Research in Pharmaceutical Sciences, 2012;7(5)
School of Pharmacy and Pharmaceutical Sciences
Isfahan University of Medical Sciences

Development of a highly efficient method for encapsulating the rat HER2/neu-derived peptide (P5) into liposomes to induce an effective tumor-specific immunity

S. Shariat[1,*], S.A. Mortazavi[1], M.R. Jaafari[2], A. Badiee[3]

[1]School of pharmacy,Shaheed Beheshti University of Medical science
[2]Nanotechnology research center,school of pharmacy, Mashhad University of Medical science
[3]Nanotechnology and Biotechnology research center,school of pharmacy, Mashhad University of Medical science

Background and Aims: The rat HER2/neu peptide (P5) is a synthetic, hydrophobic short peptide which can induce tumor –specific immunity against HER2-over expressing breast tumor in mice. Typical methods used for encapsulating peptides in lipid vesicles result in very low encapsulation efficiencies. This study was designed to develop a highly efficient method for encapsulating peptide (P5) into liposomes as a vaccine and evaluate effects of formulation variables on peptide encapsulation.
Methods: Liposomes were prepared by slow addition of succinate buffer to lipid film (DMPC, DMPG, Cholesterol) dissolved in ethanol followed by extrusion through 400nm polycarbonate filter. Peptide solution in DMSO was slowly added while vortexing. Subsequently the ethanolic dispersion was incubated at different temperatures and times and then dialyzed three times within 24 hours at 4ºC to remove excess ethanol, DMSO and un-entrapped peptide. Finally liposomes were passed through 0.45 µm microbial filter and kept at 4ºC. In this process the effects of formulation variables like incubation time (0.5, 1, 2 and 3 hrs), incubation temperature (4, 15, 25,40ºC), ethanol concentration (0, 10, 20, 30, 40, 50%) and lipid concentration (20, 40 and 60mM) on the entrapment efficiency of peptide (P5) were investigated. Particle size, polydispersity index and zeta potential of liposomes were measured by Malvern particle size analyzer. P5 concentration was determined by RP-HPLC and phospholipid concentrations were determined by Bartlett assay.
Results: The highest encapsulation ratio was 45% when the concentration of ethanol and lipid were 30% and 40mM and liposomes and peptide were incubated for 1hr at 25ºC. Liposomes were with size of 322 ± 24.3 nm, PdI of 0.14 ±0.02 and zeta potential of -48.2 ±3.7 mv.
Conclusions: These results show that Peptide (P5) can be efficiently encapsulated into liposomal carrier by using this method under optimized formulation parameters to prepare an effective peptide vaccine.

Keywords: Liposome; Peptide; Encapsulation