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Nuclear gene targeting using negatively charged liposomes

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Abstract

Oligonucleotides are a very useful tool to control gene activity. Oligos work by complementary base-pairing with target sequences either in the nucleus or in the cytosol (Zelphati, O., Szoka, F.C., Jr., 1996. Liposomes as a carrier for intracellular delivery of antisense oligonucleotides: a real or magic bullet? J. Contr. Rel. 41, 99–119). In a new approach using chimeric oligonucleotides (Yoon, K., Cole Strauss, A., Kmiec, E.B., 1996. Targeted gene correction of episomal DNA in mammalian cells mediated by a chimeric RNA–DNA oligonucleotide. Proc. Natl. Acad. Sci. USA 93, 2071–2076) conversion of single base mutations with help of intranuclear repair mechanisms maybe an advantageous method to cure genetic diseases which are based on single point mutations. These chimeric oligonucleotides are constructed in a way that they form an intramolecular double strand of DNA and modified RNA-bases. We used a fluorescent labelled pure 68-mer DNA-analogue of a chimeric oligonucleotides to follow the intracellular fate of these kind of genetic material. The oligos were complexed with protamine sulfate and coated with three different liposomal formulations. The AVE™-3 formulation shows enhanced properties compared to a classical neutral and negatively charged formulation. Nuclear localisation of oligos could only be observed with the AVE™-3 formulation. Furthermore only the negatively charged liposome formulations interact with the protamine-complexed oligonucleotides.

Keywords: Nuclear gene targeting; Liposomes; Negatively charged

Three different liposomal formulations were prepared by obtaining a lipid film-evaporation of a chloroform-solution of the lipids followed by hydration of the lipid film in Tris buffer. The multilamellar vesicles were sonicated and extruded through 50 nm Nuclepore®-membranes using the LiposoFast™ extrusion device, resulting in approximately 100 nm sized liposomes, determined by means of PCS. The lipids were fluorescently labelled by addition of rhodamine-labelled-DOPE. AVE™-3 is a negatively charged liposomal formulation with a lipid composition which is derived from the lipid membrane of an enveloped virus. This formulation has special fusogenic properties, which were shown for another liposomal formulation reconstituting the HI-viral envelope in a modified lipid composition (Chander and Schreier, 1992). Furthermore, negatively charged control liposomes and neutral control liposomes

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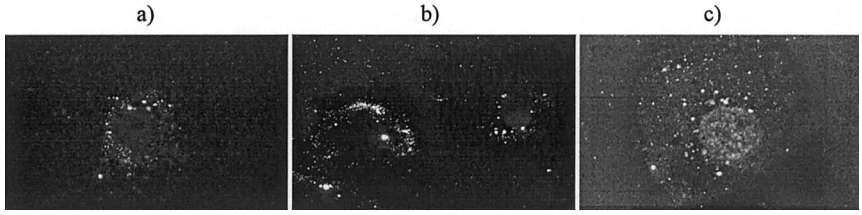

Fig. 1. Cellular distribution of fluorescence-labelled DNA: DNA-oligonucleotide–protamine-complex with a charge ratio of 3:1 (+/−) mediated by (a) neutral liposomes; (b) negatively charged control liposomes; and (c) AVE™-3.

were investigated. The oligonucleotide is a 68-mer forming an intramolecular double-strand because of four T-hairpin loops on each site of the molecule. The oligonucleotide is labelled either with FAM or fluorescein at the 5′-end. The oligonucleotide is complexed with protamine, a FDA-approved non-toxic cationic peptide which condenses DNA in the sperm of some fish-species. Recently protamine was shown to improve transfection ability of cationic lipid-based transfection systems (Sorgi et al., 1997). After incubation of complexes, the liposomal noncationic-formulations are added and the liposome-oligo-protamine construct is transferred to HepG2-Cells, cultured in six-well-plates. Serum is added after 3 h of incubation, if incubation time is longer than 5 h.

With AVE™-3 after 21 h of incubation many fluorescent nuclei can be found using a charge ratio (+/−) of 2:1 and 3:1 of cationic protamine charges to anionic DNA charges (Fig. 1c). With a charge ratio of 1:1 (+/−) the yield of fluorescent nuclei is significantly reduced and there is no fluorescence in the nucleus without protamine. Furthermore association of the liposomes with the complexed oligonucleotides can be observed indicated by the yellow colour of liposomal coated oligo-complexes. Control liposomes — either negatively charged or neutral — do not show transport of genetic material into the nucleus after 21 h on incubation with excess of cationic protamine charges (Fig. 1a and b) whereas protamine is effective in nuclear targeting of oligonucleotides. The neutral liposomal formulation does not show an interaction of the liposomes with the oligonucleotides as the green oligo fluorescence can be clearly distinguished from the red fluorescence of the rhodamine label. Oligos itself do only fluoresce very weakly in the cytoplasm of the cell. The oligos can be found in more granular structures indicating that they are still in the endosomal compartment whereas in liposomal preparations the oligos can be located in the whole cytoplasm. This means that the oligos are released from the endosome in the presence of liposomes, especially AVE™-3. An AVE™-3-formulation without addition of protamine sulfate enhances cellular uptake of oligos, too. But obviously no fluorescence can be found in the nucleus when protamine is absent.

AVE™-3 effectively transports protamine-complexed oligonucleotides into the nucleus. Neither negatively charged control liposomes nor neutral control liposomes as well as AVE™-3 in the absence of protamine induce any nuclear uptake of fluorescent labelled oligonucleotid after 21 h of incubation. Therefore AVE™-3 can be used as delivery system for protamine condensed oligonucleotides.

Liposomal coated oligos–protamine-complexes formulations show a co-uptake of negatively charged liposomes with oligos as can be seen from co-localisation of fluorescence markers in the cells. This fact enables targeting of AVE™-3 to special cell types using receptor-sensitive ligands thereby enhancing localised uptake of oligos in the cells of interest.

References

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