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Effect of cell penetrating peptide (TAT) on the complexation and transfection of siRNA

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Introduction: Gene therapy has been investigated as a strategy for the treatment of numerous diseases, such as cancer, AIDS and cystic fibrosis. In this way, post-transcriptional gene silencing by RNA interference rises as an interesting method for the target inhibition of gene expression. It should be emphasized the potential to specifically and potently knock down gene expression, however, naked siRNAs cannot reach the intracellular environment due to its high molecular weight and strongly negative charge. Then, considerable research effort is currently focused on the development of safe and effective delivery systems for these promising molecules. **Objective:** In the present work, we aim to evaluate the application of TAT peptide, a cell penetrating peptide (CPP) derived from amino acids 47-57 of the HIV TAT protein, for the delivery of a model siRNA through L929 cells. **Methods:** The systems were prepared using different proportions of TAT peptide and siRNA (10:1; 25:1 and 50:1, w/w), vortex mixed at 2000 rpm for 10 seconds and allowed to complexation during 30 min at room temperature. The systems were then characterized by zeta potential using Zetasize 3000HSa equipment and complexation efficiency by gel electrophoresis at 100V, for 20 min in TAE buffer using agarose gel (2.0% w/v) containing 10 mg/mL ethidium bromide after the addition of 20% of loading buffer (bromphenol blue (0.25 %, w/v), xylene cyanole FF (0.25 %, w/v), orange G (0.25 %, p/v), Tris-HCl buffer pH 7.5 (10 mmol/L), EDTA (10 mmol/L) and sucrose (0.65 %, w/v)). The samples were evaluated *in vitro* in L929 cell line, routinely grown in 150-cm² tissue culture flasks in DMEM cell culture medium supplemented with 1% (v/v) of an antibiotic solution containing 10,000 IU penicillin, 10 mg streptomycin and 25 µg amphotericin B per mL and 10.0% (v/v) heat inactivated FBS at 37ºC under 5% CO2 for cell viability through MTT assay(5.0×10^4cells/well) and transfection (1.0×10^6cells/well) observed in fluorescence microscope using 492 (λexc) and 517 nm (λem). **Results:** TAT peptide was able to increase the zeta potential of siRNA solution (10 µM, -70 mV), reaching positive plateau values (about 25 mV) for TAT peptide:siRNA at 50:1 (w/w). Gel retardation was also observed for increasingly amounts of TAT peptide, demonstrating that TAT peptide could effectively condense the anionic cargo at a weight ratio over 50. Studies of the cell viability of L929 mice fibroblasts revealed that TAT peptide was not harmful for this cell line, since viability rates greater than 80% were obtained. In order to investigate if the presence of TAT peptide was able to promote cell uptake, FAM-labeled siRNA was used. We found that no transfection occurred under the conditions tested which may indicate the necessity of a more complex delivery system to transfect cells. **Conclusion:** Solutions containing different proportions of siRNA and TAT peptide were prepared and characterized. It was observed increased zeta potential and electrophoresis complexation for system containing TAT: siRNA at 50:1, which indicates that TAT peptide in the absence of covalent attachment is able to interact and neutralize the negative cargo of the oligonucleotide. However, L929 cells were not transfected, which might indicate the need to associate another pharmaceutical vehicle with TAT to provide suitable cellular uptake for siRNA.

Keywords: Gene therapy, siRNA delivery, cell penetrating peptides, zeta potential, electrophoresis, L929 cell line.

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