Liposome Encapsulating Estetrol For The Treatment Of Ischemia Diseases In Premature Babies

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1. Introduction

In 2010, almost 15 million of babies in the world are prematurely borned, 11.1 % of the total amount of alive children. Despite the better midwife and neonatology techniques, and the reduction in neonatology mortality, the number of babies with motor, vision, hearing or mental deficiencies is still constant along the last twenty years.

Until now, only two neuroprotective strategies are used to reduce the adverse complications associated to this pathological condition: the hypothermia and the administration of magnesium sulfate. But both of them are not sufficient to decrease significantly the brain disease.

The estradiol (E2) and its receptor ERα have an important role in the brain development. The E2 is implicated in the proliferation, migration and differentiation of neuronal cells and it plays an important role in neuroprotection and anti-ischemic activity [1]. But, due to its collateral effects, it cannot be used in clinical practices.

The estetrol (E4), a hormone synthesized in the fetal liver from estradiol (E2) and estriol (E3) has the same pharmacological characteristics as the E2 with less collateral effects [2].

The aim of this study is to develop a new liposome formulation encapsulating E4 in order to enhance its crossing of the blood-brain barrier (BBB). Moreover, to increase its low water solubility and its percentage of encapsulation, E4 has been complexed with cyclodextrins.

2. Experimental Methods

2.1. Materials

All the lipids were purchased by Avanti Polar Lipids, inc.® (Alabaster, USA). Crysmeb® and Hydroxypropyl-β-cyclodextrin were purchased by Roquette (Lestrem, FR). E4 was provided by ESTETRA (Liège, BE).

2.2. Preparation of E4-cyclodextrin complex

Crysmeb® (CM) and Hydroxypropyl-β-cyclodextrin (degrees of substitution 0.87 and 0.63) (HPβCD ds 0.87 and HPβCD ds 0.63) water solutions were prepared at different concentrations. An excess of E4 was added to the cyclodextrin solutions and the dispersion was stirred at 140 rpm at 37°C for 24 hours. Then the insolubilized E4 was separated by filtration with a 0.2 μm polycarbonate membrane.

2.3. E4 assay in cyclodextrin solutions and liposome dispersions

The E4 encapsulation efficiency and the increased solubility of E4 were measured by high-performance liquid chromatography (HPLC) system, using a reserve-phase ZORBAX’ Rx-C8 column.
2.4. Preparation of E4 loaded liposomes

Liposomes were prepared using the thin film hydration technique [3]. The lipids, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) or L-α-phosphatidylcholine hydrogenated (HSPC) and cholesterol at different molar ratio, were dissolved in ethanol. After evaporation, the film was hydrated with 2 ml of HEPES buffer and stirred. In the case of E4 liposomes, E4 was added with the lipids while for liposomes containing the E4-CD complex, the complex was used to rehydrate the lipid film in place of the buffer. The dispersion was extruded (400 nm, 200 nm and 100 nm filters). 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethyleneglycol)-2000] (mPEG2000-DSPE) was added by post-insertion technique. Unincorporated drug was separated by ultracentrifugation. Physicochemical characteristics were then determined.

2.5. Particle size and zeta potential

The particle size, polydispersity index and ζ potential were measured using a nano ZS Zetasizer analyzer (Malvern Instruments, UK).

3. Results and Discussion

Complexes of cyclodextrins CM and HPβCD with E4 were prepared. The increased solubility, showed in figure 1 is significant.

![Figure 1. Solubility of E4 in water as a function of the cyclodextrin concentration.](image)

Due to the slightly lower solubility obtained with the HPβCD ds 0.87, only the HPβCD ds 0.63 was retained for future trials.

The stability of the complexes, at different temperatures, with a 100 mM CD concentration was tested (Figure 2).
Figure 2. Stability of the complexes Crysmeb®-E4 a) and Hydroxypropyl-β-cyclodextrin-E4 b) evaluated at 4°C, 25°C and 37°C at 100 mM of cyclodextrin concentration, respectively.

The results obtained demonstrate that the E4 complexed with Crysmeb® is unstable at the tested temperatures, while the complex HPβCD ds 0.63 - E4 is stable until 3 months at 4°C, 25°C and 37°C.

Liposomes incorporating the complexes E4 - cyclodextrin were prepared. The formulations loaded with the complex HPβCD ds 0.63 - E4 showed a good encapsulation amount (Figure 3).

Figure 3. Encapsulation efficacy (EE%) and concentration of E4 (µg/ml) of the liposome formulations.

Moreover, the ζ-potential, the size and the polydispersity index of the nanocolloids are compatible for an intravenous injection (Figure 3). At the same time their physic-chemical characteristics, like
their size less than 200 nm and a low polydispersity index may potentially allow them to cross the blood-brain barrier and reach the brain.

![Figure 3. Size and PDI of the DPPC- a), HSPC- and POPC-based b) liposomes.](image)

4. Conclusions

New liposome formulations containing estetrol were prepared. DPPC-(HPβCD 50 mM) can be considered as a promising drug delivery system to target estrogens to the brain.

References