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

Effect of degree of polyethyleneimine PEGylation on biological and cellular activity of hTERT siRNA

F. Safari[1,*], A. Tamadon[2], N. Zarghami[1], S. Abolmali[2], H. Najafi[2]

[1]Biochemistry Department, Tabriz Medical School, Tabriz University of Mediacal Sciences, Tabriz,Iran
[2]Pharmaceutics Department, Shiraz Pharmacy School , Shiraz University of Medical Sciences, Shiraz, Iran

Background and Aims: Small interfering RNA (siRNA) molecules have significant therapeutic promise for the genetic treatment of cancer. The action of gene delivery systems such as polyethyleneimine (PEI) is hindered by parameters such as inherent cytotoxicity of the carrier and insufficient protection against extracellular matrix. PEGylation technology may improve the biocompatibility of the carrier; however, may attenuate the cellular uptake. Therefore, we aimed to study the effect of degree of the polymer PEGylation on the carrier cytotoxicity and hemo compatibility, cellular association and transfection activity of hTERT siRNA in lung cancer cell.
Methods: N-hydroxysuccinimide (NHS) activated, methoxy poly ethylene glycol carboxylic acid 5KDa was grafted to branched PEI 25KDa using carbodiimide chemistry at ratio of 1, 3 and 10. The copolymer was purified by acetone precipitation. The copolymer was characterized by CHN analysis, H-NMR spectroscopy and gel filtration chromatography. Cytotoxicity of PEG-g-PEIs was determined by a validated MTT assay. The polyelectrolyte complexes of PEG-g-PEI and siRNA/DNA at different N/P ratio (0.5 – 30) was studied using ethidium bromide exclusion assay and after incubation with heparin sulfate. Moreover, the formation of the nanocomplexes was confirmed by agarose gel retardation assay. The cellular association of a large library of PEG-PEI/siRNA nanocomplexes was determined by flow cytometry of FITC-labeled oligonucleotide in A549 human lung carcinoma cells.
Results: Cytotoxicity of PEG-g-PEI was obviously lower than that of PEI. The cellular association of PEG-PEI/siRNA nanocomplexes was dependent on the charge ratio between amino groups of PEG-PEI and phosphate groups of siRNA (N/P) values and the degree of the polymer PEGylation. Flow cytometry experiments revealed that the mean fluorescence intensity was the highest for PEG-PEI/siRNA at N/P 7.5 and 15 and it was significantly higher than PEI as delivery carriers. Therapeutic activity of the transfected siRNA in comparison with control sequence is under investigation.
Conclusions: PEG-g-PEI may be a promising non-viral carrier for altering gene expression in the treatment of non small cell lung cancer with many advantages, such as relatively high gene transfection efficiency and low cytotoxicity.

Keywords: siRNA; Polyethyleneimine; Polyethylenenglycole; FACS