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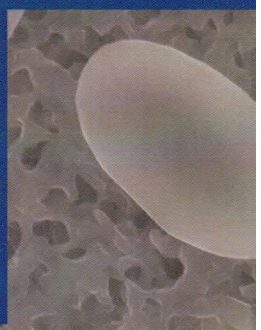

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DYNAMIN-DEPENDENT ENDOCYTOSIS IS THE MAIN PATHWAY INVOLVED IN DNA TRANSFECTION OF HEK 293 CELLS BY SILOXANE/PEI-DNA POLYPLEXES

Cristina Ana Constantinescu[1], Daniela Stan[1], Cristina Uritu[2], Mariana Pinteala[2], Maya Simionescu[1], Manuela Calin[1]

[1]*Institute of Cellular Biology and Pathology "Nicolae Simionescu" of Romanian Academy, Bucharest, Romania*

[2] *Centre of Advanced Research in Bionanoconjugates and Biopolymers, Institute of Macromolecular Chemistry "Petru Poni", Iasi, Romania*

Introduction. In order to improve the biocompatibility and transfection efficiency of polyethyleneimine (PEI), new nanoconjugates between cyclic siloxane ring (2,4,6,8-tetramethylcyclotetrasiloxane, cD4H) and PEI (2kDa branched) (cD4H–AGE–PEI) have been developed and characterized by our group and their ability to function as efficient transfection vectors has been investigated. **Materials and methods.** Different molar ratios of nitrogen from PEI to phosphate from DNA (ratio N/P: from 1 to 300) were calculated for complexes between free (PEI) or siloxane conjugated PEI (cD4H–AGE–PEI) and a plasmid pEYFP (encoding a fluorescent protein). Size of complexes and their capacity to condensate DNA have been determined by dynamic light scattering (DLS) and agarose gel electrophoresis, respectively. The cytotoxicity of cD4H–AGE–PEI and PEI complexed or not with pEYFP was determined by measuring the viability of HEK 293T cells after incubation with the media containing the complexes, using MTT assay. Transfection efficiency was evaluated after 48 hours by fluorescence microscopy and flow cytometry (513/527 nm excitation/emission wavelengths). A battery of pharmacological inhibitors of different endocytic pathways were used prior and during transfection in order to establish the internalization route(s) involved in the cellular uptake of nanocomplexes. **Results and Discussions.** 1) The size of cD4H–AGE–PEI/pEYFP complexes was around 80 nm for N/P higher than 100; 2) starting with N/P ratio of 10, the complexation of plasmid DNA with cD4H–AGE–PEI blocked the migration of DNA into gel, suggesting a very good packaging and protection of DNA by the polymeric conjugate; 3) the viability of HEK 293T cells was over 80% after incubation with all N/P ratio of cD4H–AGE–PEI/pEYFP complexes; 4) a considerable transfection efficiency for N/P ratios of cD4H–AGE–PEI /pEYFP starting with 60 compared with that of PEI/pEYFP; 5) the dynamin endocytic pathway plays the main role in cellular internalization of cD4H–AGE–PEI/pEYFP complexes; 6) the transfection efficiency is positively correlated with the polyplex-induced autophagy. **Conclusions.** The cytotoxicity and in vitro transfection study indicate that the developed cD4H–AGE–PEI conjugates is a novel gene delivery system with lower cytotoxicity and considerable transfection efficiency.

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