ELSEVIER

International Journal of Pharmaceutics 139 (1996) 69–78

international journal of pharmaceutics

Effect of cationic liposome composition on in vitro cytotoxicity and protective effect on carried DNA

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Received 29 January 1996; revised 18 April 1996; accepted 29 April 1996

Abstract

Positively charged liposomes were prepared by using three different cationic surfactants, namely cetyl-trimethyl-ammonium bromide (CTAB), didodecyl-dimethyl-ammonium bromide ($DDAB_{12}$) and dioctadecyl-dimethyl-ammonium bromide ($DDAB_{18}$). A study of the parameters influencing the in vitro toxicity of cationic liposomes on cultured cell lines was performed, demonstrating the lower cytotoxicity of $DDAB_{18}$-containing liposomes. In addition, the stability of λDNA complexed to cationic liposomes after exposure to serum exo- and endonucleases was evaluated. Our results indicated that $DDAB_{12}$ and $DDAB_{18}$ liposomes are able to efficiently protect DNA from degradation, thus representing a potential approach to deliver nucleic acid in vivo.

Keywords: Cationic liposomes; DNA stability; Cytotoxicity

1. Introduction

With the advent of molecular biology and biotechnology techniques, the use of antisense and gene transfection protocols has assumed a very important role as experimental therapy (Uhlmann and Peyman, 1990). Gene therapy is indeed emerging as a clinically viable pharmacological regimen for genetic, neoplastic and infectious diseases. Gene transfection, consisting of the intro-

Abbreviations: PC, phosphatidylcholine; CH, cholesterol; CD, cationic detergent; $DDAB_{12}$, didodecyl-dimethyl-ammonium bromide; $DDAB_{18}$, dioctadecyl-dimethyl-ammonium bromide; CTAB, cetyl-trimethyl-ammonium bromide; λDNA, lambda phage DNA.

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PII S0378-5173(96)04574-7

duction of normal exogenous genes into target cells, allows the replacement of the defective gene product, hence restoring normal cell function (Felgner et al., 1987). In addition, antisense oligonucleotides or chemically modified analogues, designed to be complementary to viral or eukaryotic mRNAs, can be used to inhibit both in vitro and in vivo protein synthesis (To and Neiman, 1992; Neckers et al., 1992). More generally, different nucleic acid molecules, including (a) mRNAs or complete genes (Mitchell and Tjian, 1989; Faisst and Meyer, 1992), (b) antisense oligonucleotides (Uhlmann and Peyman, 1990), (c) triplex-forming oligonucleotides (Alunni-Fabbroni et al., 1995) and (d) polymerase chain reaction (PCR)-generated DNA fragments (Gambari and Nastruzzi, 1994), have been considered for modulating gene transcription. The above mentioned nucleic acid molecules provide opportunities to either replace the missing/defective gene or arrest the expression of specific genes.

Nevertheless, after administration, nucleic acid molecules should remain stable in extracellular environment in order to exert a pharmacological effect. In this view, both antisense and transfection technologies require reliable and efficient systems for their delivery into target cells. On the basis of this consideration, the design of an efficient nucleic acid delivery system represents one of the key steps for these therapeutic agents (Langer, 1990). Various approaches have been proposed, such as neutral or cationic liposomes and polymeric microparticles (Janoff, 1992; Gregoriadis, 1988; Thierry et al., 1992; Cortesi et al., 1994b). Particulate systems could indeed reduce the metabolization of oligonucleotides and enhance specific cellular uptake (Felgner, 1990; Hug and Sleight, 1991). Moreover, liposomes are known to preserve the entrapped compound from enzymatic degradations and to allow targeting strategies. For the preparation of liposomes intended for nucleic acid delivery, two approaches appear particularly suitable: (a) the use of a neutral lipid composition by 'minimal volume entrapment' technique (Thierry et al., 1992) and (b) the use of cationic liposomes prepared by extrusion technique (Cortesi et al., 1994a). It should be pointed out that, in contrast with 'conventional' ones, cationic liposomes do not entrap DNA molecules within their interior. The nucleic acid molecules are bounded by ionic interactions on the surface of preformed cationic liposomes. The surfaces of these vesicles are positively charged due to the presence of quaternary ammonium detergents in the liposome composition, such as DOTMA (*N*-(1-(2,3-dioleyloxy)propyl)-*N*,*N*,*N*-trimethyl-ammonium chloride) (Felgner et al., 1987; Thierry et al., 1992), DEBDA (diisobutyl-cresoxy-ethoxy-ethyl-dimethyl-benzyl-ammonium hydroxyde) (Ballas et al., 1988) or CTAB (cetyltrimethyl-ammonium bromide) (Pinnaduwage et al., 1989). In this way, negatively charged nucleic acids complex the surface of preformed cationic liposomes. For this reason, the term 'liposome-associated DNA' should be more correctly used to describe this type of liposome.

In spite of their simple preparation protocol, cationic liposomes display some disadvantages, such as cytolitic and cytotoxic activity. Yoshihara and Nakae (1986) have demonstrated that cationic liposomes containing stearylamine showed an in vivo toxicity in rabbit. This effect was attributed to hemolysis of the erythrocytes and was directly related to the amount of stearylamine present in the liposome composition. Moreover, Felgner et al. (1987) reported that vesicles containing DOTMA, used to facilitate the fusion of liposome/DNA complex with plasma membrane, could result in a cytotoxic effect. This phenomenon is attributable to the presence of the cationic detergent, causing a disruption-solubilization process of cell membranes (Lappalainen et al., 1994).

The aims of this work were the evaluation of parameters influencing the in vitro toxicity of cationic liposomes on cultured cell lines. In addition, the study investigated the stability of DNA molecules complexed to cationic liposomes after exposure to exo- and endonucleases present in serum. In particular this report describes: (a) the preparation of positively charged liposomes by using three different cationic surfactants, namely didodecyl-dimethyl-ammonium bromide ($DDAB_{12}$), dioctadecyl-dimethyl-ammonium bromide ($DDAB_{18}$) and cetyl-trimethyl-ammonium bromide (CTAB); (b) the cytotoxic activity of the

obtained cationic liposomes on in vitro cultured human K562 erythroleukemic cells; and finally (c) the protective effect of cationic liposome complexation on lambda phage DNA (λDNA) degradation mediated by serum nucleases.

2. Experimental procedures

2.1. Materials

Egg phosphatidyl choline was purchased from Lipid Products (Surrey, England).

λ phage DNA (λDNA) was obtained from Pharmacia (Uppsala, Sweden). Cholesterol was obtained from Fluka (Bucs, Switzerland). Quaternary ammonium detergents didodecyl-dimethyl-ammonium bromide ($DDAB_{12}$) and dioctadecyl-dimethyl-ammonium bromide ($DDAB_{18}$) were purchased from Fluka, cetyl-trimethyl-ammonium bromide (CTAB) was purchased from Sigma Chemical Co.

2.2. Liposome preparation

Cationic liposomes were prepared by the reverse-phase evaporation (REV) method (Szoka and Papahadjopoulos, 1978). The aqueous phase consisted of 1 ml of water, the organic phase was a solution of egg phosphatidylcholine (PC), cholesterol (CH) and the cationic detergent (CD) in 4 ml of diethyl ether. The molar ratio of the liposome constituents was PC:CH:CD 8:2:1, mol/mol/mol.

The biphasic system was vortexed and sonicated at 0°C for 5 min in a bath-type sonicator. The ether present in the obtained stable emulsion was removed by rotary evaporation under reduced pressure at room temperature, resulting in a turbid, white liposome dispersion. In order to obtain homogeneously sized vesicles, the REV liposomes were then extruded through polycarbonate filters with different pore sizes. The formation of the liposome-DNA complex was carried on by mixing the unilamellar vesicles composed of PC, CH and CD (1 mg of total lipid in 1 ml of water) with a solution containing 0.3 μg of λDNA.

2.3. Liposome morphology

Shape and surface characteristics of the obtained liposomes were studied by freeze fracture electron microscopy. Briefly, after freezing the sample by propane jet technique, the cryofixed preparation was fractured at 108°K in a Balzen BAF 300 at 10^{-5} Pa. Photomicrographs were taken on Agfa Scientia 23D56 cut films and developed in Geratone G5C for 3.5 min at 293°K. The dimensional analysis of liposomal suspensions was performed by a photon correlation granulometer SEM F60 (SEMATech, Nice, France) equipped with a computer-driving RD goniometer and an argon ion laser, operating at a few mW of power of λ = 514 nm.

2.4. Analysis of the electrophoretic mobility of liposome-DNA complexes

VET_{100} containing increasing concentrations of cationic detergent (8:2:0.5, 8:2:1, 8:2:1.5, 8:2:2, mol/mol/mol) were mixed with λDNA. Liposome/λDNA complexes (9:1, w/w ratio) were incubated at 37°C for 5 min, then each sample was subjected to electrophoresis. Electrophoresis was carried on in a 0.8% agarose gel at constant voltage (100 mV) for 2 h in the absence or in the presence of increasing concentrations of CD. The relative band migration was determined, after staining the gels with ethidium bromide.

2.5. DNA stability studies

The stability of liposome/λDNA complexes towards fetal calf serum (FCS) containing nucleases was studied following the above protocol. λDNA (0.3 mg) (Pharmacia, Uppsala, Sweden) were complexed to different amounts of cationic liposomes, resulting in final liposomes/DNA ratios of 3:1, 9:1 and 12:1, w/w. The liposome/DNA complexes were then incubated at 37°C in a thermostatic bath. At different time intervals, comprising between 0 and 240 min, samples were withdrawn and stored at −20°C until electrophoretic analysis was performed. Electrophoresis was performed on 0.8% agarose gels containing 0.5 mg/ml ethidium bromide for 2 h at 25 mV constant current.

After electrophoresis, the λDNA bands were visualized by ultraviolet (UV) shadowing (Maniatis et al., 1982). The quantitative analysis of band intensity was performed by a computerized scanning analysis of digitalized images. For this analysis, the 'NIH Image', a public domain image processing and analysis program for the Macintosh, was used.

2.6. Cytotoxicity studies

The cytotoxicity of cationic liposomes was determined on in vitro cultured human leukemic K562 cells (Lozzio and Lozzio, 1975). Standard conditions for cell growth were a-medium (Gibco, Grand Island, NY), 50 mg/l streptomycin, 300 mg/l penicillin, supplemented with 10% FCS (Irvine Scientific, Santa Ana, CA) in 5% CO_2 at 90% humidity. Cell growth was determined by counting with a Model ZF Coulter Counter (Coulter Electronics Inc., Hielah, FL). Counts of viable cells were performed after 0.1% Trypan blue exclusion test.

3. Results and discussion

As previously stated in the introduction, the use of cationic liposomes as a delivery system for nucleic acids could result in a series of advantages over 'conventional' liposomes. Nucleic acids are not exposed to denaturing conditions possibly present in other liposome preparation protocols (i.e., sonication, organic solvents) (Felgner et al., 1987; Bennet et al., 1992; Thierry et al., 1992).

The liposome/DNA complexation can be performed just before use, by simply mixing nucleic acid to 'preformed' vesicles. Finally, both liposomes and DNA can be stored in lyophilized form and rehydrated and mixed just prior the use, resulting in a quantitative 'association' yield.

3.1. Liposome preparation

Positively charged liposomes were produced by employing a protocol based on reverse phase evaporation followed by extrusion of the liposome suspension through polycarbonate filters with homogeneous pore size. Liposomes were subjected to one extrusion cycle through two stacked 200 nm pore size filters, followed by three extrusion cycles through two stacked 100 nm pore size membranes. This choice was made on the basis of many papers that describe vesicles with an average diameter around 100 nm as good candidates for both in vitro and in vivo studies using different administration routes (i.e., intramuscular, endovenous, subcutaneous) (Janoff, 1992). The extruded liposomes were named VET_{100}, according to Mayer et al. (1986), where VET indicates vesicles produced by extrusion techniques and the subscript number the pore size of the membrane used for the extrusion.

Three different cationic detergents were alternatively used for the production of positively charged liposomes, namely CTAB, $DDAB_{12}$ and $DDAB_{18}$.

3.2. Morphological analysis

The morphological and dimensional analysis of the produced liposomes was performed by freeze-fracture electron microscopy technique and dynamic light scattering. Electron microscopy demonstrated that the liposomal suspensions were constituted of unilamellar vesicles with an average diameter reflecting the pore size of the utilized membrane. Dynamic light scattering studies demonstrated that the extruded vesicles present a narrow size distribution. In Table 1 are reported the volume-weighted and intensity-weighted particle size analyses of the three cationic liposome suspensions as determined by dynamic light scattering.

3.3. Cytotoxicity studies

In order to obtain information about the cytotoxicity of cationic liposomes, in vitro assays were performed by cultivating human erythroleukemic K562 cells in the presence of liposomes. Increasing concentrations of cationic liposomes, having a PC:CH:CD 8:2:1, mol/mol/mol composition, were added to the cell. Concentrations comprised between 0 and 40 mg/ml of liposome corresponding to 0.1–10 mM of CD were tested. The results,

Table 1
Particle size distribution of cationic liposomes produced by extrusion technique*

Cationic surfactant	Mean diameter (S.D.) (nm)	Dispersion (S.D.) (nm)	Polydispersity (S.D.)
Volume-weighted particle size analysis			
$DDAB_{18}$	114.8 (10.8)	19.4 (3.2)	0.215 (0.062)
$DDAB_{12}$	123.9 (33.0)	60.0 (9.8)	0.235 (0.069)
CTAB	104.5 (32.5)	49.5 (10.5)	0.224 (0.079)
Intensity-weighted particle size analysis			
$DDAB_{18}$	120.1 (31.0)	55.7 (9.3)	0.215 (0.062)
$DDAB_{12}$	140.0 (37.3)	67.8 (11.1)	0.235 (0.068)
CTAB	121.3 (37.7)	57.4 (12.2)	0.224 (0.079)

*Data refer to VET_{100} constituted of PC:CH:CD 8:2:1 (mol/mol/mol).
Data were obtained by using a photon correlation granulometer SEM F60 (SEMATech, Nice, France) equipped with a computer-driving RD goniometer and an argon ion laser.

reported in Fig. 1, indicate that CTAB- and $DDAB_{12}$-liposomes showed a rather pronounced cytotoxicity, with IC_{50} values of 0.88 μM and 0.85 μM, respectively (where IC_{50} is the compound concentration inhibiting 50% of the cell growth). On the contrary, $DDAB_{18}$-liposomes displayed only a low cytotoxic effect (IC_{50} > 25 mM).

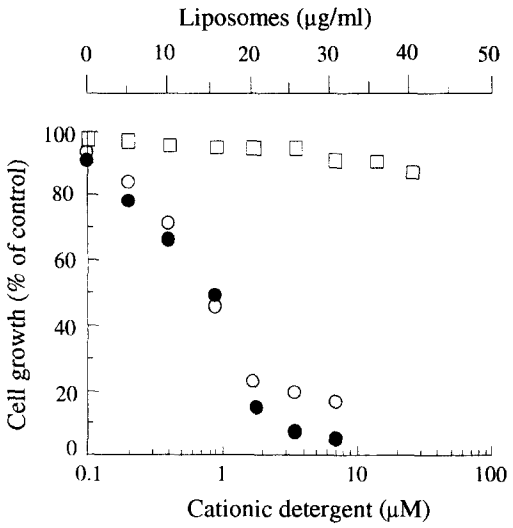

Fig. 1. In vitro cytotoxic effect of different VET_{100} cationic liposomes, containing CTAB (●), $DDAB_{12}$ (○) or $DDAB_{18}$ (□), on human erythroleukemic K562 cells. The PC:CH:CD molar ratio was 8:2:1, mol/mol/mol. Determinations were performed after 5 days of cell culture. Data represent the % of cell number/ml compared to untreated control K562 cells. The reported results are the average of 3 independent experiments, SD ≤ 7%.

Moreover, we compared the cytotoxicity of the CTAB-, $DDAB_{12}$-, and $DDAB_{18}$-liposomes with the respective cationic detergent solutions (micellar solutions). As shown in Fig. 2 (panels A and B), the curve trends of CTAB- and $DDAB_{12}$-liposome suspensions are almost superimposable with those of the respective micellar solution, whilst in the case of $DDAB_{18}$ (Fig. 2C) the two curves diverge one from the other. As clearly evident, the cytotoxicity of $DDAB_{18}$ is lower when this cationic detergent is in liposome form. Table 2 summarizes the IC_{50} values found for the three cationic detergents used as liposome suspensions or micellar solutions.

Furthermore, the influence of liposome/CD molar ratio on liposome cytotoxicity was evaluated. Four liposome compositions characterized by different PC:CH:CD molar ratios were tested, namely 8:2:0.5, 8:2:1, 8:2:1.5 and 8:2:2 (mol/mol/mol). The obtained results demonstrated that within the molar ratio interval used, no appreciable difference in the cytotoxic activity was observed. Nevertheless, in the case of CTAB and $DDAB_{18}$, one could observe a slightly increased antiproliferative effect for liposomes containing a higher proportion of cationic detergent (data not shown).

3.4. Binding migration studies

In order to evaluate the strength of the interaction occurring between DNA and liposomes and

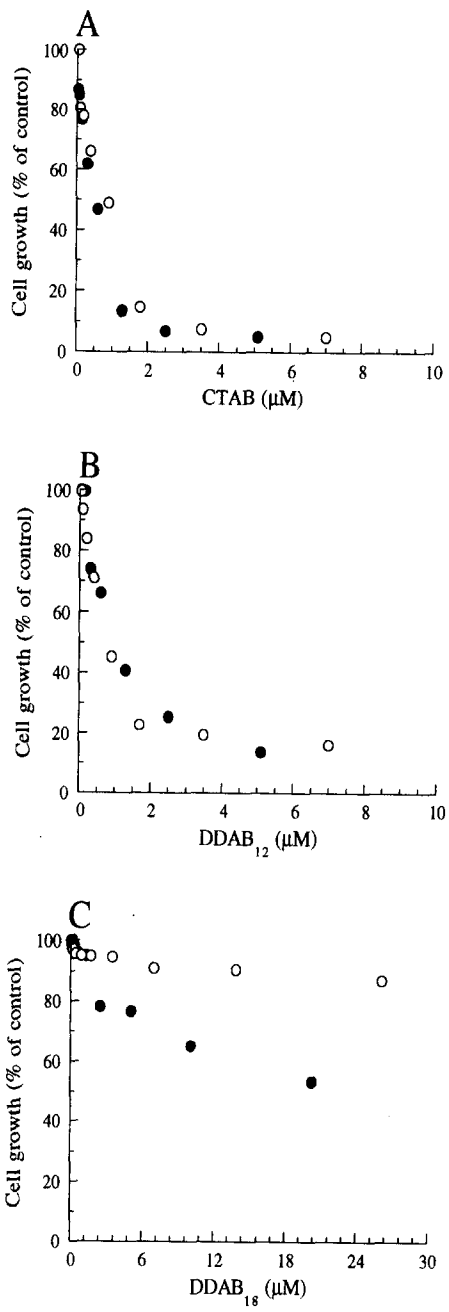

Fig. 2. In vitro cytotoxic effect of CTAB (A), $DDAB_{12}$ (B) and $DDAB_{18}$ (C) on K562 cells. Cationic detergents were tested both as liposome suspension (○) or micellar solution (●). Liposomes were VET_{100} PC:CH:CD 8:2:1, mol/mol/mol. Determinations were performed after 5 days of cell culture. Data represent the % of cell number/ml compared to untreated control K562 cells. The reported results are the average of 3 independent experiments, SD ≤ 7%.

to evaluate if different cationic detergents could cause a variation in binding strength, the following experiment was performed. Liposomes containing increased concentration of cationic detergent (see legend to Fig. 3) were incubated with λDNA (used as model nucleic acid) to a final liposome/DNA ratio of 9:1 (w/w). Samples were electrophoresed in order to determine the electrophoretic migration of DNA complexed to liposome. The results reported in Fig. 3 indicate that λDNA migration is only slightly retarded by liposome containing low quantity of cationic detergent, whilst it is evident that higher CD concentration (especially in the case of $DDAB_{18}$) cause the formation of high-molecular-weight complexes with DNA molecules. These complexes were attributed to inter-liposomal bridges formed by DNA molecules (Sternberg et al., 1994). This hypothesis was supported by the presence of non-migrating bands in the agarose gels.

3.5. Stability studies

The protective effect of liposomes was studied on degradation of λDNA catalyzed by nucleases. It is to be underlined that (a) as a model cationic liposome, we considered VET_{100} constituted by PC:CH:CD 8:2:1 mol/mol/mol and (b) as source for nucleases we used FCS at a final concentration of 10%. FCS was used since it is routinely employed in cell culture experiments. Liposome/DNA complexes 3:1 (w/w) were incubated at 37°C from 0 to 240 min in the presence of 10% FCS, samples were then loaded on agarose gels and subjected to electrophoresis. As control, the same amount of λDNA was incubated in the absence of cationic liposomes. The result of this experiment indicates that the complete degradation of free λDNA (Fig. 4) or λDNA complexed to CTAB-liposomes (data not shown) occurs within 90 min, whilst the complexation of DNA to $DDAB_{12}$-liposomes (Fig. 5) or to $DDAB_{18}$-liposomes (Fig. 6) protects λDNA from degradation, even to a different extent.

3.6. Effect of liposome charge density on DNA stability

The increase of charge density on the liposome

surface could determine the formation of a more tight liposome/DNA complex, thus increasing the protective effect of liposome toward nuclease digestion. To study the effect of liposome charge density, we incubated λDNA with a fixed amount of cationic liposomes containing increasing amounts of CD, characterized by four different PC:CH:CD molar ratios, namely 8:2:0.5, 8:2:1, 8:2:1.5 and 8:2:2, mol/mol/mol. As reported in Fig. 7, by increasing the molar ratio of CD, a more tight liposome/DNA complex is formed due to the higher charge density on the liposome surface.

This feature was evidenced by the non-migrating bands present in lanes d and e. Particularly $DDAB_{12}$- (panel B) and $DDAB_{18}$-liposomes (panel C) appear to form stronger complexes with DNA, reflecting a higher protective effect when compared to that of CTAB-liposomes.

As reported above, the formation of liposome/λDNA complex leads to high-molecular-weight aggregates that, when electrophoresed on agarose gels, result in a non-migrating band. On the basis of this consideration, we cannot formally exclude that DNA complexed to liposomes would still be integer after exposure to FCS nucleases. In order to answer this question, we performed a further experiment. Free λDNA and λDNA complexed to $DDAB_{18}$-liposome (liposome/λDNA 3:1, 9:1 and 12:1, w/w) were exposed to FCS nucleases, then DNA was phenol-extracted from liposome suspension and analyzed by gel-electrophoresis. As reported in Fig. 8, the extracted λDNA shows

Table 2
Effect of liposome and micellar cationic detergent formulations on cell growth of human erythroleukemic K562 cells

Cationic detergent	Liposome IC_{50} (mM)[a]	Micellar solution IC_{50} (mM)[a]
CTAB	0.88	0.62
$DDAB_{12}$	0.85	0.91
$DDAB_{18}$	>25	20.00

[a]IC_{50}, inhibitory concentration 50%: compound concentration (μM) required to cause a 50% inhibition of in vitro growth of K562 cells.
Data represent the average of 3 independent experiments.

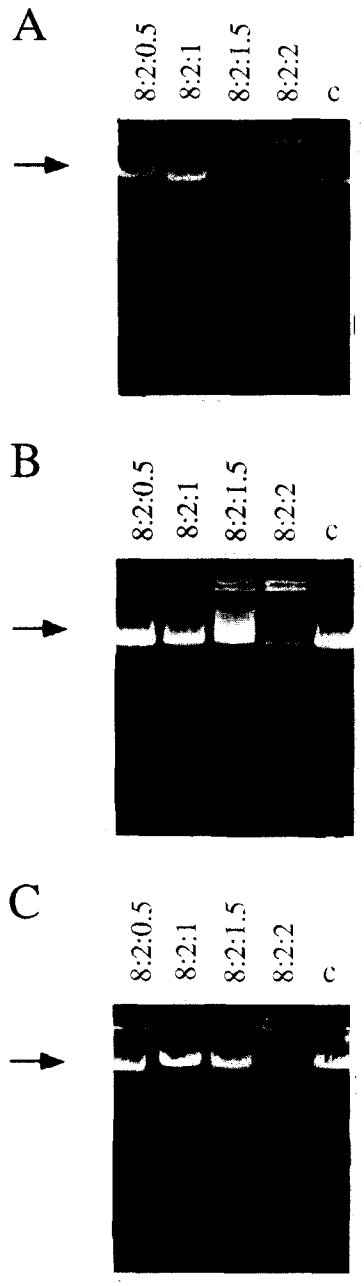

Fig. 3. Effect of cationic complexation on the electrophoretic migration of λ-phage DNA. A: CTAB; B: $DDAB_{12}$; C:$DDAB_{18}$. The following PC:CH:CD molar ratios, namely 8:2:0.5, 8:2:1, 8:2:1.5 and 8:2:2, mol/mol/mol, were used. c = free λDNA.

a single band with a molecular weight superimposable to that of undigested λDNA. This result confirms the maintenance of λDNA integrity in the presence of liposomes, whilst in their absence the λDNA completely degrades in 60 min.

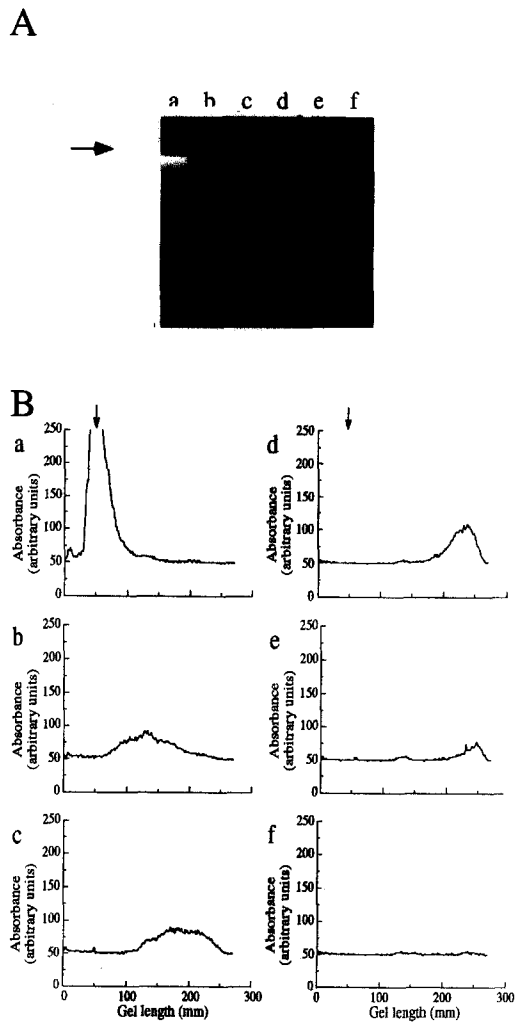

Fig. 4. Effect of FCS nucleases on free λDNA. Panel A: agarose gel electrophoresis patterns of λDNA (0.3 μg) incubated in the presence of 10% FCS at 37°C for 0 (lane a), 30 (lane b), 60 (lane c), 90 (lane d), 120 (lane e) and 240 min (lane f). Panel B: densitometric profiles of the electrophoretic migrations shown in panel A. The arrow indicates the undegraded λDNA band.

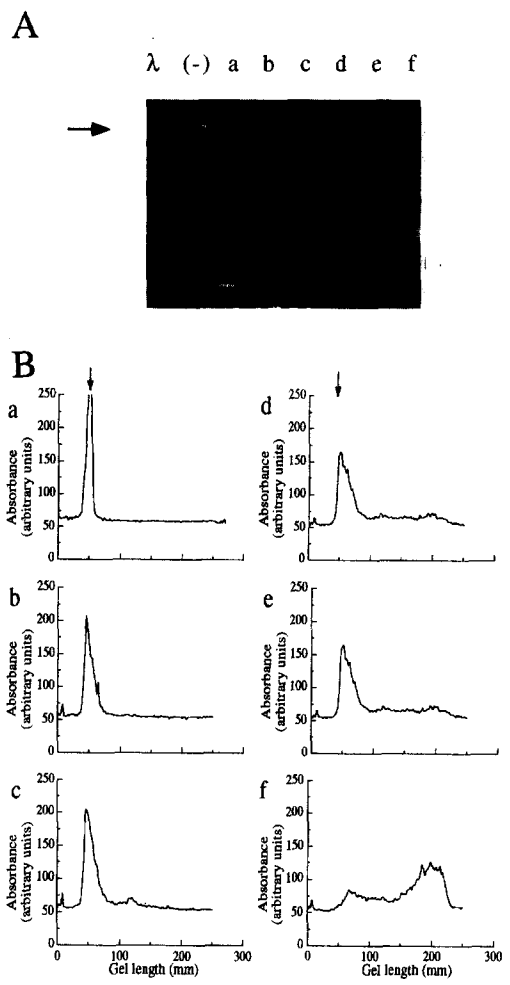

Fig. 5. Effect of FCS nucleases on λDNA complexed to $DDAB_{12}$-liposome complexation. Liposome composition was PC:CH:$DDAB_{12}$ 8:2:1, mol/mol/mol. The final liposome/λDNA ratio was 3:1, w/w. Panel A: agarose gel electrophoresis patterns of λDNA (0.3 μg) incubated at 37°C for 0 (lane a), 30 (lane b), 60 (lane c), 90 (lane d), 120 (lane e) and 240 min (lane f) (λ: untreated free λDNA; (-): untreated λDNA complexed to $DDAB_{12}$-liposome). Panel B: densitometric profiles of the electrophoretic migrations shown in panel A. The arrow indicates the undegradated λDNA band.

4. Concluding remarks

Our study indicates that the composition of cationic liposomes is of paramount importance in determining the stability of DNA complexed to

liposomes and their cytotoxicity. In conclusion, due to their low cytotoxicity and effective protection of carried DNA, $DDAB_{18}$ liposomes could be proposed as an interesting delivery system for nucleic acid administration.

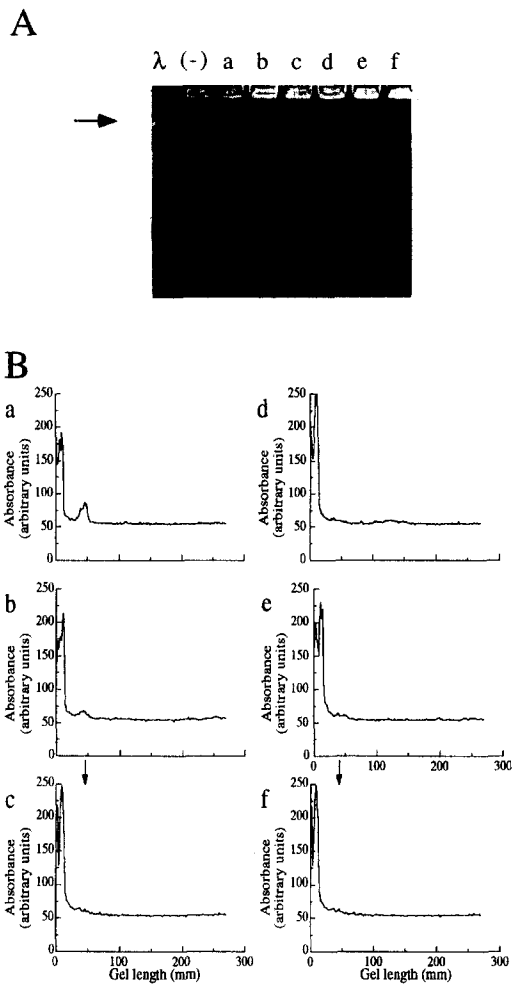

Fig. 6. Effect of FCS nucleases on λDNA complexed to $DDAB_{18}$-liposome complexation. Liposome composition was PC:CH:$DDAB_{18}$ 8:2:1, mol/mol/mol. The final liposome/λDNA ratio was 3:1, w/w. Panel A: agarose gel electrophoresis patterns of λDNA (0.3 μg) incubated at 37°C for 0 (lane a), 30 (lane b), 60 (lane c), 90 (lane d), 120 (lane e) and 240 min (lane f) (l: untreated free λDNA; (-): untreated λDNA complexed to $DDAB_{18}$-liposome). Panel B: densitometric profiles of the electrophoretic migrations shown in panel A. The arrow indicates the undegradated λDNA band.

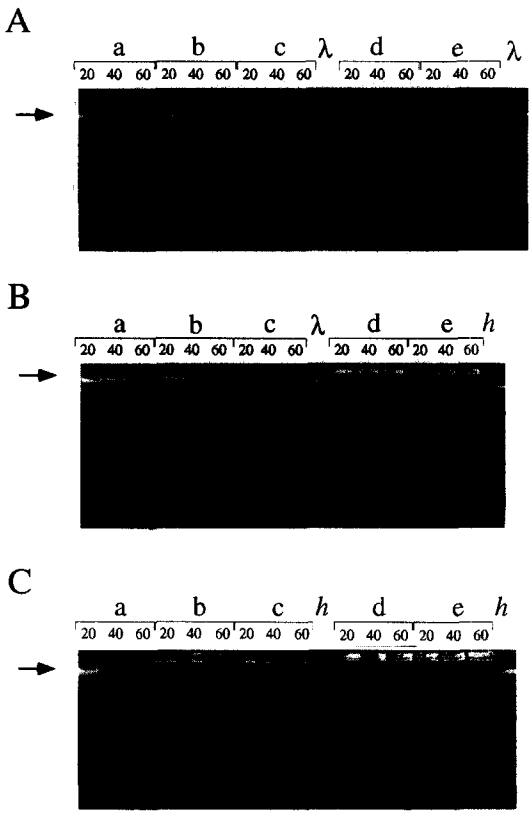

Fig. 7. Protective effect of liposome/λDNA complex on nuclease digestion. λDNA (0.3 μg) was incubated with 10% FCS in the absence (a) or in the presence (b–e) of cationic VET_{100}. A: CTAB-liposomes, B: $DDAB_{12}$-liposomes and C: $DDAB_{18}$-liposomes. Four different PC:CH:CD molar ratios were used: 8:2:0.5 (b), 8:2:1 (c), 8:2:1.5 (d) and 8:2:2 (e) mol/mol/mol. After incubation at 37°C for 20, 40 or 60 min, λDNA degradation was analysed on 0.8% agarose gels. As molecular weight marker, the migrations of untreated λDNA (l) and of *Hind*III digested λDNA (*h*) are reported.

Acknowledgements

The authors thank AIRC and ACRO for financial support.

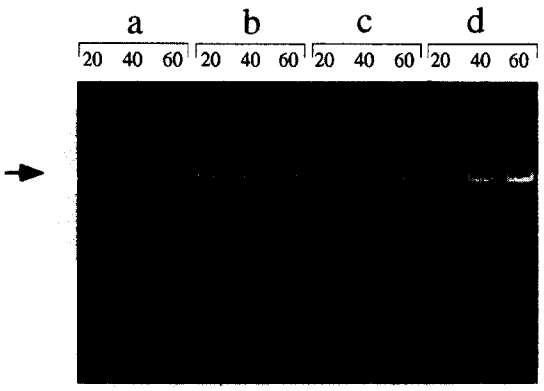

Fig. 8. Electrophoretic analysis of λDNA phenol extracted from liposome suspension. λDNA (0.3 μg) was previously incubated in the absence (a) or in the presence (b–d) of $DDAB_{18}$-liposomes at a final liposome/λDNA ratio of 3:1 (b), 9:1 (c) and 12:1 (d) (w/w). Samples were incubated at 37°C for 20, 40 and 60 min, then λDNA was extracted using a standard protocol and analyzed by agarose gel electrophoresis.

References

Alunni-Fabbroni, M., Xodo, L.E. and Manzini, G., Triplex-forming oligonucleotide as artificial gene repressors. *Minerva Biotecnologica*, (1995) 169–175.

Ballas, N., Zakay, N., Sela, I. and Loyter, A., Liposome bearing a quaternary ammonium detergent as an efficient vehicle for functional transfer of TMV-RNA into plant protoplast. *Biochim. Biophys. Acta*, 939 (1988) 8–18.

Bennet, C.F., Chiang, M.Y., Chan, H., Shoemaker, J.E.E. and Mirabelli, C.K., Cationic lipids enhance cellular uptake and activity of phosphorothioate antisense oligonucleotides. *Mol. Pharmacol.*, 41 (1992) 1023–1033.

Cortesi, R., Esposito, E., Gambari, R., Menegatti, E. and Nastruzzi, C., Liposome-associated synthetic oligonucleotides: production and in vitro release kinetics. *Pharm. Pharmacol. Lett.*, 3 (1994a) 213–216.

Cortesi, R., Esposito, E., Menegatti, E., Gambari, R. and Nastruzzi, C., Gelatin microspheres as a new approach for controlled delivery of synthetic oligonucleotides and PCR-generated fragments. *Int. J. Pharm.*, 105 (1994b) 181–186.

Faisst, S. and Meyer, S., Compilation of vertebrate-encoded transcription factors. *Nucleic Acids Res.*, 20 (1992) 3–26.

Felgner, P.L., Particulate systems and polymers for in vitro and in vivo delivery of polynucleotides. *Adv. Drug. Deliv. Rev.*, 5 (1990) 163–187.

Felgner, P.L., Gadek, T.R., Holm, M., Roman, R., Chan, H.W., Wenz, M., Northrop, J.P., Ringold, G.M. and Danielsen M., Lipofection: A highly efficient, lipid-mediated DNA-transfection procedure. *Proc. Natl. Acad. Sci. USA*, 84 (1987) 7413–7417.

Gambari, R. and Nastruzzi, C., DNA-binding activity and biological effects of aromatic polyamidines. *J. Biochem. Pharmacol.*, 47 (1994) 599–610.

Gregoriadis, G., Liposomes as drug carriers: Recent trends and progress. J. Wiley and Sons, Chichester, U.K., 1988.

Hug, P. and Sleight, R.G., Review: liposomes for the transformation of eukaryotic cells. *Biochim. Biophys. Acta*, 1097 (1991) 1–15.

Janoff, A.S., Lipids, liposomes, and rational drug design. *Lab. Invest.*, 66 (1992) 655–658.

Langer, R., New methods of drug delivery. *Science*, 249 (1990) 1527–1533.

Lappalainen, K., Urtti, A., Söderling, E., Jääskeläinen, I., Syrjänen, K. and Syrjänen, S., Cationic liposomes improve stability an intracellular delivery of antisense oligonucleotides in CaSki cells. *Biochim. Biophis. Acta*, 1196 (1994) 201–208.

Lozzio, C.B. and Lozzio, B.B., Human chronic myelogeneous leukemia cell line with positive Philadelphia chromosome. *Blood*, 45 (1975) 321–334.

Maniatis, T., Fritsch, E.F. and Sambrook, J., Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory: Cold Spring Harbor, New York, 1982

Mayer, L.D., Hope, M.J. and Cullis, P.R., Vesicles of variable size produced by a rapid extrusion procedure. *Biochim. Biophys. Acta*, 858 (1986) 161–168.

Mitchell, P.J. and Tjian, R., Transcriptional regulation in mammalian cells by sequence-specific DNA binding proteins. *Science*, 245 (1989) 371–378.

Neckers, L., Whitesell, L., Rosolen, A. and Geselowitz, D.A., Antisense inhibition of oncogene expression. *Crit. Rev. Oncogen.*, 3 (1992) 175–231.

Pinnaduwage, P., Schmitt, L. and Huang, L., Use of a quaternary ammonium detergent in liposome mediated DNA transfection of mouse L-cells. *Biochim. Biophys. Acta*, 985 (1989) 33–37.

Sternberg, B., Sorgi, F.L. and Huang, L., New structures in complex formation between DNA and cationic liposomes visualized by freeze-fracture electron microscopy. *FEBS Letters*, 356 (1994) 361–366.

Szoka, F. and Papahadjopoulos, D., Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse phase evaporation. *Proc. Natl. Acad. Sci., USA* 75 (1978) 4194–4198.

Thierry, A.R., Rahman, A. and Dritschilo, A., Liposomal delivery as a new approach to transport antisense oligonucleotides. In: Erickson, R.P. and Izant, J.G. (Eds), *Gene Regulation: Biology of Antisense RNA and DNA*, Raven Press, Ltd., New York, 1992, pp. 147–161.

To, R.Y. and Neiman, P.E., The potential for effective antisense inhibition of retroviral replication mediated by retroviral vectors. In: Erickson, R.P. and Izant, J.G. (Eds), *Gene Regulation: Biology of Antisense RNA and DNA*, Raven Press, Ltd., New York, 1992, pp. 261–271.

Uhlmann, E. and Peyman, A., Antisense oligonucleotides: a new therapeutic principle. *Chem. Rev.*, 90 (1990) 543–584.

Yoshihara, E. and Nakae, T., Cytolytic activity of liposomes containing stearylamine. *Biochim. Biophys. Acta*, 854 (1986) 530–546.