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Brain delivery of microencapsulated GDNF induces functional and structural recovery in parkinsonian monkeys

Elisa Garbayo [a, b], Eduardo Ansorena [b, c], Hugo Lana [a, b], María del Mar Carmona-Abellan [d, e], Irene Marcilla [e], José Luis Lanciego [b, e, f], María Rosario Luquin [b, d, e, **], María José Blanco-Prieto [a, b, *]

[a] Pharmacy and Pharmaceutical Technology Department, University of Navarra, 31008 Pamplona, Spain
[b] Instituto de Investigación Sanitaria de Navarra (IDISNA), 31008 Pamplona, Spain
[c] Biochemistry and Genetics Department, University of Navarra, 31008 Pamplona, Spain
[d] Neurology Department, Clínica Universidad de Navarra, 31008 Pamplona, Spain
[e] Neuroscience Division, Center for Applied Medical Research (CIMA), University of Navarra, 31008 Pamplona, Spain
[f] Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), 28031 Madrid, Spain

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ABSTRACT

Glial cell line-derived neurotrophic factor (GDNF) remains the most potent neurotrophic factor for dopamine neurons. Despite its potential as treatment for Parkinson's disease (PD), its clinical application has been hampered by safety and efficacy concerns associated with GDNF's short *in vivo* half-life and with significant brain delivery obstacles. Drug formulation systems such as microparticles (MPs) may overcome these issues providing protein protection from degradation and sustained drug release over time. We therefore sought to evaluate the efficacy and safety of GDNF delivered via injectable biodegradable MPs in a clinically relevant model of PD and to investigate the mechanism contributing to their beneficial effects. MPs were injected unilaterally into the putamen of parkinsonian monkeys with severe nigrostriatal degeneration. Notably, a single administration of the microencapsulated neurotrophic factor achieved sustained GDNF levels in the brain, providing motor improvement and dopaminergic function restoration. This was reflected by a bilateral increase in the density of striatal dopaminergic neurons 9 months after treatment. Moreover, GDNF was retrogradely transported to the substantia nigra increasing bilaterally the number of dopaminergic and total neurons, regardless of the severe degeneration. GDNF-MP injection within the putamen elicited no adverse effects such as immunogenicity, cerebellar degeneration or weight loss. MPs are therefore a safe, efficient vehicle for sustained protein delivery to the brain, supporting the therapeutic benefit of GDNF when encapsulated within MPs for brain repair. Overall, these findings constitute important groundwork for GDNF-MP clinical development.

1. Introduction

Glial cell line-derived neurotrophic factor (GDNF) is the most potent dopaminergic factor described so far [1]. Numerous studies have demonstrated that this therapeutic protein is one of the few molecules that not only protects but also repairs dopaminergic neurons [2,3]. Thus, GDNF is one of the most promising candidates for the treatment of Parkinson's disease (PD), a neurodegenerative disorder characterized by the degeneration of the dopaminergic neurons in the substantia nigra (SN) and the consequent striatal dopamine depletion. While current PD therapies aim at attenuating motor symptoms related to dopamine deficiency, a GDNF-based approach seeks to prevent or slow down the degeneration of the dopaminergic neurons, something never achieved so far.

The clinical application of GDNF for PD treatment has been hampered by safety and efficacy concerns, many related to the brain delivery strategies attempted to date [4–7]. GDNF is a protein with a short *in vivo* half-life of approximately 34 h in the

* Corresponding author. Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, Irunlarrea 1, E-31080 Pamplona, Spain.
** Corresponding author. Department of Neurology, Clínica Universidad de Navarra, Av. Pío XII 36, Pamplona 31008, Spain.
E-mail addresses: rluquin@unav.es (M.R. Luquin), mjblanco@unav.es (M.J. Blanco-Prieto).

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cerebrospinal fluid [8] and 3–4 days into the brain [9]. Furthermore, this neurotrophic factor does not cross the blood brain barrier and often presents serious side effects when administered systemically, requiring an effective drug delivery strategy to reach the brain. Therefore, in order to use GDNF effectively as a therapeutic agent for PD, it is mandatory to develop a safe and effective brain delivery system. To that end, gene and cell therapy have been widely investigated in preclinical and clinical studies with moderate success [10,11].

To address the problem of delivery, drug formulation systems such as microparticles (MPs) represent another way to overcome the current limitations of therapeutic proteins [12,13]. MPs have proved to be an efficient system to improve the outcome of therapeutic proteins in the field of prostate cancer or pediatric growth hormone deficiency, and there are numerous accepted protein drug formulations based on biodegradable MPs already on the market [12]. However, drug delivery systems for PD are still in their infancy and there is no FDA-approved protein delivery product for PD at present. Remarkably, after nearly two decades of research, most of the studies involving drug delivery systems have been performed in rodent models of the disease and only a few of them have been carried out in clinically relevant animal models of PD (reviewed in Refs. [14,15]). In this regard, a valuable therapeutic approach could be GDNF microencapsulation in bioresorbable MPs made of polylactide-co (PLGA), a biomaterial approved by the FDA with a well-established track record as vehicle for the controlled delivery of proteins [13]. MP-based drug delivery systems may protect GDNF from degradation and release the protein in a sustained and controlled manner so that drug-related side effects can be minimized. The aim of this translational study was to demonstrate the efficacy and safety of GDNF delivered via MPs in a clinically relevant non-human primate model of PD. Notably, a single administration of microencapsulated GDNF within the putamen achieved sustained improvements of motor function in animals with severe nigrostriatal degeneration associated with restoration of the dopaminergic function. In addition, the treatment with GDNF-MPs did not elicit adverse effects such as immunogenicity, cerebellar degeneration or weight loss, providing preclinical validation of this approach. In summary, we demonstrate that the developed formulation is effective and safe, thus paving the way for clinical studies.

2. Materials and methods

2.1. Materials

PLGA with a monomer ratio (lactic acid/glycolic acid) of 50:50 Resomer® RG 503H (M_w: 34 kDa) was provided by Boehringer-Ingelheim (Ingelheim, Germany). Polyethylene glycol (PEG; M_w: 400), human serum albumin (HSA), bovine serum albumin (BSA), dimethylsulfoxide (DMSO), sodium azide and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were provided by Sigma-Aldrich (Barcelona, Spain). Dichloromethane and acetone were obtained from Panreac Química S.A. (Barcelona, Spain). Poly(vinyl alcohol) (PVA) 88% hydrolyzed (M_w: 125,000) was obtained from Polysciences, Inc. (Warington, USA). GDNF EMax Immunoassay System was provided by Promega Corporation (Madison, USA). PC-12 cells were purchased (ATCC® CRL-1721™). TOPRO-3 iodide was obtained from Molecular Probes. The following primary antibodies were used: mouse α Tyrosine Hydroxylase (TH) (Millipore # MAB5280), mouse α NeuN (Chemicon International #MAB377), rabbit α GDNF (Santa Cruz Biothechnology # sc-328), goat α sex determining region Y-box 2 (SOX2) (Neuromics # GT 15,098), mouse α glial fibrillary acidic protein (GFAP) (AbD Serotec # 4650-0309), rabbit α dopamine and cyclic AMP-regulated phosphoprotein (DARP32) (Cell Signalling # 2302), goat α calretinine (CR) (Millipore # AB1550).

2.2. Preparation and characterization of MPs containing GDNF

Human recombinant glycosylated GDNF expressed and purified by our group was used in these studies [16]. GDNF-loaded PLGA MPs were prepared through a solvent extraction/evaporation method using the Total Recirculation One Machine System (TROMS) [17,18]. Briefly, the organic solution composed of 2 ml of dichlorometane:acetone (3:1) containing 100 mg of Resomer RG 503H was injected through a needle with an inner gauge diameter of 0.17 mm at a ratio of 24 ml/min into the inner aqueous phase (200 μl). The inner aqueous phase contained 400 μg of GDNF in 10 mM phosphate, 50 mM sodium chloride (PBS), pH 7.9, 5 mg of HSA and 5 μl of PEG 400. Next, the primary emulsion (W1/O) was recirculated through the system for 3 min at a flow rate of 30 ml/min. The first emulsion was then injected into 30 ml of the external aqueous phase (W2) composed of 1.5% PVA. The injection through the needle with an inner gauge diameter of 0.50 mm resulted in the formation of a multiple emulsion (W1/O/W2), which was further homogenized by circulation through the system for 4 min. The W1/O/W2 emulsion was stirred at 1000 rpm at room temperature for at least 3 h to allow solvent evaporation and MPs formation. Finally, particles were washed with ultrapure water and freeze-dried. Lyophilized MPs were stored at 4 °C until further use. Blank-MPs (non-loaded with GDNF) were prepared under the same conditions, without GDNF.

Particle size and size distribution were measured by laser diffractometry using a Mastersizer-S. The amount of GDNF entrapped into the MPs and the *in vitro* release of GDNF from MPs was quantified by western blot and ELISA as previously described [19]. Regarding GDNF content into MPs, it was quantified after extracting the neurotrophic factor from MPs with DMSO. For the *in vitro* release studies, MPs were resuspended in PBS, pH 7.4 containing 0.1% BSA and 0.02% w/w sodium azide and incubation took place in rotating vials at 37 °C. At defined times ranging from 30 min to 60 days, samples were centrifuged at 25,000×*g*, for 15 min. Release profiles were expressed in terms of cumulative release, and plotted *versus* time. Bioactivity of MP-released GDNF was evaluated *in vitro* by determining PC-12 neurite outgrowth following growth factor treatment as previously described [20]. These cells differentiate to a neural phenotype in response to neurotrophic factors such as GDNF. PC-12 cells were plated onto a 12 well culture plate at a low density, 2×10^3 cells/cm^2 in 1 ml of culture media. The culture medium was supplemented 24 h later with 50 ng of GDNF released from MPs over 24 h, which had previously been quantified by ELISA. Neurite outgrowth was visualized after 7 days in culture under phase contrast illumination with a Leika DM IRB inverted microscope connected to a Hamamatsu ORCA-ER digital camera. PC-12 cells incubated with 50 ng/ml of hGDNF were used as a positive control of the technique. PC-12 cells incubated with medium supplemented with the released medium from Blank-MPs were used as negative control of the technique. Routine testing confirmed that PC-12 cells were free of mycoplasma during the entire study period.

2.3. Experimental design

Non-human primate studies comply with the ARRIVE guidelines. Animal handling was conducted in accordance with the European Council Directive 2010/63/UE as well as in agreement with the Society for Neuroscience Policy on the Use of Animals in Neuroscience Research. The experimental design (Fig. 1) was approved by the Ethical Committee for Animal Testing of the