PL 2 - Assessment of Chitosan/Cyclodextrin Nanoparticles in Calu-3 Cells

D. Teijeiro-Osorio, C. Remuñán-López, M.J. Alonso

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Santiago de Compostela, Campus Sur s/n 15782-Santiago de Compostela, Spain

Nowadays, several non-invasive routes, such as the nasal or pulmonary, are being extensively evaluated as alternatives to the parenteral route for the systemic delivery of macromolecular drugs. Besides, significant efforts are being dedicated to design colloidal systems that act as transmucosal nanocarriers. We have recently proposed a new nanoparticulate system composed of chitosan (CS) and hidroxypropyl-β-cyclodextrin, which takes advantage of the well-known drug complexation power and stabilizing properties of cyclodextrins (CD) together with the widely reported advantages of CS in an unique delivery system.

Based on this previous information, we have developed nanoparticles composed by CS and two different anionic CD derivatives (sulfobutylether-β-CD and carboxymethyl-β-CD). CS/CD nanoparticles were prepared in presence of tripolyphosphate according to a mild ionotropic gelation method previously developed by our group, which was slightly modified to allow the CD incorporation.

In order to get a further insight into the potential of these systems as transmucosal drug nanocarriers, in the present work we explored the effect of CS/CD nanoparticles on the viability and integrity of Calu-3 cell monolayers, as well as their ability to interact with the epithelium. Calu-3 cell line was selected as model of the nasal and tracheo-bronchial epithelium because it shows features of the normal human tissues, such as tight junctions, high transepithelial electric resistance (TEER) values and mucous excretions.

MTT (3-4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) colorimetric assay was conveniently optimized and afterwards used to estimate the sensitivity of Calu-3 cells to different CS/CD nanoparticle concentrations. Interestingly, it was found that cytotoxicity of nanoparticles containing CD was significantly lower than the corresponding to the nanoparticles composed only by CS, showing an IC50 increase of app. 2-3 fold.

The effect of the formulation type on the tightness of differentiated air-liquid interface cultured Calu-3 cell monolayers was monitored by measuring TEER. Although the nanoparticles were able to significantly decrease TEER of the cell layers at the concentrations assayed, a complete reversibility of the achieved effect was observed in a few hours, following removal of the nanoparticle suspensions. This TEER recovery after the experiments implies that the cells were undamaged and functionally intact.

Confocal Laser Scanning Microscopy (CLSM) was carried out to evaluate nanoparticles uptake by differentiated cells. CS/CD nanoparticles containing fluorescein-labelled CS were used and viability and integrity results were related to the ability of nanoparticles to closely interact with the epithelium.