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Enhancement of Dendrimer-Mediated Transfection Using Synthetic Lung Surfactant Exosurf Neonatal *in Vitro*

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Pulmonary surfactants enhance adenovirus-mediated gene transfer but inhibit cationic liposome-mediated transfection in lung epithelial cells *in vitro*. This study examines the effect of the synthetic lung surfactant Exosurf on dendrimer-mediated transfection in eukaryotic cells. Exosurf significantly enhanced dendrimer-luciferase plasmid transfection in a number of cell lines and was very effective in primary cells. Luciferase expression increased up to 40-fold in primary normal human bronchial/tracheal epithelial cells (NHBE). FACScan analysis demonstrated that the transfection rate of the human T cell leukemia Jurkat cell line has significantly improved from 10 to 90% of cells at 24 h after transfection. Analysis of the components of Exosurf revealed that the nonionic surfactant tyloxapol was responsible for the enhancement of dendrimer-mediated gene transfer. The tyloxapol effect was due to increased cell membrane porosity and DNA uptake. Our results demonstrate that Exosurf and its component, tyloxapol, constitute a powerful enhancer for dendrimer-mediated gene transfer *in vitro*.

Key Words: **surfactant; exosurf; tyloxapol; dendrimer; transfection.**

Gene transfer into eukaryotic cells has been long established in studies of the regulation of gene expression. Most recent advances in gene delivery techniques, including viral and synthetic carriers, have been focused on improvement of the transfer, processing and expression of introduced genetic material for *in vivo* applications. Modified retroviral and adenoviral vectors, even though achieving high gene transfer, raise a variety of safety issues associated with recombination events and the existence of anti-viral immunity, particularly in therapeutic applications (1–5). These disadvantages of viral vectors led to the development of synthetic predominantly charged lipids and polymers. A new class of cationic spherical polymers, Starburst polyamidoamine (PAMAM) dendrimers, has been thoroughly tested *in vitro* and *in vivo* (6–9). Dendrimers complexed with plasmid DNA enhance DNA uptake and expression in a broad range of eukaryotic cell lines (7). The lack of toxicity, high transfection efficiency, and stability of complexed plasmid DNA and oligonucleotides suggest that this transfection method may have a utility for *in vivo* applications (8–11). Dendrimers, which are positively charged due to a high density of surface primary amines, bind DNA by interactions with negatively charged phosphates on the DNA molecule (7, 12). The dendrimer-DNA complexes facilitate transfer of DNA into a cell primarily by endocytosis. Furthermore, DNA in a complexed form is protected from nuclease activity while remaining transcriptionally active (9).

Natural and synthetic lung surfactants have been reported to affect gene transfer *in vitro* in cell culture and *in vivo* in the lung (13–15). Endogenous pulmonary surfactant is a mixture of phospholipids and proteins that help to lower the surface tension at the air-liquid interface in the lung (16). A synthetic, protein-free surfactant is used for replacement therapy in the course of respiratory distress syndrome (RDS) (17). Pulmonary surfactants have been reported to enhance adenovirus-mediated gene transfer *in vitro* and *in vivo* but inhibit liposome DNA transfection in cells *in vitro* (18–20). Non-ionic surfactants typically act by destabilizing the cell membrane *via* lipid and protein extraction from the cell bilayer, which in turn increases membrane fluidity and reduces restriction to molecule movement into the cell cytosol (21).

In this report, we show that the presence of the synthetic surfactant Exosurf during synthetic cationic polymer-mediated transfection *in vitro* markedly enhances the efficiency of gene expression in a variety of

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eukaryotic cells, including primary human lung and porcine vascular cells. The evaluation of the three components that form Exosurf indicates that the observed effect is entirely due to the nonionic surfactant fraction, tyloxapol. The enhancement of complex internalization and intracellular release from endosomes rather than an increase in membrane permeability underlies the improvement in transfection efficiency (22).

MATERIALS AND METHODS

Synthetic lung surfactant. The synthetic surfactant, Exosurf Neonatal, was provided by Glaxo Wellcome Co. (Research Triangle Park, NC). It is an aqueous solution of protein-free synthetic surfactant consisting of a lipid component, colfosceril palmitate (dipalitoylphosphatidylcholine = DPPC; 13.5 mg/ml); a spreading agent for DPPC, cetyl alcohol (1.5 mg/ml), a polymeric long-chain repeating alcohol; and tyloxapol (1.0 mg/ml), a nonionic surfactant which acts to disperse both DPPC and cetyl alcohol. The lyophilizate of Exosurf was resuspended in water according to the manufacturer's instructions. Exosurf solution was used at 10% to 50% (v/v) concentrations. Tyloxapol, DPPC, and cetyl alcohol were purchased from Sigma Chemical Co., St. Louis, MO, and were used at concentrations equivalent to the Exosurf dilution used.

Cell lines. Primary cell cultures of normal human bronchial epithelium (NHBE) and small airway epithelium (SAEC) were purchased and grown in serum-free SABM/SAGM medium from Clonetics Co. (San Diego, CA). Primary porcine vascular endothelial cells (YPE) were a gift from Dr. Elizabeth Nabel (University of Michigan, Ann Arbor, MI). YPE cells were maintained in Medium 199 (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS (fetal bovine serum from HyClone Lab. Inc., Logan, UT). The other cell lines—Jurkat (human acute T cell leukemia), CCD-37Lu (human normal lung fibroblast), A549 (human lung carcinoma epithelial-like), COS-1 (monkey kidney SV40 transformed fibroblast-like), Rat 2 (rat embryonal fibroblast), Clone 9 (rat normal liver epithelium) and BHK-21 (hamster kidney fibroblast-like)—were purchased from ATCC. All, except where indicated, were maintained in DMEM or RPMI 1640 medium (Life Technologies, Gaithersburg, MD) supplemented with 10% FBS serum (HyClone Lab.) and 1% penicillin-streptomycin solution (Life Technologies) at 37°C in 5% CO_2.

Plasmids. Plasmid pCF1Luc was constructed by inserting a blunt-ended Hind III–Xba I fragment of a luciferase gene from pGL3 plasmid (Promega Co., Madison, WI) into a blunt-ended Not I fragment of pCF1CAT vector (Genzyme Co., Framingham, MA) with a deleted CAT gene. Plasmid pEGFP-C1 with a green fluorescence protein (GFP) gene was purchased from Clontech Laboratories, Inc. (Palo Alto, CA). Plasmid pCF1EGFP was constructed by insertion of a blunt-ended Nhe I–Eco RI fragment of pEGFP-C1 vector containing the GFP gene into a blunt-ended Not I fragment of pCF1CAT vector without the CAT gene. Plasmid DNA was purified by two cycles of cesium chloride gradient centrifugation (23).

Cell transfection. Plasmid DNA for transfection was complexed with the Starburst dendritic polymer (G9 EDA) previously described (7). Dendrimer to DNA charge ratios were calculated based on the electrostatic charge present on each component (7). Dendrimer/DNA complexes were formed at charge ratios of 5, 10 and 20 in water at room temperature for 15 minutes before adding into the transfection medium. Transfection was performed for 3 hours in serum-free medium with or without 10% or 50% (v/v) of Exosurf or its components present at equivalent concentrations. Tyloxapol was also used during 3-hour preincubation with cells in serum-free medium, followed by washes and transfection. Cell lysates were processed for luciferase assay described below utilizing the Luciferase Assay System (Promega Co., Madison, WI). Cells were harvested at 24 to 48 hours after transfection and analyzed for expression of luciferase (Luc) or the green fluorescence protein (GFP).

TABLE 1

Transfection Efficiency in the Presence of Exosurf Measured by Luciferase Activity in Several Adherent (CCD-37LU, A549, COS-1) and Nonadherent (Jurkat) Cell Lines and Primary Adherent Cell Cultures (YPE, SAEC, NHBE)

	Luciferase expression (fold of increase)
CCD-37LU	2.4
A549	3.5
COS1	5.4
JURKAT	11.5
YPE	25.3
SAEC	8.1
NHBE	41.4

Note. Cells were transfected with pCF1Luc plasmid complexed with G9 EDA at a charge ratio of dendrimer to DNA of 10 or 20 in the presence of 10% or 50% (v/v) of Exosurf (optimal concentrations differed for different cells). The fold increase was calculated using controls transfected with dendrimer/DNA complexes in medium in the absence of Exosurf.

Luciferase assay. Luciferase activity was quantified in chemiluminescence assay. Light emission from cell extract (10 μl) incubated with 2.35×10^{-2} μmol of luciferin substrate (Promega Co.) was measured in the chemiluminometer (LB96P; EG & G/Berthold, Gaithersburg, MD) and adjusted for the protein concentration of the sample.

Protein assay. The protein concentration in the cell lysate was measured using the BCA protein assay reagent kit (Pierce, Rockford, IL) and using bovine serum albumin as a standard.

Flow cytometry analysis. Non-adherent Jurkat cells transfected with pEGFP-C1 plasmid were harvested, washed twice with PBS, and fixed in 2% paraformaldehyde for 15 minutes before analysis. Adherent YPE cells transfected with pCF1EGFP were fixed on the plate for 15 minutes with 2% paraformaldehyde, harvested, and analyzed. The green fluorescence of GFP transfected cells was determined on a flow cytometer (FACScan Becton-Dickinson, San Jose, CA) from at least 10,000 cells per sample and analyzed using CellQuest software (Becton-Dickinson) (24).

Fluorescent microscopy. The expression of green fluorescence protein (GFP) was assessed *in situ* 24 hours after transfection using an inverted fluorescent microscope (Nikon, Eclipse TE 200) at 450–480 nm excitation and 515 nm emission wavelength. Photographs were taken at 20× magnification.

Cell membrane permeability analysis. Tyloxapol at 0.5 mg/ml concentration was tested for its effect on cell membrane permeability during 3 hours of incubation with cells. Treated and untreated cells were washed twice, resuspended in serum-free medium at 2×10^5 cells/ml, and stained with fluorescein diacetate at 0.5 μg/ml and propidium iodide at 50 μg/ml (FDA and PI from Sigma Chemical Co.) as described (23). Intracellular red and green fluorescence was measured using a flow cytometer (Becton-Dickinson). A total of 10,000 cells was collected and a quadrant analysis of the dot plot was performed for each sample. Live cells stained with FDA were calculated from the lower right quadrant. Porous cells with higher membrane permeability stained red and green were calculated from the upper right quadrant. Dead cells stained with PI were calculated from the upper left quadrant and cell debris were calculated from the lower left quadrant.

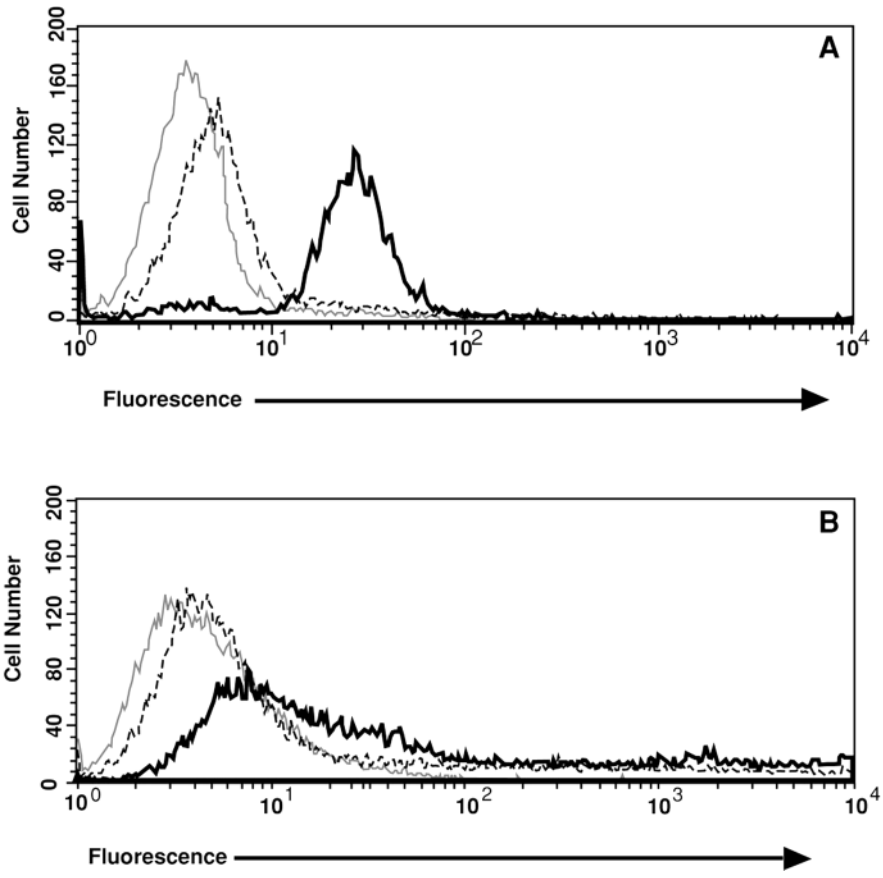

FIG. 1. Flow cytometry analysis of Jurkat cells (A) and primary vascular endothelial YPE cells (B) expressing the green fluorescence protein (GFP). Cells were transfected with either pEGFP plasmid DNA alone (thin line), or G9 EDA dendrimer/pEGFP complexes formed at a charge ratio of 10 (interrupted line), or the same complexes in the presence of 50% Exosurf (thick black line).

Lactate dehydrogenase assay. Jurkat cells were washed twice with phosphate-buffered saline (PBS, pH 7.4) and resuspended in serum-free medium containing tyloxapol from 0.25 to 2.0 mg/ml. The cells were incubated at 37°C in 5% CO_2 from 0 to 3 hours, and the supernatant was collected after centrifugation (at 400 × g for 5 min.). Supernatant was assayed for lactate dehydrogenase (LDH) activity using the LDH enzyme kit (Sigma), which allows for spectrophotometric measurement of NADH formation due to conversion of lactate to pyruvate in the presence of LDH (Milton Roy Spectronic 1001 plus, Rochester, NY). Supernatant (50 μl) was mixed by inversion with 1.0 ml of reagent solution (lactate 50 mM, NAD 7.0 mM, pH 8.9). The change in absorbance proportional to LDH activity was monitored at 340 nm wavelength at room temperature over 90 seconds.

RESULTS

Exosurf-Mediated Enhancement of Luciferase Expression

Dendrimer-mediated transfection of luciferase plasmid can be enhanced with Exosurf at 10% to 50% in a variety of cell lines and primary cells. Expression in established adherent cell lines CCD-37LU, A549, and COS-1 was enhanced 2-, 3-, and 5-fold respectively, compared with the control without surfactant (Table 1). Transfection of the non-adherent Jurkat human T cell line in the presence of Exosurf was enhanced 11-fold compared with the untreated control (Table 1). Proliferating primary cells of normal human bronchial and tracheal epithelium (NHBE) in its second or third passage were transfected 41-fold higher using Exosurf as compared to using dendrimer alone, while normal human small airway epithelial cell (SAEC) expressed at an 8-fold higher level (Table 1). Primary cells of a different origin, the porcine vascular endothelial cells (YPE) expressed luciferase at a 25-fold higher level than its surfactant-untreated control (Table 1). The synthetic surfactant Exosurf increases luciferase gene expression from the reporter plasmid in a variety of cell lines. The most significant enhancement was achieved in primary cells, regardless of their origin.

Transfection Rate Measured by GFP Expression

Another reporter gene, coding for green fluorescent protein (GFP) was employed to demonstrate the rate of transfection represented by the number of transgene expressing cells. Exosurf (50%) increased the number of G9

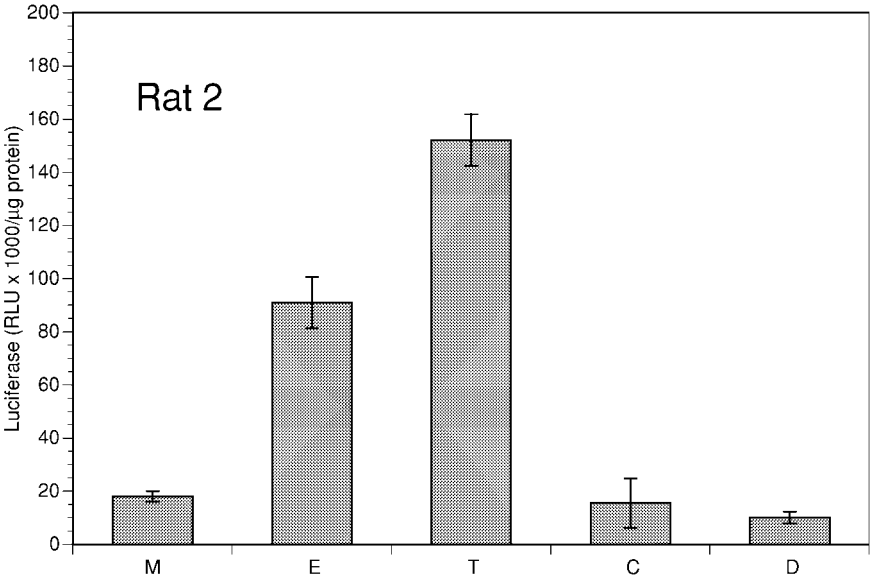

FIG. 2. Luciferase expression in Rat 2 cells after transfection with dendrimer/pCF1Luc plasmid in the presence of Exosurf and its individual components. The complexes were formed at a charge ratio of 20. Exosurf (E) was used at 50%. The components, DPPC (D), tyloxapol (T) and cetyl alcohol (C) were used at concentrations equivalent to the amount present in 50% Exosurf. Transfection in serum-free medium (M) was used as a control. Relative light unit (RLU) is a measured light emission and it is adjusted per μg of cell protein.

EDA dendrimer/EGFP DNA transfected Jurkat cells to 89.9% (8.8-fold increase over the complex alone) within 24 hours after transfection as compared with 10.25% of cells transfected with the complex alone and 3.0% of cells transfected with naked plasmid DNA (Fig. 1A). The expression was maintained in 75% of Jurkat cells treated with Exosurf at 42 hours (not shown). The same concentration of Exosurf increased the number of YPE-positive cells transfected with G9 EDA dendrimer/EGFP DNA complexes to 55.5% (3.3-fold increase over the complexes alone) in 24 hours as compared with 16.7% for the complexes alone and 5% of positive cells transfected with naked DNA (Fig. 1B). In both cell lines of different origin and cell type, Exosurf increased the number of GFP expressing cells. The differences in both histogram profiles can be attributed to their different origin and the degree of clonality of Jurkat cell line and the primary endothelial YPE cell culture.

Expression Mediated by Exosurf Components

The three components of Exosurf—DPPC, cetyl alcohol, and tyloxapol—were tested for their ability to enhance expression of luciferase in Rat 2 cells transfected with G9 EDA dendrimer/pCF1Luc DNA complexes. The concentrations used for each component reflected the amounts present in 50% (v/v) Exosurf. Tyloxapol was the only compound increasing expression comparable to the level observed for Exosurf (Fig. 2). Moreover, the DPPC treatment showed even some inhibition of dendrimer/DNA transfection compared with complexes in medium alone (Fig. 2). The transfection efficiency of dendrimer-mediated gene transfer using tyloxapol is similar to that of Exosurf. This effect was confirmed in other cell lines including A549, CCD-37Lu, BHK-21, Clone 9, NIH3T3, Jurkat, EL4 and CHO-21 (data not shown).

GFP Expression in Situ

During dendrimer-mediated transfection, Exosurf and its component tyloxapol enhance the rate of expression of luciferase or GFP reporter protein (Table 1, Figs. 1, 2, 3). The observed increase in GFP expression in the Jurkat cell line and primary YPE cells for Exosurf (Fig. 1) was also confirmed *in situ* in transfection with G9 EDA dendrimer/pCF1EGFP plasmid in the presence of tyloxapol. It resulted in a high percentage of Jurkat and YPE cells expressing GFP protein (Fig. 3). Localization of the GFP protein in the analyzed non-adherent Jurkat cell line and the primary adherent YPE cell culture does not change upon treatment with tyloxapol. In both, however, the number of cells expressing GFP and the intensity of fluorescence reflecting the amount of GFP dramatically increase when dendrimer-mediated transfection is performed in the presence of tyloxapol (Fig. 3).

Cell Membrane Permeability

One of the main properties of surfactants is the ability to alter the cell membrane structure. The effect of tyloxapol, a nonionic surfactant component of Exosurf, on cell transfection and expression indi-

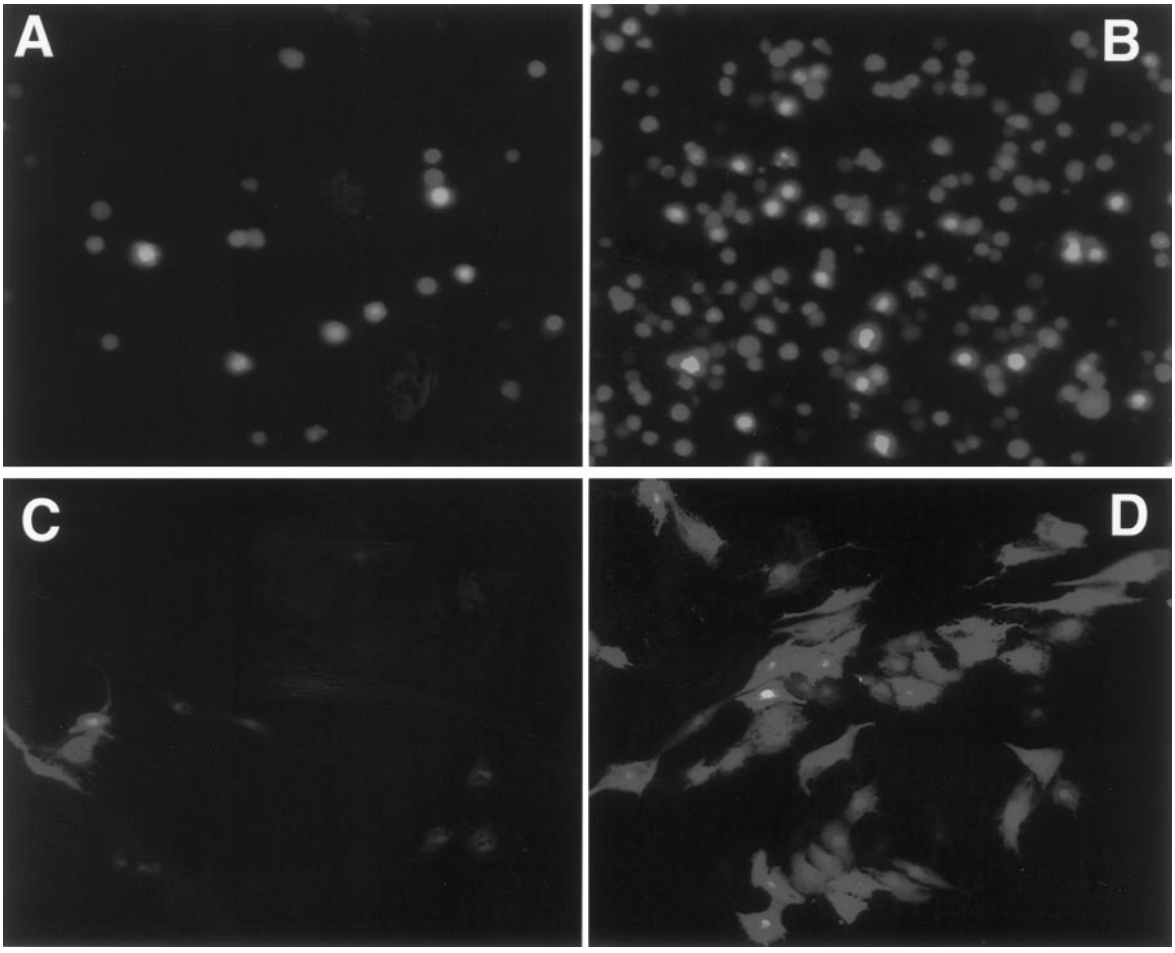

FIG. 3. Transfection efficiency of Jurkat and YPE cells expressing GFP (green fluorescence protein) *in situ*. (A) Jurkat cells were transfected with pCF1EGFP DNA/G9 EDA dendrimer complexes only. (B) Jurkat cells transfected with DNA/Dendrimer complexes in the presence of 0.5 mg/ml tyloxapol. (C) YPE cells transfected with DNA/Dendrimer complexes only. (D) YPE cells transfected with DNA/Dendrimer complexes in the presence of 0.5 mg/ml tyloxapol. Dendrimer/DNA complexes were formed at a charge ratio of 10 (Jurkat) and 20 (YPE). A similar number of cells is present in the background of A and B or C and D. Magnification: 20×.

cated a potential mechanism involving cell membrane permeability. The flow cytometry analysis of the Jurkat cells showed that tyloxapol does not affect the permeability of the cell membrane (Table 2). This analysis indicates that in the presence of tyloxapol the viability of the Jurkat cells slightly decreased from 90.1% to 83.7% with a simultaneous increase in dead cells from 5.48% to 9.86%, and an increase in cell debris (Table 2). The effect of tyloxapol on expression was also tested after preincubation of BHK-21 cells with tyloxapol before transfection and compared with transfection performed in the presence of tyloxapol. Transfection with G9 dendrimer/luciferase plasmid DNA in both instances increased luciferase expression over the control with the complexes in medium alone. The increase was about 5 times higher when the cells were pre-incubated with tyloxapol for 3 hours before transfection with the complexes or about 12 times higher when transfection was performed for 3 hours in the presence of tyloxapol (Fig. 4). This suggests somewhat transient effect of tyloxapol that results in about 2-fold lower

TABLE 2

Tyloxapol Effect on the Viability and Membrane Permeability of Jurkat Cells

	Tyloxapol (−)	Tyloxapol (+)
Live normal cells (%)	90.1	83.7
Live porous cells (%)	0.53	0.44
Dead cells (%)	5.48	9.86
Cell debris (%)	3.89	6.03

Note. The percentages were calculated from the flow cytometry analysis of dot plots of green (FDA) and red (PI) fluorescence. The total numbers of cells were obtained for live cells (green), live porous cells (red and green), dead cells (red) and cell debris in the absence (−) and presence (+) of 0.5 mg/ml of tyloxapol.

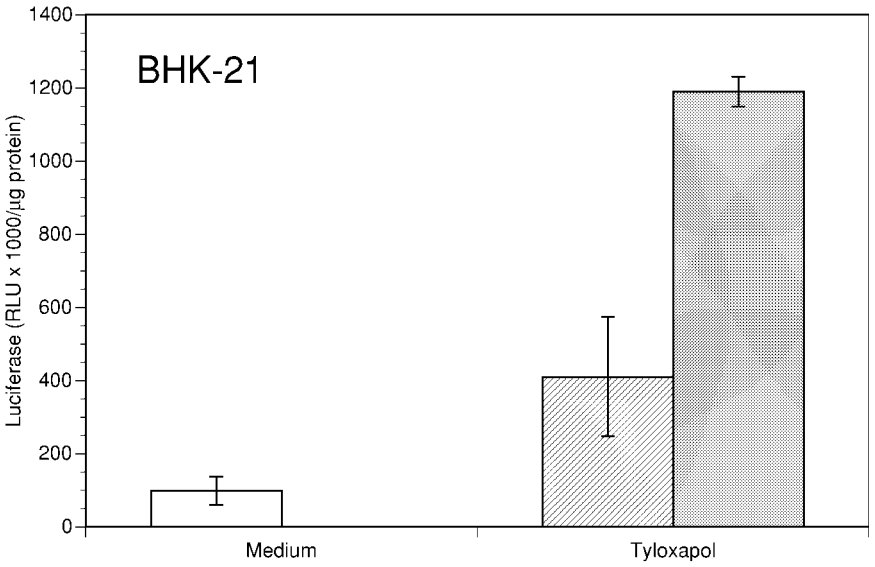

FIG. 4. Luciferase expression in BHK-21 cells after transfection with pCF1Luc plasmid DNA/G9 dendrimer complexes. Tyloxapol (0.5 mg/ml) was present during 3-hour transfection (grey bar) or pre-incubated with cells for 3-hours, and removed before transfection (striped bar). The control transfection was performed in the absence of tyloxapol (white bar). Dendrimer/DNA charge ratio of 20 was used. Relative light unit (RLU) is a measured light emission, and it is adjusted per μg of cell protein.

expression as compared with tyloxapol present during transfection (Fig. 4). As shown in the following figure, the luciferase expression is minimally dependent on the tyloxapol concentration (0.25 to 2.0 mg/ml) during transfection and increases with longer transfection time from 0.5 to 3.0 hours (Fig. 5A).

The effect of tyloxapol on the cell membrane was measured using lactate dehydrogenase (LDH) enzyme assay (Fig. 5B). The leakage of this cytosolic enzyme is indicative of pore formation in the cell membrane and provides a measure of cell membrane porosity or cell lysis. Jurkat cell incubation with tyloxapol at concentrations from 0.25 to 1.0 mg/ml for up to 2 hours did not produce significant LDH release compared to the control without tyloxapol (Fig. 5B). The highest concentration of tyloxapol (2.0 mg/ml) resulted in significant LDH release, beginning at 2 hours ($P > 0.05$). Higher release occurred at 3 hours and also in the lower concentration (1.0 mg/ml) of tyloxapol (Fig. 5B).

DISCUSSION

Our studies demonstrate that the synthetic surfactant Exosurf significantly enhances transfection mediated by cationic dendritic polymers in a variety of eukaryotic cells. The greatest enhancement of transfection was observed in primary cells including human lung (NHBE) and porcine vascular endothelial cells (YPE) that are difficult to transfect with most non-viral vectors. The expression of transfected luciferase reporter genes in these primary cells increased 41.4- and 25.3-fold, respectively, and appears to be a direct reflection of the increased uptake of plasmid DNA, and the processing of the luciferase gene product (Table 1). Significant enhancement was also observed in the transfection of the non-adherent lymphoid Jurkat cell line (11.5-fold; Table 1), rarely achieving greater than 1% of the cells expressing a transgene when transfected by non-viral methods (6, 7). The high level of luciferase expression presented for nine other cell lines of varying origin and tissue source was further supported by analysis of the expression of the green fluorescence protein (GFP) reporter gene (Table 1, Figs. 2, 4). The combination of cationic polymer with Exosurf results in green fluorescence protein (GFP) expression in approximately 90% of Jurkat and 55% of YPE cells (Fig. 1). The number of Jurkat cells expressing the green fluorescence protein (GFP) increased about 9-fold (Fig. 1A), and the number of positive GFP-expressing YPE cells increased about 3-fold (Fig. 1B). The differences in fluorescence histogram profiles relate to the high degree of clonality of the Jurkat cell line and the non-clonal character of the primary vascular endothelial (YPE) cells. The *in situ* analysis of GFP protein expression confirmed these results and indicated that the enhancement is due to increases in the number of transfected cells (Fig. 3). The apparent differences in the enhancement of luciferase and GFP expression, especially in the YPE cells, may be attributed to the transcription and processing of the two reporter genes and to variations in sensitivity of the different methods of detection. We have further clarified that the Exosurf enhancement of dendrimer-

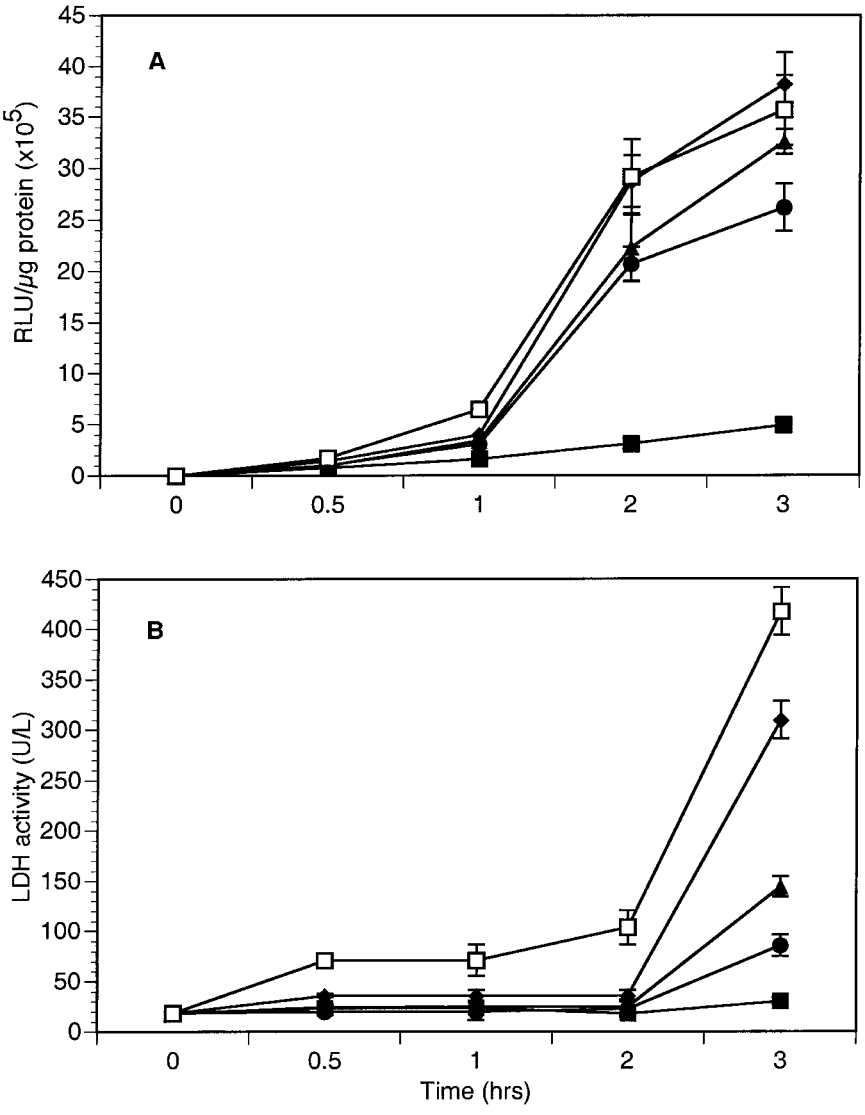

FIG. 5. Effect of tyloxapol on luciferase expression (RLU/μg protein) in transfected COS-1 cells (A) and lactate dehydrogenase (LDH) activity (U/L) release in Jurkat cells (B). Tyloxapol was used at concentrations of 0.25 (●), 0.50 (▲), 1.0 (◆), and 2.0 (□) mg/ml. The incubation was carried for up to 3 hours. The controls were performed in the absence of tyloxapol (■). The values are the means ± s.d. of 3 replicates.

mediated transfection is due to its nonionic surfactant component, tyloxapol (Fig. 2). Tyloxapol can be substituted for Exosurf and achieve equivalent enhancements in transfection, while no other component or combination of components can enhance transfection better than tyloxapol. Even transient preincubation of cells with tyloxapol before transfection with dendrimer/DNA complexes is sufficient to increase the expression of luciferase (Fig. 4). Therefore, the nonionic surfactant tyloxapol markedly enhances transfection with dendrimer/DNA complexes, and is especially effective in aiding the transfection of primary cells.

The mechanism of tyloxapol mediated transfection enhancement is not completely defined. Surfactants have the ability to disrupt the lipid bilayer of cell membranes, and we have hypothesized that tyloxapol increases cellular or nuclear membrane permeability (21). However, tyloxapol does not markedly alter cell permeability, as measured by dual staining with propidium iodide and fluorescein diacetate, at the concentrations which enhance luciferase or GFP expression in Jurkat cells (Table 2) (25). Only at high concentrations of tyloxapol (1.0 and 2.0 mg/ml) and longer incubation periods ($\geq$3 hours) is there increased cell lysis as demonstrated by LDH release assay (26) (Fig. 5B). This argues against membrane disruption, but it is possible that tyloxapol mediates an increase in the formation of membrane pores that do not lead to cell lysis but can facilitate passive uptake of polymer/DNA complexes. This can be postulated by a direct comparison of the transfection assay with the LDH release assay where luciferase expression approaches the maximal level at

2-hour time point much earlier than observed in the absence of tyloxapol (Figs. 5A and 5B). The enhancement also can occur through the selective destablization of the endosome membrane (low pH environment), since endosome trapping of transferred genetic material is a well known barrier in gene transfection (22, 27). The tyloxapol-mediated enhancement of the dendrimer-DNA complex release from endosome into the cytosol during transfection is supported by the fact that the level of expression essentially does not depend on the concentration of tyloxapol but rather significantly increases with extended incubation time (Fig. 5A). Therefore, the increase in transfection efficiency in the presence of intermediate concentrations of tyloxapol may be due in part to an increase in the cell or endosomal membrane porosity (Figs. 2, 3, 5).

Another potential mechanism of tyloxapol enhancement of transfection is in facilitating active uptake of plasmid DNA. We have previously reported that dendrimer-DNA complexes are at least partially internalized to the cell through an energy-dependent endocytosis because inhibitors of cellular metabolism and specific inhibitors of endocytosis inhibit the uptake of these complexes (7). The presence of an enriched GFP signal and the increase in the number of transgene expressing cells suggests a generalized increase in cellular uptake of DNA complexes leading to high transfection levels (Fig. 3). Furthermore, the preincubation of cells with tyloxapol before transfection indicates that tyloxapol can transiently activate certain cell membrane properties and facilitate DNA uptake even after its removal from the medium (Fig. 4). In addition to affecting endocytosis, tyloxapol may affect the physicochemical properties of the dendrimer-DNA complex. Our studies on the properties of the dendrimer-DNA complex and transfection efficiency indicate that the soluble, low-density fraction of complexes is the most efficient fraction in cell transfection (28), and tyloxapol may enhance transfectional properties of this component.

In conclusion, Exosurf and its component tyloxapol greatly enhance gene transfer using dendrimers in eukaryotic cells, and are especially effective in the transfection of primary cells. The high rates of transfection indicate a unique mechanism for tyloxapol in enhancing dendrimer-mediated transfection and strongly suggest that this combination of materials can allow new strategies for cells that are difficult to transfect by non-viral methods. Although the exact mechanism of the tyloxapol or Exosurf effect is not clear, our results suggest that it could be due to increased membrane porosity and/or endocytosis, and a subsequent increase in DNA uptake and its release. The possible mechanisms involved in this process deserve further study.

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