Convocatoria de ayudas para la realización de proyectos coordinados en el marco de IBEROS. Anualidad 2017

Proyecto concedido

DATOS GENEREALES:

Título proyecto: Intracellular delivery of an antibody with nanogels
Entidades participantes (mínimo 2 entidades): INEB University of Santiago Compostela
Grupos de investigación: Nanomedicines and Translational Drug Delivery R+D in drug dosage forms and drug delivery systems (R+D Pharma)
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OBJETIVOS DEL PROYECTO (máximo 100 palabras):

Current antibody-based therapy is restricted to the extracellular milieu. However, antibodies can potentially function within the cell to inhibit many intracellular processes such as signaling cascades or protein-protein interactions. The problem lies in delivering antibodies to the intracellular environment. Nanogels are nanoparticles capable of harboring hydrophilic biotherapeutics, such as antibodies, and delivering their payload inside cells.
Our main objective is to formulate pH/redox dual-sensitive dextran nanogels that can safely harbor a therapeutic antibody and characterize their capability to deliver the antibody inside cells.

PLAN DE TRABAJO:

Brief Introduction:
Nanogels composed of pH/redox dual-sensitive dextran (DEX-SS) and harboring the hydrophilic drug methotrexate (MTX) (MTX@DEX-SS) have recently been developed in our lab [1]. These nanogels displayed higher MTX release at pH 5.0 compared to pH 7.4, and MTX release was also higher in the presence of the antioxidant glutathione (GSH). These results demonstrate that MTX release should be stimulated when the nanogels are internalized within a cell where lower pH is first encountered within the endosomal compartment and where GSH levels are high.

Task I – CET@DEX-SS nanogel production, physical characterization and CET release:
Here, we will exploit our established and robust protocol of DEX-SS nanogel production for the encapsulation of a model therapeutic antibody, Cetuximab (CET) (anti-EGFR). As both MTX and CET are hydrophilic, it is expected that our protocol should need minimal, if any, optimization to accomodate CET. CET@DEX-SS nanogels will be characterized by dynamic light scattering and laser Doppler anemometry. CET encapsulation efficiency and release kinetics over time at pH 5.0 and 7.4, with or without GSH, will be monitored by ELISA.

Task II – Monitoring of cellular internalization of CET:
In this task, we aim to quantify the presence of CET within cells after incubation with CET@DEX-SS nanogels. HeLa cells will be used as the human cell model as they were used in the previous study [1] and express EGFR on their surface [2] for future quality controls experiments to determine if CET maintains its receptor-binding capability after nanogel encapsulation and subsequent release. Intracellular CET will be monitored by Western blotting and/or ELISA from cell lysates obtained from HeLa cells incubated with CET@DEX-SS nanogels and appropriate controls.

Task III – Cytotoxicity of CET@DEX-SS nanogels:
Here, we will determine the cytotoxicity of the CET@DEX-SS nanogels and appropriate controls at different concentrations on HeLa and Balb 3T3 cells by MTT reduction as performed in the previous study [1].

References:
[1] Curcio M, Diaz-Gomez L, Cirillo G, et al. pH/redox dual-sensitive dextran nanogels for enhanced intracellular drug delivery. Eur. J. Pharm. Biopharm. 2017;117:324-32.
[2] Zhang F, Wang S, Yin L, et al. Quantification of epidermal growth factor receptor expression level and binding kinetics on cell surfaces by surface plasmon resonance imaging. Anal Chem. 2015;87:9960-5.