Development of triazine-based cationic lipid for gene delivery

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Introduction

Gene therapy, because of its aim to eradicate causes rather than symptoms of diseases, is believed by many to be the therapy of the future.

The main objective of this study was to evaluate the efficiency of a new cationic lipid in delivering genes in vitro and to develop its applicability for gene therapy.

Materials and Methods

We designed and synthesized a conceptually new cationic lipid based on a heterocyclic scaffold bearing a C-14 hydrophobic alkyl chain (compound **4**).

Gel retardation assay for compound **4** was performed in 0.75% agarose gel using increasing charge ratios (CR).

Cell lines were seeded at a density of $3x10^5$ per 25 cm^2 flask. The plasmid DNA coding for Green Fluorescent Protein (GFP, 2 µg) was added to Compound **4** at CR6 (0.36 mM) in dH_2O, and kept at r.t. for 30 min before use. Lipoplexes were added to cells either in OptiMEM®, replaced after 4 h with complete DMEM or directly in complete DMEM. GFP expression was estimated by FACS after 48 h. Lipofectamine™ 2000 (Invitrogen, Milan, Italy), was used as positive control according to manufacturer's instructions.

To calculate critical micellar concentration (cmc) increasing amounts of compound 4 were added to an aqueous solution of *N*-phenyl-1-naphthylamine 10 μM and fluorescence was recorded.

Results and Discussion

The new triazine-based transfectant features very simple preparation (Fig. 1) from inexpensive materials.

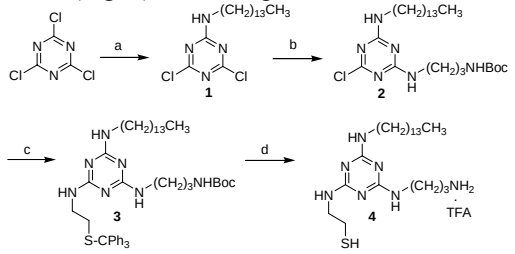

Figure 1. Key: (a) $CH_3(CH_2)_{13}NH_2$, $NaHCO_3$, acetone/H_2O, r.t. (61%). (b) $H_2N(CH_2)_3NHBoc$, $NaHCO_3$, acetone/H_2O, 60°C (82%). (c) $Ph_3CS(CH_2)_2NH_2 \cdot HCl$, DIPEA, benzene, 120°C, sealed vial (76%). (d) TFA/DCM (1:4), Et_3SiH, 5°C (89%)

By electrophoretic gel retardation assay we demonstrated that lipoplexes were completely formed at a CR≥6. Indeed lower CRs were inefficient in transfecting, as a very likely consequence of ineffective lipoplex formation. No micelles should be present during DNA condensation. The cmc of molecule **4** was found to be ca. 30 mM, well above the concentration used in all the assays. SEM analysis showed heterogeneous lipoplex shape and size.

We tested toxicity and transfection efficiency of compound **4** (CR6, OptiMEM®) on different cell lines. Compound **4** showed a comparable or lower toxicity but higher transfection efficiency than Lipofectamine™ 2000 (Fig. 2).

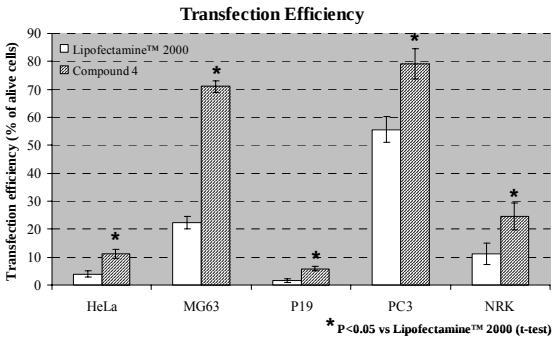

Figure 2. Transfection efficiency of Compound 4 *vs* Lipofectamine™ 2000 in different cell lines.

Moreover, transfection assays were carried out directly in DMEM. Compound **4** showed the same toxicity but higher transfection efficiency than in OptiMEM®, well above Lipofectamine™ 2000 (Fig. 3). This allows simplification of the transfection procedure and paves the way for future applicability for *in vivo* transfections.

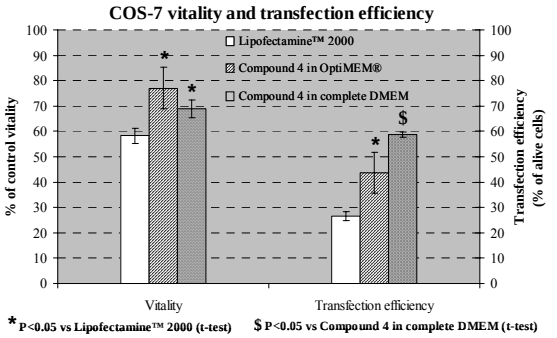

Figure 3. Vitality and transfection efficiency of Lipofectamine™ 2000 and Compound 4 in OptiMEM® and in DMEM in COS-7.

Conclusions

The easy synthesis in multi-gram amounts from non expensive and commercially available starting materials, combined with a user-friendly and effective protocol for cell transfection render this triazine-based compound **4** a very attractive reagent for gene delivery applications.