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Gene Transfer Mediated by YKS-220 Cationic Particles: Convenient and Efficient Gene Delivery Reagent

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A monocationic lipid, YKS-220, with a symmetrical and biodegradable structure can be used as an effective gene transfer vector in a cationic particle form (not a cationic liposome form), and is obtained by diluting an ethanol solution of YKS-220 and DOPE (1:5, molar ratio) with an aqueous medium. This preparation method is more convenient than that for cationic liposomes. YKS-220 cationic particles showed a heterogeneous large mean diameter of 4.4 μm. An obvious size change was not observed when plasmid DNA was added. The transfection activity of YKS-220 cationic particles was comparable to those of YKS-220 liposomes and DOSPA liposomes (LipofectAMINE™), and even higher than that of DOGS (TRNSFECTAM). Interestingly, the YKS-220 cationic particle/DNA complexes were resistant to the neutralizing effect of serum. All of these findings indicate that YKS-220 cationic particles are a convenient and efficient gene delivery reagent.

Key words: cationic liposomes, cationic particles, transfection.

Extensive efforts have been directed toward the development of novel cationic lipids as effective lipofection vectors. In these studies, it was found that the structure of the head group of the cationic lipid was very important for its transfection activity (*1-5*). Cationic lipids can be classified into two kinds according to the structure of the head group: monocationic lipids, such as DOTMA (*6*) and DC-chol (*7*), and polycationic ones, such as DOSPA (*8*) and DOGS (*9*).

It is well known that amphipathic lipids are driven in water to form well-defined structures, such as liposomes and micelles, through hydrophobic and hydrophilic interactions (*10, 11*). The sizes and structures of the lipid vesicles depend on the preparation method and the structural features of the component lipids. In general, monocationic or polycationic lipids can form cationic liposomes with a neutral phospholipid for gene transfection, such as DOTMA liposomes (Lipofectin®), DOSPA liposomes (LipofectAMINE™), and YKS-220 liposomes (Transome™). Alternatively, cationic micelles formed from polycationic lipids, such as DOGS, are also effective for gene transfection.

It is also well known that cationic transfection vectors sometimes lose, completely or partially, their transfection activity in the presence of serum (*4*). It has been supposed that serum proteins with a negative charge bind to cationic lipoplexes (lipidic particle/DNA complexes) and impede their association with cells.

In the previous papers (*12, 13*), we reported the synthesis of a novel monocationic lipid, YKS-220, with a symmetrical and biodegradable structure, and that cationic liposomes composed of YKS-220 and DOPE showed high transfection efficiency and low cytotoxicity. Here, we report that YKS-220 cationic particles prepared by a simple method showed effective transfection activity in the absence and presence of serum.

MATERIALS AND METHODS

Materials—The YKS-220 cationic lipid was prepared according to the procedure reported previously (*12*). The structures of YKS-220 and other cationic lipids are shown in Fig. 1. DOPE, DOGS (TRANSFECTAM), and luciferase expression plasmids pGVC and pGVC2 were purchased from Wako Pure Chemical Industries, Osaka. LipofectAMINE™ and OPTI-MEM® were purchased from GIBCO BRL, NY, USA. The luciferase expression vector, pRSVL (*14*), was kindly provided by Dr. M. Nakanishi (Research Institute for Microbial Disease, Osaka University).

Preparation of YKS-220 Cationic Particle/DNA Complexes—An ethanol solution (25 μl) of lipids (YKS-220: 0.83 nmol, DOPE: 4.17 nmol), and plasmid DNA (0.25 μg) were separately diluted with 50 μl of medium (OPTI-MEM®) and then mixed. The mixture (YKS-220 cationic particle/DNA complexes) was allowed to stand for 15 min at room temperature and then used for the following transfection procedure.

Transfection Procedure—Cells were seeded at a density of 2.5×10^4 on 48-well plates, and then incubated at 37°C for 18 h in Ham's F-12 medium for CHO cells, and DMEM

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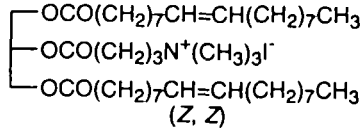
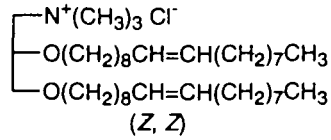
Abbreviations: DOPE, dioleoylphosphatidylethanolamine; RLU, relative light units; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle medium; YKS-220, *N*-[3-[2-(1,3-dioleoyloxy)propoxycarbonyl]propyl]-*N,N,N*-trimethylammonium iodide; DOGS, dioctadecylamidoglycylspermine; DOSPA, 2,3-dioleyloxy-*N*-[2-(sperminecarboxamido)ethyl]-*N,N*-dimethyl-1-propanaminium trifluoroacetate; DOTMA, *N*-[1-(2,3-dioleyloxy)propyl]-*N,N,N*-trimethylammonium chloride; DC-chol, 3β-[*N*-[(*N'*,*N'*-dimethylamino)-ethane]carbamoyl]cholesterol.

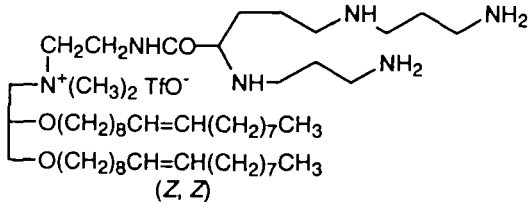


YKS-220

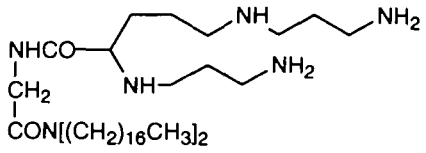


DOTMA (Lipofectin®)

Fig. 1. **The structures of YKS-220 and other cationic lipids.**



DOSPA (LipofectAMINE™)



DOGS

for COS, HepG2, and HeLa cells, supplemented with 10% FBS and 2% antibiotics under 5% CO_2. The plates were washed twice with PBS, and then 400 μl of OPTI-MEM® medium and a 100 μl solution of YKS-220 cationic particle/DNA complexes, prepared as described above, were added. After incubation for 24 h at 37°C, luciferase activity was measured using a luciferase assay system (PicaGene; Toyo Ink, Tokyo) and a luminometer (Lumat LB9501; EG & G Berthold, Bad Wildbad, Germany).

Size Determination of YKS-220 Cationic Particles—The sizes of YKS-220 cationic particles or YKS-220 cationic particle/DNA complexes were determined with a submicron laser light scattering instrument, LPA/3000/3100 (Otsuka Electronics, Osaka). The concentration of cationic particles was 0.1 mM and the charge (+/−) of YKS-220 cationic particle/DNA complexes was 1.1.

RESULTS

Optimal Transfection Conditions for YKS-220 Cationic Particles—The transfection conditions for YKS-220 cationic particles were optimized as to the ratio of YKS-220 to DOPE, and the ratio of DNA to YKS-220 cationic particles. At first, we investigated the effect of the DOPE content in cationic particles. The transfection activities of cationic particles composed of various ratios of YKS-220 lipid to DOPE (1:1 to 1:7, molar ratios) were examined as to by the expression level of the luciferase gene encoding a marker plasmid, pRSVL. As shown in Fig. 2, initially, increasing amounts of DOPE resulted in increased luciferase activity, however, further increases in the DOPE content resulted in a decrease in luciferase expression. Moreover, little luciferase activity was detected when DOPE alone was used (data not shown). From these results, it was deduced that the optimal molar ratio of YKS-220 to DOPE in cationic particles was 1:5. Next, the effects of the concentrations of DNA and YKS-220 cationic particles (YKS-220:DOPE= 1:5) on transfection efficiency were examined, as shown in Fig. 3. Regardless of the amount of DNA used, the lipid concentration of 10 μM gave the highest luciferase activity under our experimental conditions. The ratios of positive to negative charges for these samples are 1.1, 0.6, 0.3, and 0.2.

Effect of FBS on Gene Transfer by YKS-220 Cationic Particles—It is well known that cationic transfection vectors sometimes lose their transfection activity when serum is included in the culture medium (*4*). In fact, OPTI-MEM®, a serum-free medium that is enriched with various growth factors, is commonly used as a culture medium in transfection experiments. We investigated the transfection activity of YKS-220 cationic particles in the presence of serum. As the amounts and kinds of serum proteins vary greatly depending upon the serum lot, three serum lots were used to evaluate the transfection activity of YKS-220 cationic particles. As shown in Fig. 4, CHO cells were transfected with 0.5 μg of pGVC plasmid complexed with 20 nmol YKS-220 cationic particles (YKS-220:DOPE, 1:5 molar ratio) in OPTI-MEM® or Ham's-F12 medium supplemented with 10% of serum. Although the transfection efficiency of YKS-220 cationic particles varies depending upon the serum lot, it is comparable to that in OPTI-MEM®. This indicated that the transfection activity of YKS-220 cationic particles was not lost when 10% FBS was included in the incubation medium during the transfection.

Transfection Efficiency of YKS-220 Cationic Particles in Some Cell Lines—The transfection efficiency of YKS-220 cationic particles was assessed in the COS, HeLa and HepG2 cell lines. For comparison, we studied those in YKS-220 and DOSPA liposomes, and DOGS as well. The experiments were carried out by applying various transfection reagents and pRSVL DNA to cells in a serum-free medium according to the procedure described above. The results are shown in Table I. The transfection efficiency of YKS-220 cationic particles was comparable to or higher than that of YKS-220 cationic liposomes. Except for in the case of HeLa cells, YKS-220 cationic particles showed better transfection efficiency than DOSPA liposomes did (LipofectAMINE™), which are known to be one of the most effective transfection vectors to date. Moreover, YKS-220 cationic particles were more effective than DOGS in all cell lines examined here. These results clearly indicate that the monocationic lipid, YKS-220, is useful for transfection in a cationic particle form as well as a cationic liposome form.

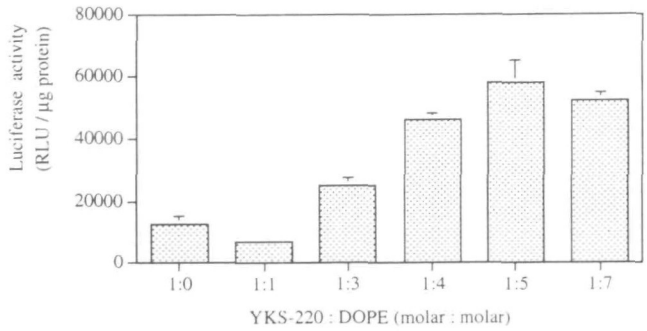


Fig. 2. **Effect of the proportion of helper lipid DOPE on YKS-220 cationic particle transfection activity.** COS cells were incubated with complexes comprising YKS-220 cationic particles (5 nmol) and pRSVL (0.25 μg). Luciferase activity was assayed according to the procedure described under "MATERIALS AND METHODS." Values are expressed as means ± SD (n = 3).

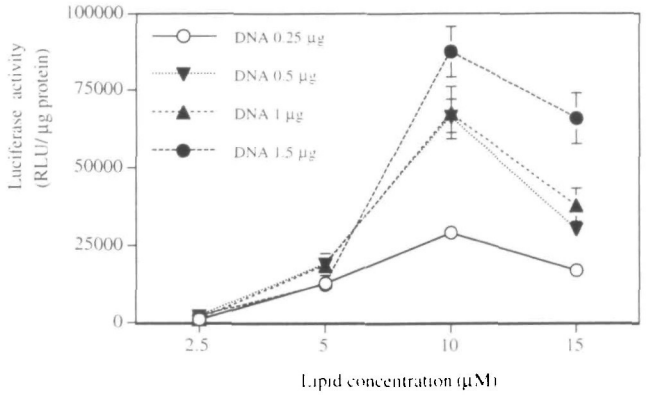


Fig. 3. **Effects of the amounts of YKS-220 cationic particles and plasmid DNA on the transfection activity.** COS cells were incubated with complexes containing the indicated amounts of YKS-220 cationic particles and pRSVL. Luciferase activity was assayed according to the procedure described under "MATERIALS AND METHODS." Values are expressed as means ± SD (n = 3).

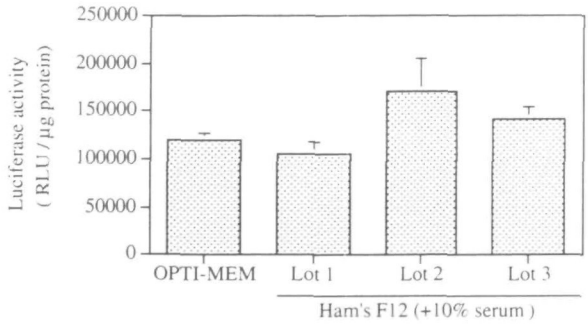


Fig. 4. **Effect of serum on transfection activity.** CHO cells were seeded onto 6-well plates at 1×10^5 cells per well. YKS-220 cationic particles (10 nmol) and pGVC2 (0.5 μg) were separately diluted in 50 μl of Ham's F12 (−FBS), and then mixed. The mixtures were added to cell wells containing 1.9 ml of Ham's F12 (+FBS). Luciferase activity was assayed according to the procedure described under "MATERIALS AND METHODS." Lot 1, Filton No. 55701; Lot 2, Bio Whittaker No. 5M0861; Lot 3, Bio Whittaker No. 3M14171. Values are expressed as means ± SD (n = 3).

TABLE I. **Luciferase expression in various cell lines transfected with YKS-220 cationic particles, YKS-220 cationic liposomes, DOSPA liposomes or DOGS.**

Transfection reagent	Luciferase activity ($\times 10^4$ RLU/μg protein, mean ± SD)		
	COS	HepG2	HeLa
YKS-220 cationic particles	8.7 ± 1.0	3.5 ± 0.3	0.4 ± 0.1
YKS-220 liposomes (Transome™)	6.2 ± 1.7	4.5 ± 1.4	0.1 ± 0.02
DOSPA liposomes (LipofectAMINE™)	3.1 ± 0.1	3.2 ± 1.2	2.2 ± 0.3
DOGS (TRANSFECTAM)	0.6 ± 0.1	0.5 ± 0.1	0.2 ± 0.1

Cells were seeded into 6-well plates at 1×10^5 cells per well. pRSVL (0.5 μg) and YKS-220 cationic particles (20 nmol), YKS-220 cationic liposomes (20 nmol), DOSPA liposomes (10 nmol), or DOGS (8 nmol) was separately diluted in 50 μl of OPTI-MEM® medium and then mixed. The mixtures were added to cell wells containing 1.9 ml of OPTI-MEM®. Luciferase activity was assayed according to the procedure described under "MATERIALS AND METHODS." Values are expressed as means ± SD (n = 3).

DISCUSSION

The cationic lipid, YKS-220, can be formulated with DOPE in either a liposome or a cationic particle form for the transfer of a gene into cells. DOPE is generally used as a helper lipid in lipofection vectors due to its non-bilayer (H_{II} phase) forming activity, that can promote membrane fusion (*15*). We found that the inclusion of DOPE in YKS-220 cationic particles greatly increased the transfection efficiency, while DOPE or YKS-220 alone, respectively, showed almost no transfection activity. This behavior is just like that of conventional cationic liposomes that contain DOPE as a helper lipid (*16, 17*).

YKS-220 cationic particles showed a heterogeneous large mean diameter of 4.4 μm, that is much greater than the mean diameter of about 0.1–0.3 μm of conventional cationic liposomes (*7, 18, 19*), as summarized in Table II. The subsequent addition of DNA produced YKS-220 cationic particle/DNA complexes of a mean diameter of 2.4 μm, that was much greater than the 0.1–0.5 μm mean diameter of conventional cationic liposome/DNA complexes (*19, 20*). Thus far, two contradictory relationships between the size of lipoplexes and their transfection efficiency have been proposed. One is based on the fact that cationic lipoplexes enter a cell *via* an endocytotic pathway (*21–23*). It is thought to be advantageous to use particles of less than 0.1 μm in size in transfection *via* endocytosis (*24*). On the contrary, the other one is based on the lability of the cationic lipoplexes (*25, 26*). The larger particles are likely to be disrupted, the plasmid DNA thus being released into the cytoplasm; thus, the large size of cationic lipoplexes was thought to be more favorable for transfection. Our present results are consistent with the latter assumption.

YKS-220 cationic particles were found to be resistant to the neutralizing effect of serum (Fig. 4). In contrast, little transfection activity was detected in the presence of 10% serum when YKS-220 liposomes were used (data not shown). This indicated that YKS-220 cationic particles could tolerate the inhibitory effect of the serum more than YKS-220 cationic liposomes. This may be attributed to the large size of YKS-220 cationic particle/DNA complexes.

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