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Preparation and characterization of poly(lactic-co-glycolic acid) microspheres loaded with a labile antiparkinson prodrug

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ABSTRACT

L-Dopa-α-lipoic acid (LD–LA) is a new multifunctional prodrug for the treatment of Parkinson's disease. In human plasma, LD–LA catechol esters and amide bonds are chemically and enzymatically cleaved, respectively, resulting in a half-life time of about fifty minutes. In the present work, the unstable LD–LA was entrapped into biodegradable poly(lactic-co-glycolic acid) (PLGA) microspheres designed as depot systems to protect this prodrug against degradation and to obtain a sustained release of the intact compound. The microspheres were prepared by an oil-in-water emulsion/solvent evaporation technique and the effect of formulation and processing parameters (polymer concentration in the organic solvent, volumes ratio of the phases, rate of the organic solvent evaporation) on microspheres characteristics (size, loading, morphology, release) was investigated. Also emphasis was given on the stability of the drug before and after release as well as on the underlying mass transport mechanisms controlling LD–LA release. Interestingly, when encapsulated in appropriate conditions into PLGA microspheres, the labile prodrug was stabilized and released via Fickian diffusion up to more than one week.

1. Introduction

Parkinson's disease (PD) is a progressive, neurodegenerative disorder which is characterized by the loss of dopaminergic neurons of the substantia nigra pars compacta with a subsequent reduction of dopamine (DA) neurotransmitter. PD affects about 1% of the population above the age of sixty, and its major clinical symptoms include movement disorders, anxiety, depression and dementia (Dauer and Przedborski, 2003; Duvoisin, 1987; Tanner, 1992; Gibb, 1992; Blandini and Greenamyre, 1999). The aetiology of PD has a multifactorial origin including oxidative stress and brain iron dysregulation (Dauer and Przedborski, 2003; Blandini and Greenamyre, 1999; Andersen, 2004; Ben-Shachar et al., 1991). The current chemotherapy is essentially symptomatic using exogenous L-Dopa (LD), the direct precursor of DA, as drug (Blandini and Greenamyre, 1999; Andersen, 2004; Fahn, 2006). LD administration is however associated with three important problems: (i) LD metabolism generates a variety of cytotoxic reactive oxygen species (ROS) that contribute to the progression of the disease; (ii) LD has a poor bioavailability in the CNS; (iii) during chronic treatment, patients become sensitive to LD plasma level fluctuations (Dauer and Przedborski, 2003; Bindoli et al., 1992; Di Stefano et al., 2001). In order to overcome these problems, a multifunctional prodrug (LD–LA, Fig. 1) containing LD and natural lipoic acid moiety (LA) has been recently synthesized (Di Stefano et al., 2006). Lipoic acid, which readily crosses the blood–brain barrier (BBB) and subsequently accumulates in neurons, has both antioxidant and iron-chelating properties. This means that it is active in reducing auto-oxidation of catecholamines (accelerated by transition metals) and in scavenging the ROS generated by LD metabolism (Ben-Shachar et al., 1991; Smith et al., 2004; Packer et al., 1997). LD–LA activity against Parkinson's disease symptoms and its antioxidant efficiency have been demonstrated using both in vitro and in vivo studies (Di Stefano et al., 2006). LD–LA therefore represents a good example of a multifunctional drug for treating central nervous system diseases. Moreover, in comparison with LD, oral administration of LD–LA prolonged the plasma LD concentration and this prodrug can therefore be particularly beneficial in the treatment of motor fluctuation, directly related to LD plasma level fluctuations. However, LD–LA catechol esters and amide bond are cleaved both chemically and enzymatically, resulting in a plasma half-life of about fifty minutes. Therefore multiple administrations per day are required to keep effective brain levels of LD (Di Stefano et al., 2006).

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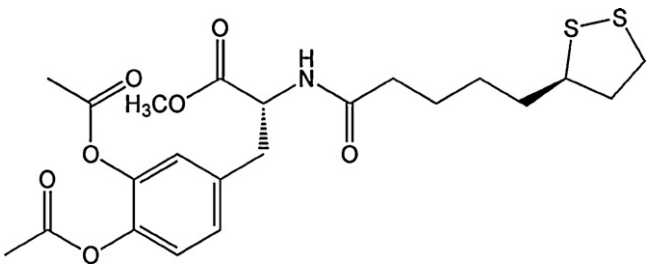

Fig. 1. LD–LA (methyl O-acetyl-3-(acetyloxy)-N-{5-[(3R)-1,2-dithiolan-3-yl]-pentanoyl}-L-tyrosinate) molecular structure.

In the present work, the labile prodrug LD–LA was entrapped into biodegradable polymeric microspheres to protect this prodrug from enzymatic and chemical degradation and to obtain a sustained release of the intact compound. Poly(lactic-co-glycolic acid) (PLGA), was used to prepare prodrug-loaded microparticles because this polymer has a good biocompatibility and is biodegradable (Anderson and Shive, 1997; Fournier et al., 2003; Jain, 2000; De Luca et al., 1993; Varde and Pack, 2004). Therefore it has been used for the development of sustained drug formulations of low molecular weight compounds as well as therapeutic proteins and plasmid DNA (Varde and Pack, 2004; Giovagnoli et al., 2008; Fernández-Carballido et al., 2004; Elkharraz et al., 2006; Janoria and Mitra, 2007; Ye et al., 2010; Jiang et al., 2005; Abbas et al., 2008). Due to the lipophilic character of LD–LA, PLGA microspheres were formulated using an oil-in-water emulsion/solvent evaporation method (Jain, 2000; Wischke and Schwendeman, 2008). The effect of formulation and processing parameters on the microspheres properties (size, loading, release) was investigated. Also emphasis was given on the stability of the drug before and after release as well as on the underlying mass transport mechanisms controlling LD–LA release.

2. Materials and methods

2.1. Materials

PLGA 50:50 Purasorb 5004A (intrinsic viscosity 0.41 dL/g) with free carboxyl end-groups was obtained from Purac Biomaterials. Polyvinyl alcohol (PVA, degree of hydrolysis 88%, molecular weight ranging from 13,000 to 23,000) was purchased from Aldrich Chemical Company. HPLC grade dichloromethane (DCM) and methanol were obtained from Biosolve BV. Dimethyl sulfoxide (DMSO, absolute) was obtained from Fluka. Formic acid, acetic acid anhydrous, NaOH, NaH_2PO_4 monohydrat, Na_2HPO_4 dihydrat, and NaCl (all pro-analysi grade) were obtained from Merck. NaN_3 (99.5%) was obtained from Sigma. The LD–LA prodrug was synthesized as previously described (Di Stefano et al., 2006).

2.2. Preparation of the microspheres

LD–LA loaded PLGA microspheres were prepared by an oil-in-water emulsion/solvent evaporation technique (o/w, ESE) (Wischke and Schwendeman, 2008). Briefly, 0.5 mL of oil phase (o-ph) was prepared by dissolving both PLGA and LD–LA in DCM in different concentrations and drug/polymer ratios (200, 400, 800 mg of PLGA per mL of DCM; drug/polymer ratio of 2%, 5% or 20% (w/w)). For the o-ph with the higher polymer concentration, the solutions were sonicated for 30 min at room temperature. The o-ph was emulsified (30 s, room temperature, 25000 min^{-1}) with 0.5, 2.5 or 5 mL of aqueous phase (aq-ph) consisting of PVA 2% (w/w) in water (previously saturated, or not, with DCM) by using an IKA Ultra-Turrax T8 homogenizer. In order to allow DCM evaporation and microsphere solidification, the resulting emulsion was then added drop wise to a PVA aqueous solution (0.5% (w/w); 2.5 or 5 mL), the hardening bath (hb), and magnetically stirred for two hours at room temperature. Alternatively, the emulsion was stirred without dilution (Table 1). The microspheres were collected by centrifugation at room temperature (3 min, 3000 × *g*), subsequently washed twice with water, flash frozen with liquid N_2 and freeze-dried overnight.

2.3. Microspheres characterization

The size and size distribution of the microspheres were measured using an Accusizer 780 (Optical particle sizer, Santa Barbara, California, USA). Scanning electron microscopy (S.E.M.) using a Phenom (FEI Company, The Netherlands) microscope was employed to study the size, porosity and morphology of the microspheres. Modulated differential scanning calorimetry (DSC) analyses were performed on prodrug, on empty and on prodrug loaded microspheres by means of a TA Instruments DSC Q2000. About 5 mg of material was loaded into aluminium pans, which were sealed, and subsequently equilibrated at −20 °C for 5 min. They were then heated from −20 to 120 °C at a heating rate of 1 °C/min, subsequently cooled to −20 °C (5 °C/min) and then heated again to 120 °C (1 °C/min). The glass transition temperature (T_g) was determined from the thermogram recorded during the second heating cycle.

The drug loading of the microspheres was determined by HPLC quantification of the prodrug extracted from the microspheres. Briefly, 5 mg of dried microspheres was dissolved in 0.5 mL of DMSO, followed by the addition of 1 mL of cold methanol to precipitate the PLGA. Next, the samples were centrifuged (30 min, 20,000 × *g*, 4 °C), the supernatants were filtered and methanol was evaporated under vacuum to obtain DMSO solutions of the

Table 1
Characteristics of the different prodrug loaded microspheres formulations. Data are expressed as mean ± SD ($n = 3$), hb denotes hardening bath.

Formulation	PLGA concentration in o-ph (mg per DCM mL)	DCM pre-saturation of aq-ph	Phases volume ratio (o-ph/aq-ph/hb)	TDL (%)[a]	EDL (%)[b]	LE (%)[c]	Volume–weight diameter (μm)
F1	200	No	1/1/10	2	1.5 ± 0.2	59 ± 3	16 ± 8
F2	200	No	1/1/10	5	3.2 ± 0.3	46 ± 2	11 ± 6
F3	200	No	1/1/10	20	14 ± 1	46 ± 5	13 ± 9
F4	400	No	1/1/10	5	3.4 ± 0.2	47 ± 2	30 ± 13
F5	400	No	1/1/10	20	14 ± 1	37 ± 3	21 ± 11
F6	400	Yes	1/5/5	5	3.2 ± 1	50 ± 5	34 ± 18
F7	400	Yes	1/10/0	5	2.7 ± 0.9	37 ± 8	30 ± 15
F8	800	Yes	1/5/5	5	2.7 ± 0.1	25 ± 1	26 ± 20
F9	800	Yes	1/10/0	5	2.6 ± 0	15 ± 8	28 ± 23

[a] TDL (theoretical drug loading) expressed as ratio between mass of drug and mass of polymer used in formulation.
[b] EDL (effective drug loading) expressed as mass ratio of drug entrapped in microspheres.
[c] LE (loading efficiency) expressed as mass ratio of drug entrapped in microspheres and drug used to prepare them.