Cationic Lipid Nanoparticles for ocular delivery of epigallocatechin gallate

J.F. Fangueiro[1], T. Andreani[1,2,3], A. Parra[4], A. M. Silva[2,3], A.C. Calpena[4,5], M.A. Egea[5,6], M.L. Garcia[5,6], E.B. Souto[1,7]

[1]Faculty of Health Sciences, Fernando Pessoa University, Porto, Portugal; [2]Centre for Research and Technology of Agro-Environmental and Biological Sciences (CITAB), Vila Real, Portugal, [3]Department of Biology and Environment, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal; [4]Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain; [5] Institute of Nanoscience and Nanotechnology, University of Barcelona, Barcelona, Spain; [6]Department of Physical Chemistry, Faculty of Pharmacy, University of Barcelona, Barcelona, Spain; [7]Institute of Biotechnology and Bioengineering, Centre of Genomics and Biotechnology, Vila-Real, Portugal
joanafpf@ufp.edu.pt

Introduction: Epigallocatechin gallate (EGCG) is a natural product and the major polyphenolic constituent found in green tea. Use of this molecule has been reported for several biomedical therapies e.g. anti-cancer, anti-inflammatory, anti-diabetic, anti-bacterial and anti-aging [1,2]. The antioxidant activity of EGCG is also useful for several ocular diseases, including retinopathy that is mainly caused by an anti-angiogenesis process. The purpose of this work is the encapsulation of EGCG in lipid nanoparticles (LN) to improve the poor bioavailability and high biodegradation sensitivity of the drug. Since EGCG is a molecule susceptible to undergo degradation, epimerization and other degradation processes that disable their pharmacological activity, the encapsulation in lipid matrices could improve and overcome this essential feature [3]. The use of LN could be a safe and biocompatible carrier for ocular drug delivery [4]. Since ocular mucosa has an anionic nature, the use of cationic LN is important to promote LN adhesion to ocular mucosa and prolong drug retention time [5]. Two cationic lipids, namely quaternary salts of ammonium (CTAB and DDAB) were used to develop LN dispersions and their physicochemical parameters, lipid crystallization and polymorphism, long-term stability and *in vitro* and *in vivo* toxicity were analyzed.

Materials and Methods: Initially, a full factorial design was applied in Blank-LN obtained by a double emulsion methodology [5]. Therefore, a suitable concentration of cationic lipids, namely CTAB and DDAB, was studied in the best formulation from the factorial design. EGCG was incorporated in the inner aqueous phase of the formulations and further studies were carried out. Physicochemical characterization was performed regarding the mean particle size (Z-ave), polydispersity index (PI) and zeta potential (ZP) by photon correlation spectroscopy (PCS, Zetasizer Nano ZS, Malvern, UK) and Laser diffraction (LD, Mastersizer 2000, Malvern, UK). Lipid crystallization and polymorphism of LN dispersions was assessed by differential scanning calorimetry (DSC, Mettler DSC 823e, Toledo, Spain) and by X-ray diffraction (X'Pert PRO, PANalytical). EGCG-loaded LN were also analyzed by Transmission electronic microscopy (TEM) to visualize their shape and confirm their size. The physical stability of prepared LN dispersions was assessed with an optical analyzer TurbiscanLab® (Formulation, France). The release study was carried out over 24 h using the dialysis bag method. In this *in vitro* release model, the sample was separated from the medium by means of a membrane (cellulose membrane MW cutoff 12,000 Da, Iberlabo, Spain). The system was held at 37°C to mimic *in vivo* conditions, stirred continuously, and at selected time intervals collected samples were analyzed by RP-HPLC. The samples withdrawn were replaced by the medium maintaining sink conditions. Transcorneal and transscleral permeation studies were performed in fresh removed eyes from New Zealand Rabbits using Franz diffusion cells with a permeation area of 2.54 cm^2. The receptor compartment was filled with receptor medium maintaining a temperature of 37±0.5°C with constant agitation to maintain sink conditions, while in the donor compartment the EGCG-loaded LN were placed in contact with the outer surface of the eye. Aliquots of samples were collected at various time intervals during 24 hours (n=3). After 24h, the eye was properly cleaned and the extraction of drug was performed by sonication of the corneas and

sclera with the receptor medium under controlled temperature. All samples were analyzed by RP-HPLC .*In vitro* tests were performed using the HET-CAM (Hen's Egg Test Chorioallantoic Membrane) in egg embryos and in Human retinoblastoma cell line Y-79 exposing the LN dispersions and the EGCG to the cells by the Alamar blue assay. *In vivo* Draize test was performed in nine adult New Zealand male rabbits (Panlab, Spain).

Results and Discussion: The physicochemical properties of LN obtained from the experimental design [5] were analyzed, i.e. the effects of the selected variables on LN dispersions characteristics (Z-Ave, PI and ZP). For drug ocular administration, the values of Z-Ave and PI should be the lowest as possible, since dispersions should be well tolerated in the ocular mucosa avoiding eye irritation and the transport and uptake from the cornea is facilitated. After selection of the suitable cationic lipid concentration (0.5%wt in the total formulation), EGCG was incorporated in the inner aqueous phase of the LN dispersions produced by double emulsion. The results showed dispersions with submicron size (<300nm). DCS analysis for the bulk Softisan®100 was compared to those obtained for LN dispersions. These results indicate that incorporation of EGCG accelerated the polymorphic transitions of bulk lipid upon crystallization due the decrease of the recrystallization index. This emphasizes the fact that EGCG affects the thermal properties of LNs. X-ray analysis confirmed the presence of a β´modification of complex triglyceride for Softisan®100 that remains for LN dispersions. This transition is relatively small due the lowest values of enthalpy presented by the LN dispersions compared with the bulk lipid. A possible explanation for the reduction of crystallinity of the LN dispersion is the coexistence of lipid being present in the α modification and also due to the colloidal particle size offering insufficient number of diffraction levels. These differences can be attributed to the high surface to volume ratio of LN dispersions. The evaluation of physical stability of prepared LN dispersions could predict a good physical stability of developed LN dispersions. A small destabilization phenomenon (little fluctuations in BS signal) in the end of the cell is observed. However, this deviation does not mean an instable formulation, because is lower than 2% in the LN dispersions. The *in vitro* release study of the developed LN dispersions evidenced a controlled release, being all drug out of the systems after 12h which means that the drug is delivered at a rate proportional to the concentration gradient. Concerning EGCG transcorneal and transscleral permeation from LN dispersions, the results demonstrated that these carriers deliver the encapsulated drug at a constant rate with enhanced accumulation into the corneal and scleral tissue, able to prolong the ocular residence time. Even though the mechanisms by which this phenomenon occurs are not completely understood, this study provides clear evidence that LN have a targeting and prolonged release effect with great potential. *In vitro* HET-CAM showed no signs of ocular irritancy or vascular processes (coagulation, lysis, hemorrhage). *In vivo* Draize test also comprove the safety of the EGCG-LN dispersions showing no ocular toxicity. LN dispersions were also exposed to Human retinoblastoma cell line Y-79, to achieve the maximum concentration of LN dispersions that could be used and prevent cell damage.

Conclusions: In conclusion, the cationic LN dispersions developed could be a promising and useful carrier for EGCG. The stabilization of this sensitive molecule was achieved by encapsulation in LN and proved to reach inner ocular tissues with safety and providing biocompatibility. It is thus anticipated the enhanced anti-angiogenesis activity of EGCG in the eye when loaded in LN.

References

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