Development of liposomal formulations for Relaxin 2: Effect of lipid membrane composition on liposome properties and Relaxin activity

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ABSTRACT

Relaxin-2 is a member of the insulin superfamily of peptides[1,2] and is now known as a multifunctional hormone with pleiotropic action. It is mainly involved in the maintenance of reproduction and pregnancy, while also possesses angiogenic and other cardioprotective actions. As a peptide, it has low stability in blood due to proteolytic degradation. A potential strategy to prolong the half-life of peptides and improve their stability in blood is their incorporation into liposomal nanocarriers. The purpose of this study was to develop different liposomal formulations for encapsulation of Relaxin-2, by investigating the Dehydrated-Rehydrated Vesicle (DRV)[3] technique. Different lipid membrane compositions were used, and all liposomal formulations were compared for their physicochemical properties and encapsulation efficiency. Finally, the bioactivity of liposomal relaxin was tested in HEK293 (Human Embryonic Kidney) cells, transiently transfected with Relaxin-2 receptor (RXFP1) and analyzed for cAMP (Cyclic Adenosine Monophosphate) detection with competitive ELISA immunoassay. Our results demonstrate higher encapsulation percentages for the liposomal formulations without phosphatidyl glycerol (PG). Comparing the physicochemical properties (vesicle size distribution and zeta-potential), liposomal vesicles consisting of PG indicated higher size and negative charge. Biologically, liposomal relaxin retained its bioactivity which was found to be significantly higher compared to the respective activity of free peptide (recovered after size exclusion chromatography). The identification of an optimal method for peptide encapsulation in liposomes, that will retain the bioactivity of the peptide, is crucial for future development of effective liposomal nanocarriers for sensitive biomolecules.

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