Stealth Lipid Nanoparticles as Tools for the Administration of Astaxanthin Aimed to the Treatment of Alzheimer's Disease

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Background

Alzheimer's disease (AD) is a neurodegenerative disorder associated with marked oxidative stress at the level of the brain. Recent studies indicate that increasing the antioxidant capacity could represent a very promising therapeutic strategy for AD treatment [1]. Astaxanthin (AST), a powerful natural antioxidant, could be a good candidate for AD treatment [2], although its use in clinical practice is compromised by its high instability. In order to overcome this limit, our attention focused on the development of AST-loaded lipid nanoparticles (SLNs) able to improve AST bioavailability in the brain. Solid lipid nanoparticles (SLNs) are well-known nanocarriers representing a consolidated approach for the delivery of lipophilic compounds, but their systemic use is limited due their short half-life. One of the most used strategy to prolong the systemic residence of these carriers is based on the modification of the nanoparticle surface with the surfactant polysorbate 80 (p80) and the realization of "stealth" systems (SSLNs) able to avoid the defense line represented by the macrophages [3].

Objective

The aim of research project is to find an efficient formulation strategy for obtaining stable and homogeneous stealth SLNs loaded with astaxanthin (AST), a natural powerful antioxidant, for the treatment of Alzheimer's disease through parenteral administration.

Methods

AST SSLN preparation

SLNs were prepared using a slightly modified *solvent-diffusion* technique [4]. The aqueous phase was constituted of hydroxypropyl-methyl cellulose (HPMC), soy lecithin, poloxamer 188 and water heated at 70° C. Briefly, stearic acid and AST (1 mmol) were solubilized to ethanol (70 °C) and the mixture was stirred to obtain a dispersion. This lipid phase was added to the aqueous phase, afterwards the mixture was emulsified at 15,000 rpm, 70°C for 8 min, subjected to ultrasonication for 10 min and then let cooling at 4°C. In order to modify the surface of the nanoparticles, p80 (20% w/w with respect to the lipid weight) was added to the SLN stirring at 250 rpm for 30 min. Blank SLNs were prepared by the same procedure without the addition of AST.

DSC analysis

DSC analysis was carried out using a Mettler Toledo STAR system equipped with a DSC-822 calorimetric cell. Each sample (120 µL) was loaded into a 160 µL aluminum crucible, hermetically sealed and submitted to DSC analysis, under an atmosphere of dry nitrogen. DSC analysis was carried out using a heating scan from 5°C to 85° C (2° C/min) and a cooling scan from 85°C to 5°C (4° C/min), for at least three times.

Oxygen Radical Absorbance Capacity (ORAC) Assay

The antioxidant capacity of AST, free and encapsulated into SLN and SSLN, was measured using ORAC assay [5]. During this test, the decay in fluorescein fluorescence due to peroxyl-radical formation by the peroxyl radical generator AAPH (2,20-azobis 2-amidinopropane, dihydrochloride) is monitored over time. FL solution containing AAPH was used as the positive control and FL solution without AAPH as negative control. The assay was performed at 37°C on a VICTORWallac 1420 Multilabel Counters fluorimeter set with excitation filter 540 nm and emission filter 570 nm.

UV Stability Assay

600 µL of each sample were dispensed into a well of a 24-well plate, covered with a quartz plate and placed on a brass block embedded on ice for UVA exposure [6]. Samples were exposed from above to 15 min UVA irradiation. After exposure, 200 µL were taken and extracted in 1 mL ethyl acetate by vortexing for 2 min, followed by brief centrifugation. The UV/Vis spectrum of the organic solution was then measured on a UV Varian Cary 50 spectrophotometer.

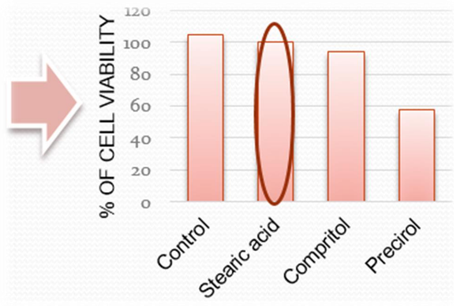
In vivo assay

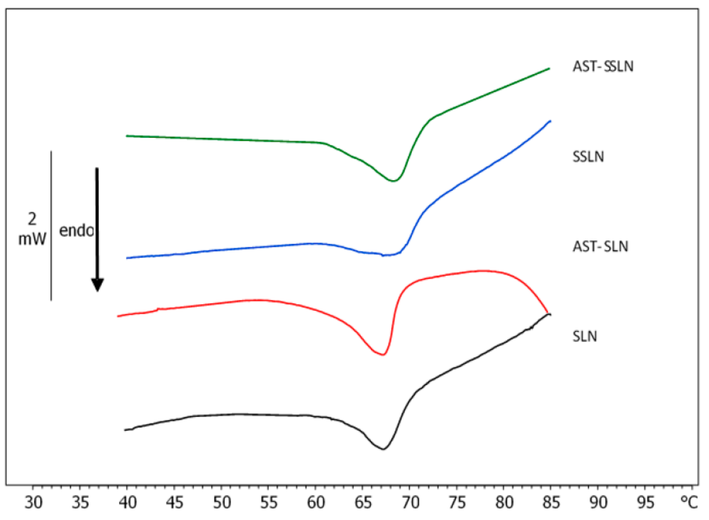
The pharmacological activity of the formulation has been evaluated by *in vivo* assay using transgenic mice TgCRND8 that express two mutated human APP genes implicated in AD [7]. The treatment was performed with unloaded (n=5 mice Tg e 5 wt, 0.1 mL/10 g; untreated mice) and AST-loaded SLN (n=5 mice Tg e 5 wt, 40 mg/kg) injected intraperitoneally for three weeks. At the end of the treatment memory performance was investigated by the behavioral test: the Step down inhibitory passive avoidance task.

Results And Discussion

SLNs were formulated using stearic acid as the lipid phase. This choice was confirmed by *the in vitro* assay able to determine the potential cytotoxic effects of different blank SLNs formulated using different lipid on adult primary stem cells (DPSCs). As reported in the table, this system shows a good mean particle size suitable for parenteral administration.

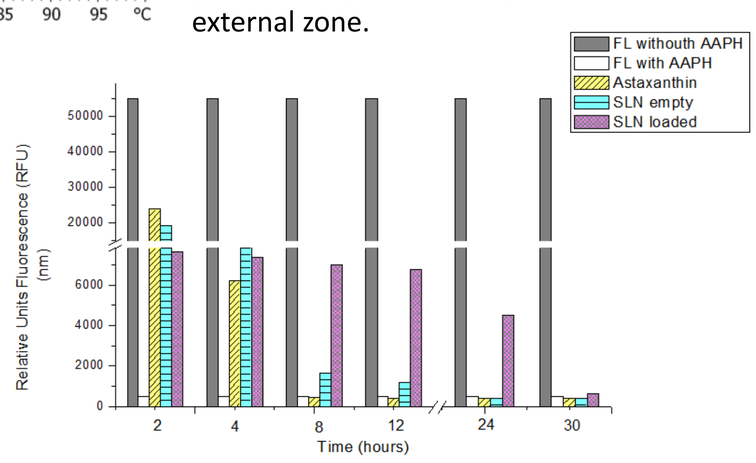
Formulation	Z-Ave [nm ± SD]	PDI [-] ± SD	ZP [mV ± SD]
Blank SLN	136.0 ± 0.2	0.27 ± 0.1	-20.4 ± 0.2
AST-SLN	111.1 ± 0.2	0.33 ± 0.2	-16.2 ± 0.2
Blank p80SLN	154.2 ± 0.3	0.29 ± 0.2	-18.2 ± 0.3
AST-p80SLN	130.8 ± 0.2	0.32 ± 0.3	-13.0 ± 0.4

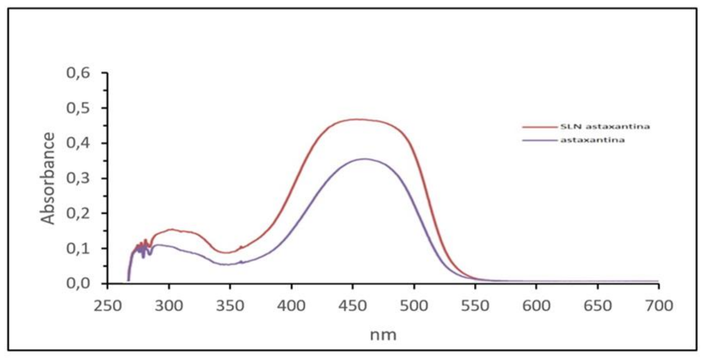


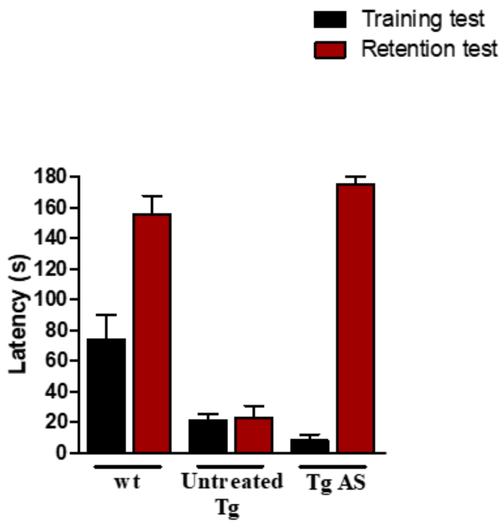


The calorimetric curve of unloaded SLN is characterized by a main peak at about 67.5°C. In the presence of AST, the calorimetric peak retains its shape and temperature, but it becomes larger; this can be due to a homogenous distribution of AST on the SLNs structure. Instead the calorimetric curve of SSLN is characterized by a main peak at about 68.5°C and a shoulder at lower temperatures. This is evidence of a non-homogeneous distribution of the SSLNs components: p80 could preferentially localize in the external zone.

The antioxidant capacity of AST free and once encapsulated into lipid nanoparticles was determined by ORAC assay. As reported in the graph, at 12 hs AST-SSLN had a much higher fluorescence value (6770 nm) than free AST (620 nm). These results suggest that the encapsulating of AST into SSLNs preserved the antioxidant capacity of AST for a longer time (24 hs) and probably maintains its stability. Therefore, this result confirms the key role of encapsulation in preserving and therefore increasing the antioxidant activity of powerful active compounds. The protective effect of SLN was further demonstrated by UV stability assay confirming that the lipid shell protected AST from photodegradation.







As reported in the graph, initially memory performance showed no significant differences during the training test (TT) between treated and untreated animals. However, in the 24 hs retention test (RT) step-down, the latencies recorded for Tg mice were significantly reduced, the administration of drug-loaded SLN significantly improved the performance of Tg mice to a higher level than wt mice. Moreover, the cognitive deficit was completely recovered. Therefore, the formulation could be regarded as a promising carriers for the treatment of CNS disorders, through systemic administration.

References

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