Transfection of human endothelial cells

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Received 7 February 1997; accepted 28 May 1997

Abstract

Objective: The introduction of recombinant genes into endothelial cells provides a method to study specific gene products and their effect on cell function. In addition, endothelial cells can be used for implantation into vessels or prosthetic vascular grafts. Because transfection efficiencies in human endothelial cells have been low, it is important to develop improved gene transfer techniques. Therefore, several transfection methods were optimized and transfection efficiencies were determined. Methods: Transfection by particle-mediated gene transfer (biolistics) or by cationic liposomes was optimized and compared to calcium phosphate and DEAE-dextran. Transfection efficiency was determined using either a β-galactosidase or placental alkaline phosphatase reporter gene. The effect of promoter strength was analyzed by transfecting plasmids with either the Rous sarcoma virus RSV promoter or cytomegalovirus CMV promoter regions.

Results: Optimal conditions for particle-mediated gene transfer utilized gold particles of 1.6 μm diameter, a target distance of 3 cm, helium pressures of 8.96 MPa (1300 psi) and cell confluence of 75%. Transfection with different cationic liposomes demonstrated that one compound, N-(3-aminopropyl)-N,N-dimethyl-2,3-(bis-dodecyloxy)-1-propaniminium bromide/dioleoyl phosphatidylethanolamine (γAP-DLRIE/DOPE), was optimal for gene transfer when 5 μg of DNA and 10 to 20 μg of lipid was used. With both gold particles and γAP-DLRIE/DOPE, the alkaline phosphatase reporter was more efficient than β-galactosidase using comparable promoters and polyadenylation sites. CMV regulatory elements were more efficient than the RSV promoter in optimizing gene expression. Optimal gene transfer efficiency was 20.28% of cells with γAP-DLRIE/DOPE, 3.96% with biolistics, 2.09% with calcium phosphate and 0.88% with DEAE-dextran. Conclusions: Gene expression is detectable in a high percentage of human endothelial cells after liposome-mediated transfection when expression is controlled by a strong promoter. Particle-mediated transfection is less efficient under these conditions, but more effective than liposomes when expression is driven by a relatively weak promoter. Calcium phosphate and DEAE-dextran are less useful. © 1997 Elsevier Science B.V.

Keywords: Endothelium; Human; Transfection; Gene expression; Particle bombardment; Cationic liposomes

1. Introduction

Transfection of endothelial cells can be used to examine the function of recombinant genes in this cell type. Moreover, transfection of endothelial cells has potential therapeutic applications, as genetically modified endothelial cells may be used for implantation into vessels [1] or prosthetic vascular grafts [2]. However, transfection efficiencies of human endothelial cells are generally low, which may be related to an intrinsic resistance of these cells to take up foreign genes or a high sensitivity to toxic effects exerted by the substances used for transfection.

Bombardment of cells and tissues with DNA-coated gold particles for transfection of recombinant genes has been demonstrated in vitro as well as in vivo [3–5]. The method can be applied for gene transfer into bacteria [6], yeast [7], plant cells [8] and mammalian cells [9]. Indeed, it has been used successfully to reduce tumor growth by introducing cytokine genes into neoplastic tissue [10] or to induce immunity by delivery of influenza antigen genes into the epidermis [11]. Importantly, the method is also suited for the introduction of recombinant genes into such...
cell types as lymphocytes or macrophages which are difficult to transfect by other methods [12,13].

Cationic liposomes form complexes with negatively charged polymessenger RNA [16], antisense oligonucleotides [17] and proteins [18] by mammalian cells. Depending on the cell type, the method can result in higher transfection rates than calcium phosphate or DEAE-dextran [19], both of which have been used for transfection of bovine endothelial cells [20,21].

The present study was therefore designed to investigate whether particle-mediated and liposome-mediated gene transfer into human umbilical vein endothelial cells (HUVEC) is feasible, to optimize transfection efficiency for these methods, and to compare them with calcium phosphate and DEAE-dextran.

2. Methods

2.1. Cell culture

Endothelial cells from human umbilical vein (HUVECs) segments were isolated by collagenase digestion [22] and cultured in Medium 199 (25 mM HEPES) supplemented with fetal bovine serum (20%), penicillin (50 U/ml), streptomycin (50 µg/ml), glutamine (2 mM) (all from Gibco BRL, Gaithersburg, MD), heparin (90 µg/ml; Sigma Chemical Company, St. Louis, MO) and endothelial cell growth factor (20 µg/ml; Boehringer Mannheim, Indianapolis, IN). Cultures were passaged in Falcon Primaria tissue culture dishes coated with gelatin (1%; Sigma Chemical Company, St. Louis, MO) and endothelial cell growth factor (20 µg/ml; Boehringer Mannheim, Indianapolis, IN). All experiments were performed with cells at passage 2. For some experiments, the cells were stimulated with phorbol-12-myristate-13-acetate (PMA), lipopolysaccharide (LPS), human recombinant tumor necrosis factor (TNFα) or ionomycin (all from Sigma Chemical Company, St. Louis, MO).

2.2. Plasmids

pRSVβgal was prepared by inserting an expression cassette with the Escherichia coli β-galactosidase gene [23] into the polylinker of pBR322. The reporter gene was placed downstream of the Rous sarcoma virus long terminal repeat (RSV LTR) and was followed by the simian virus 40 (SV40) polyadenylation signal. pRSVhpAP was constructed similar to pRSVβgal using a heat stable human placental alkaline phosphatase (hpAP) gene [24]. In pCMVhpAP the hpAP gene was ligated downstream of the cytomegalovirus (CMV) promoter combined with a 5′ untranslated region and intron sequences from the CMV immediate early gene. In this plasmid the hpAP gene was followed by the bovine growth hormone (BGH) polyadenylation signal. pCMVCAT was constructed similar to pCMVhpAP using the chloramphenicol acetyl trans-ferase (CAT) gene [25]. All plasmids were prepared by double-banded cesium chloride purification [26].

2.3. Transfections

Particle-mediated transfections were performed using gold beads with a diameter of 1.0 or 1.6 µm (Bio-Rad, Hercules, CA). The beads were coated with plasmid DNA by precipitation with calcium chloride (Fisher Scientific, Pittsburgh, PA) and spermidine (Sigma Chemical Company, St. Louis, MO) [3,27]. The coated beads were spread onto macrocarrier discs which were accelerated by a helium discharge in a vacuum chamber (Biolistic PDS-1000/He particle delivery system, Bio-Rad, Hercules, CA) [27,28]. Accelerated macrocarrier discs were arrested by a stopping screen while the coated beads could pass the screen and penetrate the endothelial cell membrane leading to transfection. Important parameters determining the velocity of particles are chamber vacuum and helium pressure; in addition, particle size and target distance (flight distance of the gold beads from the macrocarrier to the cell layer) are major determinants of transfection efficiency [29]. Therefore, the effect of these four parameters on transfection of endothelial cells was determined during the optimization process. Besides helium pressure and chamber vacuum, the velocity of particles is affected by the distance from the power source to the macrocarrier as well as by the flight distance of the macrocarrier. Thus, these parameters might also influence efficiency of particle-mediated gene transfer; however, with increasing velocity, both of these parameters lead to enhanced variability in flight orientation as well as transfection efficiency [29]. Thus, both parameters were kept constant at 8 mm each; this value generally results in optimal velocity with small variability and is optimal for transfection of mammalian cells [29,30].

500 µg of gold beads coated with 833 ng of DNA were used per bombardment [31]. As the precipitation pattern of the DNA during the coating process is a major source of variability, the beads used for transfection of an experimental group were treated as a block by coating in the same reaction and spreading onto the appropriate number of macrocarrier discs [29]. Once spread on the discs, the beads were used within 1 h [29]. Endothelial cells were plated on 60-mm culture dishes and used for transfection at 75% confluence. Prior to placing the dishes into the vacuum chamber, the culture media were removed in order to prevent deceleration of the gold beads. As soon as the transfection was accomplished the cells were covered with fresh media. No damage attributable to this procedure was observed, as the cells were left without media for less than 1 min. The transfection was performed under sterile conditions in a cell culture hood. No infection of bombarded cells was detected within two days after gene transfer.

For liposome-mediated transfections, the DNA liposome mixture was prepared in polystyrene tubes. The
DNA for 1 ml of mixture was diluted in final 100 μl Opti-MEM (GIBCO BRL, Gaithersburg, MD). N-(3-
aminopropyl)-N,N-dimethyl-2,3-bis(dodecyl-oxyl)-1-prop-
anininium bromide/dioleoyl phosphatidyl ethanolamine (γAP-DLRIE/DOPE; Vical Inc., San Diego, CA) was
diluted identically in a separate tube. The 100 μl with DNA and those with liposome were then added to 800 μl Opti-MEM to result in a total volume of 1 ml and mixed by gentle pipetting. The mixture was left at room temperature for 10 min and then used for transfection.

Endothelial cells were plated on 6-well cell culture dishes and used for gene transfer at 75% confluence. Pilot experiments demonstrated that efficiency of HUVEC transfection by cationic liposomes was comparable from 40 to 80% confluence, and 75% confluence was used for all subsequent experiments. The cells were rinsed two times in Opti-MEM and exposed to the DNA liposome complexes for 60 min. Then the liposomes were removed and the cells were rinsed two times in normal media.

Pilot studies were performed to determine an optimal incubation time of γAP-DLRIE/DOPE in HUVECs. Incu-
bation periods of 30 min to 4 h were evaluated and optimal transfection efficiency was observed at one h. Incubation for 2 to 4 h resulted in toxicity and diminished cell viability. In all experiments, the expression of recombinant genes was studied by the appropriate reporter gene assay 48 h after transfection.

Transfections by calcium phosphate and by DEAE-de-
xtran were performed as described [26]. In all experiments, the expression of recombinant genes was studied by the appropriate reporter gene assay 48 h after transfection.

2.4. Reporter gene assays

To analyze reporter gene activity, the cells were rinsed twice with phosphate-buffered saline (PBS), fixed in 1.25% glutaraldehyde (Sigma Chemical Company, St. Louis, MO) for 5 min and rinsed again with PBS. β-galactosidase activity was assessed by overlaying the cells with X-Gal solution (5-bromo-4-chloro-3-indolyl-β-D-galactoside; 20 mg/ml; RPI, Mt. Prospect, IL) for 4 h at 37° [32]. No β-galactosidase activity was detected in nontransfected endothelial cells under these conditions. hpAP activity was determined by incubating the cells in NBT/BCIP solution (nitroblue tetrazolium chloride/5-bromo-4-chloro-3-indolylphosphate p-toluidine; 40 mg/ml; GIBCO BRL, Gaithersburg, MD) for 30 min at 37°. Alkaline phosphatase activity not related to the recombinant placental isoyme was inactivated prior to staining by keeping the cells in PBS at 65° for 30 min [33]. No alkaline phosphatase activity was detected in nontransfected endothelial cells under these conditions. CAT assays were performed as described [25]. 5 μg of protein from cell lysates were incubated with 14C-chloramphenicol (Amersham, Arlington Heights, IL) and acetyl coenzyme A (Pharmacia Biotech Inc., Piscataway, NJ) at 37° for 30 min. Acetylated chloramphenicol was separated chromatographically and measured with a scintillation counter.

The number of cells expressing β-galactosidase or hpAP controlled by the RSV promoter was determined microscopically at 40-fold magnification. Counting was facilitated by a grid attached to the bottom of the dishes. The total number of cells per dish was evaluated after trypan blue exclusion. This allowed to calculate the percentage of stained cells per dish. The percentage of cells expressing hpAP after transfection of the p1012hpAP plasmid by calcium phosphate or DEAE-dextran was determined in the same manner. As the number of stained cells was higher when the pCMVhpAP was transfected by either gold particles or cationic liposomes, the percentage was calculated for these conditions by determining the number of stained cells as well as the number of all the cells on four random microscopic fields at 100-fold magnification. Counting was fa-
ilitated by a grid placed into one of the oculars.

2.5. Statistics

Data are expressed as mean ± standard error of the mean (S.E.M.) from 4 to 6 experiments. Statistical comparisons were performed using Student’s t-test for unpaired observations or analysis of variance with Dunnett t-test correction for multiple comparisons. A two-tailed p value of less than 0.05 was considered to indicate a significant difference.

3. Results

3.1. Particle-mediated gene transfer

After bombardment, the cells at the periphery of the dishes were transfected and remained morphologically intact. In contrast, those in the center of the dishes were severely damaged. Consistent with this observation, the number of intact cells as assessed by trypan blue exclusion was reduced on bombarded dishes when compared to that on nontransfected dishes (291250 ± 6259 per dish without bombardment; 79750 ± 6985 per dish with bombardment; n = 4). Covering the cells with a media layer (40 μm) did not improve cell survival; moreover, transfection was less efficient under these conditions (80% of control values; n = 4). Similarly, bombardment of suspended cells (2 × 10⁷ cells/ml) led to reduced transfection rates (40% of control values; n = 4).

While the cells could be transfected with 1.6 μm beads, no gene expression was detected with 1.0 μm beads (Fig. 1). A target distance of 3 cm resulted in much higher transfection rates than distances of 6 cm or more (Fig. 1). Based on these results, the effect of target distance on transfection rates was examined from 2.5 to 4.5 cm (Table.
Fig. 1. Particle-mediated gene transfer into HUVECs using pRSVβgal. Bombardment with 1.6 μm gold beads resulted in β-galactosidase activity, while 1.0 μm beads did not. At 3 cm, the number of transfected cells per dish was 178, 213 and 192 for helium pressures of 7.6, 10.7 and 13.8 MPa (1100, 1550 and 2000 psi, respectively). At 6 cm, this number was much lower (7, 20 and 5 cells per dish for helium pressures of 7.6, 10.7 and 13.8 MPa, respectively). Transfection was even less efficient at distances above 6 cm (distance 9 cm: 1, 7 and 3 cells per dish for helium pressures of 7.6, 10.7 and 13.8 MPa, respectively). Helium pressure did not have a major effect on transfection efficiency from 7.6 to 13.8 MPa.

Table 1: Particle-mediated transfection of human umbilical vein endothelial cells

<table>
<thead>
<tr>
<th>Distance</th>
<th>Pressure (MPa)</th>
<th>Vacuum (cm Hg)</th>
<th>Confluence (%)</th>
<th>Stained cells/dish (mean ± S.E.M.; n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5</td>
<td>10.7</td>
<td>38</td>
<td>75</td>
<td>309 ± 92</td>
</tr>
<tr>
<td>3.0</td>
<td>10.7</td>
<td>38</td>
<td>75</td>
<td>480 ± 105</td>
</tr>
<tr>
<td>3.5</td>
<td>10.7</td>
<td>38</td>
<td>75</td>
<td>290 ± 81</td>
</tr>
<tr>
<td>4.0</td>
<td>10.7</td>
<td>38</td>
<td>75</td>
<td>258 ± 25</td>
</tr>
<tr>
<td>4.5</td>
<td>10.7</td>
<td>38</td>
<td>75</td>
<td>95 ± 22</td>
</tr>
<tr>
<td>3.0</td>
<td>7.6</td>
<td>38</td>
<td>75</td>
<td>434 ± 123</td>
</tr>
<tr>
<td>3.0</td>
<td>9.0</td>
<td>38</td>
<td>75</td>
<td>464 ± 129</td>
</tr>
<tr>
<td>3.0</td>
<td>10.7</td>
<td>38</td>
<td>75</td>
<td>323 ± 70</td>
</tr>
<tr>
<td>3.0</td>
<td>13.8</td>
<td>38</td>
<td>75</td>
<td>183 ± 30</td>
</tr>
<tr>
<td>3.0</td>
<td>9.0</td>
<td>25</td>
<td>75</td>
<td>382 ± 113</td>
</tr>
<tr>
<td>3.0</td>
<td>9.0</td>
<td>38</td>
<td>75</td>
<td>411 ± 112</td>
</tr>
<tr>
<td>3.0</td>
<td>9.0</td>
<td>50</td>
<td>75</td>
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</tr>
<tr>
<td>3.0</td>
<td>9.0</td>
<td>38</td>
<td>75</td>
<td>364 ± 92</td>
</tr>
<tr>
<td>3.0</td>
<td>9.0</td>
<td>38</td>
<td>60</td>
<td>402 ± 89</td>
</tr>
<tr>
<td>3.0</td>
<td>9.0</td>
<td>38</td>
<td>90</td>
<td>224 ± 97</td>
</tr>
</tbody>
</table>

1). 2.5 cm was chosen as the shortest distance because the design of the particle-delivery system did not allow to move the dishes closer to the stopping screen. Helium.

Fig. 2. Liposome-mediated transfection of HUVECs using pCMVCAT. Combination of 1 μg/ml γAP-DLRIE/DOPE with 0.5, 1.0, 3.0, 5.0, 7.0 and 10.0 μg/ml plasmid DNA resulted in 0.55 ± 0.17, 2.38 ± 0.63, 7.07 ± 1.87, 11.82 ± 3.39, 7.42 ± 2.24 and 6.41 ± 1.93% acetylated chloramphenicol, respectively (upper left panel; n = 6). Transfection was more efficient with 5 μg/ml γAP-DLRIE/DOPE (10.68 ± 5.53, 25.10 ± 10.75, 37.89 ± 7.54, 45.80 ± 9.72, 42.58 ± 7.72 and 34.07 ± 7.10% acetylated chloramphenicol (upper middle panel; n = 6). This effect was even more pronounced with 10 μg/ml γAP-DLRIE/DOPE (3.71 ± 2.59, 35.29 ± 15.08, 62.64 ± 8.01, 74.89 ± 8.56, 80.87 ± 3.24 and 66.58 ± 8.30% acetylated chloramphenicol (upper right panel; n = 6). However, there was no further improvement with 15 μg/ml γAP-DLRIE/DOPE (2.16 ± 1.26, 25.22 ± 13.75, 77.54 ± 4.53, 75.06 ± 7.11, 70.27 ± 8.20 and 62.5 ± 7.71% acetylated chloramphenicol; lower left panel; n = 6) nor 20 μg/ml γAP-DLRIE/DOPE (1.48 ± 0.84, 21.06 ± 13.81, 54.33 ± 12.54, 89.63 ± 4.47, 71.54 ± 11.64 and 63.81 ± 8.98% acetylated chloramphenicol; lower right panel; n = 6).
Pressure was kept constant at 10.7 MPa (1550 psi), chamber vacuum at 38.1 cm (15 inches) Hg and cell confluence at 75%. Maximal transfection efficiency was observed at target distance 3.0 cm, while fewer cells were transfected at 2.5 cm as well as 3.5 cm or higher (Table 1). Although the influence of helium pressure seemed to be less pronounced than that of bead diameter or target distance (Fig. 1) the effect of pressure on transfection efficiency was tested from 7.58 to 13.8 MPa (1100 to 2000 psi). These experiments were performed at target distance 3.0 cm, chamber vacuum 38.1 cm Hg and cell confluence 75%. Transfection was maximal at 1300 psi and almost as efficient at 7.58 MPa. While transfection rates were only slightly diminished at 10.7 MPa, they were markedly lower at higher pressures (Table 1). The importance of chamber vacuum was analyzed from 25.4 to 50.8 cm (10 to 20 inches) Hg. Target distance was kept at 3.0 cm, helium pressure at 8.96 MPa and cell confluence at 75%. Changes in chamber vacuum did not affect transfection rates (Table 1). The influence of cell confluence was assessed from 60 to 90%. Target distance was kept at 3.0 cm, helium pressure at 8.96 MPa and chamber vacuum at 38.1 cm Hg. There was no difference in transfections at 60 or 75% confluence; however, transfections became less efficient at 90% confluence (Table 1). Thus, optimal conditions for particle-mediated transfection of endothelial cells were bead size 1.6 μm, target distance 3 cm, helium pressure 8.96 MPa, chamber vacuum 38.1 cm Hg, and cell confluence 75%.

Endothelial expression of hpAP after particle-mediated transfection was compared to that of β-galactosidase. Expression of both genes was studied using the pRSVhpAP and pRSVβgal plasmids. Transfection efficiency was higher for hpAP compared to β-galactosidase (Fig. 3, left panel). We next compared gene expression of the same reporter gene, hpAP, using 2 viral promoters, RSV and CMV. Transfection efficiency in HUVECs was significantly increased with pCMVhpAP compared with pRSVhpAP. The percentage of transfected cells per dish using gold particles was 3.96 ± 0.37% for pCMVhpAP, 1.81 ± 0.28 for pRSVhpAP, and 0.59 ± 0.14 for pRSVβgal, n = 4; p < 0.05, analysis of variance with Dunnett t-test, left panel).

The efficiency of particle-mediated gene transfer into human lymphocytes is enhanced by stimulating the cells with various agonists [13]. To assess whether a similar effect is seen in human endothelial cells, these cells were stimulated with one of the following substances: phorbol-12-myristate-13-acetate (10 ng/ml) [34], lipopolysaccharide (1 μg/ml) [35], human recombinant IL-1α (10 U/ml) [36], human recombinant TNF-α (100 U/ml) [37] or ionomycin (1 μM) [34]. Stimulation with each of these substances was performed for 24 or 48 h either before, during or after transfection. No improvement of transfection efficiency was observed in any of these experiments (n = 2; data not shown).

### 3.2. Liposome-mediated gene transfer

Optimal conditions for liposome-mediated transfections were established using a CAT reporter gene. When 1 μg/ml γAP-DLRIE/DOPe was complexed with 0.5, 1.0, 3.0, 5.0, 7.0 and 10.0 μg/ml DNA (lipid to DNA ratios of 1:0.5 to 1:10), CAT activity increased in a concentration-dependent manner with maximal conversion at 5 μg/ml DNA (Fig. 2, upper left panel). With 5 μg/ml γAP-DLRIE/DOPe, the transfections were more efficient for all DNA concentrations tested with maximal activity was still observed at 5 μg/ml DNA (Fig. 2, upper right panel). This effect was even more pronounced with 10 μg/ml γAP-DLRIE/DOPe (Fig. 2, middle left panel). However, transfection efficiency did not improve further with 15 μg/ml γAP-DLRIE/DOPe (Fig. 2, middle right panel) nor 20 μg/ml γAP-DLRIE/DOPe (Fig. 2, lower panel).

Using γAP-DLRIE/DOPe liposomes, the transfection efficiency in HUVECs was higher for hpAP compared to β-galactosidase when plasmids with the same promoter were used (Fig. 3, right panel). The transfection efficiency was significantly increased with the pCMVhpAP plasmid compared to the pRSVhpAP plasmid. With cationic liposomes, the transfection efficiency was 20.28 ± 1.38% for pCMVhpAP, 0.07 ± 0.007 for pRSVhpAP, and 0.03 ± 0.002 for pRSVβgal, n = 6; p < 0.01, analysis of variance with Dunnett t-test).

### 3.3. Other transfection methods

Calcium phosphate and DEAE-dextran were less efficient (calcium phosphate: 2.09 ± 0.33% cells per dish;
DEAE-dextran: 0.88 ± 0.21% cells per dish; \( n = 6 \), but better than DNA alone (0.08 ± 0.01% cells per dish) (Fig. 4).

4. Discussion

The present study demonstrates that HUVECs can be transfected efficiently. Transfection rates are determined by the vector, promoter and reporter gene. The data with particle-mediated gene transfer are consistent with the observation that this method is useful for some cell types otherwise difficult to transfect [38]. The most important parameters were particle size and target distance, while helium pressure, chamber vacuum and cell confluence did not significantly influence transfection efficiency. This optimization pattern is very similar to results obtained with CHO and COS-7 cells [30]. The decline in transfection rates at 90% confluence is consistent with the observation that transfection is more efficient when cells are growing in log phase.

The efficiency of liposome-mediated gene transfer was determined by varying both the concentration of DNA and the concentration of \( \gamma \)AP-DLRIE/DOPE. To quantitate the stoichiometry of interaction of cationic liposome with DNA, the molar ratio of cationic liposome to nucleotide can be calculated based on the molecular weight of \( \gamma \)AP-DLRIE (594 g/mol) and the average molecular weight of a single nucleotide (330 g/mol) resulting in optimal transfection at a molar ratio of cationic lipid to nucleotide of 0.5 to 1.0. Each molecule of \( \gamma \)AP-DLRIE contains a polar head with two positive charges, while each nucleotide contains one negatively charged phosphate group. The molar lipid:nucleotide ratios can thus be expressed as electrovalent lipid:nucleotide ratios resulting in optimal transfection at a ratio of 1.0 to 2.0. Thus, the best conditions for transfection with \( \gamma \)AP-DLRIE/DOPE were created with a neutral or weakly positive DNA liposome complex, which is consistent with the observation that an excess of positive charges facilitates interaction of the DNA liposome complex with negatively charged cell surface molecules [39]. The usefulness of \( \gamma \)AP-DLRIE/DOPE for transfection of human endothelial cells reflects the large potential of liposome-mediated gene transfer, as systematic structural changes during the last few years led to the discovery of new liposome formulations with advantageous properties for different applications [40].

The lower number of endothelial cells staining for \( \beta \)-galactosidase as compared to alkaline phosphatase cannot be related either to the transfection procedure nor to the plasmid properties since the observation was made after particle-mediated as well as liposome-mediated gene transfer and the plasmids were identical except for the reporter gene itself. The difference may be related to alterations in gene expression due to methylation of the retroviral promoter and surrounding sequences [32,41] or to a different sensitivity of the staining method. The higher number of cells staining for alkaline phosphatase after transfection of pCMVhpAP as compared to pRSVhpAP underscores the importance of a strong promoter for transfection studies.

Although the efficiency of particle-mediated gene transfer into HUVECs is higher than calcium phosphate or DEAE-dextran, it is lower than that achieved with the cationic liposome \( \gamma \)AP-DLRIE/DOPE. Moreover, particle bombardment requires specialized equipment. Thus, it is not suitable for routine transfection of endothelial cells. On the other hand, liposome-mediated gene transfer has several advantages such as high efficiency and simple preparation. Moreover, although adenoviral gene transfer may result in higher efficiency than cationic liposomes, preparation of recombinant adenoviruses is technically more complex. Therefore, new cationic liposomes such as \( \gamma \)AP-DLRIE/DOPE may be the method of choice for transfection of HUVECs.

Acknowledgements

This work was funded by grants from the National Institutes of Health. FCT was supported by the Swiss Society of Internal Medicine (Conrad Gessner Stipendium), the Ciba Geigy Jubiläums Stiftung and the Swiss National Science Foundation. EGN is an Established Investigator of the American Heart Association.

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