

FORMATION AND CHARACTERISATION OF NON-TOXIC ANIONIC LIPOSOMES FOR DELIVERY OF THERAPEUTIC AGENTS TO THE PULMONARY AIRWAYS

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The pulmonary route offers huge potential for delivering biotherapeutics not only to the lung itself but to other body organs as well. Before any liposomal formulation could be utilised for pulmonary drug delivery some critical points should be addressed, namely toxicity of the liposomal formulation, stability of the liposomes, drug entrapment efficiency and size variation of the formulation. Toxicity of some liposomes seems to be caused by the application of organic solvents or detergents during their manufacture, or employing cationic agents in their structure. Recent developments in the field have made it possible to prepare liposomes without using any organic solvent or detergent, examples of which are the polyol dilution method [1], and the heating method [2]. Anionic liposomes were prepared by the conventional thin-film hydration method and the heating method in which the lipids are brought to high temperatures (up to 120°C) in the presence of glycerol (3% v/v) [2-3]. In order to reduce the sizes of the vesicles the liposomal suspensions were extruded through polycarbonate filters of 100 or 400nm pore sizes. A gene transfer vector was constructed with the liposomes prepared by the heating method (HM-liposomes), as previously reported for the unilamellar and multilamellar liposomes prepared by conventional techniques [4-6]. Incorporation of 5FU, as a water soluble model drug, was achieved by adding the drug to the lipids before the heating step. Encapsulation efficiencies of 70.3% (± 1.5) and 38.4% (± 1.8) were obtained for DNA and 5FU respectively. The size and morphology of the lipid vesicles were studied by light scattering and a variety of optical and electronic microscopic techniques. HM-liposomes extruded through 100nm filters were found to be monodisperse up to two months after their preparation and storage under N₂ at 4°C. A size enlargement was observed for the liposomes upon storage under the above mentioned conditions. Microscopic studies revealed formation of spherical bilayer structures with homogenous size range, and stabilities of at least eight months. The high-resolution imaging technique of Scanning Tunnelling Microscopy (STM) has been applied for two and three dimensional morphological investigation of the liposomes and to study the effect of storage on their structure. STM also enabled measuring the diameter and the bilayer

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thickness of the vesicles with and without DNA and Ca^{2+} . Light scattering measurements revealed that incorporation of plasmid DNA and Ca^{2+} to anionic HM-liposomes confers mean size enlargements of between 2.0 and 3.8. This indicates successful incorporation of DNA to HM-liposomes and has also been previously documented for conventional liposomes [4-6]. Presence of DNA in the liposomal vector was also confirmed by agarose gel electrophoresis studies. The transfection efficiency and the cytotoxicity of the liposomal vector was determined using a plasmid carrying a β -galactosidase reporter gene in a human bronchial epithelial cell line (16HBE14o). Cell viability was evaluated after 24h using the Neutral Red Uptake (NRU) and MTT toxicity assays. The reason for applying two toxicity assays is due to the fact that some liposomal formulations have been viewed as non-toxic if only one of the NRU or MTT methods was used to assess the toxicity. Results indicated no toxicity for the 100nm and 400nm liposomes prepared by the heating method. Conventional liposomes, however, decreased cell viability by 5-17% (100nm vesicles) and 4-17% (400nm vesicles) and were significantly more toxic ($P < 0.01$) than liposomes prepared by the heating method. Gene transfer studies have revealed a low transfection efficiency for HM-liposomes compared with that of commercially available DOTAP reagent. The mechanisms underlying the toxicity of conventional liposomes as well as the barriers to successful liposomal gene and drug delivery to the lung will also be discussed.

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