siRNA and also silencing of the BCR-ABL protein in K562 CML cells. Tat-LK15 peptide [1], a fusion of Tat and membrane lytic peptide LK15, was used to non-covalently complex siRNA targeting the BCR-ABL mRNA (b3a2 isoform). Complexation of siRNA by Tat-LK15 was studied using fluorescence correlation spectroscopy (FCS) in the presence of the intercalating dye YOPRO-1. Cy5 labelled siRNA was used to study uptake in K562 cells using flow cytometry and confocal microscopy. The reduction in BCR-ABL protein levels was observed by Western blot. Results were compared with K562 cells transfected with lipofectamine/siRNA complexes. MTT assay was performed to study the cytotoxicity of the Tat-LK15/siRNA complexes. The YOPRO-1 competitive binding assay revealed efficient condensation of siRNA by Tat-LK15 and Lipofectamine™ at charge ratios higher than 3:1 (less than 10% of YOPRO-1 labelled siRNA). Flow cytometry studies using varying amounts of siRNA showed an increase in intracellular existence of Cy5-siRNA also leading to an increase in percentage positive transfected cells. Confocal microscopy confirmed the increase in intracellular localization upon transfection with higher amount of siRNA 4 hours and 24 hours post-transfection. Finally RNAi was observed using siRNA, which resulted in 70–80% reduction in BCR-ABL protein levels at lower concentrations. However, silencing observed using siRNA did not last longer than 48 hours. Cytotoxicity studies show that Tat-LK15/siRNA complexes are not toxic when lower concentrations of siRNA are used. Here, we show that Tat-LK15 can be a potential vector in delivering siRNA targeting genes of clinical significance.

Reference

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A56

Carbon nanotube-dendron series for siRNA delivery: mechanisms of cellular internalisation

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Carbon nanotubes have been attracting attention as tools for various biomedical applications. Chemical surface functionalization of multi-walled carbon nanotubes (MWNT) has shown remarkably increased aqueous solubility and debundling of nanotube aggregates that makes this material a promising candidate for biological applications. In this work, a series of dendron-MWNT derivatives were synthesized as potential vectors for siRNA delivery [1]. To elucidate the mechanism of cellular internalization characteristics of the dentron-MWNT:siRNA complexes, a fluorescence probed, non-coding siRNA sequence was used and its nanotube-mediated cytoplasmic delivery was studied in comparison to that by cationic liposomes. siRNA delivered by the dendron-MWNT was found throughout the cytoplasm including the nucleus. The siRNA delivered by cationic (DOTAP:cholesterol) liposomes was co-localized with endosomal markers indicating primarily an endocytosis pathway for internalization as previously described in the literature. The cellular transport of the siRNA was significantly increased with higher dendron generations conjugated on the nanotube surface at physiological conditions (37 °C) as well as under endocytosis-inhibiting conditions (4 °C). This work demonstrated that clathrin-coated endocytosis is a contributing but not the major pathway for the cellular internalization of the dentron-MWNT:siRNA complexes and could offer a great advantage via direct cytoplasmic delivery of siRNA for effective gene silencing.

Reference

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A57

Cellular internalisation of humanized IgG antibody changes by functionalization onto multi-walled carbon nanotubes

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Antibodies have been extensively used as anti-neoplastic therapeutics clinically and preclinically as they allow for therapeutic and specific targeting to specific cell receptors. The humanized CTMO1 IgG antibody was raised against the membrane-associated antigen of human milk fat globules (HMFG) derived from the anti-HMFG mouse monoclonal antibody CTMO1, but with similar affinity to the polymorphic epithelial mucin-1 (MUC-1). Anticancer drugs derived from murine HMFG1 have been under development in phase III clinical trial [1]. Carbon nanotubes have remarkable physicochemical properties offering an array of interesting features. In the context of this study, their large surface area offered a template for conjugation with a variety of monoclonal antibodies. Multi-walled carbon nanotubes (MWNT) were chemically functionalized with humanized CTMO1 IgG. The MWNT-IgG constructs were observed to target MUC-1 positive cells, but were retained at the plasma membrane with limited internalization. In contrast, a time-dependent cell surface binding and internalization was observed for the humanized CTMO1 IgG alone. The co-localization of the fluorescently labeled IgG with markers of specific cellular compartments was also studied using confocal laser scanning microscopy, to determine its mechanism of cellular uptake and trafficking pathway. The results here indicated that the size and aggregation state of the MWNT-IgG constructs played a determinant role in their interaction with cells. The design and development of CNT-antibody con-