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STEROIDAL SAPOGENINS TO ENHANCE LIPID NANOCARRIER ENABLED TRANSFECTION EFFICIENCY

Biological Science

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ABSTRACT

Cationic lipids are effective non-viral vectors for gene therapy, though improved clinical vectors are needed. This study explores the use of steroidal alkaloids—Solasodine, Tomatine, and Tomatidine—as helper lipids to enhance nucleic acid transfection. Liposomes combining Ester linker-based lipid (1 mM) and Cholesterol (1 mM) with varying molar ratios of these alkaloids were tested in HEK 293 and SKHEP-1 cell lines. Among the formulations, ECS1:1:0.4 was the most efficient in HEK 293, while ECS1:1:0.3 outperformed others in SKHEP-1, surpassing Lipofectamine™ 3000. These findings highlight Solasodine as a promising enhancer of cationic lipid-mediated transfection for gene therapy applications.

KEYWORDS

Gene therapy, Sapogenins, Cationic lipids

INTRODUCTION

Cationic lipids are widely used as non-viral vectors for gene delivery, but many still lack efficient transfection performance [1]. Success depends on forming stable lipid–DNA complexes (lipoplexes), cellular uptake, and efficient intracellular release [2]. Steroidal alkaloids like Solasodine, Tomatidine, and Tomatine, derived from Solanaceae plants, share structural features with cholesterol, allowing them to integrate into liposomal bilayers [3], [4]. Their amphiphilic nature enhances membrane stability, fluidity, and permeability, improving drug encapsulation, protection, and delivery [5]. These compounds also offer inherent therapeutic properties—anti-cancer, anti-inflammatory, and antimicrobial—which may synergise with gene delivery applications [6] [7]. In this study, we investigated their potential as co-lipids to enhance liposomal transfection. We formulated three liposome sets by combining Ester-based cationic lipid (E), cholesterol (C), and varying ratios of Tomatine (T), Tomatidine (Td), or Solasodine (S), and evaluated their transfection efficiency in HEK 293 and SKHEP-1 cells.

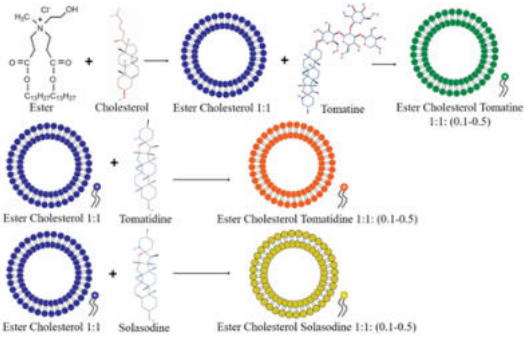

Fig. 1: Schematic representation of lipids, co-lipids, and liposomes

MATERIALS AND METHODS

Reagents And Cell Culture: pEGFP-N1 plasmid and Ester lipid (E) were provided by Dr. S. Marepally (Centre for Stem Cell Research, Vellore). Cholesterol, Tomatidine, Tomatine, and Solasodine were from Sigma-Aldrich. Lipofectamine™ 3000 was from ThermoFisher. Heparin, antibiotics, agarose, and chamber slides were from HiMedia, India. SKHEP-1 and HEK 293 cells (NCCS, Pune) were cultured in DMEM with 10% FBS at 37°C, 5% Co_2.

Liposome And Plasmid Preparation: pEGFP-N1 plasmid was amplified in E. coli DH5α and purified (A260/A280 ≈ 1.9). Three liposome sets with 0.1–0.5 mM Tomatine, Tomatidine, or Solasodine were prepared with 1 mM Ester lipid and Cholesterol each. Lipids were dissolved in chloroform, dried under nitrogen, vacuum-desiccated (6 h), hydrated overnight with sterile water, vortexed to form MLVs, then sonicated to obtain SUVs.

DNA Binding Assay: (a) Gel retardation assay: Lipid-pDNA complexes (lipid: DNA charge ratios 1:1 to 8:1) were incubated 20 min in 1 M HEPES (pH 7.4), mixed with loading dye, and run on 1.5% agarose gel (80 V, 45 min) in TAE buffer; bands visualized by Bio-Rad Gel Doc XR+. (b) Heparin displacement assay: Same as above with 1 µg heparin added; incubated 30 min before gel loading.

Transfection Assay: HEK 293 cells (30,000/well) were seeded in 24-well plates. Lipoplexes (0.5 µg pEGFP + 3.6 nmol liposomes in 100 µL DMEM) were incubated 30 min, added to cells, incubated 3 h at 37°C/5% CO_2, then supplemented with DMEM + 20% FBS to 10% final FBS. eGFP expression was assessed 48 h later by fluorescence microscopy and FACS at lipid:DNA ratios 2:1 and 4:1.

Cytotoxicity Assay: MTT assay was performed on HEK 293 cells (10,000/well) seeded in 96-well plates with DMEM + 10% FBS. After 24 h, lipoplexes were added, followed by MTT (5 mg/mL) 4 h later. After another 4 h, DMSO (150 µL) was added, and absorbance measured. Viability (%) = [(A540 treated – background) / (A540 untreated – background)] × 100.

RESULTS & DISCUSSION

Table 1: Liposomal Compositions (in mM)

	Ester	Cholesterol	Tomatine
EC 1:1	1	1	
ECT 1:1:0.1	1	1	0.1
ECT 1:1:0.2	1	1	0.2
ECT 1:1:0.3	1	1	0.3
ECT 1:1:0.4	1	1	0.4
ECT 1:1:0.5	1	1	0.5
			Tomatidine
ECTd 1:1:0.1	1	1	0.1
ECTd 1:1:0.2	1	1	0.2
ECTd 1:1:0.3	1	1	0.3
ECTd 1:1:0.4	1	1	0.4
ECTd 1:1:0.5	1	1	0.5
			Solasodine
ECS 1:1:0.1	1	1	0.1
ECS 1:1:0.2	1	1	0.2
ECS 1:1:0.3	1	1	0.3
ECS 1:1:0.4	1	1	0.4
ECS 1:1:0.5	1	1	0.5

To evaluate the effects of sapogenins on liposomal formulations, we prepared three sets of liposomes by doping 1 mM Ester-based cationic lipid (E, with two myristic acid tails) and 1 mM Cholesterol (C) with varying molar ratios (10–50%) of Tomatine (T), Tomatidine (Td), and Solasodine (S). This yielded five formulations per set: ECT1:1:0.1–0.5, ECTd1:1:0.1–0.5, and ECS1:1:0.1–0.5. All were compared to a control EC formulation (E:C = 1:1) without sapogenin (Table 1).

To optimise transfection conditions, we evaluated DNA binding efficiency of liposomes—ECT, ECTd, and ECS—at increasing molar concentrations (0 to 0.5) with a constant pDNA amount (0.5µg,

pEGFP-N1) across lipid:DNA charge ratios from 8:1 to 1:1. All formulations showed strong binding, completely inhibiting pDNA migration on 1% agarose gel (Fig. 2), indicating full encapsulation. To assess lipoplex stability, a heparin displacement assay was performed. Heparin, a negatively charged glycosaminoglycan, was used to mimic competitive interactions in biological environments. All lipoplexes resisted heparin-induced displacement, confirming strong pDNA binding and stability (Fig. 3), in agreement with gel retardation results.

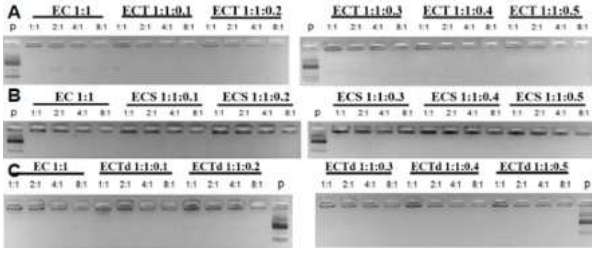

Fig. 2: Gel Retardation Assay of ECT (A) and ECTd (B), and ECS (C)

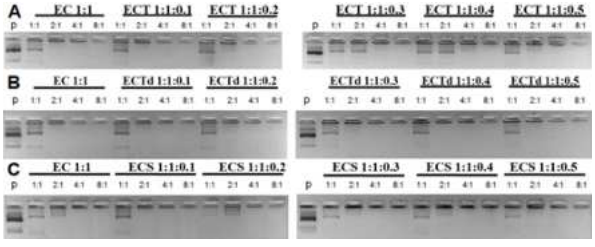

Fig. 3: Heparin Displacement Assay of ECT (A) and ECTd (B), and ECS (C)

Following DNA binding efficiencies studies, we evaluated cytotoxicity/safety profiles of all three sets of liposomes in HEK 293 representative cell lines, using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT)-based cell viability assay. As illustrated in Fig. 4, liposomes were found to be non-cytotoxic (>70% cell viability) for all three sets of liposomes across the lipid/DNA charge ratios 1:1−4:1 (except at 8:1 lipid/DNA charge ratio, which showed ~60-70 % cell viability).

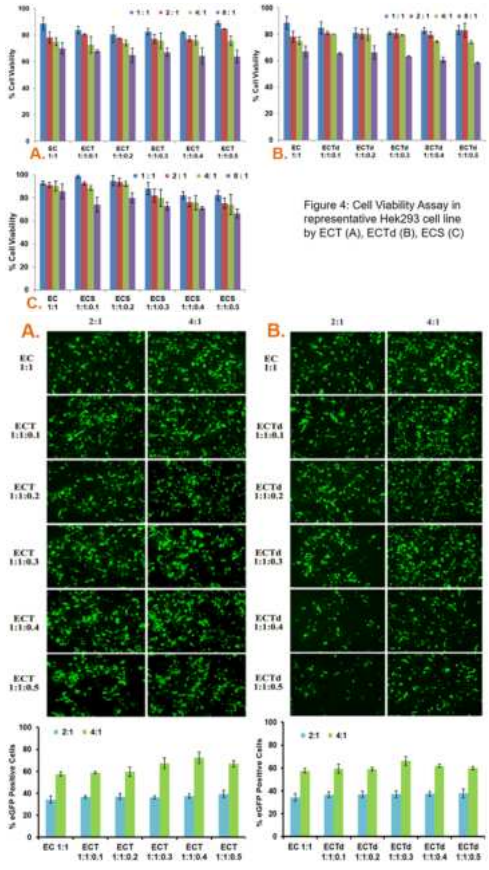

Fig. 5: Fluorescent Microscopic Images of transfected Hek 293 cell line by ECT (A) and ECTd (B) liposomes and their respective percentage eGFP positive cells measured through FACS.

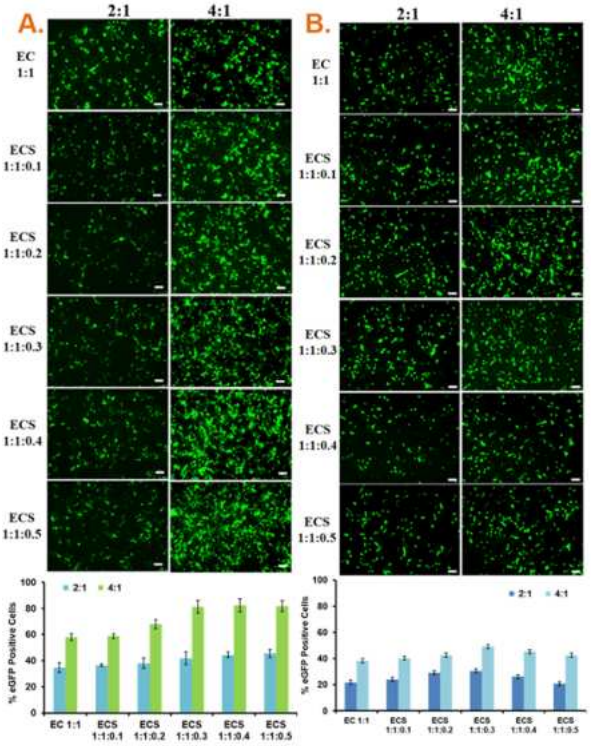

Fig. 6: Fluorescent Microscopic Images of transfected Hek 293 (A) and SKHEP-1 (B) cell lines by Ester Cholesterol Solasodine liposomes and their respective percentage eGFP positive cells measured through FACS.

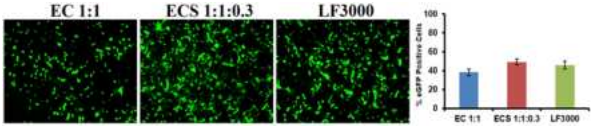

Fig. 7: Fluorescent Microscopic Images of Ester Cholesterol (1:1), Ester Cholesterol Solasodine (1:1:0.3), & Lipofectamine™ 3000 (LF3000) mediated Transfection in SKHEP-1 cell line and their respective percentage eGFP positive cells measured through FACS.

We assessed transfection efficiency of ECT and ECTd liposomes (ratios 1:1:0.1 to 1:1:0.5) using pEGFP-N1 plasmid in HEK 293 cells across lipid:DNA charge ratios from 1:1 to 8:1. Among these, ECT1:1:0.4, ECTd1:1:0.3, and ECS1:1:0.4 showed the highest transfection efficiencies in their respective sets. At 4:1 ratio, EC1:1, ECTd1:1:0.3, ECT1:1:0.4, and ECS1:1:0.4 achieved 59.8%, 66.25%, 72.45%, and 82.45% efficiency, respectively (Fig. 5, 6). Further evaluation of ECS liposomes in SKHEP-1 cells identified ECS1:1:0.3 as most effective, with 49.3% transfection at 4:1 ratio—slightly outperforming Lipofectamine™ 3000 (45.8%) (Fig. 7). Solasodine, as a helper lipid, consistently enhanced transfection in both HEK 293 and SKHEP-1 cells, highlighting its potential as a superior co-lipid for gene delivery.

CONCLUSION

In this investigation, we aimed to improve the efficiency of nucleic acid transfection reagents by doping three steroidal sapogenins-Tomatine (T), Tomatidine (Td), and Solasodine (S)-into cationic liposomes. Each sapogenin was incorporated at varying molar concentrations into liposomes containing 1 mM Ester lipid (E) and 1 mM Cholesterol (C), generating three liposomal sets. Among the formulations, ECT1:1:0.4, ECTd1:1:0.3, and ECS1:1:0.4 demonstrated superior transfection performance within their respective sets in HEK 293 cells. Of these, ECS1:1:0.4 emerged as the most effective overall in HEK 293. Interestingly, ECS1:1:0.3 proved to be the best transfection reagent in SKHEP-1 cells, slightly outperforming the commercial Lipofectamine™ 3000 (LF3000). In conclusion, Ester-Cholesterol-Solasodine (ECS) liposomes show

strong potential as effective, non-viral gene delivery systems. Solasodine, a biocompatible sapogenin, enhances cationic lipid-mediated transfection and merits further investigation in both in-vitro and in-vivo disease models.

Conflict Of Interest

None

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