Analysis of new Polglumyt® derivatives as nanocarrier for efficient intracellular siRNA delivery

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Polglumyt® is a proprietary highly purified form of glycogen, which is composed of D-glucose molecules linked by α(1→4) bonds with branches every 5-10 glucose units linked by α(1→6) bonds. Polglumyt® has a roughly spherical macromolecular structure where surface and bulk have identical chemical composition. Glycogen structure can be seen as a natural dendrimeric structure. The high degree of branching confers Polglumyt® a high water solubility and a very low viscosity.

Polglumyt®, selectively modified, could be an alternative to dendrimers and synthetic hyperbranched polymers, which have been extensively studied in the last 10-15 years as delivery agents for a variety of pharmaceutical applications including the delivery of genetic material and the solubilization of poorly soluble drugs.

Polglumyt® Polycationic Derivatives (PPDs) were synthesized and investigated as carriers for genetic material delivery. The chemical-physical properties and the toxicity were determined and the derivatives were characterized by gel electrophoresis and cellular uptake studies.

The cytotoxicity of PPDs was determined for 0.01-1 mg/mL polymer concentrations in human monocyte-derived MonoMac-6 and human colon adenocarcinoma HT29 cell lines using an ATPlite assay.

siRNA-PPD complex formation at different ratios as well as stability in the presence of RNases or serum was evaluated by agarose gel electrophoresis.

The internalization of polymers/siRNA oligo complex into the cells and their ability to silence specific target genes have been investigated. Complex formation was evaluated for 16 different siRNA-PPD complexes. Among them 8 PPDs were able to retain siRNA in the range of 5-20% wt nucleic acid/PPD ratio.

The cytotoxic activity of all siRNA-PPD complexes was evaluated by FACS analysis and no cytotoxic effects were observed when cells were transfected with siRNA-PPD complexes.

The capability of six polymer complexes to protect siRNA from RNase enzymatic activity was evaluated, both with purified RNases and in mouse or foetal bovine serum endogenous RNases.

In the presence of purified RNases, naked siRNA was gradually degraded over time, while siRNA complexed with all tested cationic polymers derivatives was effectively protected at any tested time: a maximum amount of about 20% siRNA was degraded after 60 minutes incubation when complexed to derivatives polymers, while 70-90% naked siRNA was degraded under the same conditions.

In the presence of mouse serum, naked siRNA was gradually degraded over time, while in foetal bovine serum naked siRNA was almost totally degraded after 1 hour of incubation. Complexation of siRNA with cationic polymer derivative MPP3 protected siRNA from nuclease degradation until 24 h after incubation both in mouse serum and foetal bovine serum.

To verify the uptake of FITC-labelled PPD/siRNA complexes into cells and to confirm that the complexes reached the cytoplasmic target, an immunofluorescence analysis (IF) of transfected cells has been performed by using confocal microscopy. IF analysis revealed that all siRNA-PPD complexes were internalized by the cells and a partial degree of silencing was obtained.

Our results indicate that the tested Polglumyt derivatives can be efficiently internalized but further chemical modifications should be introduced in order to increase their gene silencing efficacy.

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- Sato A, Choi SW, Hirai M, Yamayoshi A, Moriyama R, Yamano T, Takagi M, Kano A, Shimamoto A, Maruyama A. Polymer brush-stabilized polyplex for a siRNA carrier with long circulatory half-life. J Control Release. 2007;122(3):209-16.