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Lipid nanocarriers containing a levodopa prodrug with potential antiparkinsonian activity

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ABSTRACT

This paper describes the production, characterization and in vivo activity of lipid nanocarriers (LN) containing a levodopa prodrug (LD-PD) with therapeutic potential in Parkinson's disease. LD is the mainstay of the pharmacotherapy of Parkinson's disease. However, after a good initial response, motor fluctuations, dyskinesia and loss of efficacy, develop over time, partly due to oscillations in plasma and brain levels of the drug. LD-PD was produced with the aim of prolonging the pharmacological activity of LD. To improve solubility, and simultaneously provide a long lasting release and therapeutic efficacy, the prodrug was formulated in tristearin/lecithin LN. The obtained formulation was homogeneous in particle size and remained stable for up to 2 months from preparation. For the three different tested LD concentrations, namely 1.25, 2.5 and 5.0 mg/ml, the morphological characterization revealed no substantial differences between unloaded and LD-PD loaded LN. The calorimetric test showed an interaction between the lipid phase and the loaded prodrug. In vitro studies using the dialysis method and enzymatic degradation procedure showed that the LD-PD loaded LN provided a controlled prodrug release. Finally, two behavioural tests specific to akinesia (bar test) or akinesia/bradykinesia (drag test) performed in 6-hydroxydopamine hemilesioned mice (a model of Parkinson's disease) demonstrated that the LD-PD loaded LN attenuated parkinsonian disabilities, showing a slightly reduced maximal efficacy but a longer lasting action (up to 24 h) than an equal dose of LD. We conclude that LD-PD loaded LN may represent a future LD formulation useful in Parkinson's disease therapy.

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1. Introduction

Parkinson's disease is a neurodegenerative disorder associated with the loss of dopamine neurons in the nigrostriatal system [1]. Current therapy for Parkinson's disease is essentially symptomatic and the gold standard is the natural isomer of the immediate precursor to dopamine, L-3,4-Dihydroxyphenylalanine (L-dopa, LD) [2]. LD is readily transported across the blood–brain barrier (BBB) and converted to dopamine by aromatic L-amino acid decarboxylase. After a good initial response, various complications develop over the course of long-term therapy with LD [3]. Dyskinesia, in particular, is thought to partly depend on the oscillation of plasma levels of the drug, as they appear to be reduced with longer acting LD formulations or dopaminomimetics [4]. In fact, LD is sensitive to peripheral decarboxylation as well as chemical and enzymatic oxidation.

Drug delivery systems (DDS) represent an opportunity in the development of effective treatments for Parkinson's disease since they are able to improve both the pharmacological and therapeutic properties of conventional and new drugs. DDS can be either biodegradable or non-biodegradable, depending on the material used for their preparation. In addition, two main classes of materials should be individuated, namely, polymers and lipids. Among DDS, nanoparticles seem to be effective in facilitating the delivery of small molecules to the brain [5–10].

As an example of polymeric nanoparticles, it has been demonstrated that dopamine-loaded chitosan nanoparticles can improve dopamine

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transport across the cells [11]. Moreover, LD encapsulated in chitosan nanoparticles and incorporated in poloxamer gel for intranasal delivery has been shown to significantly increase the drug content in the brain [12].

To our knowledge, there are few papers in the literature concerning lipid-based nanoparticles with a specific focus on possible Parkinson's treatment. For instance, one of our previous studies on bromocriptine demonstrated that solid lipid nanoparticles are able to prolong the uptake of bromocriptine and increase its half-life, reducing dyskinesia in rats effectively [13].

Another interesting study conducted by Fernandes and Patravale [14] demonstrated that lipid nanocarriers of LD obtained using supercritical solvents in surfactant solutions can be successfully prepared with a homogenous distribution and more than 90% encapsulation efficiency, thereby contributing to improved stability of the drug against atmospheric oxidation. However, no in vivo experiments were performed with these lipid nanocarriers [14].

The dispersal phase of lipid nanoparticle dispersions is typified by a matrix of crystalline solid lipids, which protects encapsulated molecules from degradation and modulates their release [15–17]. Indeed, lipid nanoparticles seem to allow brain penetration of otherwise non-transportable drugs, masking their physico-chemical characteristics [18–23]. They represent a good delivery system for drug administration offering several clinical advantages, such as the increase of drug bioavailability, the reduction of drug dosage and side effects, and the improvement of patient quality of life [24–26].

Another largely employed strategy to prolong the pharmacological activity of LD and enhance its absorption is typified by the synthesis of LD-based prodrugs. Among these prodrugs, Di Stefano and collaborators have synthesized some compounds providing a relatively slow and constant release of LD in rat and human plasma. Unfortunately, these compounds were characterized by poor water solubility that limited systemic administration [27–29].

In particular, methyl O-acetyl-3-(acetyloxy)-N-[(2E)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]-L-tyrosinate (LD-PD; Fig. 1) was obtained by joining 3,4-diacetyloxy-L-dopa methyl ester with caffeic acid [27–30]. LD-PD showed a good pharmacological profile, but was rapidly degraded in human plasma (half-life of about 6.53 min). A therapeutic advantage of this prodrug might be the targeted delivery of an antioxidant molecule (caffeic acid) to specific cells (such as dopamine neurons) where cellular stress is associated with the pathology [30]. This therapeutic approach appears to be unexplored in the field of Parkinson's disease. Preliminary in vitro and in vivo studies evaluating the chemical and enzymatic properties of this molecule have revealed that LD-PD is stable in aqueous solutions and improves the release of LD and dopamine into the brain [27].

Taking the considerations described above together, our purpose in this study was to investigate the use lipid nanocarriers (LN) as an alternative biocompatible delivery system for administering an LD prodrug. Specifically, a characterization of the LN preparation containing LD-PD in terms of its morphology, dimensions, structural properties and drug distribution was firstly performed. Then, the ability of the LN containing LD-PD to attenuate motor deficits in 6-hydroxydopamine (6-OHDA) hemilesioned mice (a model of Parkinson's disease) was determined in vivo.

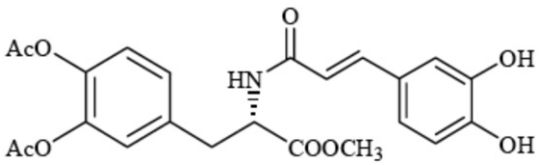

Fig. 1. Chemical structure of LD-PD.

2. Materials and methods

2.1. Materials

Stearic triglyceride (tristearin) was provided by Fluka (Buchs, Switzerland). Lutrol F 68, methyl-oxirane polymer (75;30) (poloxamer 188) was a gift from BASF ChemTrade GmbH (Burgbernheim, Germany). Phospholipon® 90G (P90G), a highly purified soybean lecithin containing at least 90% phosphatidylcholine, was supplied by Rhône-Poulenc-Rorer (Germany). Labrasol®, caprylocaproyl macrogol-8 glycerides, was purchased from Gattefossé (France). Six-hydroxydopamine (6-OHDA) hydrochloride was purchased from Tocris Bioscience (Bristol, UK). LD methyl ester and benserazide were purchased from Sigma (Sigma-Aldrich, AB, Italy). LD and 0 benserazide were dissolved in saline solution (NaCl 0.90% w/v) just prior to use.

2.2. Lipid nanoparticles' preparation

LNs were prepared by stirring and ultrasonication [13]. Briefly, 0.8 g of tristearin and 0.005 g of soybean phosphatidylcholine were melted at 70 °C. The fused lipid phase was added to 0.2 g of Labrasol®, and the mixture obtained was dispersed in 19 ml of poloxamer 188 solution (2.5% w/w). In turn, LD-PD at 2.5%, 5.0% or 10% by weight with respect to the solid phase was added to the molten mixture of tristearin/soybean phosphatidylcholine/Labrasol®. Afterwards, the mixture was added to the aqueous phase. The obtained emulsion was subjected to ultrasonication (Microson™, Ultrasonic cell Disruptor) at 6.75 kHz for 15 min and then cooled down to room temperature by placing it in a water bath at 22 °C. LN dispersions were stored at room temperature.

2.3. Characterization of lipid nanoparticle dispersions

2.3.1. Photon correlation spectroscopy (PCS)

Submicron particle size analysis was performed using a Zetasizer 3000 PCS (Malvern Instr., Malvern, England) equipped with a 5 mW helium neon laser with a wavelength output of 633 nm. Glassware was cleaned of dust by washing with detergent and rinsing twice with water for injections. Measurements were made at 25 °C at an angle of 90°. Data were interpreted using the "method of cumulants" [31].

2.3.2. Sedimentation field flow fractionation (SdFFF) analysis

An SdFFF system Model S101 (FFFractionation, Inc., Salt Lake City, UT, USA) was employed to determine the size distribution of particles (PSD) by converting the data to graphical results [32]. The mobile phase was deionized water produced by a Milli-Q water (Millipore S.p.A., Vimodrone, Milan, Italy) pumped at 2.0 ml/min and monitored on each run. Fifty microlitre samples were injected through a 50 μl Rheodyne loop valve.

The automatic collection of the fractions (every 90 s) was performed using a Bio Rad Model 2110 fraction collector (Bio Rad laboratories, UK) positioned at the end of the SdFFF system. The volume of each fraction was 3 ml.

2.3.3. Cryo-transmission electron microscopy (Cryo-TEM)

Samples were vitrified as previously described [13,33], and transferred to a Zeiss EM922 transmission electron microscope for imaging using a cryoholder (CT3500, Gatan). The temperature of the sample was kept below −175 °C throughout the examination. Specimens were examined with doses of about 1000–2000 e/nm^2 at 200 kV. Images were digitally recorded by a CCD camera (Ultrascan 1000, Gatan) using an image processing system (GMS 1.4 software, Gatan).

2.3.4. FTIR studies

The spectra of pure LD-PD, excipients containing 10% of LD-PD, empty LN, and LD-PD loaded LN were run on a Perkin-Elmer FTIR

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