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Solid lipid nanoparticles loaded with lipoyl–memantine codrug: Preparation and characterization

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ABSTRACT

Solid lipid nanoparticles (SLNs) are considered very attractive drug-delivery systems (DDS) able to enhance the efficacy of some therapeutic agents in several pathologies difficult to treat in a conventional way. Starting from these evidences, this study describes the preparation, physicochemical characterization, release, and *in vitro* cytotoxicity of stealth SLNs as innovative approach to improve solubility and absorption through the gastrointestinal tract of lipoyl–memantine (LA–MEM), a potential anti-Alzheimer codrug.

Physico-chemical properties of LA–MEM loaded SLNs have been intensively investigated. Differential scanning calorimetry (DSC) was used to clarify the state and crystalline structure of the formulation. The results obtained from particles size analysis, polydispersity (PDI), and zeta potential measurements allowed the identification of the optimized formulation, which was characterized by a drug-lipid ratio 1:5, an average intensity diameter of 170 nm, a PDI of 0.072, a zeta potential of −33.8 mV, and an entrapment efficiency of 88%. Moreover, *in vitro* stability and release studies in both simulated gastric fluid (SGF) and simulated intestinal fluid (SIF), and preliminary *in vitro* cytotoxicity studies revealed that LA–MEM loaded SLNs could represent potential candidate for an *in vivo* investigation as DDS for the brain since it resulted devoid of citotoxicity and able to release the free codrug.

1. Introduction

Nanotechnological approaches are often used to improve the pharmacokinetic profile of drugs such as poor gastro-intestinal absorbtion, low drug solubility, and rapid metabolism but also to ensure an efficient CNS delivery of many compounds. Among various colloidal systems, solid lipid nanoparticle (SLNs) have roused special interest due to many features that make them particularly intriguing as alternative carriers in the field of drug delivery (Kanwar et al., 2009). In combination with the narrow sizes, sterical properties obtained in stealth SLNs result particularly advantageous to increase the blood circulation time of the particulate, thus reducing RES uptake and extending the contact time between the BBB and the drug, which can therefore be caught in the brain when the target is represented by CNS (Üner and Yener, 2007). Such properties can improve bioavailability of drugs through several mechanisms: (1) augmenting the drug solubility and permeability, (2) overcoming the first-pass effect and the P-gp efflux, and (3) enhancing the stability in the GI tract (Esposito et al., 2008). Several drug-loaded SLN formulations in brain targeting and delivery showed a very low cytotoxicity or resulted to be not-cytotoxic (Patel et al., 2013). However, the biocompatibility assessment seemed to be tough due to the variable composition of SLN formulations that differs in nature, percentage of lipids and loaded molecules (Silva et al., 2012).

Recently, our research has been focused on the development of innovative therapeutic strategies to reduce the disease progression and improve the quality of life of patients suffering from Parkinson's (PD) (Di Stefano et al., 2009; Cingolani et al., 2000) and Alzheimer's (AD) diseases (Sozio et al., 2009, 2013a; Pinnen et al., 2011; Cacciatore et al., 2012, 2013). To date, AD treatments mainly exploit a symptomatic approach based on the use of

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cholinesterase inhibitors for patients with mild to moderate AD, and *N*-methyl-D-aspartate (NMDA) receptor antagonists, such as memantine (3,5-dimethyl-1-adamantanamine, MEM), for patients with moderate to severe AD (Anand et al., 2014; Parihar and Hemnani, 2004). According to the multifactorial hypothesis of AD, glutamate excitotoxicity, oxidative stress (OS), depletion of antioxidant defense systems, metal dyshomeostasis, and protein misfolding and aggregation are considered the main causes of neuronal loss in selected brain regions (Di Stefano et al., 2011). Taking into account the role played by OS and over-stimulation of glutamate NMDA receptors in causing neuronal damage, we previously reported the synthesis of lipoyl–memantine (LA–MEM) (Fig. 1), a new potential anti-Alzheimer codrug obtained by joining MEM with a natural neuroprotective agent, (*R*)-α-lipoic acid (LA) (Sozio et al., 2013b). Despite of its good capabilities to inhibit Aβ (1–42) aggregation and scavenge free radicals, LA–MEM showed poor water solubility (0.010 mg/mL) to permit an oral administration.

This paper is focused on the preparation and characterization of a new SLN formulation containing LA–MEM codrug to increase its solubility in g.i. fluids and favor its intestinal absorption. High lipophilic compounds, such as LA–MEM (Log *P* = 4.20), have often been selected to be incorporated into SLNs due to their high drug loading and entrapment efficiency (Al Haj et al., 2008). The new formulation was characterized evaluating particle size, zeta potential, surface morphology, drug loading capacity, drug encapsulation efficiency, and stability in simulated gastric (SGF) and intestinal (SIF) fluids. Few studies are reported in literature about the potential toxicity of SLN formulations (Nassimi et al., 2010), thus *in vitro* citotoxicity of LA–MEM loaded SLNs was assessed against mouse N2a neuroblastoma (NB) and primary human whole blood (PHWB) cells using MTT and lactate dehydrogenase (LDH) assays, respectively. Total antioxidant capacity (TAC) and total oxidative stress (TOS) levels were also determined to valuate oxidative alterations.

2. Material and methods

2.1. Chemicals

LA–MEM was synthesized as we previously reported (Sozio et al., 2013b). Stearic acid, Brij 78 and acetone were purchased from Sigma–Aldrich (St. Louis, MO, USA). Sucrose was obtained from Alfa Aesar (MA, USA), Cremophor® ELP was obtained from BASF (Ludwigshafen, Germany). NaCl, NaOH, KH_2PO_4 and all other solvents of chromatographic grade were obtained from Fisher (Loughborough, UK). MilliQ water was produced by a MilliQ Direct-Q UV3 Millipore system (Merck, Darmstadt, Germany). Chromafil® PET-120/25 filters (Duren, Germany) were used for all the formulations.

2.2. Preparation of empty and LA–MEM loaded stealth solid lipid nanoparticles

SLNs were prepared according to a slight modification of the emulsification–evaporation–solidifying method previously reported (Sjöström et al., 1995; Shahgaldian et al., 2003). For the blank SLN, stearic acid (SA) (33.36 mg) was completely dissolved in 8 mL of acetone under mild magnetic stirring at 40 ± 2 °C. Concurrently, a solution of surfactant (Brij 78) in deionized water, with different lipid:surfactant ratio (1:0.4 and 1:1), was prepared and heated at 75 ± 2 °C. The organic phase was added dropwise to the aqueous phase under mechanical stirring (1000 rpm) to obtain the lipid emulsion which was subsequently concentrated to 11.2 mL evaporating the solvents mixture at 75 ± 2 °C. The translucent emulsion system was quickly added to 11.2 mL of cold deionized water on ice bath under mechanical stirring (1000 rpm). The obtained solidified nanoparticles suspension was adjusted to total 25 mL with cold distilled water. The drug-loaded nanoparticles were prepared with the same procedure adding LA–MEM to the organic phase with different drug:lipid ratio (1:2.5, 1:5, 1:10, 1:20). The final suspension was filtered with 1.20 μm filters to remove the unentrapped drug.

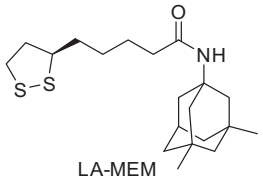

Fig. 1. Chemical structure of LA–MEM codrug.

2.3. Dynamic light scattering and zeta potential measurement

Intensity mean hydrodynamic size, polydispersity index (PDI), and zeta potential of the particles were measured on a Malvern Zetasizer-NanoZS with a He–Ne laser with a wavelength of 632.8 nm. The measurements were carried out at a scattering angle of 173° using disposable sizing cuvette and keeping the temperature at 25 °C throughout the experiments. All measurements were repeated three times ($n = 3$) and the results are given as the effective diameter, also called *Z*-average diameter, and the PDI as a parameter of the particle size distribution. The measurements were performed at 25 °C, in triplicate ($n = 3$) and the average values were calculated. The electrophoretic mobility was determined in an aqueous medium with a Smoluchowski approximation.

2.4. Lyophilization of SLNs

SLNs dispersed in water were subjected to lyophilization to obtain dried formulations. Two different cryoprotectants were tested to avoid coalescence of SLNs (Schwarz and Mehnert, 1997; Cavalli et al., 1997), 0.8 mL of sucrose (5% w/v, 10% w/v) and 0.8 mL of trehalose (5% w/v, 10% w/v) aqueous solutions were mixed with 2 mL of SLNs water suspension; the mixture was then frozen at −20 °C overnight. The lyophilization process was carried out using a VirTis Advantage freeze dryer. The freeze-drying protocol employed was the following:

Freezing: shelf temperature −38 °C for 120 min.

Primary drying (sublimation): step 1: shelf temperature −30 °C, vacuum 200 mTorr for 120 min; step 2: shelf temperature −10 °C, vacuum 200 mTorr, for 120 min. Secondary drying (desorption): shelf temperature: 0 °C, vacuum 200 mTorr, duration 20 h. Post heat: shelf temperature: 10 °C, vacuum: 200 mTorr, duration 240 min.

2.5. Morphology evaluation by transmission electron microscopy

Morphological examination of the freshly prepared SLNs was performed using a transmission electron microscope (TEM). A drop of SLNs suspension was placed on a copper grid and stained with 1% (w/v) of uranyl acetate. The sample was viewed under TEM (FEI CM120 BioTwin instrument). Images were recorded using an AMT 5 Mp digital camera.

2.6. Differential scanning calorimetry

Differential scanning calorimetry (DSC) was performed on DSC Q2000 (TA instruments, LLC, USA). The instrument was calibrated with indium (Tm = 156.6, ΔHf = 28.71 J/g) according to the