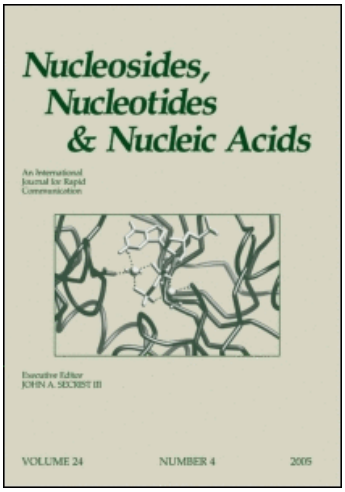

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LIPOPLEX-MEDIATED STABLE GENE TRANSFER INTO HeLa CELLS

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LIPOPLEX-MEDIATED STABLE GENE TRANSFER INTO HeLa CELLS

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ABSTRACT

Unilamellar cationic liposomes containing phosphatidylcholine, L-α-phosphatidyl-DL-glycerol, cholesterol and N,N-dimethylaminopropylaminyl succinyl cholesterol in lipoplexes with plasmid ptkNEO transfected HeLa cells efficiently in the presence of G418.

The introduction of cationic liposomes based on 3β[N-(N′,N′-dimethylaminoethane)-carbamoyl] cholesterol (1) (DC-Chol) and dioleoylphosphatidylethanolamine (DOPE) has led to successful non-viral gene therapy protocols in human trials (2,3). In related work liposomes containing N,N-dimethylethylenediaminyl succinyl cholesterol (A) and DOPE have transfected several cell lines with the plasmid pUCSV2CAT (4). Replacement of the co-lipid DOPE with dioleoylphosphatidylcholine (DOPC) however resulted in poor transfection activity. Recent work has shown that cationic liposomes constituted with the helper lipid cholesterol (C) and small amounts of polyethylene glycol/phospholipid conjugates form stable complexes with DNA (5) and achieve high transfection activities *in vivo* (6).

We have prepared N,N-dimethylaminopropylaminyl succinyl cholesterol (B), a higher homologue of A, from the N-hydroxysuccinimide ester of cholesteryl hemisuccinate and N,N-dimethylaminopropylamine in 68% overall yield. MS m/z 570 (M^+). B was shown in a lipid impregnated paper DNA-binding assay to bind

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Table 1. The Binding of pBR322 Plasmid DNA to Liposomes

Liposome Composition	Lipid Mole Ratios	Liposome Associated DNA Relative to ST:PC (1:4)%
ST:PC	1:4	100
ST:PC:B	1:4:2	76.8
PC:PG:C	4:1:5	14.5
PC:PG:C:B	4:1:5:1	71

λDNA at least 4 times more avidly than PC, dipalmitoylphosphatidylcholine and DOPE, while C showed no affinity for DNA.

Four unilamellar liposome suspensions (200–800 nm vesicle diameter) containing combinations of B, C, PC, stearylamine (ST) and L-α-phosphatidyl-DL-glycerol (PG) were prepared by a modified reverse evaporation procedure. Control liposomes were of the following composition and lipid mole ratios (7): **1**, ST:PC(1:4); **2**, PC:PG:C (4:1:5) and two further preparations contained the cationic cholesterol derivative B : **3**, ST:PC:B (1:4:2), **4**, PC:PG:C:B (4:1:5:1).

The binding of plasmid DNA to liposomes was determined in an ultracentrifugation assay in which liposomes (5 mg) were incubated with pBR322 DNA (1 μg) for 20 minutes before sedimenting lipoplexes at 100 000 $\times$ g. Table 1 reveals that preparation **1**, with its inherent cationic nature, bound DNA well while **2** exhibited only modest DNA binding. Inclusion of B into liposomes based on **1** and

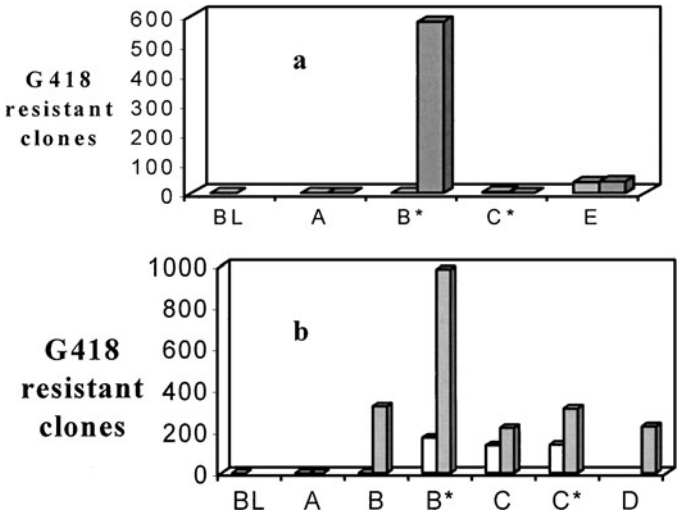

Figure 1. Comparative liposome-mediated transfection of HeLa cells with ptkNEO DNA. BL = blank, no lipid, no DNA; A = no lipid; B = PC:PG:C liposomes; B* = PC:PG:C:X liposomes; C = ST:PC liposomes; C* = ST:PC:X liposomes; D = DOTAP; E = calcium phosphate. **a**, 60 μg liposome lipid and 2 μg or 6 μg (dark grey) ptkNEO DNA. **b**, 4 μg plasmid DNA and 40 μg or 160 μg (grey) liposome lipid except DOTAP (100 μg).

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2 reduced moderately the DNA binding capacity of stearylamine derived liposomes while that of **4** was considerably greater than that of **2**.

Transfections were carried out in HeLa cells with the expression plasmid ptkNEO. Lipoplexes formed by combining appropriate aliquots of liposome preparations with plasmid DNA were incubated with cells in 25 cm^2 flasks bathed in HEPES buffered saline (1.5 mL, pH 7.4). After 4 hours at 37°C complete medium was added (5 ml) and 24 hours later cells were plated 1:3 (Fig. 1a) or 1:2 (Fig. 1b). Cultures were maintained in the presence of G418 at 800 μg/mL for 4 days and thereafter at 400 μg/mL until the unambiguous appearance of resistant clones and the death of non-resistant cells.

Liposomes **1**–**4**, each in two different combinations with ptkNEO DNA (4 μg, Fig. 1b) transfected more efficiently at the higher lipid concentration (160 μg) while preparations containing B performed best. In a separate experiment to compare the transfection efficiencies of the B containing lipoplexes at lipid: DNA ratios of 60 μg: 2 μg and 60 μg (Fig. 1a), 580 clones were recorded for the PC:PG:C:B/DNA complex at the higher DNA concentration while no resistant clones were detected with the ST:PC:B/DNA lipoplex at the same concentrations. It is interesting to note here that cationic liposomes containing ST have shown toxicity in rabbits (8).

In conclusion, we have shown that unilamellar liposomes prepared from B and the lipids PC, PG and C mediate high levels of transfection in the human cervical carcinoma HeLa cell line and may be suitable for further study in whole organisms.

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