Colloids and Surfaces B: Biointerfaces 155 (2017) 238–247

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Nanoencapsulation strategies for the delivery of novel bifunctional antioxidant/σ1 selective ligands

Claudia Carbone[a,*], Emanuela Arena[a], Veronica Pepe[a], Orazio Prezzavento[a], Ivana Cacciatore[b], Hasan Turkez[b,c], Agostino Marrazzo[a], Antonio Di Stefano[b], Giovanni Puglisi[a]

[a] Department of Drug Sciences, University of Catania, v.le A. Doria 6, 95100, Catania, Italy
[b] Department of Pharmacy, University G. d'Annunzio Chieti-Pescara, Via dei Vestini 31, 66100, Chieti, Italy
[c] Department of Molecular Biology and Genetics, Erzurum Technical University, Erzurum 25240, Turkey

ARTICLE INFO

Article history:
Received 24 November 2016
Received in revised form 7 April 2017
Accepted 10 April 2017
Available online 12 April 2017

Keywords:
Nanoparticles
NLC
NC
Turbiscan
Nanotoxicity
Total antioxidant capacity
Total oxidant status
Sigma receptors
Lipoic acid

ABSTRACT

Nowadays sigma-1 receptors are considered as new therapeutic objectives for central nervous system neurodegenerative diseases. Among different molecules, alpha lipoic acid has been identified as a natural potent antioxidant drug, whose therapeutic efficacy is limited by its many drawbacks, such as fast metabolism, poor bioavailability and high physico-chemical instability. Alfa-lipoic acid derivatives have been recently developed demonstrating their neuroprotective activity and effectiveness in different types of oxidative stress. In this work, two derivatives containing an amide or an ester functional group with different lipophilicity, were selected for their important affinity for sigma-1 receptors. Herein, in order to improve the in vitro stability and antioxidant effectiveness of alpha-lipoic acid derivatives, we focused our efforts in the nanoencapsulation strategies. Aqueous-core nanocapsules for the delivery of the hydrophilic compound and nanostructured lipid carrier for the lipophilic derivative, were properly designed and prepared using a direct or inverse eco-friendly organic solvent-free procedure. All nanosystems were characterized in terms of mean size, polydispersity, stability, morphology, encapsulation efficiency and in vitro release profiles. In order to evaluate the nanocarriers biocompatibility and antioxidant effectiveness, in vitro biological studies (cell viability, total antioxidant capacity and total oxidative status) were developed on primary human whole blood cell cultures, on both unloaded and derivatives-loaded nanodevices.

1. Introduction

In the last decade there has been a renewed interest from the research world in natural compounds, with particular attention to their nanoencapsulation into drug delivery systems (DDS), as demonstrated by the increasing number of scientific publications (Supplementary Fig. 1). Natural compounds are considered as a potential reservoir of innovative therapeutic strategy to human health, with the prospect of integrating and sometimes replacing conventional drugs with a consequent improvement of the patient's compliance and reduction of side effects both on human health and the environmental impact [1–3]. Different natural molecules such as melatonin, curcumin, quercetin, idebenone, ferulic acid and lipoic acid, have been successfully investigated for the potential treatment of human neurodegenerative diseases related to high levels of oxidative stress [4–13]. Alpha lipoic acid (ALA) is an amphiphilic potent natural antioxidant with a central role in energy metabolism, important neuroprotection properties and recently showed to have antimutagenic and anticarcinogenic

Abbreviations: ALA, alpha lipoic acid; BS, back scattering; Brij® 98, polyoxyethylene (20) oleyl ether, Oleth-20; Cutina CP, cetyl palmitate; Cryo–TEM, cryogenic transmission electron microscopy; Da, PDI after freeze-thawing; Db, PDI before freeze-thawing; DDAB, dimethyldioctadecylammonium bromide; DDS, drug delivery systems; ΔT, variation of transmission; EE%, percentage of encapsulation efficiency; FDA, food and drug administration; HWBC, human whole-blood culture; HPLC, high performance liquid cromatography; IPS, isopropyl stearate; LDH, lactate dehydrogenase assay; MTT, 3(4,5-dimethyl-thiazol-2-yl)2,5-diphenyl-tetrazolium bromide; NC, aqueous-core nanocapsule; NLC, nanostructured lipid carrier; Pa, particles diameter after freeze-thawing; Pb, particles diameter before freeze-thawing; P1, hydrophilic compound; P2, lipophilic compound; PCS, photon correlation spectroscopy; PHWB, primary human whole blood; PDI, polidispersity index; PIT, phase inversion temperature; PLA, polylactic acid or polylactide; ROS, reactive oxygen species; S.D., standard deviation; Span® 80, sorbitan monooleate; $_{\sigma 1}$R, sigma 1 receptor; T, transmission; TAC, total antioxidant capacity; TAGS, turbiscan AG Station; TCT, caprylic/capric triglyceride, Tegosoft® CT; Tegin O, gliceryl monooleate; TOS, total oxidant status; TSI, turbiscan stability index; Tween® 80, polysorbate 80; Zave, mean particle size; ZP, zeta potential; W/O, water in oil.

* Corresponding author at: Department of Drug Sciences, University of Catania, Viale A. Doria 6, 95125, Italy.
E-mail address: ccarbone@unict.it (C. Carbone).

http://dx.doi.org/10.1016/j.colsurfb.2017.04.016
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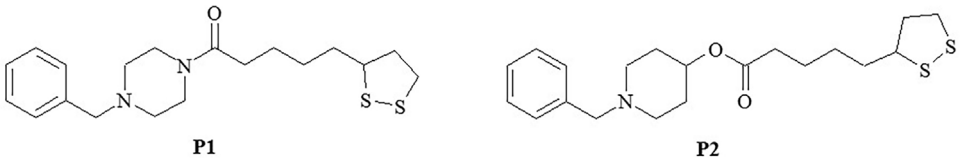

Fig. 1. Bifunctional ligands with σ_1R affinity and antioxidant activity obtained by linking ALA and an appropriate aminic moiety, based on the piperazine (P1) or piperidine (P2) scaffold.

activities [14–16]. Unfortunately, the limits of ALA related to its metabolism, poor bioavailability and high instability, led to serious problems in the possibility of exploiting this compound as an effective drug [17]. We previously reported a series of new bifunctional ligands with sigma 1 receptor (σ_1R) affinity and antioxidant activity, based on combining the ALA with an appropriate amine scaffold [18]. Among the synthesized compounds, the derivatives **P1** and **P2** (Fig. 1) displayed an agonist activity [18,19].

Recently, some researchers reported the involvement of σ_1R in protection against oxidative stress, highlighting their critical role in neuroprotection mechanism. In particular, it has been found that σ_1R knockdown promotes an accumulation of reactive oxygen species (ROS), whereas their activation or up-regulation agonists-mediated induces a suppression of oxidative stress [20–24]. Herein, a further improvement of the *in vitro* antioxidant activity was observed for our compounds compared to ALA. In particular, **P2** was found to significantly increase the total antioxidant capacity (TAC) at high concentrations (25–50 μM) without important variations in terms of total oxidant status (TOS), whereas **P1** did not alter the TAC and TOS levels at all tested concentrations [19]. However, the presence of both the lipophilic function and the ester or amidic group could represent a drawback for the compounds stability. On the basis of these consideration, in order to improve the stability and *in vitro* antioxidant properties of derivatives **P1** and **P2**, we exploited the nanoencapsulation delivery strategies [25,26]. Nanoencapsulation of actives, through surface nanoparticles modification with cationic lipids, has been reported as a promising strategy to improve the physico-chemical stability, enhance the interaction with biological sites, thus enhancing the biological activity of drug molecules [27–33]. Thus, we designed, prepared and characterized two different positively charged nanoparticulate systems for brain delivery: aqueous-core nanocapsules (NC) for the delivery of the hydrophilic **P1** and nanostructured lipid carrier (NLC) for the lipophilic **P2**, respectively. NC and NLC were prepared by eco-friendly procedures taking advantage of biocompatible and biodegradable materials approved by Food and Drug Administration (FDA) [34,35–39]. All nanodevices were characterized in terms of mean particles size, polidispersity, zeta potential, *in vitro* release and stability, exploiting the Turbiscan® technology. *In vitro* biological studies on primary human whole blood (PHWB) were performed on both the unloaded and the loaded nanodevices, at different concentrations, so as to collect information on their cell viability and efficiency.

2. Experimental section

2.1. Materials and methods

Polylactic acid or polylactide (PLA, MW 10,000–18,000), Polysorbate 80 (Tween® 80), Sorbitan monooleate (Span® 80), Didecyldimethylammonium bromide (DDAB) and 3(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) were bought from Sigma (Milan, Italy). Gliceryl Monooleate (Tegin O), Isopropyl stearate (IPS) and Polyoxyethylene (20) oleyl ether (Oleth-20) were purchased from ACEF (Piacenza, Italy) while Caprylic/Capric Triglyceride (Tegosoft® CT, TCT) was obtained from Farmalabor (Bari, Italy). Cetyl palmitate (Cutina CP) was a gift from Galenitalia Spa (Roma, Italy). Acetonitrile, methanol, acetic acid and water used in the HPLC procedures were of LC grade and were bought from Merck (Milan, Italy). Regenerated cellulose membranes (Spectra/Por CE; Mol. Wet. Cutoff 3000) were supplied by Spectrum (Los Angeles, CA, USA). Cell culture medium (Chromosome Medium B) and phytohemagglutinin were purchased from Biochrom (Leonorenstr. 2-6, D-12247, Berlin).

2.2. Nanodevices preparation

2.2.1. Nanostructured lipid carriers

A low energy organic solvent-free phase inversion procedure (PIT method) was exploited for the preparation of the NLC, as previously reported [9,31]. Briefly, the aqueous phase (water) and the oil phase constituted by surfactants and lipid matrix (CP and IPS at ratio 4:1) were separately heated at ~85 °C; then the aqueous phase containing 8.7% w/w of Oleth-20 and 4.4% w/w of Tegin O, was added drop by drop, at constant temperature and under agitation, to the oil phase. The mixture was cooled to 60 °C, successively subjected to three thermal cycling (85–60 °C) and then cooled to room temperature under slow and continuous stirring. Compound **P2** (0.4 mg/ml) was loaded in NLC.

2.2.2. Aqueous-core nanocapsules

NC were prepared exploiting the deposition of the polymer at the interface of a W/O nanoemulsion, prepared by the reversed PIT method as previously described [27]. The oily phase constituted in 1 mg/ml of PLA in TCT and the aqueous phase containing the surfactants (6.55% w/w of Span® 80 and 2.18% w/w of Brij® 98), were separately heated (85 °C) under continuous stirring (1000 rpm). The aqueous phase was added to the oily phase at constant temperature and under agitation. According to PIT method, the mixture was cooled to 60 °C, successively subjected to three heating cycling (85–60 °C) and then cooled to room temperature under slow and continuous stirring for 24 h. DDAB (0.25 or 0.5% w/w) was added to the oily phase during the nanoparticles preparation to obtain positively charged NLC and NC. In order to remove the excess of surfactants and obtain a water suspension, all nanodevices were purified by ultracentrifugation (10000 rpm, 60 min, 12–22 °C, Beckman model J2-21 Centrifuge). Compound **P1** (0.4 mg/ml) was loaded in NC.

2.3. Physico-chemical characterization

The particle size was determined by photon correlation spectroscopy (PCS) which yields the mean particle size (Zave) and the polydispersity index (PDI), which provides the width of the particle sizes distribution. PCS was performed using a Zetasizer Nano S90 (Malvern Instruments, Malvern, UK) at a detection angle of 90°, at 25 °C, with a 4 mW He–Ne laser operating at 633 nm. Each value was measured at least in triplicate. The results are shown as mean ± standard deviation (SD). The zeta potential values (ZP), which reflects the electric charge on the particle surface, was determined using the same equipment described previously at 25 °C. All samples were analyzed 24 h after the preparation and for the mea-