroprotective effects in two different PD animal models, as confirmed by the significantly improved locomotor activity, reduced dopaminergic neuronal loss and enhanced monoamine neurotransmitter levels of the lesioned rats (33, 34).

3.2 LOCAL DDS IN PD

The direct drug injection to the brain was first used owing to the difficulty of targeting specific brain regions via the vascular route. Drug delivery directly to the brain can circumvent the BBB and release the drug directly to an intracranial target. DDS can be injected by stereotaxy in discrete, precise and functional brain areas without damaging the surrounding tissue. Treatment strategies aimed at local drug delivery have been markedly used since the advent of polymer-based particulate systems (Table 2 and Figure 2).

3.2.1 DDS FOR DOPAMINE DELIVERY

In order to restore striatal DA content, Pillay *et al.* developed an intracranial nano-enabled scaffold device (NESD) for DA site-specific delivery [35]. The NESD was modulated through biometric simulation and computational prototyping to produce a binary crosslinked alginate scaffold embedding stable DA-loaded cellulose acetate phthalate NP. The NESD improved DA delivery to the brain in a rat model. DA concentrations in plasma were minimal and could reduce the profile of side-effects compared to orally administered L-DOPA due to peripheral conversion of L-DOPA to DA.

3.2.2 DDS FOR NEUROTROPHIC FACTOR DELIVERY

The potential of GDNF to alleviate symptoms and to slow or even halt PD progression is widely acknowledged [11, 12]. Equally, it is generally accepted that successful translation of GDNF to clinical practice will first require resolving the crucial delivery issues posed by NF. Different strategies including direct gene transfer using

recombinant virus (For review see [12]) and polymeric MP has been proposed to improve protein delivery to the brain [11, 36]. All research using GDNF-PLGA-MP in rat PD models reported animal functional recovery together with increase in striatal DArgic innervation [36, 37]. In the next step, our recent studies evaluating GDNF-MP efficacy in a primate PD model demonstrated that long-term controlled brain delivery of microencapsulated GDNF induced functional and structural recovery of the dopaminergic nigrostriatal pathway (Unpublished results). No apparent safety concern after GDNF-MP putaminal delivery was observed. None of the macaques showed weight loss, a common side effect observed after GDNF administration. These studies demonstrated that MP are an efficient vehicle for sustained neurotrophic factor delivery to the brain.

In order to regulate the NF release from MP, Gujral *et al.* developed PLGA/collagen MP that encapsulated GDNF fused with collagen binding peptide [38]. MP with an outer layer of PLGA and an inner layer of collagen were able to control the release of the NF from the MP. Promising results were obtained *in vitro*, but this strategy needs *in vivo* validation before being applied in clinical settings.

Combinatorial approaches delivering several growth factors at relevant therapeutic times and locations without toxic side effects have started to be researched. For instance, Herran *et al*. [39] investigated the neuroregenerative potential of PLGA microencapsulated VEGF, GDNF and their combination in a severely lesioned rat model. Both GDNF alone and the combination of GDNF and VEGF demonstrated regenerative effects. In a different study, Lampe *et al.* [40] assessed protein distribution and glial response after dual GDNF and BDNF delivery to the brain via PLGA MP patterned within a degradable polyethylene glycol (PEG)-based hydrogel. PEG-based hydrogels loaded with BDNF or GDNF locally released the respective NFs and were biocompatible with the brain tissue. Further studies are required to characterize this strategy's effectiveness in brain regeneration.

PLGA-MP encapsulating NF has also been proposed as scaffold for stem cell therapy/delivery. Recently, the efficacy of MIAMI cells, a mesenchymal stem cell subpopulation conveyed by neurotrophin-3 releasing MP, was assessed in a PD rat model. Functional recovery together with nigrostriatal pathway protection/repair was observed [41].

Plasmid DNA encoding for GDNF was compacted into DNA NP using polycations to form colloidally stable NP. NP were then injected into the denervated striatum of rats with unilateral 6-OHDA lesion in order to enhance grafted DA neuron survival. Data suggest that compacted pGDNF could transfect cells to overexpress GDNF protein at levels that provide support for grafted stem cells [42].

3.2.3 OTHER DDS

The sialic acid was embedded in a poly (N(2-hydroxypropyl) methacrylamide hydrogel, developed to interact with the damaged neural tissue and to promote tissue formation and axonal regeneration. The sialyl lactose hydrogel was characterized *in vitro* and implanted into the brain in an experimental PD model to examine its biofunctionality [43]. The hydrogel provided a three-dimensional substrate for cell migration, tissue remodeling and angiogenesis, demonstrating great potential.

4 CONCLUDING REMARKS

There is an urgent need to develop both symptomatic and disease-modifying treatments, the neuroprotective ones currently being considered the most important. However, most of the new drugs under clinical trials are related to last generation dopaminergic agonist administration. In addition, in the research field of DDS for systemic delivery, a considerable proportion of the recent studies still deal with the treatment of motor symptoms and only a few studies focus on the reversal of the neurodegenerative process (Figure 2).

New extended-release formulations are a promising approach to improve L-DOPA therapy. In this respect, research efforts have focused on new strategies to more effectively deliver drugs to the CNS. DDS might improve L-DOPA efficacy as well as the value of the new molecules currently being tested in clinical trials. However, while the efficacy of some DDS is currently being assessed in clinical trials for cancer and some of them have reached the market, DDS for PD are still in their infancy. It is remarkable that most of the DDS studies discussed here have been performed in rodent models of the disease and only a few of them have been carried out in preclinical animal models, like parkinsonian monkeys, which are the most clinically relevant of all available models. At present, the more advanced innovative treatments for PD are transdermal patches and extended release tablets. Thus, although initial progress has

been made, significant additional work is required before one of these promising strategies will be available in clinical practice.

It is likely that future approaches in research on PD treatment will combine continuous dopaminergic stimulation obtained with L-DOPA derivatives or DA agonist, together with antioxidant molecules and a cocktail of different NF, probably all of them combined in a smart DDS conjugated with specific ligands to deliver all these protected compounds in a controlled and extended manner in the basal ganglia.

New animal models able to completely recapitulate all the disease features, and a general consensus to use the same model to compare novel formulation efficacy, are also needed. Additionally, new clinical trial designs that include objective end-points for studies testing the efficacy of neuroprotective agents are required. To this end, innovative clinical trial designs have been proposed such as the delayed-start and futility designs. Finally, a better understanding of PD's etiology together with the development of effective biomarkers would also help us to make progress in developing new forms of treatment.

REFERENCES

[1]. Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. Neuron 2003 ;39(6):889-909.

[2]. Pandya M, Kubu CS, Giroux ML. Parkinson disease: not just a movement disorder. Cleveland Clinic Journal of Medicine 2008;75(12):856-64.

[3]. Chaudhuri KR, Rizos A, Sethi KD. Motor and nonmotor complications in Parkinson's disease: an argument for continuous drug delivery? Journal of neural transmission 2013; 10.1007/s00702-013-0981-5.

[4]. Stocchi F. Continuous dopaminergic stimulation and novel formulations of dopamine agonists. Journal of neurology 2011;258(Suppl 2):S316-22.

[5]. Obeso JA, Olanow W. Continuing efforts to obtain continuous delivery of levodopa. Movement disorders 2011;26(12):2149-50.

[6]. Gabathuler R. Blood-brain barrier transport of drugs for the treatment of brain diseases. CNS & Neurological Disorders Drug Targets 2009;8(3):195-204.

[7]. Misra A, Ganesh S, Shahiwala A, Shah SP. Drug delivery to the central nervous system: a review. Journal of Pharmacy & Pharmaceutical Sciences 2003;6(2):252-73.

[8]. Meissner WG, Frasier M, Gasser T, Goetz CG, Lozano A, Piccini P, et al. Priorities in Parkinson's disease research. Nature Reviews Drug Discovery 2011;10(5):377-93.

[9]. Olanow CW, Rascol O, Hauser R, Feigin PD, Jankovic J, Lang A, et al. A double-blind, delayed-start trial of rasagiline in Parkinson's disease. New England Journal of Medicine 2009;361(13):1268-78.

[10]. Ramaswamy S, Soderstrom KE, Kordower JH. Trophic factors therapy in Parkinson's disease. Progress in Brain Research 2009;175:201-16.

[11]. Garbayo E, Ansorena E, Blanco-Prieto MJ. Brain drug delivery systems for neurodegenerative disorders. Current Pharmaceutical Biotechnology 2012;13(12):2388-402.

[12]. Coune PG, Schneider BL, Aebischer P. Parkinson's disease: gene therapies. Cold Spring Harbor Perspectives in Medicine 2012;2(4):a009431.

[13]. Sherer TB, Chowdhury S, Peabody K, Brooks DW. Overcoming obstacles in Parkinson's disease. Movement disorders 2012;27(13):1606-11.

[14]. De Giglio E, Trapani A, Cafagna D, Sabbatini L, Cometa S. Dopamine-loaded chitosan nanoparticles: formulation and analytical characterization. Analytical and Bioanalytical Chemistry 2011;400(7):1997-2002.

[15]. Trapani A, De Giglio E, Cafagna D, Denora N, Agrimi G, Cassano T, et al. Characterization and evaluation of chitosan nanoparticles for dopamine brain delivery. International Journal of Pharmaceutics 2011;419(1-2):296-307.

[16]. Malvindi MA, Di Corato R, Curcio A, Melisi D, Rimoli MG, Tortiglione C, et al. Multiple functionalization of fluorescent nanoparticles for specific biolabeling and drug delivery of dopamine. Nanoscale 2011;3(12):5110-9.

[17]. Peura L, Malmioja K, Huttunen K, Leppanen J, Hamalainen M, Forsberg MM, et al. Design, Synthesis and Brain Uptake of LAT1-Targeted Amino Acid Prodrugs of Dopamine. Pharmaceutical Research 2013; 10.1007/s11095-012-0966-3.

[18]. D'Aurizio E, Sozio P, Cerasa LS, Vacca M, Brunetti L, Orlando G, et al. Biodegradable microspheres loaded with an anti-Parkinson prodrug: an in vivo pharmacokinetic study. Molecular Pharmacology 2011;8(6):2408-15.

[19]. Ren T, Yang X, Wu N, Cai Y, Liu Z, Yuan W. Sustained-release formulation of levodopa methyl ester/benserazide for prolonged suppressing dyskinesia expression in 6-OHDA-leisoned rats. Neuroscience Letters 2011;502(2):117-22.

[20]. Yang X, Zheng R, Cai Y, Liao M, Yuan W, Liu Z. Controlled-release levodopa methyl ester/benserazide-loaded nanoparticles ameliorate levodopa-induced dyskinesia in rats. International Journal of Nanomedicine 2012;7:2077-86.

[21]. Sharma S, Lohan S, Murthy RS. Formulation and characterization of intranasal mucoadhesive nanoparticulates and thermo-reversible gel of levodopa for brain delivery. Drug Development and Industrial Pharmacy 2013; 10.3109/03639045.2013.789051.

[22]. Wang A, Wang L, Sun K, Liu W, Sha C, Li Y. Preparation of rotigotine-loaded microspheres and their combination use with L-DOPA to modify dyskinesias in 6-OHDA-lesioned rats. Pharmaceutical Research 2012;29(9):2367-76.

[23]. Tian J, Du G, Ye L, Yu X, Zhang J, Wang H, et al. Three-month subchronic intramuscular toxicity study of rotigotine-loaded microspheres in Cynomolgus monkeys. Food and Chemical Toxicology 2013;52:143-52.

[24]. Regnier-Delplace C, Thillaye du Boullay O, Siepmann F, Martin-Vaca B, Degrave N, Demonchaux P, et al. PLGA microparticles with zero-order release of the

labile anti-Parkinson drug apomorphine. International Journal of Pharmaceutics 2013;443(1-2):68-79.

[25]. Tsai MJ, Huang YB, Wu PC, Fu YS, Kao YR, Fang JY, et al. Oral apomorphine delivery from solid lipid nanoparticles with different monostearate emulsifiers: pharmacokinetic and behavioral evaluations. Journal of Pharmaceutical Sciences 2011;100(2):547-57.

[26]. Azeem A, Talegaonkar S, Negi LM, Ahmad FJ, Khar RK, Iqbal Z. Oil based nanocarrier system for transdermal delivery of ropinirole: a mechanistic, pharmacokinetic and biochemical investigation. International Journal of Pharmaceutics 2012;422(1-2):436-44.

[27]. Md S, Khan RA, Mustafa G, Chuttani K, Baboota S, Sahni JK, et al. Bromocriptine loaded chitosan nanoparticles intended for direct nose to brain delivery: Pharmacodynamic, Pharmacokinetic and Scintigraphy study in mice model. European Journal of Pharmaceutical Sciences 2012;48(3):393-405.

[28]. Carroll RT, Bhatia D, Geldenhuys W, Bhatia R, Miladore N, Bishayee A, et al. Brain-targeted delivery of Tempol-loaded nanoparticles for neurological disorders. Journal of Drug Targeting 2010;18(9):665-74.

[29]. Fernandez M, Barcia E, Fernandez-Carballido A, Garcia L, Slowing K, Negro S. Controlled release of rasagiline mesylate promotes neuroprotection in a rotenone-induced advanced model of Parkinson's disease. International Journal of Pharmaceutics 2012;438(1-2):266-78.

[30]. Hu K, Shi Y, Jiang W, Han J, Huang S, Jiang X. Lactoferrin conjugated PEG-PLGA nanoparticles for brain delivery: preparation, characterization and efficacy in Parkinson's disease. International Journal of Pharmaceutics 2011;415(1-2):273-83.

[31]. Wen Z, Yan Z, Hu K, Pang Z, Cheng X, Guo L, et al. Odorranalectin-conjugated nanoparticles: preparation, brain delivery and pharmacodynamic study on Parkinson's disease following intranasal administration. Journal of Controlled Release 2011;151(2):131-8.

[32]. Kurakhmaeva KB, Djindjikhashvili IA, Petrov VE, Balabanyan VU, Voronina TA, Trofimov SS, et al. Brain targeting of nerve growth factor using poly(butyl cyanoacrylate) nanoparticles. Journal of Drug Targeting 2009;17(8):564-74.

[33]. Huang R, Han L, Li J, Ren F, Ke W, Jiang C, et al. Neuroprotection in a 6-hydroxydopamine-lesioned Parkinson model using lactoferrin-modified nanoparticles. Journal of Gene Medicine 2009;11(9):754-63.

[34]. Huang R, Ke W, Liu Y, Wu D, Feng L, Jiang C, et al. Gene therapy using lactoferrin-modified nanoparticles in a rotenone-induced chronic Parkinson model. Journal of the Neurological Sciences 2010;290(1-2):123-30.

[35]. Pillay S, Pillay V, Choonara YE, Naidoo D, Khan RA, du Toit LC, et al. Design, biometric simulation and optimization of a nano-enabled scaffold device for enhanced delivery of dopamine to the brain. International Journal of Pharmaceutics 2009;382(1-2):277-90.

[36]. Garbayo E, Montero-Menei CN, Ansorena E, Lanciego JL, Aymerich MS, Blanco-Prieto MJ. Effective GDNF brain delivery using microspheres, a promising strategy for Parkinson's disease. Journal of Controlled Release 2009;135(2):119-26.

[37]. Garbayo E, Ansorena E, Lanciego JL, Blanco-Prieto MJ, Aymerich MS. Long-term neuroprotection and neurorestoration by glial cell-derived neurotrophic factor microspheres for the treatment of Parkinson's disease. Movement disorders 2011;26(10):1943-7.

[38]. Gujral C, Minagawa Y, Fujimoto K, Kitano H, Nakaji-Hirabayashi T. Biodegradable microparticles for strictly regulating the release of neurotrophic factors. Journal of Controlled Release 2013; 10.1016/j.jconrel.2013.03.031.

[39]. Herran E, Ruiz-Ortega JA, Aristieta A, Igartua M, Requejo C, Lafuente JV, et al. In vivo administration of VEGF- and GDNF-releasing biodegradable polymeric microspheres in a severe lesion model of Parkinson's disease. European Journal of Pharmaceutics and Biopharmaceutics 2013; 10.1016/j.ejpb.2013.03.034.

[40]. Lampe KJ, Kern DS, Mahoney MJ, Bjugstad KB. The administration of BDNF and GDNF to the brain via PLGA microparticles patterned within a degradable PEG-based hydrogel: Protein distribution and the glial response. Journal of Biomedical Materials Research Part A 2011;96(3):595-607.

[41]. Delcroix GJ, Garbayo E, Sindji L, Thomas O, Vanpouille-Box C, Schiller PC, et al. The therapeutic potential of human multipotent mesenchymal stromal cells combined with pharmacologically active microcarriers transplanted in hemi-parkinsonian rats. Biomaterials. 2011;32(6):1560-73.

[42]. Yurek DM, Flectcher AM, Kowalczyk TH, Padegimas L, Cooper MJ. Compacted DNA nanoparticle gene transfer of GDNF to the rat striatum enhances the survival of grafted fetal dopamine neurons. Cell Transplantation 2009;18(10):1183-96.

[43]. Woerly S, Fort S, Pignot-Paintrand I, Cottet C, Carcenac C, Savasta M. Development of a sialic acid-containing hydrogel of poly[N-(2-hydroxypropyl)

methacrylamide]: characterization and implantation study. Biomacromolecules 2008;9(9):2329-37.

FIGURE LEGENDS

Figure 1: Schematic representation of the drug delivery systems under investigation for Parkinson's disease.

Figure 2: Pyramid representation of the percentage of studies published in the last five years using local or systemic drug delivery systems for Parkinson's disease.

Table 1: Studies published in the last 5 years using systemic drug delivery systems for Parkinson's disease treatment.

Drug	DDS	Material	Targeting ligand	Administration route	Animal model PD	Ref
Dopamine	NP	Chitosan				14
Dopamine	NP	Chitosan		Intraperitoneal		15
Succinyl Dopamine	Nanotool		Galactose			16
Dopamine	Molecular carrier		Aminoacid derivatives	Intraperitoneal		17
L-dopa-α-lipoic acid	MP	PLGA		Subcutaneous		18
Levodopa methyl ester + Benserazide (decarboxylase inhibitor)	MP	PLGA		Subcutaneous	6-OHDA	19
Levodopa methyl ester + Benserazide (decarboxylase inhibitor)	NP	PLGA		Subcutaneous	6-OHDA	20
Levodopa	NP thermoreversible gel	Chitosan		Intranasal		21
Rotigotine (agonist)	MP	PLGA		Intramuscular	6-OHDA	22

Rotigotine (agonist)	MP	PLGA		Intramuscular		23
Apomorphine (agonist)	MP	PLGA				24
Apomorphine (agonist)	Solid lipid nanoparticles	Tripalmitin hydrogenated soybean phosphatidilcholine		Oral	6-OHDA	25
Ropinirole (agonist)	Nanoemulsion			Transdermal	6-OHDA	26
Bromocriptine (agonist and antioxidant)	NP	Chitosan		Intranasal	Haloperidol	27
Tempol (antioxidant)	NP	PLGA	Ab OX-26 Transferrin receptor			28
Rasagiline (MAO-B inhibitor)	MP	PLGA		Intraperitoneal	Rotenone	29
Urocortin	NP	PEG-PLGA	Lactoferrin	Intravenous	6-OHDA	30
Urocortin	NP	PEG-PLGA	Odorranalectin	Intranasal	6-OHDA	31
NGF	NP	Poly butylcyanocrylate	Polysorbate-80	Intravenous	MPTP	32

GDNF	NP	PANAM/PEG	Lactoferrin	Intravenous	6-OHDA	33
GDNF	NP	PANAM/PEG	Lactoferrin	Intravenous	Rotenone	34

Table 2: Studies published in the last 5 years using systemic drug delivery systems for Parkinson's disease treatment.

DRUG	**MATERIAL**	**ANIMAL MODEL**	**REF**
Dopamine	Alginate scaffold embedding stable DA-loaded cellulose acetate phatalate nanoparticles	Healthy Sprague-Dawley rats	35
GDNF	PLGA	6-OHDA rat model	36 and 37
GDNF fused with collagen binding peptide	PLGA/collagen	In vitro	38
GDNF and BDNF	PLGA microparticles within a PEG-based hydrogel	In vitro	40
GDNF and VEGF	PLGA	6-OHDA rat model	39
Neurotrophin-3 and MIAMI stem cells	PLGA microparticles with a biomimetic surface	6-OHDA rat model	41
Plasmid GDNF	DNA compacted by polycations to form colloidally stable nanoparticles	6-OHDA rat model	42
Sialic Acid	Poly (N(2-hydroxypropyl) methacrylamide	6-OHDA rat model	43