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Synergism in gene delivery by small PEIs and three different nonviral vectors

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Abstract

We have reported earlier that a combination of low-molecular weight polyethylenimines (PEIs) with the cationic liposome, Dosper, results in a synergistic increase in the transfection efficiency. Now we have investigated whether this synergism is a general mechanism seen with other transfection reagents as well. Therefore, we have combined the low-molecular weight PEIs (MW 700 and 2000) with Dotap (a monocationic liposome), Lipofectamine (a combination of neutral and polycationic liposome), and Superfect (a dendrimer). The highest synergism was achieved with Lipofectamine and PEIs in the SMC cells, or with Dotap and PEIs in the C6 cells. Superfect did not induce any synergism. The combinations did not cause any changes in DNA condensing ability measured with ethidium bromide exclusions. The proton pump inhibitor, bafilomycin A1, had similar effects in both cell lines. Interestingly, the combination of Dosper (a positive control) and PEI caused the most effective transfection synergism in the presence of serum, although Lipofectamine, with or without PEIs, was a very potent reagent demonstrating the best transfection efficiency in the absence of serum. It is suggested that the PEI/Dosper-mediated synergism in the transfection efficiency may be a general mechanism for liposomal transfection reagents, although the effects can vary depending on cell lines.

Keywords: Transfection; Plasmid; Polyethylenimine; Liposome; Dendrimer; Gene delivery

1. Introduction

In gene therapy, viral or nonviral vectors are used to deliver the therapeutic gene to the body. Although viral vectors are efficient, their use is limited due to side-effects, e.g. cytotoxicity (Mulligan, 1993). Nonviral vectors, i.e. plasmids, are also widely used. Since plasmid DNA is a large and negatively charged molecule, it poorly penetrates into cells and transfection is usually enhanced by combining DNA with different vectors, e.g. dendrimers (Tang et al., 1996), polymers (Perez et al., 2001) and cationic liposomes (Zabner et al., 1995) which are used to aid the transport of the transgene into cells. Polyethylenimines (PEIs) are commonly used, since they have many advantageous properties for gene transfection, e.g. substantial buffering capacity (for review, see Godbey et al., 1999). In general, PEIs have a wide distribution of molecular weights (Bieber and Elsässer, 2001). At present, the belief is that mainly larger PEIs are effective in transfection, although small PEIs can also

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be used in combination with other reagents (Petersen et al., 2002; Lampela et al., 2002).

Superfect consists of activated-dendrimer molecules with a defined and flexible architecture. The formation of complexes between DNA and dendrimers is based on a charge interaction. Most of the plasmid DNA is contracted into isolated toroids (Bielinska et al., 1997), and the complexed DNA is protected against degradation. There is a correlation between transfection activity and the degree of flexibility of dendrimers (Tang et al., 1996). Furthermore, almost all of transfection is carried by low-density, soluble complexes which represent less than 20% of the total complexed DNA (Bielinska et al., 1999). Unfortunately, the DNA–dendrimer complexes that typically induce high levels of cell transfection also induce high levels of vesicle leakage (Zhang and Smith, 2000). Dendrimers have been conjugated with cyclodextrins (Arima et al., 2001), which change cellular association as well as intracellular trafficking of DNA, resulting in enhancement of gene expression.

Dotap (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium methylsulfate) is a monocationic lipid, that has been used in gene delivery. In aqueous solutions Dotap forms liposomes, small cationic spherical vesicles where the aqueous space is encapsulated by a lipid bilayer. Cationic Dotap liposomes bind electrostatically to negatively charged DNA, resulting in the formation of lipid-coated DNA complexes with a cationic charge, where DNA is condensed (for review, see Mönkkönen and Urtti, 1998).

Lipofectamine is a combination of two lipids: polycationic DOSPA (2,3-dioleyloxy-*N*-[2(spermine-carboxamido)ethyl]-*N*,*N*-dimethyl-1-propanaminium trifluoroacetate) and neutral DOPE (dioleoyl phosphtidylethanolamine). DOPE itself is inactive as a transfection reagent, but it increases the transfection efficiency, when combined with cationic lipids (Felgner et al., 1994) by assuming non-bilayer structures, which destabilize the endosome membrane (Farhood et al., 1995).

We have shown previously that a combination of small PEIs (MW 700 and 2000) and polycationic liposome Dosper could result in a synergistic increase in transfection efficiency (Lampela et al., 2002). In this present study, we have studied whether this synergism can also be achieved when combining small PEIs with dendrimer (Superfect), monocationic liposome (Dotap) and Lipofectamine, which is a combination of polycationic and neutral lipids.

2. Materials and methods

2.1. Materials

Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum, penicillin–streptomycin and Lipofectamine were purchased from Invitrogen (UK), Superfect from Qiagen (USA), Dosper, Dotap and X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) from Roche (Germany) and polyethylenimines and ONPG (*o*-nitrophenol-β-D-galactopyranoside) from Sigma-Aldrich (USA). The bovine papilloma virus plasmid (TKBPVlacZ) (Lampela et al., 2001) was obtained from Dr. Mart Ustav. All other chemicals were of cell culture and molecular biology quality.

2.2. Cell culture

Rabbit smooth muscle (SMC) and rat glioma (C6) cells were kind gifts from Drs. Seppo Ylä-Herttuala and Antero Salminen, respectively. The cells were grown in an incubator (Revco RCO3000TVBA, USA) at 37 °C and 5% CO_2 with DMEM containing 9% heat-inactivated fetal bovine serum and penicillin (90 U/ml)–streptomycin (90 μg/ml).

2.3. Preparation of DNA complexes

The transfection efficiencies of low-molecular weight PEIs (MW 700 and 2000) and commercially available transfection reagents were examined as such and in different combinations. PEI/DNA ratios are expressed as the PEI amine nitrogen/DNA phosphate ratio (N/P) (Boussif et al., 1995). PEIs were used in N/P ratios of 0, 2.5 and 5 and commercially available transfection reagents at reagent/DNA ratios between 0 and 30 (w/w), depending on a reagent. The Dosper/DNA ratio (7.5) was based on our earlier work (Lampela et al., 2002), and was used as a positive control.

First, BPVTKlacZ plasmid was diluted to a final concentration of 1 μg/30 μl in NaCl–HEPES. PEI solutions were diluted likewise in NaCl–HEPES. After a 10 min incubation, the PEI solutions were combined

with the plasmid DNA dilution and incubated for another 10 min to achieve N/P ratios of 2.5 and 5. To ensure that there were equal volumes in all samples, NaCl (150 mM)–HEPES (20 mM) were added to mixtures without PEI.

Superfect was used as such, but other commercially available transfection reagents were diluted in NaCl–HEPES according to the supplier's instructions (Dosper and Lipofectamine 1/5, Dotap 3/10). Dotap and Dosper were also sonicated for 10 min prior dilution. After 15 min incubation, the diluted transfection reagents were added to DNA–PEI mixtures (or "PEI 0" mixtures) at DNA ratios mentioned above.

2.4. Transfection

Cells were seeded in 24-well plates at a density of 70,000 (SMC) or 100,000 (C6) cells per well in a final volume of 1 ml. Cells were grown to approximately 70% confluency. Immediately prior to transfection, the growth medium was substituted with fresh DMEM without serum and antibiotics (1 ml per well) (in some experiments, DMEM containing serum was used). Then, the transfection mixtures were pipetted dropwise to the cell cultures. Cells were exposed to transfection agents for 6 h, after which the medium containing trasfection mixtures was replaced with normal growth medium.

2.5. β-Galactosidase assay

At 48 h incubation after transfection, the cells were washed with PBS, lysed with 150 μl of lysis reagent (25 mM glycylglycine, 15 mM $MgSO_4$, 4 mM EGTA, 1% Triton X-100, 1 mM DTT, 1 mM PMSF), and centrifuged at 13,000 rpm for 5 min (Eppendorf Centrifuge 5415C, Eppendorf-Netheler-Hinz, Germany). The β-galactosidase activity was measured with an ONPG assay from the supernatant: 1 μl (SMC) or 5 μl (C6), 10 μl (SMC) or 30 μl (C6), 20 μl (SMC) or 30 μl (C6), 1 μl (SMC) or 5 μl (C6) of the supernatant for Lipofectamine, Superfect, Dotap and Dosper-mediated transfection, respectively, up to 100 μl of H_2O and 100 μl of ONPG solution (2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 1.33 mg/ml ONPG in 0.2 M sodium phosphate buffer) were pipetted in a 96-well plate and incubated up to 1 h at room temperature. After this, the reaction was stopped with 1 M Na_2CO_3. During the incubation, the β-galactosidase enzyme cleaves the β-bond from ONPG resulting in the formation of the yellow *o*-nitrophenol molecule. Samples were analyzed by measuring absorbance at 405 nm with the Bio-Tek Elx-800 microplate reader (Bio-Tek Instruments, USA) and KC-3 PC-program.

2.6. Toxicity

SMC and C6 cells were grown in 96-well plates, and transfected with the same concentrations as in the 24-well plates. Transfection was performed using 150 ng of DNA in 150 μl of serum-free growth medium. After 48 h incubation, 150 μl of serum-free growth medium was changed into the wells, and 10 μl of MTT solution (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl tetrazolium bromide, 5 mg/ml) were added. The cells were then incubated for up to 1 h (C6) or 1.5 h (SMC), and then 100 μl of SDS–DMF buffer (200 μg/ml SDS, 50% dimethylformamide, pH 4.7) was added. After 24 h incubation, the absorbance was read at 570 nm with the Bio-Tek Elx-800 microplate reader.

2.7. Effect of bafilomycin A1

Subconfluent SMC and C6 cells were transfected with BPVTKlacZ plasmid. After changing the growth medium to serum-free transfection medium, the endosome proton pump inhibitor, bafilomycin A1 (a specific inhibitor of vacuolar type H^+-ATPase) diluted in DMSO or plain DMSO was pipetted into wells. After a 10 min incubation period, transfection and analysis were carried out as described above.

2.8. X-gal analysis

The number of transfected cells was measured with X-gal staining. The cells were incubated 24 h after transfection. Then, the cells were washed with PBS, fixed with 4% paraformaldehyde (15 min, RT) and washed again twice with PBS prior incubation in the X-gal staining solution (X-gal 1 mg/ml, $MgCl_2$ 2 mM, 5 mM $K_3Fe(CN)_6$, 5 mM $K_4Fe(CN_6)\cdot 3H_2O$, 0.01% sodiumdeoxycholate, 0.02% Nonidet P-40) for 3 h at +37 °C. β-Galactosidase cleaves X-gal substrate resulting in the formation of the blue color of 3,5′-dichromo-4,4′-dichloroindigo molecule in the

transfected cells. After the staining, the cells were washed with PBS, photographed with Nikon Coolpix E995 CDD digital camera (Nikon Corp., Japan) under Nikon Eclipse TE 300 microscope (Nikon Corp.), and the percentage of blue-colored cells was counted from the photographs.

2.9. DNA condensation

The ability of PEIs and commercially available transfection reagents to condense DNA was studied by ethidium bromide (EtBr) fluorescence spectroscopy. The experiments were performed using 96-well plates and a microplate fluorescense reader (Bio-Tek FL500) at 530 nm excitation and 590 nm emission wavelengths. BPVTKlacZ in NaCl–HEPES (pH 7.4) was pipetted to 96-well plate (0.6 μg plasmid DNA per well) and ethidium bromide (4 μg/ml) was added. Then the fluorescence was read and different amounts of PEI were added. After a 10 min incubation, the fluorescence was read again and then, Dotap/Lipofectamine/Superfect was added and after a further 10 min incubation, the fluorescence was read again. The decrease in the fluorescence is attributable to condensation of DNA.

3. Results

3.1. Transfection efficacy: β-galactosidase expression

3.1.1. SMC cells

There were major differences in the level of β-galactosidase expression (Fig. 1). The best expression without combination was achieved with Lipofectamine (10), then with Dosper (7.5), Superfect (30) and Dotap (10) (β-galactosidase levels being 6560 ± 128 mU/mg, 1840 ± 146 mU/mg, 1810 ± 200 mU/mg and 405 ± 22 mU/mg protein, respectively). When PEI 700 (N/P 2.5) was combined with Lipofectamine (LF/DNA ratio 5), β-galactosidase activity increased up to 15,200 ± 155 mU/mg protein. However, PEI tended to decrease β-galactosidase expression when combined with Superfect or Dotap.

3.1.2. C6 cells

In general, β-galactosidase activities were lower in C6 cells than in SMC cells (Fig. 1). The best expression without combination was again achieved with Lipofectamine (15–20), then with Dosper (7.5), Dotap (10) and Superfect (β-galactosidase levels being 4940 ± 368 mU/mg, 620 ± 105 mU/mg, 231 ± 33 mU/mg and 155 ± 14 mU/mg protein, respectively). When PEI was combined with Lipofectamine, the best result (6100 ± 424 mU/mg protein) was achieved with a low Lipofectamine/DNA ratio (10). Combination of PEI 2K/5 and Dotap (10) increased β-galactosidase activity to 790 ± 85 mU/mg protein, while PEI had a mainly negative effect on Superfect-mediated transfection. An increase in the Dotap/DNA ratio above 10 did not cause any further increase in the transfection efficiency (data not shown).

Transfection in serum-containing medium completely eliminated any β-galactosidase activity in C6 cells with Dotap and Lipofectamine with or without PEI (data not shown). In SMC cells, addition of serum decreased β-galactosidase activities as well, though less than has seen in C6 cells. The best result was seen with the Dosper 7.5–PEI 700/2.5 combination, which resulted in approximately 1360 ± 143 mU/mg protein β-galactosidase activity when transfected with serum. This is of the same magnitude as the activities of PEI 25K/8 alone or Dosper (1300 ± 196 mU/mg and 1510 ± 447 mU/mg protein, respectively) when transfected in serum-free medium. Plain Dosper or Lipofectamine with or without PEI had only a negligible expression, when transfected with serum (data not shown). The toxicity of the most effective reagent Lipofectamine and the control reagent Dosper were studied using the MTT toxicity assay. The viability of cells was always more than 75% of control (data not shown).

3.2. Transfection efficacy: number of transfected cells

X-gal was used in SMC and C6 cells to see how the synergism could change the number of transfected cells. The best combinations in both cell lines (Lipofectamine in SMC and Dotap in C6) and Dosper (the control reagent) were studied. In SMC cells, the number of transfected cells increased from 13% (Dosper) up to 37% (Dosper–PEI 700/2.5) (Fig. 2A). These results are in agreement with our previous report (Lampela et al., 2002). The cells trans-

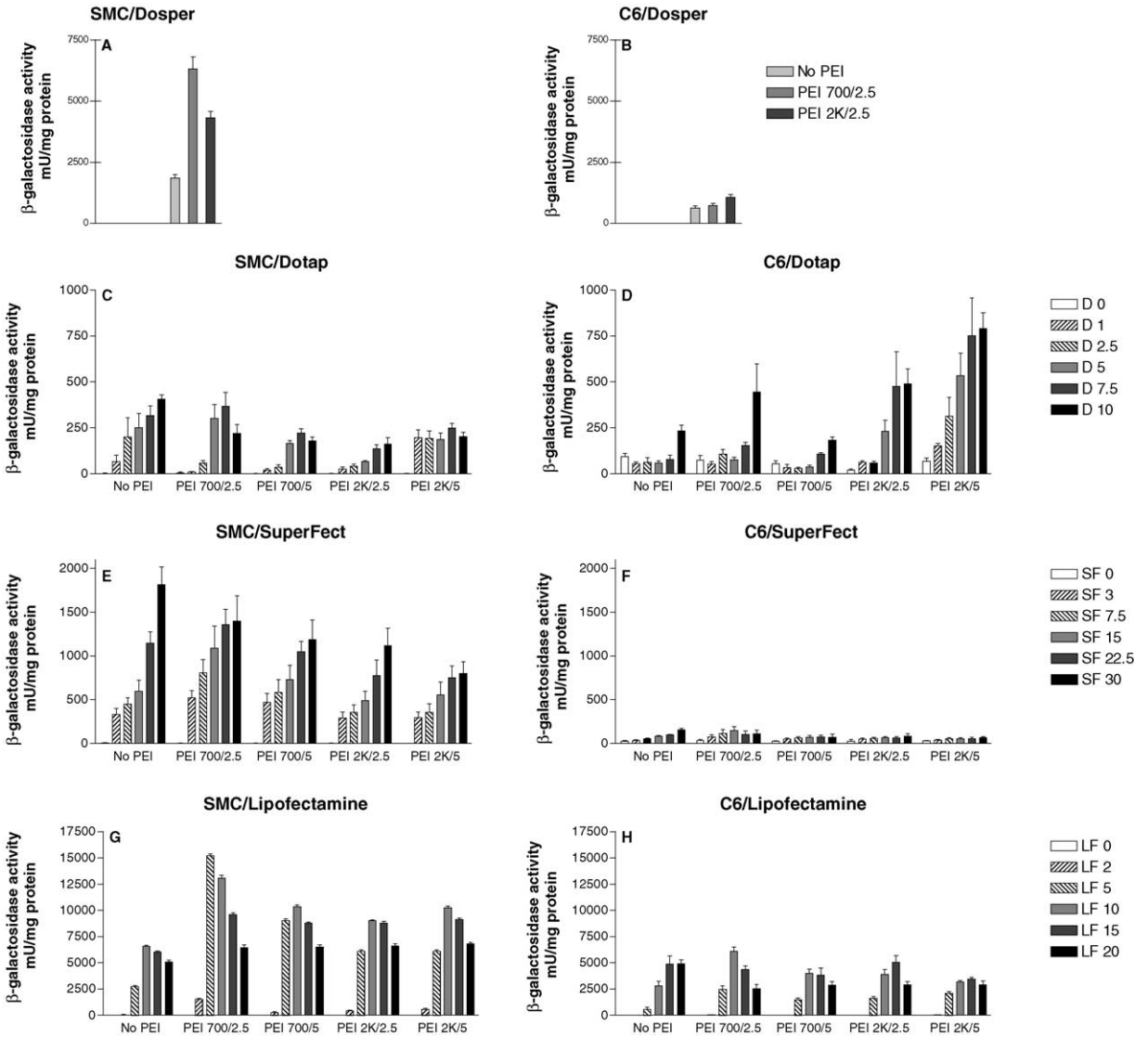

Fig. 1. Transfection efficiencies of Dosper (A–B), Dotap (D) (C–D), Superfect (SF) (E–F) and Lipofectamine (LF) (G–H) with or without PEIs in SMC and C6 cells. The cells were transfected with 1 μg of TKBPVlacZ plasmid at different reagent/DNA ratios as indicated, or DNA was condensed with PEI before the addition of reagents. The cells were incubated in the transfection solution for 6 h and then in the growth medium for 42 h. β-Galactosidase activity was measured with the ONPG assay. The values are the means of β-galactosidase activity/mg protein ± S.E. ($n = 4$–6).

fected with Lipofectamine resulted in a 10 and 17% transfection efficiency without or with PEI 700/2.5, respectively.

In C6 cells, Dosper resulted in 18% transfection, and combining Dosper with PEI 2K/2.5 did not markedly increase the transfection efficiency (Fig. 2B). Dotap alone caused less than 1% transfection, while the combination of Dotap and PEI 2K/5 resulted in 3.4% transfection.

3.3. Effect of bafilomycin A1

The combinations that worked the best synergism in both cell lines (Lipofectamine and Dotap) were

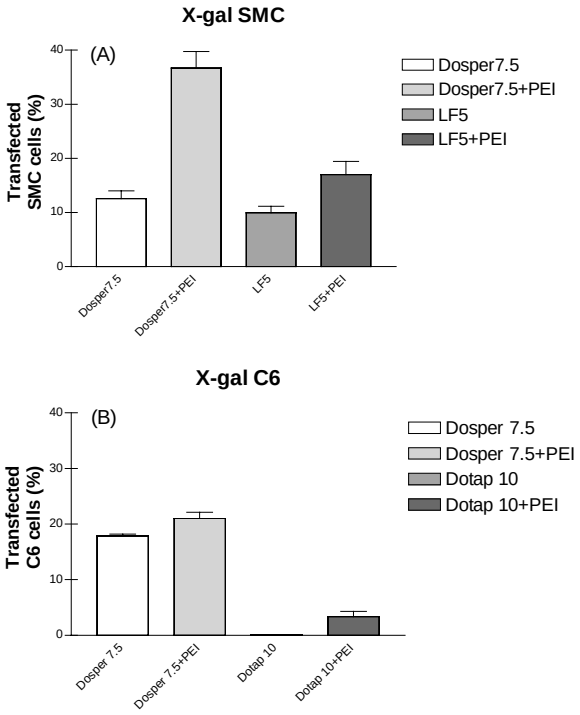

Fig. 2. Effect of PEI on the number of transfected cells. SMC (A) and C6 (B) cells were transfected with 1 μg of TKBPVlacZ plasmid with Lipofectamine, Dotap or Dosper, or DNA was condensed with PEIs before the addition of Lipofectamine, Dotap or Dosper. The cells were incubated in the transfection solution for 6 h and then in the growth medium for 18 h. After the incubation, the cells were washed with PBS and fixed with 4% paraformaldehyde for 15 min. Then the cells were stained with X-gal (1 mg/ml) for 3 h at 37 °C and washed with PBS. The percentage ± S.E. of blue-stained cells was calculated ($n = 4$–6).

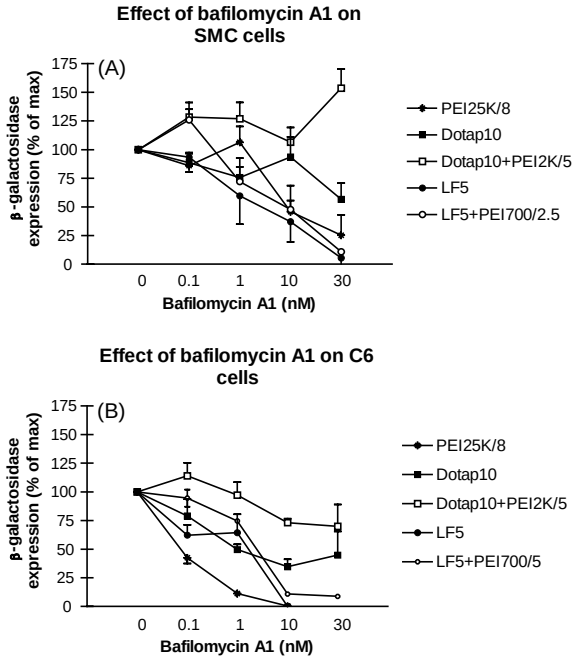

Fig. 3. Effect of bafilomycin A1 on the gene transfection in SMC (A) and C6 (B) cells. Serum-free transfection medium was changed to subconfluent cells, and different amounts of bafilomycin A1 were included into the wells. After 10 min incubation, transfection solutions were added into the wells. The cells were incubated in the transfection solution for 6 h and then in the growth medium for 42 h. β-Galactosidase activity was measured by the ONPG assay. The values are the means of β-galactosidase activity per milligram protein ± S.E. ($n = 3$–6).

used in the bafilomycin A1 experiments. In both cell lines, addition of bafilomycin decreased transfection. In SMC cells, Lipofectamine exhibited approximately the same results with or without PEI 700, resulting in approximately 10% transfection in 30 nM bafilomycin (Fig. 3A). The control reagent, Dotap, resulted in at least 56% expression at 30 nM bafilomycin, and combining PEI with Dotap further decreased sensitivity to bafilomycin A1. Bafilomycin decreased also PEI 25K-mediated transfection. In C6 cells, bafilomycin decreased the expression by PEI 25K to the greatest extent, and the expression was completely blocked at 10 nM bafilomycin (Fig. 3B). Lipofectamine-mediated transfection with or without PEI was also sensitive to bafilomycin. Dotap was less sensitive to bafilomycin, and the Dotap–PEI combination was the most resistant to bafilomycin of all of the studied combinations.

3.4. DNA condensation

The ability of the reagents to condense DNA was studied with ethidium bromide. When reagents bind to DNA, they expel EtBr from DNA resulting in a decrease in the ethidium bromide fluorescence. Lipofectamine was the most effective at condensing DNA, resulting in condensation to less than 50% from the original value, even at the lowest Lipofectamine/DNA ratio used (2) (Fig. 4). Superfect was almost as effective as Lipofectamine in condensing DNA (approximately 50% from the original). Dotap was the least effective. Condensation increased with the increase of Dotap/DNA ratio, and was approximately 60% at

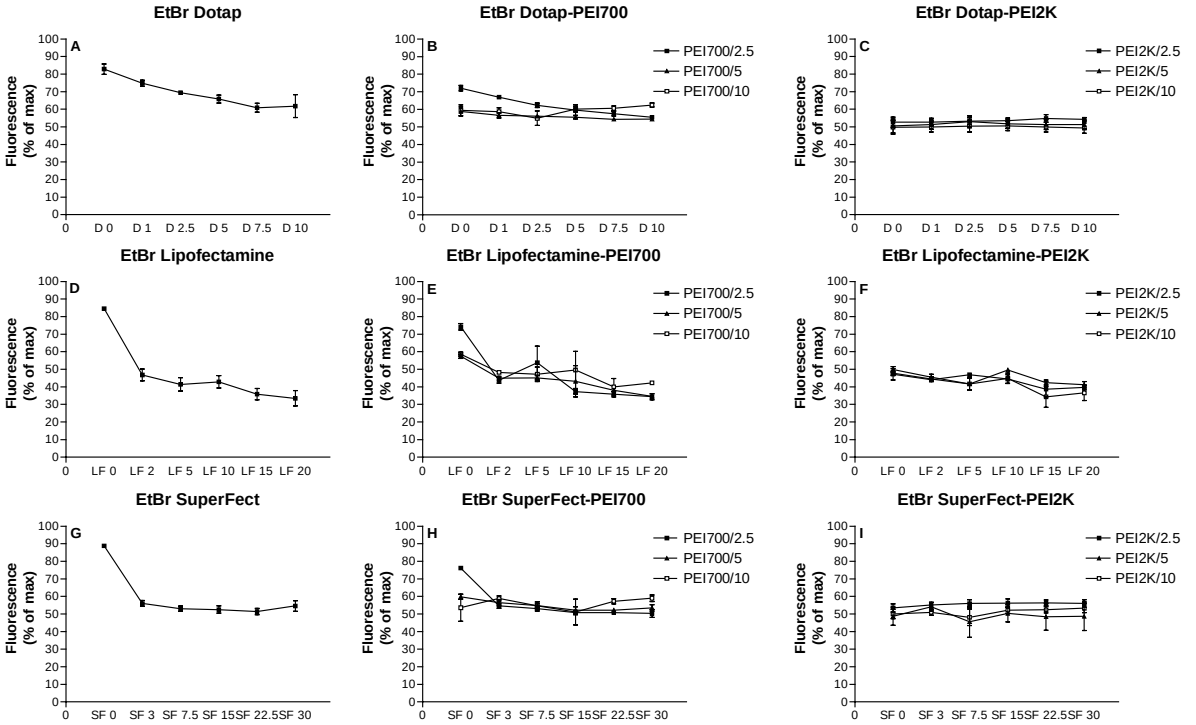

Fig. 4. DNA condensation by Dotap (A–C), Lipofectamine (D–F) and Superfect (G–I) with or without PEI. PEI 700 or PEI 2K was added at different N/P ratios to DNA, and reagents were then added to the wells. The values are percentage of the ethidium bromide fluorescence of plain DNA ± S.E. ($n = 4$).

its best (Dotap/DNA 7.5–10). Pre-condensation of DNA with PEI (700 or 2K) had only a minor effect in experiments with Lipofectamine and Superfect, but caused a somewhat higher condensation rate with Dotap.

4. Discussion

We have shown previously (Lampela et al., 2002), that the combination of low-molecular weight PEIs and the Dosper liposome can cause a synergistic increase in β-galactosidase activity. This is presumably due to intracellular kinetics (Lampela et al., 2003). Here, we have studied, whether this kind of synergy could be generalized to other transfection reagents as well. We selected three different reagents for this study; a monocationic liposome Dotap, a dendrimer Superfect and a combination of polycationic and neutral lipid, i.e. Lipofectamine. The polycationic liposome, Dosper, was used as a positive control (Lampela et al., 2002).

Initially, we studied if different reagents exhibit any synergism when combined with small PEIs (MW 700 or 2000). There were differences in the two cell lines studied. In general, SMC cells were transfected more efficiently than C6 cells. Lipofectamine was clearly the best reagent for both cell lines. When Lipofectamine was combined with PEIs, the β-galactosidase activities were more than doubled in SMC cells, while the increase was less extensive in C6 cells. Dosper was the next best reagent in both cell lines. Furthermore, addition of PEI increased Dosper-mediated transfection, again this phenomenon was more evident in SMC cells than in C6 cells. The combination of Dotap and PEIs revealed differences between the cell lines studied; while pre-condensation of DNA with PEI 2K increased the β-galactosidase activity by up to threefold in C6 cells, the combination of PEI and Dotap resulted in an inhibition of β-galactosidase activity in SMC

cells. In Superfect-mediated transfection, addition of PEIs had only negative effects on β-galactosidase activity in both cell lines. These changes in transfection cannot be explained by toxicity; the most effective reagents Lipofectamine and Dosper were not toxic in the MTT assay.

The buffering capacity of PEI is suggested to be crucial if one wishes to achieve a high efficiency in gene delivery (Merdan et al., 2002). Bafilomycin A1 is a specific inhibitor of vacuolar type H^+-ATPase, and it has been shown to decrease PEI-mediated transfection by inhibiting the lysosomal proton pump (Kichler et al., 2001; Merdan et al., 2002). We studied the effects of bafilomycin A1 on transfection of the best reagents (Lipofectamine in SMC, Dotap in C6 cells). PEI 25K was sensitive to bafilomycin especially in C6 cells. In both cell lines, Dotap was the most resistant to bafilomycin, though addition of a small PEI caused a slight increase in bafilomycin sensitivity. Dotap is known to be less affected by bafilomycin, since its protonation state is not significantly altered in the pH range 5–7.5 (Kichler et al., 2001). Lipofectamine was more sensitive to bafilomycin than Dotap, and addition of a small PEI had only minor effect on bafilomycin sensitivity. Since the bafilomycin sensitivity profiles were similar in both cell lines despite the different synergy, the buffering capacity does not seem to account for the observed synergism.

The number of transfected cells and the total gene expression do not seem to correlate completely. X-gal staining was used in both cell lines to examine, whether the increase of the β-galactosidase activity was due to an increase in the number of cell transfected. As seen with Dosper–PEI combination, the increase of β-galactosidase activity is explained by an increased number of transfected SMC cells (Fig. 2, Lampela et al., 2002). The same effect was found in Lipofectamine/PEI-transfected SMC cells. However, the overall number of transfected cells was lower in Lipofectamine-transfected cells than in Dosper-transfected cells, but the β-galactosidase activity in Lipofectamine-transfected cells was significantly higher. This suggests that the Lipofectamine-transfected cells were producing more protein than the Dosper-transfected cells. In C6 cells, Dosper alone and the Dosper–PEI combination did not markedly differ in their number of transfected cells (Fig. 2B). As seen in Fig. 1B, this Dosper–PEI combination exhibited no major synergism. However, the combination of Dotap and PEI 2K did increase the number of transfected cells from less than one percent up to 3.4% accounting for the synergism seen in Fig. 1D. These results suggest that in the gene transfection, the number of transfected cells and the amount of expressed protein are both at least partly regulated by different parameters, e.g. the uptake of the complex and the intracellular kinetics of the complex. Also, the detection limits of different methods may contribute to the differences seen in the results.

The synergism caused by low-molecular weight PEIs seems to depend on the cell line and the transfection reagents used. In this study, we selected three different lipids (Dosper, Dotap and Lipofectamine) and one polymer (Superfect). Dosper and Lipofectamine contain polycationic lipids, while Dotap is monocationic. We studied the condensation of DNA with the combinations, and found no major differences that could explain our findings. On the other hand, Dotap–PEI combination produced the best increase of β-galactosidase activity in C6 cells. Therefore, the mechanism of synergism seems to be associated with the chemistry of the reagents as well as to the cellular mechanisms of transfected cells. We have reported earlier (Lampela et al., 2003), that combining PEI with Dosper had no effect on cellular uptake, but rather appeared to be mediated via intracellular kinetics. This may be the case also with Lipofectamine and Dotap. Superfect was the only reagent whose transfection efficiency decreased when PEI was used. Both PEIs and Superfect are polymers, which have primary, secondary and tertiary amino nitrogens, resulting in buffering capacity. Since the DNA complexes formed by PEI or Superfect may have similar natures, the combination of PEI and Superfect may not cause any transfection synergism.

The synergism of gene transfection was determined both in the absence and the presence of serum in SMC and C6 cells. This is interesting since one major difference between in vivo and in vitro transfection is the presence of serum in vivo. The anionic compounds present in serum often complex with liposomes and other positively charged transfection reagents, resulting in decreased transfection efficiency (Dodds et al., 1998; Ghosh et al., 2000; Faneca et al., 2002). It has also been reported that dodecylation of primary

amino groups of PEI 2K can enhance the transfection efficiency in the presence of serum (Thomas and Klibanov, 2002). As expected, serum decreased the efficiency of all of the reagents. The only combination that caused higher expression with serum was the Dosper–PEI combination in SMC cells, and in this case the expression was at the same level as that produced by plain Dosper or PEI 25K without serum. Also, PEI 25K has been reported to be effective in vivo (Abdallah et al., 1996; Boletta et al., 1997; Bragonzi et al., 2000). Since the combination of PEI and Dosper caused significantly higher expression compared to PEI 25K in the presence of serum, this combination would be worthy of testing in in vivo transfection studies.

In conclusion, we have demonstrated that the synergy presented earlier (Lampela et al., 2002) with low-molecular weight PEIs and Dosper is not universal. The synergism was achieved with cationic lipids, but not with a polyamidoamine dendrimer. The level of synergism also varied in different cell lines. This synergy must be optimized in each case and it may result in a significant increase of transfection efficiency, sometimes even in the presence of serum. The mechanisms involved in the synergy will require further elucidation.

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