Mangiferin-loaded nanostructured lipid carriers (NLC) with antioxidant activity

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Background

Ophthalmic drug delivery, especially for the treatment of the posterior eye segment diseases, is still a great challenge. The efficacy of topical administration into the eyes by conventional solutions and suspensions is limited by a series of pre-corneal drug removal mechanisms and physiological barriers [1,2] which oppose to trans-corneal drug absorption [3]. Therefore, frequent instillations of eye drops are necessary to achieve the expected therapeutic effect; this, especially in chronic therapy, decreases the patient compliance [4]. Nowadays, there is an increasing need to find efficient and patient-friendly formulations for the retinal diseases related with oxidative stress, such as diabetic retinopathy and macular degeneration. Therefore, increasing attention is being paid on antioxidants. Among the natural antioxidant, mangiferin (2-b-D-glucopyranosyl-1, 3, 6, 7-tetrahydroxyxanthone; MGN) is an interesting natural compound, primarily extracted from the leaves, stem barks and fruits of *Mangifera indica L.*, showing anti-inflammatory and antioxidant activities for the potential treatment of eye diseases. Despite these important effects, however, the use of MGN in clinical practice is strongly compromised by an unfavorable pharmacokinetic profile due to the low water solubility and the lack of stability that limit the development of conventional eye drops [5].

Objective

The aim of the present work was the preparation of a stable nanostructured lipid carrier (NLC) system for the delivery of MGN to the back of the eye. The limits of MGN (poor solubility and high instability) justify its nanoencapsulation into drug delivery systems.

Methods

MGN-NLC was prepared by high shear homogenization coupled to ultrasound (HSH-US) method [6] slightly modified. Briefly, MGN (0.1% w/v) was dissolved in an oil phase containing Miglyol (0.4 g) and Compritol (0.6 g) and the mixture was stirred at 80°C to obtain a dispersion. The melted lipid phase was dispersed in the hot (80°C) surfactant solution (Lutrol F68 0.4%w/v) by using a high-speed stirrer for 10 min. The obtained pre-emulsion was ultrasonified for 8 min. The hot dispersion was then cooled by dilution in 25 mL of additional water at 4°C. Unloaded NLC was prepared by the same procedure without adding MGN.

NLC characterization

The average particle size and the polydispersity index of the NLC were measured by Dynamic Light Scattering (DLS). Samples were prepared diluting 100 µL of NLCs suspension with 900 µL of distilled water. Morphological and structural characteristics of MGN-NLC prepared were investigated using TEM (JEOL JEM-101).

Oxygen radical absorbance assay (ORAC)

The antioxidant activity of MGN-NLC, MGN solution and unloaded NLC was evaluated by ORAC assay [7]. Data were obtained using an OPTIMA FLUOstar microplates reader with a fluorescence filter (excitation 485 nm, emission 520 nm). Fluorescein solution (3 µM) was prepared in 75 mM phosphate buffer (pH 7.4). The assay was carried out at 37°C, using Trolox (0.1 mM) as the control standard and phosphate buffer (pH 7.4) with fluorescein as the blank. Samples (MGN-NLC, MGN solution and unloaded NLC) were diluted in phosphate buffer (1:1, 1:3, 1:10, 1:20, 1:50, 1:75, 1:100 and 1:150) and then they were deposited (70 µL) in triplicate in the well of the microplate with fluorescein solution (100 µL). Moreover, eight calibration solutions using Trolox (3, 5 10, 15, 20, 30, 50 and 100 µM, in phosphate buffer) were added in triplicate (70 µL). After thermostating for 15 min at 37°C, 30 µL of AAPH solution was added and fluorescence measurement was begun. The fluorescence was recorded every 2 min for 180 min.

Ocular Tolerability: HET-CAM assay

The potential ocular irritancy and/or damaging effects of unloaded NLC and MGN-NLC formulations were evaluated using the Hen's Egg Test on Chorio-Allantoic Membrane (HET-CAM) assay. The HET-CAM test was carried out using fertilized hen's eggs incubated at 37 °C and a relative humidity of 60% during 9 days. On the ninth day, the eggshell was pierced and the inner membrane was wet with 0.9% NaCl for 30 min (37°C). Afterwards, the inner egg membrane was carefully removed and unloaded NLC and MGN-NLC (100 µL) were dropped on the CAM. 0.9% NaCl and 0.1 N NaOH solutions (300 µL) were used in duplicate as negative and positive controls, respectively. The vessels of CAM were observed during 5 min to the irritation score (IS).

Hemocompatibility assay

In vitro hemolysis assays were performed for unloaded NLC and MGN-NLC using freshly drawn human whole blood. Briefly, blood samples (5 mL) were diluted with 0.9% NaCl (145 mL). Samples (100 µL; dilution 1:5) were added in diluted blood (1 mL), while positive and negative controls were incubated with Triton X-100 (4%) and phosphate buffer (PBS), respectively. After incubation (37°C) at 100 rpm for 1 hour, the samples were centrifuged (Sigma 2-16P; Sigma Laboratory Centrifuges, Germany) at 10000 rpm for 30 min and released hemoglobin was monitored by measuring the absorbance of the supernatant (150 µL) at 540 nm using a microplates reader (FLUOstar Optima, BMG Labtech, USA) and the percentage of hemolysis was calculated.

Corneal permeability assay

Mangiferin corneal permeability assay was carried using fresh bovine eyes from which corneas were isolated and mounted on vertical diffusion Franz cells. Receptor chambers were filled with a propylene glycol:water (40:60) solution and placed in a bath at 37 °C, while the donor chambers were completely emptied and filled with 2 mL of MGN-NLC formulation. The amount of mangiferin permeated was quantified using a JASCO HPLC fitted with a C18 column (Waters Symmetry C18, 5 µm, 4.6 × 250 mm). The analysis was carried out by isocatric elution using 0.1% formic acid:acetonitrile (87:13) as a mobile phase at 1.5 ml/min and 26°C. The injection volume was 100 µL and the UV detector was set at 254 nm (retention time 12 min) [8]. The standard solutions were 0.075-1 µg/mL of mangiferin in ethanol:propylenglycol:water (10:40:50).MNG content in the samples was calculated from a validated calibration curve.

Results And Discussion

MGN-loaded NLC showed a mean diameter of 148.9 ± 0.1 nm, a PDI value around 0.21 ± 0.02 and zeta potential value of -23.5 mV (Table 1), predicting a good long-term stability for the formulation [9]. DLS data were in agreement with TEM images.

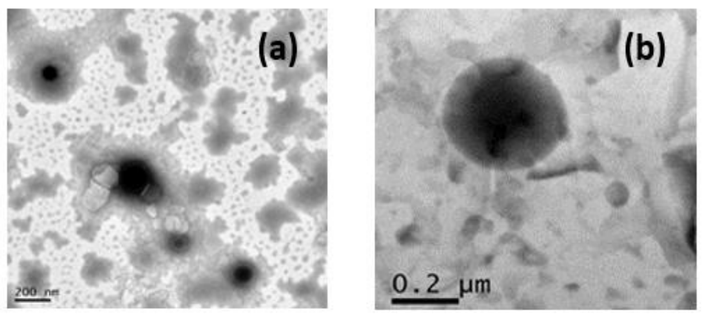

Fig. 1 *(Figures a-b) Transmission electron microscopy images of MGN-NLC. The scale bar represents 200 nm.*

Furthermore, the antioxidant activity of MGN-NLC (6494 ± 186 µM TE/g) was higher than the free compound (3521 ± 271 µM TE/g). This confirmed that the encapsulation of the drug was able to preserve and increase its activity.
Potential ocular irritation effects of MGN-NLC and unloaded NLC were determined on the chorioallantoic membrane (CAM) of fertilized hen eggs. No damage to the blood vessels on the CAM surface after a 5 min period of contact with MGN-NLC was detected, as reported in Fig. 2. The IS was 0.0, as occurred with the negative control (0.9% NaCl). Thus, MGN-NLC can be classified as non-irritant (IS < 1), since no hemorrhage, lysis or coagulation was observed.

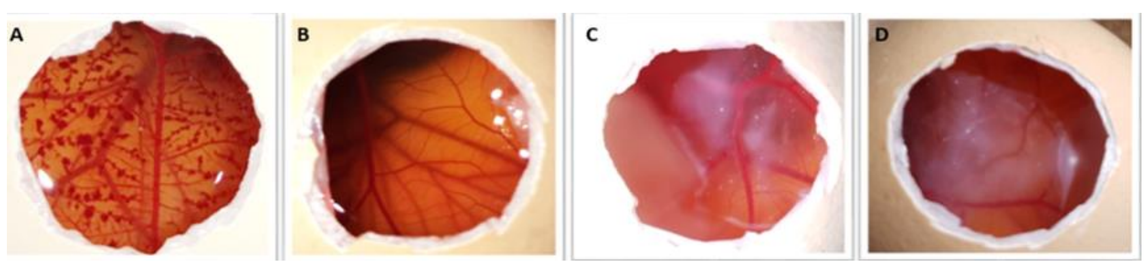

Fig. 2 *Pictures of membrane (CAM) after application of: (A) 0.1 M sodium hydroxide (NaOH) solution (positive control), (B) 0.9 wt% saline sochorioallantoic lution (negative control), (C) unloaded NLC and (D) MGN-NLC.*

Moreover, the formulations showed adequate hemocompatibility: the percentage of hemolysis for unloaded NLC and MGN-NLC were 11.16 ± 3.12% and 8.10 ± 0.95%, respectively.
Benefits of encapsulating MGN in NLC were evaluated in terms of capability to promote ocular permeability through the cornea. A lag time of ca. 2 hours was observed in agreement with reports for other drugs [10,11], which is understandable considering the compact layered structure of the cornea. After 6 h contact, a relevant amount of MGN accumulated in the cornea, 1.86 (s.d. 0.51) µg/cm2, and a larger amount permeated, 2.75 (s.d. 1.18) µg/cm2.

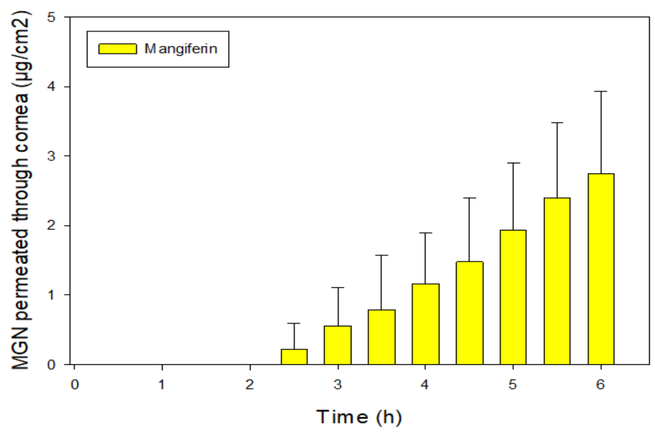

Fig. 3 *Accumulated amounts of MGN permeated through bovine cornea for 6 hours.*

References

[1] Del Amo E.M. et al., 2008; [2] Bucolo C. et al., 2012; [3] Puglia C. et al., 2015; [4] Battaglia L. et al., 2016; [5] Pignatello R. et al., 2011; [6] Brugè F. et al., 2013; [7] Lucas-Abellan et al., 2011; [8] Naveen P. et al., 2017; [9] Muller R.H. et al., 2002; [10] Lorenzo-Veiga B. et al., 2019; [11] Varela-Garcia A. et al., 2018.