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Conventional and Galactosylated Liposomal Formulations of Ellagic Acid for the Modulation of Cellular Senescence

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INTRODUCTION & AIM

Ellagic acid (EA) is a natural polyphenol mainly found in *Punica Granatum L.,* known for its antioxidant and anti-senescence effects. Despite its therapeutic potential in age-related diseases, including neurodegenerative disorders such as Alzheimer's disease (AD), hepatorenal, cardiovascular, metabolic, and cancer-related conditions [1], its application is limited by poor aqueous solubility, low bioavailability, and limited biological stability [2].

To address these issues, liposomes (LPs) were developed as biocompatible carriers to enhance EA solubility and bioactivity. Senescent cell targeting was achieved by functionalizing LPs with galactosylceramide, a galactose derivative cleaved by β-galactosidase (β-gal) overexpressed in senescent cells [3].

Both conventional LP (LP-EA) and galactosyled LP (Gal-LP-EA) were fully characterized, evaluating their physiscochemical properties, release behavior, and stability profile during one-month storage. Finally, considering the relevance of AD among age-related conditions, the ability of LPs to improve EA permeability across the blood-brain barrier (BBB) was evaluated *in vitro* using Parallel Artificial Membrane Permeability Assay (PAMPA).

METHOD

LP-EA and Gal-LP-EA were prepared using the thin-film hydration method with phosphatidylcholine and cholesterol. Galactosylceramide was incorporated into the lipid phase to obtain the β-gal-responsive targeting formulation. Empty LPs and EA-loaded LPs were characterized in terms of particle size (nm), polydispersity index (PdI), and ζeta potential (mV) by Dynamic and Electrophoretic Light Scattering (DLS/ELS), while morphology was confirmed by Transmission Electron Microscopy analysis (TEM). From a chemical point of view encapsulation efficiency (EE%) was determined by HPLC-DAD [4] using the dialysis bag method. Physicochemical stability was assessed for 30 days at +4 °C. The *in vitro* release profile of LPs was investigated in PBS:EtOH (70:30) at 37 °C for 48h and compared with a solution of free EA. In the end, passive permeability of EA, LP-EA, and Gal-LP-EA across a brain-mimicking BBB was evaluated using the PAMPA assay, with the membrane functionalized with a 2% (w/v) Porcine Polar Brain Lipid solution in n-dodecane [5].

RESULTS & DISCUSSION

Encapsulation of EA in LPs successfully increased its aqueous solubility of approximately 56-fold compared to the free compound. The DLS analysis confirmed the presence of a homogeneous system with narrow size distribution and appropriate PdI values. The presence of EA did not affect the physical characteristics of the system. The subsequent galactosylceramide coating did not negatively affect particle stability or EE%, as reported in Table 1.

Sample	Size (nm)	PdI	Z-pot (mV)	EE%
Empty-LP	82.19±0.33	0.20±0.00	-17.75±0.54	
LP-EA	113.2±0.91	0.21±0.00	-24.57±0.43	78.20±0.18
Gal-LP-EA	110.8±0.17	0.21±0.02	-24-04±0.04	80.44±0.62

Table 1. Physical characterization of empty LP, EA-LP and Gal-LP-EA. Data are expressed as mean ± SD of n=3 experiments.

Free EA solution exhibited an initial burst effect followed by a rapid release. LP-EA and Gal-LP-EA provided a sustained and controlled release over 48 h. The amount of EA released from LP-EA at 1h was 15.29% and the plateau was reached at 48h, with a percentage of 30%. Similarly, Gal-LP-EA showed a rapid liberation in the first hours (25% at 1h), which continued steadily until 48 hours (40%), as shown in Figure 1. The controlled and gradual drug liberation is essential for maintaining consistent therapeutic levels of the active compound. LP-EA and Gal-LP-EA exhibited first-order release kinetics.

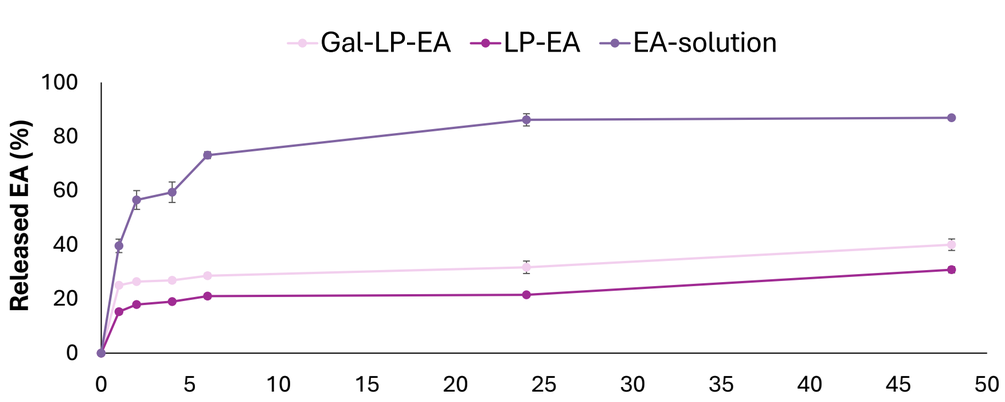

Figure 1. *In vitro* release profile of EA from solution, LP-EA and Gal-LP-EA in PBS:EtOH (70:30). Values are expressed as mean ± SD of n=3 expreriments.

LP-EA and Gal-LP-EA were stored at +4°C for one month. The formulations proved to be stable with minimal variations in size, PdI and ζeta potential (Figure 2). From a chemical point of view EE% stayed with values around 80% for both the formulations.

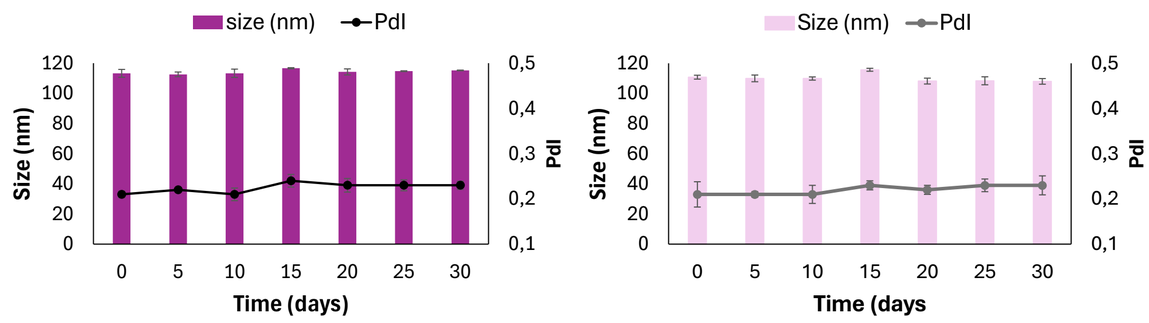

Figure 2. LP-EA (left) and Gal-LP-EA (right) physical stability study at 4°C. Data are expressed as mean ± SD of n=3 experiments.

EA displayed low passive membrane permeability coefficient (Pe) as a free compound. LPs significantly enhanced permeation across the BBB, with Pe values approximately one order of magnitude higher than the free solution. The amount of EA permeated expressed as µg/cm² was also considerably greater for LPs, confirming the superior transport efficiency. Recovery values >80% for all the formulations supported the assay reliability.

Time	Pe (cm/s)					
	EA-sol	SD	LP-EA	SD	Gal-LP-EA	SD
4h	1.72E-06	2.95E-07	1.58E-06	1.93E-07	1.26E-06	7.28E-08
8h	7.44E-07	3.18E-08	1.12E-06	5.99E-08	1.02E-06	1.02E-07

Table 2. Pe values referred to EA solution, LP-EA and Gal-LP-EA. Data are expressed as mean ± SD of n=3 experiments.

CONCLUSION

Liposomal encapsulation enhanced the solubility, stability, and sustained release of EA. Gal-LP-EA preserved these properties, appearing to be a promising nanomedicine platform for the treatment of age-related diseases.

FUTURE WORK

LP-EA and Gal-LP-EA will be tested in senescent cells to assess EA effectiveness in counteracting senescence through SA-β-Gal staining and the targeting capability provided by the coating.

Neuroprotective and anti-aggregation effects will be further evaluated in SH-SY5Y cells and in transgenic Alzheimer's (AD) animal models since senescent phenotypes have been observed in neurons, astrocytes, and microglia in AD.

References

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