Abstract. Background: The aim of this study was to investigate the transfection efficiency of cationic vectors (polyethylenimine; PEI25K and lipofectamine™), electroporation and their combination in the human cancer cell lines, Raji human lymphoma and KB human oral carcinoma. Materials and Methods: Raji human lymphoma and KB human oral carcinoma cell lines were transfected with pcDNA3-CMV-Luc at various N/P ratios of cationic vectors and voltages of electroporation, as well as with a combination of the cationic vectors and electroporation. Results: The major findings were: (a) cationic vectors or electroporation alone increased transfection efficiency; (b) cationic vectors inhibited the transfection efficiency by electroporation. Conclusion: Our results demonstrate that cationic vectors and electroporation are feasible and efficient in transfecting human cancer cell lines. However, a combination of cationic vectors and electroporation is ineffective.

Viral vectors have been intensively investigated for gene delivery and have been demonstrated to have high transfection efficiencies. However, they carry safety risks with immunogenicity, oncogenicity and inflammatory potential (1, 2). Non-viral gene delivery systems have been developed to avoid potential problems inherent in viral gene vectors. Among non viral vectors are cationic polymers, cationic lipids and electroporation.

Cationic vectors have been investigated as possible carriers of nucleic acids in gene delivery with particular attention having been given to complexes of DNA with cationic polymers (polyplexes), such as polyethylenimine (PEI) and those with cationic liposomes (lipoplexes), such as DOTAP. PEI is considered to be one of the most effective cationic polymers for gene delivery. PEI/DNA complexes are bound to proteoglycans on cell surfaces and subsequently undergo endocytosis (3). After uptake, the high proton-buffering capacity of PEI results in rapid osmolysis of the endosomes and the PEI/DNA complexes escape into the cytosol (4) and are subsequently transported into the nucleus (5). Cationic liposomes are a self-assembled nanosystem. The positively-charged structure binds to the negatively-charged outer membrane of most cells. Subsequently, they enter the cells by adsorptive endocytosis. Positively-charged complexes have been described as being able to completely condense DNA and to mediate transfection, both in vitro and in vivo (6-8).

Electroporation is a process by which an electrical pulse is used to create transient pores in cellular membranes, combined with electrophoresis of DNA into cells, thereby increasing the efficiency of uptake of exogenous DNA (9, 10). This study evaluates the potential of PEI, lipofectamine, electroporation and their combination for gene transfer in human cancer and lymphoma cell lines.

Materials and Methods

Materials. Polyethylenimine (PEI, branched, MW ~25 kDa) was purchased from Aldrich (Milwaukee, WI, USA). Lipofectamine™ (a 3:1 liposome formulation of the polycationic lipid 2,3-dioleyloxy-N(2(sperminecarboxamido)ethyl)-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA) and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) in membrane-filtered water was purchased from Invitrogen (Carlsbad, CA, USA). Luciferase assay reagents were purchased from Promega (Madison, WI, USA). Bicinchoninic acid (BCA) protein assay reagent was obtained from Pierce (Rockford, IL, USA). Plasmid Maxiprep Kit was from Qiagen (Santa Clarita, CA, USA). All tissue culture media and supplies were purchased from Gibco BRL (Rockville, MD, USA).
**Plasmid preparation.** pcDNA3-CMV-Luc plasmid DNA encoding the firefly luciferase reporter gene under control of the cytomegalovirus enhancer/promoter was used. Plasmid DNA was isolated and purified from DH5-α E. coli using the Qiagen plasmid purification kit (Qiagen, Santa Clarita, CA, USA). DNA concentration and purity were quantified by measurement of UV absorbance at 260 nm and 280 nm (OD260/OD280 ~ 1.9) on a Spectrophotometer (UV-160U; Shimadzu, Tokyo, Japan). The size and integrity of plasmids were confirmed by gel electrophoresis (0.8% agarose gel) in Tris acetate-EDTA buffer (TAE, pH 8.0; Qiagen).

**Complex formation between cationic vectors and plasmid DNA.** pcDNA3-CMV-Luc was mixed with cationic vectors (PEI or lipofectamine) of different N/P ratios in HEPES buffer (20 mM HEPES pH 7.4). PEI/DNA complexes were prepared at N/P ratios of 1.93, 7.75, 15.5 and 23.25, and Lipofectamine/DNA complexes were prepared at N/P ratios of 2.72, 6.82, 13.64 and 27.28 by adding the DNA solution to the cationic vector solution. The cationic vector/DNA complexes were incubated for 15 min at room temperature. Complex formation was confirmed by gel electrophoresis on a 0.8% agarose gel compared with a DNA marker, λDNA/HindIII. The gel electrophoresis was performed in Tris acetate-EDTA buffer at 100 V for 45 min. UV transillumination of the gel was employed with ethidium bromide to visualize the DNA.

**Cell culture.** Raji (human Burkitt lymphoma cell line) and KB (human oral carcinoma cell line, a subline of HeLa) were grown in RPMI-1640 with glutamine supplemented with 10% fetal bovine serum (FBS), 50 µg/ml streptomycin and 50 µg/ml penicillin, and were kept in a humid incubator with 5% CO₂ at 37°C. The cells were split 1 day prior to transfection.

**Transfection by cationic vectors.** Raji or KB cells were seeded at 5x10⁵ and 1x10⁶ cells, respectively, in 250 µl of RPMI-1640 on 24-well plates. Cationic vector/DNA complexes were prepared as described above. Cells were incubated with PEI/DNA or lipofectamine/DNA complex solutions in 250 µl of RPMI for 4 h in a humid incubator with 5% CO₂ at 37°C. After 4 h incubation, the transfection medium was then removed and the cells were incubated for a further 24 h in fresh medium containing 10% FBS.

**Transfection by electroporation.** The cells were suspended in RPMI at a concentration of 2x10⁵ cells/ml. A volume of 250 µl of cell suspension was mixed with 1 µg of pcDNA3-CMV-Luc in a sterile eppendorf tube. The cell suspension was then pipetted into a sterile electroporation cuvette (0.4-cm gap width, Eppendorf; Hamburg, Germany). Electroporation was performed with the Gene Pulser® Transfection Apparatus (Bio-Rad, CA, USA). Four different voltage settings of 250, 500, 750 and 1,000 Vcm⁻¹ were applied at a capacitance of 500 µF and a resistance of 400 Ω. After electroporation, the cells were transferred from the cuvette and plated on 24-well plates in complete RPMI-1640 medium. After 24 h, the cells were analysed for luciferase activity, as described below.

**Transfection by a combination of cationic vectors and electroporation.** The combination of cationic vectors and electroporation was performed at the optimal conditions for electroporation and N/P ratio for PEI and lipofectamine. The conditions used were as follows: For KB cells, PEI/DNA complex at an N/P ratio of 7.75, lipofectamine/DNA complex at an N/P ratio of 6.82 and an electroporation voltage of 750 Vcm⁻¹; For Raji cells, PEI/DNA complex at an N/P ratio of 7.75, lipofectamine/DNA complex at an N/P ratio of 6.82 and an electroporation voltage of 1,000 Vcm⁻¹.

The cell suspension was mixed with cationic vectors/DNA complexes in a sterile eppendorf tube. The cell suspension was then pipetted into a sterile electroporation cuvette. Electroporation was then performed. The cells were then transferred from the cuvette and plated on 24-well plates in complete RPMI1640 medium. After 24 h, the cells were analysed for luciferase activity as described below.

**Luciferase activity assay.** The medium was removed from each well and the cells were rinsed two times with cold PBS. Lysis buffer (Glo lysis buffer; Promega) was added to each well and plates were incubated for 10 min. The cell lysate was then centrifuged at 12,000 xg for 3 min. The luminescence was measured on a Mini-Lum luminometer (Bioscan, Inc., Washington DC, USA) immediately after mixing cell lysate with luciferase substrate (Promega). The transfection efficiency was defined as relative light units standardized with protein concentration determined using the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL, USA) using bovine serum albumin as a standard.

**Statistical analysis.** Statistical significance of differences in transfection efficiencies were examined using one-way analysis of variance (ANOVA) followed by an LSD post hoc test. The significance level was set at p<0.05.

**Results**

**Transfection by cationic vectors.** The transfection efficiency of PEI in Raji cells was low at the majority of the N/P ratios tested. The highest transfection efficiency was observed at an N/P ratio of 7.75 and found to be 4-fold compared to that of the control and naked DNA. The transfection efficiency decreased when the N/P ratio was greater than 7.75 (Figure 1a). In KB cells, PEI had high transfection at most N/P ratios tested. The transfection efficiency reached the sufficient gene transfection at an N/P ratio of 7.75 and found to be 3,500-fold compared to the control and naked DNA and highest at an N/P ratio of 15.5 (Figure 1b).

In Raji cells, lipofectamine had lowest transfection efficiency at an N/P ratio of 2.72 which increased at higher N/P ratios. The highest transfection efficiency, observed at an N/P ratio of 6.82, was 2.6-fold compared to the naked DNA (Figure 2a). In KB cells, the transfection efficiency reached its highest level at an N/P ratio of 6.82 and was 3,200-fold higher compared to the naked DNA. Further increase in the N/P ratio above 6.82 resulted in no further increase in transfection efficiency (Figure 2b). The N/P ratio of the cationic vector/DNA complexes at which the cells can be transfected was used in the combination study.

**Transfection by electroporation.** The voltage applied to the cells ranged from 250 to 1,000 Vcm⁻¹. Voltage applied at 1,000 Vcm⁻¹ was found to maximize the expression of...
pcDNA3-CMV-Luc in Raji cells. The maximal transfection efficiency was about 10-fold higher than that obtained without electroporation (Figure 3a). The transfection efficiency in KB cells by electroporation increased with the increasing the voltage from 250 to 750 Vcm\(^{-1}\) (Figure 3b). The best result was obtained at 750 Vcm\(^{-1}\) where the transfection efficiency was 500-fold higher than that obtained without electroporation. At a high pulse field strength of 1,000 Vcm\(^{-1}\), the transfection efficiency dropped.

Transfection by a combination of cationic vectors and electroporation. The transfection efficiency in KB and Raji cells could be enhanced by PEI, lipofectamine and electroporation. In order to determine whether the combination of PEI or lipofectamine and electroporation further enhanced the transfection efficiency in human cancer cells, the DNA was complexed with PEI or lipofectamine. The cells were pretreated with PEI/DNA or lipofectamine/DNA complexes and subjected to electroporation. The condition used for the combination was selected to obtain the highest transfection efficiency.

In Raji cells, the transfection efficiency of the electroporation alone was 58-fold higher than that of naked DNA. The transfection efficiency of the combination of PEI and electroporation was 25-fold lower than that of electroporation alone. The combination of lipofectamine and electroporation reduced transfection efficiency 19-fold compared to the electroporation alone (Figure 4a).

Similar results were found in KB cells. The transfection efficiency of the combination of PEI and electroporation was 83-fold lower than that of electroporation alone. The combination of lipofectamine and electroporation reduced transfection efficiency 14-fold compared to the electroporation alone (Figure 4b).

**Discussion**

Improvement of the delivery efficiency of genes into human cancer cells and the development and optimization of...
transfection methods has increasingly become an important research objective. In this study, the transfection efficiency of three non-viral methods of gene delivery and their combination were investigated. pcDNA3-CMV-Luc encoding luciferase was delivered to two different cell lines using naked DNA, PEI, lipofectamine, electroporation and a combination of PEI or lipofectamine and electroporation. N/P ratios of cationic vectors and electroporation voltage were examined to obtain optimal transfection efficiency. It was found that transfection efficiency in KB and Raji cell lines could be enhanced by cationic vectors: PEI and lipofectamine, and electroporation, but not by the combination either of PEI or lipofectamine and electroporation.

Complexes between PEI or lipofectamine and DNA are formed because of strong electrostatic interactions between the positively-charged carrier and negatively-charged DNA. The net positive charge of formed lipoplexes and polyplexes facilitates their interaction with negatively-charged cells and improves transfection efficiency. The N/P ratio of cationic vectors was examined to obtain optimal transfection efficiency.

It was found that the transfection efficiency increased with increasing N/P ratio, but at higher N/P ratios, the transfection efficiency was constant or decreased. This could be due to increased toxicity of the vectors to the cells. We observed that the pattern of transfection efficiency was different in KB and Raji cells, and the enhancement of transfection efficiency was achieved effectively in the KB cell line and relatively well in the Raji cell line. This shows that the promising transfection vectors found in certain cell types are not necessarily the optimal vectors for other cell types. A previous report showed that the cationic vector transfection efficiency varied according to the N/P ratio and cell lines used (6).

Transfection using electroporation with various voltages gave different optimal transfection efficiencies in cell lines. In Raji cells, an increase in the transfection efficiency was not observed at a voltage strength of 250 and 500 V cm⁻¹. Electroporation treatment at the voltage strength of 750 V cm⁻¹ resulted in significantly better transfection efficiency and even better at 1,000 V cm⁻¹ ($p<0.05$). This is because the high voltage electroporation provides a high level of cell permeabilization and a driving force for transporting DNA into cells. The transfection efficiency increased with the

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**Figure 3.** Transfection efficiency using electroporation at voltages of 250, 500, 750 and 1,000 V cm⁻¹ in (a) Raji cells and (b) KB cells. Results are the mean of three separate experiments. Error bars represent SD. Differences values * vs ** were statistically significant ($p<0.05$, ANOVA, LSD). RLU indicates relative light unit.

**Figure 4.** Transfection efficiency of naked DNA, PEI/DNA and lipofectamine/DNA complexes followed by electroporation in (a) Raji cells and (b) KB cells. Results are the mean of three separate experiments. Error bars represent SD. RLU indicates relative light unit; EP, Electroporation; PEI, Polyethylenimine; LFM, Lipofectamine.
voltage strength due to the increased DNA uptake into cells. In KB cells, the transfection efficiency increased with increasing voltage strength (250 and 750 V cm⁻¹), but decreased at the highest voltage strength (1,000 V cm⁻¹) (p < 0.05). The decrease in the transfection efficiency at the high voltage strength could be due to a decrease in cell viability (57%; results not shown).

The observed differences in the transfection efficiency and the optimal N/P ratios of cationic vectors and voltage strength of electroporation depend on the cell types. Our findings suggest that the N/P ratio and electroporation condition could not be generalized, and have to be optimized for each cell type and vector or transfection method used.

A combination of non viral vectors, such as PEI and cationic liposome has been shown to exert a synergistic effect (11). A previous study demonstrated gene packaging with lipofectin and salmon protamine inhibiting transfection by electroporation (12). In this study, the transfection efficiency using electroporation significantly decreased using cationic vectors, both with PEI and lipofectamine, in KB and Raji cell lines (p < 0.05). The inhibitory effect of cationic vectors on the transfection efficiency by electroporation could be due to the fact that the size of cationic vector/DNA complexes is larger than the transient pore produced by electroporation. Therefore, the transportation of DNA is hindered, resulting in a decrease in transfection efficiency. Another possibility is that the positive charge of cationic vectors affects the negative charge of the plasmid DNA, and, therefore, alters the electrophoretic mobility of DNA during electroporation.

Conclusion

Our results demonstrate that cationic vectors or electroporation alone increased transfection efficiency. The N/P ratio and electroporation conditions could not be generalized and have to be specified for each cell type and vector or transfection method. A combination of cationic vectors and electroporation was ineffective.

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