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Cationic cholesterol with a hydroxyethylamino head group promotes significantly liposome-mediated gene transfection

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Abstract A novel cationic cholesterol derivative with a hydroxyethylamino head group, cholesteryl-3β-carboxyamidoethylene-*N*-hydroxyethylamine (II), has been synthesized and used for liposome-mediated gene transfection. The cationic liposomes containing the derivative (II) facilitated greatly pSV2CAT gene transfection into mouse NIH3T3 and L929 cells in the absence of serum. The transfection efficiency was much higher than those by the cationic liposomes containing cationic derivatives with a dialkylamino head group (I, III or IV). Further, the efficiency by the cationic liposomes with the derivative (II) was not so much decreased in the presence of serum. This suggested that a novel cationic cholesterol derivative (II) should be very promising in liposome-mediated gene transfection of plasmid and antisense DNA into target cells.

Key words: Cationic liposome; Cationic cholesterol; Gene transfection; Plasmid DNA; Serum; Hydrophilicity

1. Introduction

Cationic liposomes have proven to be useful tools for delivery of plasmid DNA and antisense DNA into mammalian cells [1–10]. For the experiments of such cationic liposomes, cationic cholesterol derivatives were justified by their high transfection efficiency and low toxicity [5–8,10]. In these experiments two kinds of cationic cholesterol derivatives were used. One was a derivative of cholesterol ester and the other was cholesteryl carboxyamido. In our recent experiments we have shown that the cholesteryl carboxyamido derivatives were usually more effective than the cholesterol ester derivatives in transfection of pSV2CAT gene into cultured cells and that zeta potential (positive charge) of cationic liposomes was one of the important factors to control gene transfection. Of the eight cationic liposomes containing a different cationic cholesterol derivative, either cholesterol ester or cholesteryl carboxyamido, the liposomes which contained a cholesteryl carboxyamido derivative (I) (see Fig. 1) showed the highest transfection efficiency [10]. Further, substitution of a dimethylamino head group of the cationic cholesterol derivatives with a diethylamino or a diisopropylamino head group showed significant decreases in transfection efficiency. This suggested that the increased hydrophobicity of the amino head group of cholesterol derivatives decreases the efficiency of gene transfection.

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Abbreviations: CAT, chloramphenicol acetyltransferase; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine; SUV, small unilamellar vesicle; FCS, fetal calf serum; SFM, serum free medium

Here, we have synthesized a novel cholesterol derivative which contained a hydrophilic amino head group, cholesteryl-3β-carboxyamidoethylene-*N*-hydroxyethylamine (II) in Fig. 1, and used it for gene transfection. By this novel cholesterol derivative with a secondary amino head group we have studied transfection of plasmid pSV2CAT DNA into mammalian cultured cells in the absence or in the presence of serum. Transfection efficiency of pSV2CAT was calculated from chloramphenicol acetyltransferase (CAT) activity. The results showed that cationic liposomes made by the derivative (II) increased transfection efficiency more significantly than the cationic liposomes containing cationic cholesterol derivatives with a dialkylamino head group did.

2. Materials and methods

2.1. Materials

1,2-dioleoyl-sn-glycero-3-phosphatidylethanolamine (DOPE) was purchased from Sigma (St. Louis, MO). The syntheses of cholesteryl-3β-carboxyamidoethylene-*N*-hydroxyethylamine (II) were done by the following procedure. Cholesteryl-3β-carboxylic acid was condensed with *N*-hydroxyethylethylenediamine by carbonyldiimidazole in CH_2Cl_2 under nitrogen atmosphere overnight and the residue was recrystallized from ethylacetate. The compound was m.p. 148–150°C. The syntheses of other cationic cholesterol derivatives with a dimethylamino head group were done by the similar procedures described in our previous paper [10]. Plasmid pSV2CAT was given by Prof. Kikuo Onozaki (Nagoya City University).

2.2. Liposome preparation

DOPE was combined with cationic cholesterol derivatives in chloroform and dried with N_2 gas to remove chloroform solvent. A mol ratio of 3:2 was used for DOPE/cholesterol derivatives [5,10]. The dried lipid film was vacuum desiccated for at least half an hour and suspended by vortexing in distilled water and the samples were sonicated in a bath type sonicator (Branson model B1200) to generate small unilamellar vesicles (SUVs) [10]. The diameter of the cationic liposomes was measured using a multiangle light scattering instrument (Otsuka Electronics). The diameters of cationic liposomes were usually between 200 nm and 350 nm. However, the diameters of the cationic liposomes containing cholesteryl-3β-carboxyamidoethylene-*N*-hydroxyethylamine (II) were 1–2 μm and they were not SUVs. The liposome suspension was turbid and then it was difficult to measure zeta potential by laser Doppler spectroscopy [10,11].

2.3. Cell culture and transfection

NIH3T3 cells were cultured in RPMI1640 medium from Gibco (Grand Island, NY) and L929 cells were cultured in DM-160AU medium (Kyokuto Pharm., Tokyo), both of which were supplemented with 10% FCS (Bio Whittker, MD). COS-7 cells were cultured in D-MEM medium from Gibco supplemented with 10% FCS [10]. RPMI1640, DM-160AU and D-MEM media contained antibiotics: 100 U/ml penicillin and 100 μg/ml streptomycin. Plasmid pSV2CAT DNA was complexed to cationic liposomes in SFM101 (Nissui) or in SFM101 with 10% FCS at room temperature for 15 min and then the complex was incubated with target cells for 4 h at 37°C. Then the cells were washed and cultured for another 40 h in a growth medium at 37°C before the CAT assay [10].

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2.4. Transfection activity

The CAT assay was done by following the previous paper [12]. Cell extracts were made 44 h after transfection by sonicating washed, pelleted cells in 100 μl of 0.25 M Tris-hydrochloride (pH 7.8). After the cells were spun for 10 min (at 12000 rpm) in an Eppendorf microfuge at 4°C, the supernatants were collected and assayed for enzyme activity. The assay mixture contained 25 μl of cell extract, 0.1 μCi of [^{14}C]chloramphenicol (54 μCi/mmol; Amersham Life Science, Buckinghamshire) in 25 μl of 0.25 M Tris-hydrochloride (pH 7.5) and 5 μl of 10 mM acetylcoenzyme A. All of the reagents except coenzyme A were preincubated together for 5 min at 37°C. After equilibration was reached at 37°C, the reaction was started by adding coenzyme A. The CAT reaction was performed at 37°C for 1 h using 40–60 μg protein of cell lysates [10,12].

3. Results

3.1. Effects of cholesterol with a hydroxyethylamino head group

Cationic liposomes made by the cholesterol derivative (II) and DOPE promoted significantly gene transfection of plasmid pSV2CAT into NIH3T3 cells. As shown in Fig. 2 the efficiency of transfection by the liposomes with the derivative (II) was much higher than that of the cationic liposomes with the derivative (I), which had been the highest of the eight cationic liposomes in our previous paper [10]. Fig. 2a and b show the transfection efficiency of plasmid pSV2CAT into mouse NIH3T3 and L929 cells, respectively. In L929 cells the CAT activity of the liposomes with the derivative (II) was almost four times as high as that of the liposomes with the derivative (I). In the case of COS-7 cells the CAT activity of the liposomes with the derivative (II) was not so significantly different from that by the liposomes with derivative (I) (Fig. 2c).

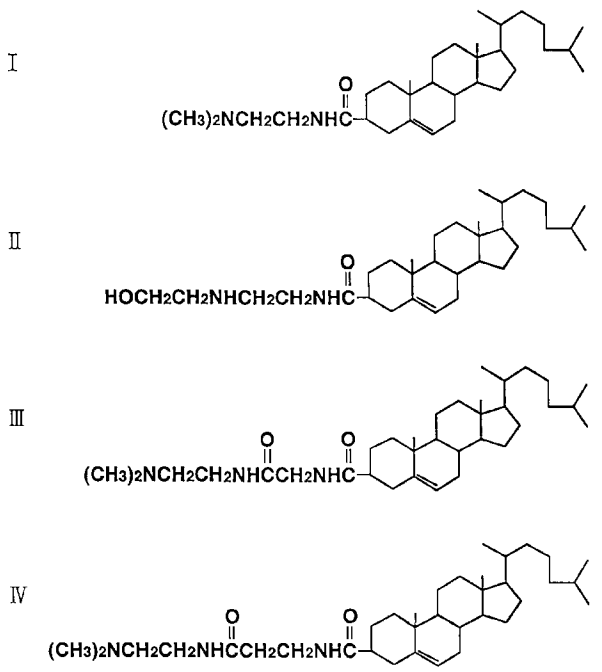

Fig. 1. The structure of cationic derivatives of cholesterol with an amino head group. Cholesteryl-3β-carboxyamidoethylene-*N*,*N*-dimethylamine (I), cholesteryl-3β-carboxyamido ethylene-*N*-hydroxyethylamine (II), cholesteryl-3-carboxyamidomethylenecarboxyamidoethylene-*N*,*N*-dimethylamine (III), and cholesteryl-3β-carboxyamidoethylenecarboxyamidoethylene-*N*,*N*-dimethylamine (IV).

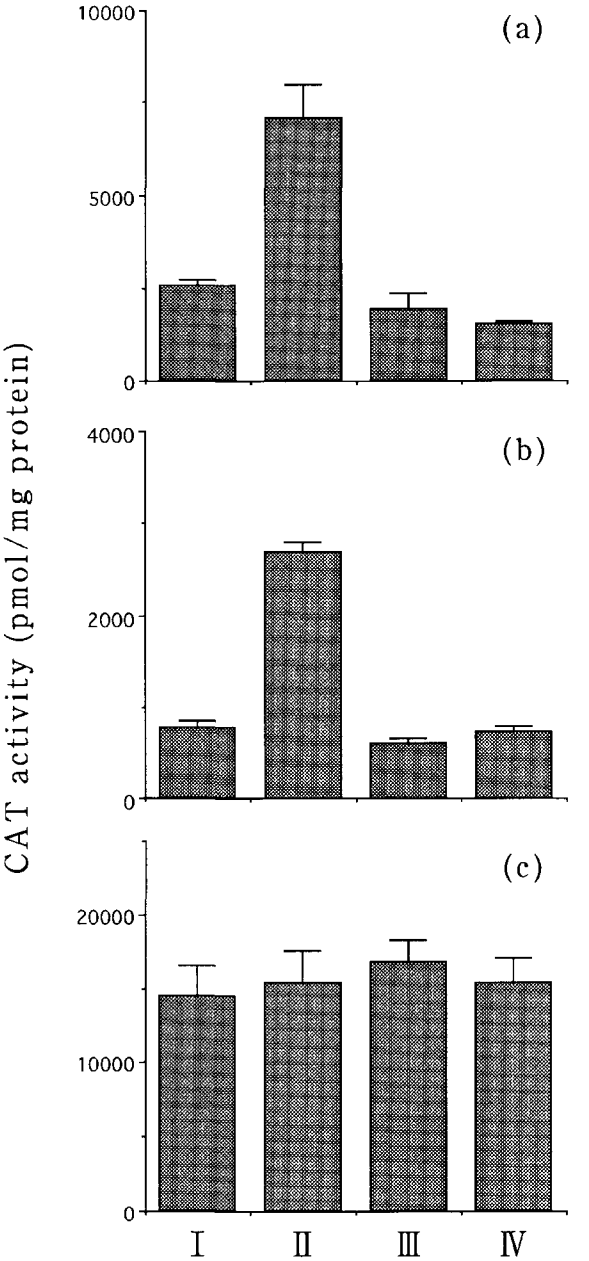

Fig. 2. Effects of cationic liposomes with a different cholesterol derivative on transfection efficiency. Vertical axes show the CAT activity which was measured following the standard procedures described in Section 2. Bars are standard deviations. (a) NIH3T3, (b) L929, (c) COS-7 cells.

3.2. Cholesterol with a dialkylamino head group

In the present experiments we further synthesized derivatives of cholesteryl carboxyamido with a dialkylamino head group (III and IV) as shown in Fig. 1. The transfection efficiency by these two new liposomes was not so significantly different from the efficiency by the liposomes with the derivative (I) (see Fig. 2). This indicated that the cationic liposomes containing the cholesterol derivative with a hydroxyethylamino head group (II) had much higher transfection efficiency than the liposomes containing cholesterol derivatives with a dialkylamino head group did. It seemed that the increased hydrophilicity in the head group of the derivative (II) promoted gene transfection more efficiently.

Table 1
The effects of serum on the transfection efficiency by cationic liposomes with a cationic cholesterol derivative

Derivative	CAT activity (pmol/mg protein)	
	without FCS	with 10% FCS
(I)	2600 ± 180	250 ± 20
(II)	7000 ± 920	3000 ± 60
(I) to (II) ratio in percentage	37%	8%

3.3. Diameters of cationic liposomes

The solution of the cationic liposomes made by the derivative (II) was turbid. The diameters of the liposomes were determined to be 1–2 μm by a multiangle light scattering instrument [10]. They were longer than those of the cationic liposomes containing cholesterol derivatives with a dimethylamino head group. The diameters of the latter were usually 200–350 nm. Reducing the time of sonication we prepared large liposomes (1–2 μm) from the cationic liposomes of cholesterol derivatives (I, III and IV). However, the larger sizes of the liposomes did not increase the CAT activity but they rather decreased it. Then, we supposed that a specific physical property (the increased hydrophilicity of an amino head group) of the cholesterol derivative (II) contributed to the efficiency of gene transfection greatly.

3.4. Effects of serum

Lastly, we checked the effects of serum on the gene transfection by the cationic liposomes. The transfection efficiency was apparently decreased in the presence of 10% FCS as shown in Table 1. However, the decrease was not so great in the case of the derivative (II) as in the case of the derivative (I). For example, the derivative (I) to the derivative (II) ratio in percentage was 37% and 8% in the absence and in the presence of serum, respectively.

4. Discussion

In the present paper we have described the use of cationic liposomes containing cationic cholesterol with a hydroxyethylamino head group to facilitate the delivery of plasmid DNA into mammalian cells. Transfection efficiency by the cationic liposomes with the derivative (II) was significantly higher than those by the cationic liposomes containing the cholesterol derivatives with a dialkylamino head group (I, III or IV) both in the absence and in the presence of serum. This suggested that the increased hydrophilicity of the amino head group of the derivative (II) should promote gene transfection efficiently.

In our preliminary experiments antisense *c-myc* DNA was also able to be transfected efficiently into cultured cells by the cationic liposomes with the derivative (II). We measured the intracellular distribution of fluorescein-conjugated *c-myc* antisense DNA in NIH3T3 cells after 4 h incubation (37°C) with complexes of antisense DNA and cationic liposomes. Fluorescein-conjugated *c-myc* antisense DNA was found mostly in the nucleus. The fluorescence intensity in NIH3T3 cells was much higher than that of liposomes with the derivative (I). Thus, a novel cationic cholesterol derivative (II) described in the present experiments is very promising in liposome-mediated gene transfection of plasmid and antisense DNAs into target cells.

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