Understanding the siRNA Delivery with Lipophilic Polymers in Chronic Myeloid Leukemia (CML) for therapeutic purposes

+[1]Valencia-Serna, J; [1]Gül-Uludağ, H; [2]Jiang, X; [1,3,4]Uludağ, H.
[1]Department of Biomedical Engineering, University of Alberta, Edmonton, AB, Canada; [2]Terry Fox Laboratories, BC Cancer Agency, Vancouver, BC, Canada; [3]Department of Chemical & Materials Engineering, University of Alberta, Edmonton, AB, Canada; [4]Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, AB, Canada.

Introduction:

Chronic Myeloid Leukemia (CML) is a disease of the hematopoietic stem cells characterized by the rearrangement of the chromosome 9 and 22 that gives raise to the Bcr-Acl fusion oncogene. This gene is translated into a protein with abnormal constitutive tyrosine kinase activity, which causes uncontrolled proliferation and differentiation in the hematopoietic system (1). Due to improved knowledge of the CML at a molecular level, RNA interference (RNAi) has become a promising therapy that can be used for down-regulating the production of specific proteins for treatment in CML. In order to implement this technology, however, a carrier that internalizes the siRNA moieties through the cell membrane and delivers them into the cytosol in a safe and efficient manner is needed. Lipid substitutions of low molecular weight (MW) polyethyleneimine (PEI) were used in this study to investigate the efficacy of these polymers in silencing Green Fluorescent Protein (GFP)-positive K562 model cells. The most suitable lipid-substituted polymers for GFP silencing CML cells was then evaluated for targeting Bcr-Abl protein and induce apoptosis. This will help us to have a better understanding of the structural characteristics of the siRNA carriers needed for the design of novel therapies for CML disorders.

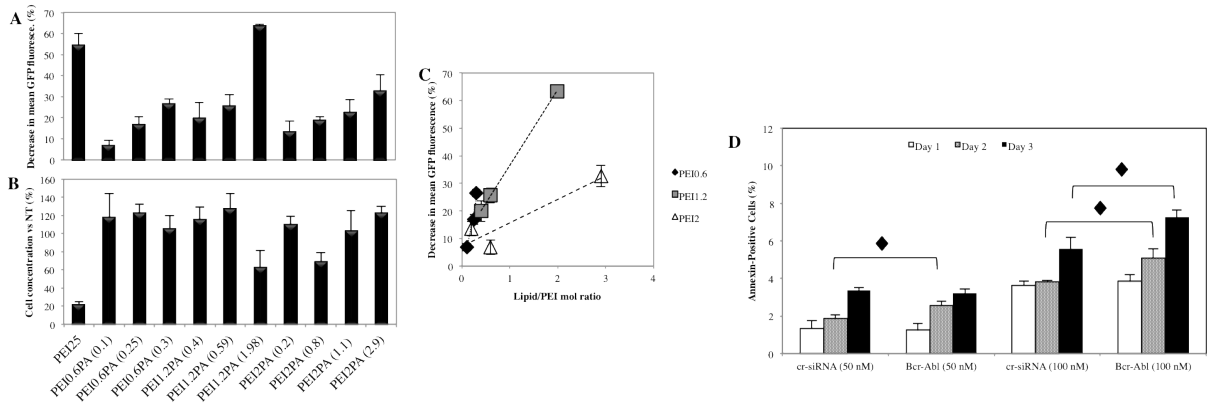
Materials and Methods:

PEIs of MW of 0,6, 1,2 and 2 kDa that were substituted with Palmitic Acid (PA) at three degrees of lipid substitutions as well as the commercial PEI of MW 25kDa were used as siRNA carriers. The synthesis of the lipophilic polymers has been described before (2). Complexes were prepared with scrambled and anti-GFP siRNA at a polymer:siRNA ration of 8:1 in 150nM NaCl solution and incubated for 30 min. GFP–expressing CML cells (GFP-K562 cells) were used as cell model and were cultured 24 h prior transfection in RPMI medium with 10% of serum and 1% of streptomycin/penicillin and were exposed to the complexes at a 72 nM siRNA concentration. 72 h after transfection, decrease in GFP fluorescence as well as cell concentration were assessed by flow cytometry. Wild type K562 cells were exposed to complexes prepared with PEI1.2PA and anti-Bcr-Abl siRNA at polymer:siRNA of 4:1 and at a siRNA concentration of 100nM. Annexin-FITC was used to stain cells in order to quantify the cells that are undergoing early apoptosis by flow cytometry after 1, 2 and 3 days post-transfection.

Results and Discussion:

GFP silencing with PEI25 gave a 54% decrease in the mean GFP fluorescence (Fig. 1A); however, an 80% reduction (compared with no-treated (NT) cells) of the cells treated with this polymer suggests was also found (Fig. 1B). On the other hand, among the PA-substituted PEIs the polymer that had its best performance was 1.2PEIPA (lipid substitution of 1.98 PEI/PA) showing a 63% decrease in the mean GFP fluorescence and a milder effect on the cell concentration decreasing it by 40% (Fig. 1A, B). A strong correlation between the extend of lipid substitution and the GFP silencing was found with all the PEIs used. Moreover, the GFP silencing is also dependent on the PEI used; the lower the MW of the PEI the greater the increase in GFP silencing at increasing lipid substitutions.

Based on the percent of Annexin-positive cells, an increase of early apoptosis was seen at a 50 nM siRNA concentration on day 2 and at 100 nM on day 2 and 3 post-transfection in comparison with the complexes prepared with scrambled siRNA (Fig. D).



Conclusions:

PA-substitutions on PEIs of low MW (especially with 1.2PEI with 1.98 PEI/PA) increased the GFP silencing and induced smaller changes in cells concentration in comparison with the commercial 25PEI polymer. The induction of the apoptosis by RNA interference using the described liphophilic polymers demonstrated the potential of these lipophilic polymers to target specific mRNA in order to have a therapeutic effect in CML disorders.

References:

1. Sloma I, Jiang X, Eaves AC, Eaves CJ. Insights into the stem cells of chronic myeloid leukemia. Leukemia. Nature Publishing Group; 2010 Sep 23;24(11):1823–33.

2. Remant Bahadur KC, Landry B, Aliabadi HM, Lavasanifar A, Uludağ H. Lipid substitution on low molecular weight (0.6–2.0kDa) polyethylenimine leads to a higher zeta potential of plasmid DNA and enhances transgene expression. Acta Biomaterialia. 2011 May;7(5):2209–17.

Acknowledgements:

Alberta Cancer Foundation, Natural Sciences and Engineering Research Council of Canada and, Canadian Institutes of Health Research