FOLATE-RECEPTOR MEDIATED GENE DELIVERY BY LIPO-POLYMERIC NANOPARTICLES

García, L. and Tros de Ilarduya, C.
Department of Pharmacy and Pharmaceutical Technology, School of Pharmacy, University of Navarra, Pamplona, Spain.
lgarcia9@alumni.unav.es

Nanomedicine may be defined as the monitoring, repair, construction and control of human biological systems at the molecular level using engineered nanodevices and nanostructures. Many products of biotechnology are enormously useful in near-term medical application.

The objective of this study is the development, optimization and characterization of a novel nanostructure (lipopolyplexes) composed of polyethylenimine (PEI), cationic liposomes and DNA, which can deliver genetic material into liver tumour cells. We studied the association of folic acid with nanoparticles to prepare targeted complexes, that can deliver genetic material in a best way.

Nanoparticles were prepared by first mixing a solution of different types of PEI with plasmid DNA at N/P ratio of 4 and after 15 minutes of incubation cationic liposomes were added in order to prepare complexes at molar ratio of 17/1 (lipid/DNA). Targeted-lipopolyplexes were prepared by the addition of folic acid to nanoparticles and incubating for 15 minutes at room temperature. Complex sizes and surface charges were determined by dynamic light scattering in a Zetasizer-nano particle analyzer. These nanoparticles were positively charged and the complex sizes were around 200 nm. The addition of folic acid to lipopolyplexes resulted on slightly higher complex sizes (300 nm). The size of condensed DNA is critical for in vivo delivery, because the particle size influences not only the biodistribution but also the efficiency of cellular uptake through endocytosis [1]. The ability of the polymer (PEI) to condense DNA was studied by measuring the decrease in the ethidium bromide fluorescence upon its expulsion from DNA. Condensation assays by ethidium bromide exclusion, revealed that PEI was effective in condensing DNA at N/P ratio of 4.

In vitro studies were performed with HepG2 cells in the presence of 60% fetal bovine serum (FBS). DNA expression was measured by a luminometer and the results were expressed as ng of luciferase per mg of protein. Cell viability was determined by Alamar blue assay and was calculated according to the formula $(A_{570} - A_{600})$ of treated cells x 100/ $(A_{570} - A_{600})$ of control cells. Nanoparticles formed with linear PEI were more effective than branched PEI in transfecting liver cancer cells, showing branched PEI of low molecular weight better efficacy compared to high molecular weight PEI (Figure 1). It is important also to note, that these systems showed improved gene delivery to cancer cells compared to conventional lipidic or polymeric systems, and that they were able to transfect in the presence of high concentration of serum (60%). The association of folic acid conjugated to lipopolyplexes via electrostatic interactions enhances significantly the transfection of cells. In summary, these nanoparticles have a low toxicity and good transfection performance *in vitro*. Studies on the suitability of this non-viral vector for gene transfer *in vivo* are in progress.

References:

[1] Sun, X., Zhang, H.W., Zhang, Z.R. Transfection efficiency of pORF lacZ plasmid lipopolyplex to hepatocytes and hepatoma cells. World J. Gastroenterol. **10**(4) (2004), 531-534.

Figures:

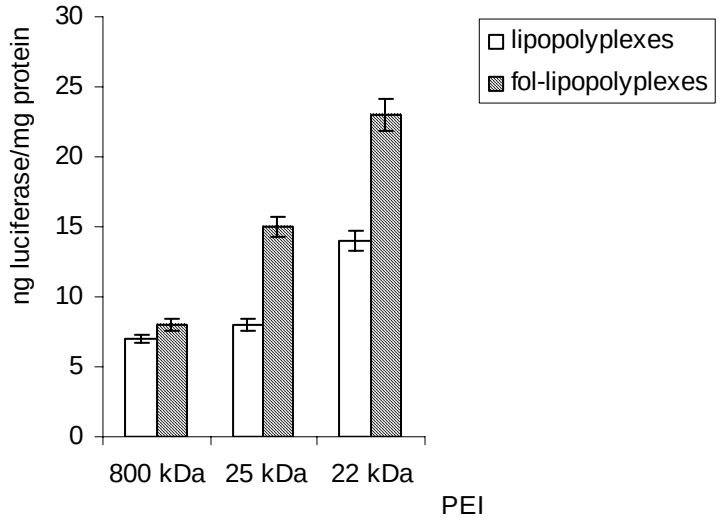

Figure 1. In vitro transfection activity