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Dexamethasone conjugated poly(amidoamine) dendrimer as a gene carrier for efficient nuclear translocation

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Abstract

Nuclear membrane is one of the main barriers in polymer-mediated intracellular gene delivery. It was previously reported that glucocorticoid receptor dilated the nuclear pore and translocated into nucleus when it bound to its ligand, glucocorticoid. This suggests that the transport of DNA into nucleus may be facilitated by glucocorticoid. In this study, a glucocorticoid, dexamethasone, was conjugated to polyamidoamine (PAMAM) dendrimer and the effect of the conjugation was investigated. The PAMAM-Dexamethasone (PAM-Dexa) was synthesized by the one-step reaction using Traut's reagent. PAM-Dexa/plasmid DNA complex was completely retarded at a 1/1 weight ratio (polymer/DNA) in a gel retardation assay. PAM-Dexa protected DNA from DNase I for more than 60 min. PAM-Dexa/plasmid DNA complex showed the highest transfection efficiency to 293 cells at a 0.8/1 weight ratio. At this ratio, PAM-Dexa had higher transfection efficiency than PAMAM. Especially in the presence of serum during the transfection, the transfection efficiency of PAM-Dexa was higher than that of PAMAM or PEI by one order of magnitude. In addition, more PAM-Dexa/DNA complexes were observed in the nucleus region than PAMAM/DNA from the confocal microscopy studies. These results indicated that the technique with dexamethasone might be useful for the gene delivery using polymeric gene carriers and the development of efficient polymer vectors.

Keywords: Dexamethasone; Glucocorticoid; Gene delivery; Dendrimer; Transfection

1. Introduction

Non-viral gene delivery systems such as polymer and liposome have been developed as an alternative for viral gene delivery system to overcome intrinsic problems of viral vectors. Although non-viral carriers have advantages such as non-immunogenecity, low cytotoxicity, and low cost (Lee and Kim, 2002), low transfection efficiency compared to viral carriers limits its application to clinical gene therapy. Therefore, diverse approaches have been undertaken to potentiate non-viral gene carriers. It was previously suggested that there were three main barriers in the intracellular delivery of genetic materials using polymeric carriers (Nishikawa and Huang, 2001). The three main barriers are cellular membrane, endosomal membrane, and nuclear membrane. It was reported that the cellular uptake could be facilitated through receptor-mediated endocytosis by conjugation of cell-specific ligands to polymeric carriers (Wagner et al., 1998). In addition, TAT-derived peptides, protein transduction domains (PTP) or membrane translocalization signals (MTS) have been used to increase the cellular uptake of genetic materials (Futaki, 2002; Tung and Weissleder, 2003; Henry, 2003). For endosomal escape of polymer/DNA complex, endosome-disrupting peptides have been extensively investigated (Wagner et al., 1992; Plank et al., 1994). To increase nuclear transport of polymer/DNA complex, nuclear localiza-

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tion signal (NLS) peptides have been conjugated to polymeric carriers (Cartier and Reszka, 2002; Chan and Jans, 1999, 2002; Jensen et al., 2003; Keller et al., 2003; Munkonge et al., 2003). NLS-conjugated polymer enhanced the nuclear transport and transfection efficiency. NLS facilitates energy-dependent transport of large molecules by dilating nuclear pore up to 25 nm in diameter.

Glucocorticoid receptor is a nuclear receptor, which is mainly located in cytoplasm in the absence of its ligand. When the glucocorticoid receptor binds to the ligand, the receptor–ligand complex translocates from cytoplasm to nucleus (Adcock and Caramori, 2001). In addition, the receptor dilates the nuclear pore up to 60 nm during the translocation process, which is favorable for translocation of polymer/DNA complex into nucleus (Shahin et al., 2005). Therefore, the transport of polymer/DNA complex into nucleus may be facilitated by conjugating glucocorticoid to polymer carriers.

Polyamidoamine (PAMAM) dendrimers contain large number of surface amines that are generation-dependent and are capable of forming polymer/DNA complex through the electrostatic interaction with polyanions, such as nucleic acids. Thus, PAMAM dendrimers have been developed as polymeric gene carriers because of their well-defined structure, ease of control of surface functionality, and relatively high gene transfection efficiency.

In this study, we conjugated dexamethasone, a potent glucocorticoid, to PAMAM dendrimer to facilitate nuclear translocation of polymer/DNA complex and increase the transfection efficiency. PAMAM-Dexamethasone (PAM-Dexa) was characterized in terms of physicochemical properties, cytotoxicity, and in vitro transfection efficiency. As control polymeric gene carriers, polyethylenimine (PEI) and polyamidoamine (PAMAM) dendrimer were used in the transfection experiments. The intracellular localization of PAMAM/DNA and PAM-Dexa/DNA complexes was also performed and compared by confocal microscopy.

2. Materials and methods

2.1. Materials

PAMAM G4 (Starburst), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), *N*,*N*-dimethylsulfoxide (DMSO), and Rhodamine B isothiocyanate (RITC) were purchased from Sigma–Aldrich Korea (Seoul, Korea). Dexamethasone mesylate was purchased from Steraloids Inc. (Newport, RI). Traut's reagent was from Pierce (Iselin, NJ). Luciferase assay kit was from Promega (Madison, WI). 4,6-Diamidino-2-phenylindole, dihydrochloride (DAPI) was purchased from Molecular Probes (Invitrogen). Fetal bovine serum (FBS), 100× Antibiotic–antimycotic agent, and Dulbecco's modified Eagle's medium (DMEM) were purchased from GIBCO (Gaithersburg, MD).

2.2. Synthesis of PAM-Dexa

The conjugation reaction was performed as reported previously with some modification (Gruneich et al., 2004). The

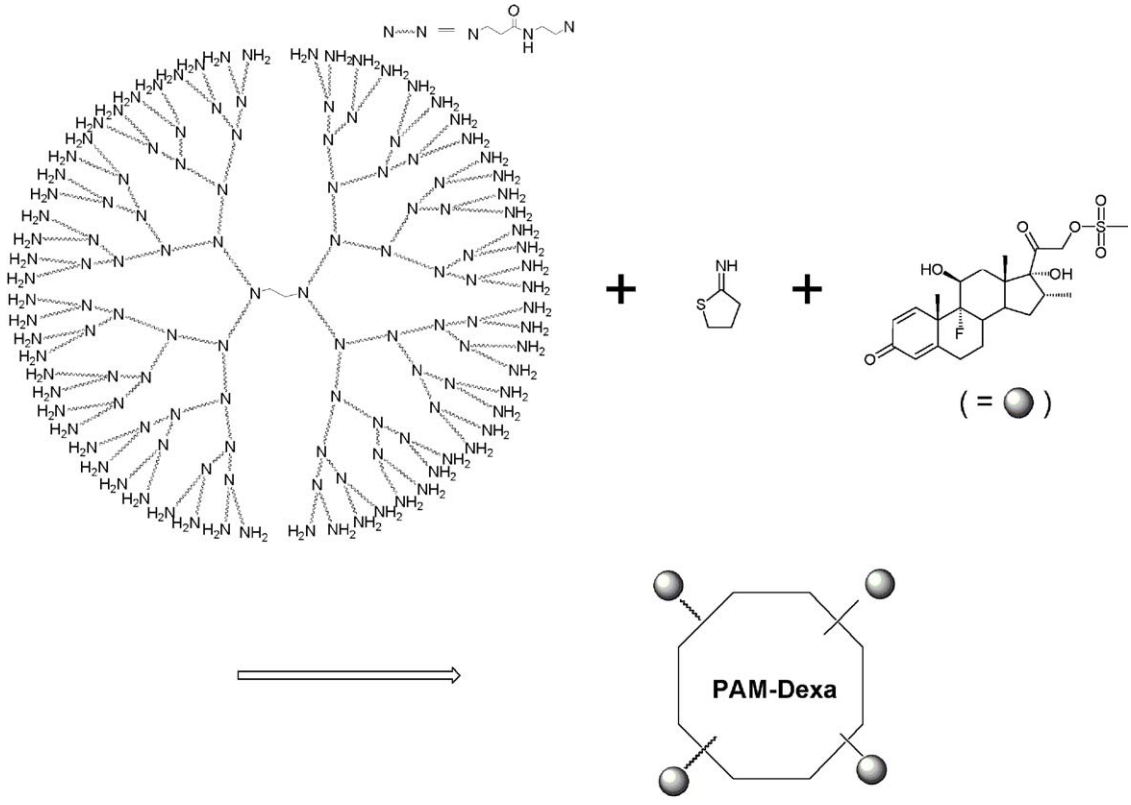

Fig. 1. Synthesis scheme of PAM-Dexa.

overall synthesis scheme is depicted in Fig. 1. Briefly, dexamethasone coupling to PAMAM was performed with 4 equiv. of Traut's reagent, and 4 equiv. of dexamethasone mesylate in anhydrous DMSO for 4 h at room temperature. To the reaction mixture, same volume of water was added and it was dialyzed against pure water and filtered through 0.45 μm syringe-type filter to remove insoluble impurities. Then the product was obtained after freeze-drying, yielding white crystalline powder. The yields for the products were usually over 90%.

2.3. Preparation of plasmid

pCMV-Luc was constructed by inserting the HindIII/XbaI firefly luciferase cDNA fragment from pGL3-control vector into the HindIII/XbaI site of pcDNA3 (Han et al., 2001). The pCMV-Luc was transformed in *Escherichia coli* DH5α and amplified in terrific broth media at 37 °C overnight. The amplified pCMV-Luc was purified by using the Maxi plasmid purification kit (Qiagen, Valencia, CA). The purity and concentration of the purified pCMV-Luc were determined by ultraviolet (UV) absorbance at 260 nm. The optical density ratios at 260–280 nm of these plasmid preparations were in the range of 1.7–1.8.

2.4. Gel retardation assay

PAM-Dexa/pCMV-Luc complexes were prepared at various weight ratios and left for 20 min at room temperature for complex formation. The complexes were electrophoresed on a 1% (w/v) agarose gel for 30 min at 100 V. The gel was stained with ethidium bromide (0.5 μg/ml) for 30 min and illuminated on a UV illuminator to show the location of the DNA.

2.5. DNase I protection assay

Ten micrograms of pCMV-Luc was mixed with 8 or 16 μg of PAM-Dexa in 500 μl of PBS. After complex formation, DNase I (10 unit, Promega, Madison, WI) was added to the complex solution and the reaction mixture was incubated at 37 °C. One hundred microlitres of the sample was taken at 30 or 60 min after incubation and mixed with 100 μl of 2× stop solution (80 mM EDTA and 2% SDS) to dissociate pCMV-Luc form PAM-Dexa. The DNA was analyzed by agarose gel electrophoresis.

2.6. Zeta-potential and size measurements

The zeta-potential values and size of PAM-Dexa/pCMV-Luc were determined by the Malvern Zetasizer 3000HAs system (Malvern Instruments Ltd., Worcestershire, UK) using the PCS 1.61 software. PAM-Dexa/pCMV-Luc complexes were formed at a final concentration of 5 μg/ml plasmid DNA in HBS (10 mM Hepes, 1 mM NaCl, pH 7.4) for zeta-potential experiments and in water for size measurements, respectively.

2.7. Cell culture and in vitro transfection

Human embryonic kidney 293 cells and mouse neuroblastoma Neuro2A cells were grown in DMEM with 10% FBS. The cells were routinely maintained on plastic tissue culture dishes (Falcon Co., Becton Dickenson, Franklin Lakes, NJ) at 37 °C in an incubator with a humidified atmosphere containing 5% CO_2/95% air. All media routinely contained 1× antibiotic–antimycotic agent. For the transfection studies, the cells were seeded at a density of 2.5×10^5 cells/well in six-well flat-bottomed microassay plates (Falcon Co., Becton Dickenson, Franklin Lakes, NJ) 24 h before transfection. PEI/pCMV-Luc complexes were prepared at a 5/1 N/P ratio. PAMAM/pCMV-Luc complexes were prepared at various weight ratios to optimize transfection condition. The PAM-Dexa/pCMV-Luc complexes were also prepared at various weight ratios. Before transfection, the medium was replaced with 2 ml of fresh DMEM with or without FBS. Then, the polymer/pCMV-Luc complexes were added to the cells. The amount of pCMV-Luc was fixed at a 2 μg/well. The cells were then incubated for 4 h at 37 °C in a 5% CO_2 incubator. After 4 h, the transfection mixtures were removed and 2 ml of fresh DMEM medium containing FBS. The cells were incubated for an additional 44 h at 37 °C.

To inhibit the translocation of the dexamethasone induced glucocorticoid receptor into nucleus, the cells were pre-treated with 1,2-bis-(*o*-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid-acetoxymethyl ester (BAPTA-AM). The medium was replaced with 2 ml of DMEM containing 10% FBS and 10 μM BAPTA-AM 1 h before transfection. Then, polymer/pCMV-Luc complexes were added to the cells. The cells were then incubated for 4 h at 37 °C in a 5% CO_2 incubator. After 4 h, the transfection mixtures were removed and 2 ml of fresh DMEM medium containing FBS and 10 μM BAPTA-AM. The cells were incubated for an additional 44 h at 37 °C.

2.8. Luciferase assay

After transfection, the cells were washed with PBS twice, and 200 μl of reporter lysis buffer (Promega, Madison, WI) was added to each well. After 15 min of incubation at room temperature, the cells were harvested and transferred to microcentrifuge tubes. After 15 s of vortexing, the cells were centrifuged at 11k rpm for 3 min. The protein concentrations of the extracts were determined by using Pro-measure protein assay kit (Intron Biotechnology, Seoul, Korea). Luciferase activity was measured in terms of relative light units (RLU) using a Luminometer (TD-20/20, Turner Designs, Sunnyvale, CA). The final values of luciferase were reported in terms of RLU/mg total protein.

2.9. Cytotoxicity assay

Evaluation of cytotoxicity was performed by the 3-[4,5-dimethylthiazol]-2,5-diphenyltetrazolium bromide (MTT) assay. The cells were seeded at a density of 2×10^4 cells/well in 96-well microassay plates (Falcon Co., Becton dickenson, Franklin Lakes, NJ), and incubated for 24 h before transfection. PEI/pCMV-Luc complex was prepared at a 5/1 N/P ratio and PAMAM/pCMV-Luc was formulated at a 4/1 weight ratio. PAM-Dexa/pCMV-Luc complex was prepared at a 0.6/1, 0.8/1, or 1/1 weight ratio. The medium was replaced with fresh DMEM medium without FBS before transfection. The

polymer/pCMV-Luc complexes were added to the cells. The amount of pCMV-Luc was fixed at 0.2 μg/well. After the incubation at 37 °C for 4 h, the transfection mixture was replaced with 100 μl of fresh DMEM medium supplemented with 10% FBS. The cells were incubated for an additional 44 h at 37 °C. After the incubation, 24 μl of 2 mg/ml MTT solution in PBS was added. The cells were incubated for an additional 4 h at 37 °C and then MTT-containing medium was aspirated off and 150 μl of DMSO was added to dissolve the formazan crystal formed by live cells. Absorbance was measured at 570 nm. The cell viability (%) was calculated according to the following equation:

$$\text{Cell viability (\%)} = \frac{\text{OD}_{570(\text{sample})}}{\text{OD}_{570(\text{control})}} \times 100$$

where the $\text{OD}_{570(\text{sample})}$ represents the measurement from the wells treated with polymer/plasmid DNA complex and the $\text{OD}_{570(\text{control})}$ represents the measurements from the wells treated with PBS buffer only.

2.10. Preparation of rhodamin-labeled pCMV-Luc and confocal microscopy

Rhodamine B isothiocyanate (RITC) and pCMV-Luc were mixed in 0.2 M sodium carbonated-buffered solution (pH 9.7) at 4 °C for 12 h at both the concentration of 1 mg/ml. The residual RITC was separated by gel filtration of a MicroSpin G-25 column (Sigma–Aldrich). The RITC-labeled pCMV-Luc was obtained by ethanol precipitation.

The RITC-labeled plasmid DNA was complexed with PAMAM or PAM-Dexa and incubated with 293 cells. 293 cells were seeded at a density of 5×10^4 cells/well in 24-well plates (Falcon) 1 day before transfection. PAMAM/pCMV-Luc complexes were prepared at a 4/1 weight ratio (polymer/plasmid DNA) and PAM-Dexa/pCMV-Luc complexes were prepared at a 0.8/1 weight ratio. The amount of pCMV-Luc was fixed at 1 μg/well. Each polymer/pCMV-Luc complex was added to the cells and incubated for 4 h in 10% FBS-containing media. After replacing the old media with fresh ones, the cells were further incubated for an additional 24 h at 37 °C for confocal microscopy experiments (Radiance 2000, Bio-Rad). The locations of nucleus were detected using DAPI reagent (Molecular Probes).

3. Results

3.1. Synthesis of PAM-Dexa

Dexamethasone was conjugated to PAMAM dendrimer by the method reported previously with slight modification (Fig. 1; Gruneich et al., 2004). PAMAM (G4) was dissolved in 1.8 ml of anhydrous DMSO with four-fold molar excess of Traut's reagent and dexamethasone-21-mesylate. The reaction was allowed to proceed for 3.5 h at room temperature and quenched by the addition of excess amount of deionized water. The reaction mixture was dialyzed at 4 °C for 3 days (MWCO 3,400) against pure water and filtered through a 0.45 μm syringe-type filter. It was further freeze dried and white product was obtained. The product was solubilized in DMSO-d_6 for ^{1}H NMR analysis (300 MHz). The peak intensity ratio was calculated between the primary amine protons of PAMAM dendrimer (128 H, $\delta = 7.8$–8.4 ppm) and the two methyl protons at 13′ and 16′ position of the five-numbered ring of dexamethasone ($\delta = 0.7$–0.9 ppm), and it was confirmed that about four dexamethasones were conjugated to PAMAM dendrimer.

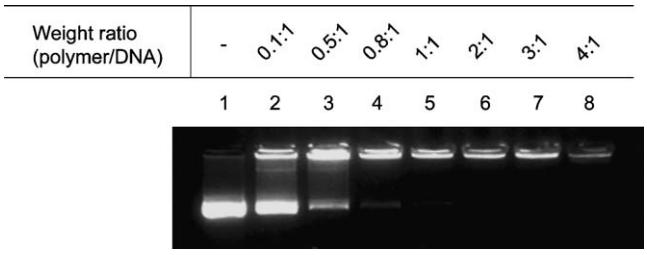

Fig. 2. Gel retardation assay PAM-Dexa/pCMV-Luc complexes were prepared at various weight ratios. Increasing amounts of PAM-Dexa were added to DNA. The mixtures were incubated at room temperature for 30 min and electrophoresed on 1% (w/v) agarose gel.

3.2. Analysis of complex formation and DNase I protection by agarose gel electrophoresis

To verify the complex formation of PAM-Dexa with plasmid DNA, gel retardation assay was performed at various weight ratios of polymer/plasmid DNA complexes. PAM-Dexa/pCMV-Luc complexes were prepared at various weight ratios and analyzed in a 1% (w/v) agarose gel. Most of pCMV-Luc was retarded at a 0.8/1 weight ratio of PAM-Dexa/plasmid DNA complex. The electrophoresis of PAM-Dexa/plasmid DNA complex at this weight ratio showed faint DNA band (Fig. 2, lane 4). The complete retardation of pCMV-Luc was observed at a 1/1 or higher weight ratio (Fig. 2, lanes 5–8).

DNase I protection assay was performed to confirm that PAM-Dexa protected plasmid DNA from nuclease. The PAM-Dexa/pCMV-Luc complex was formed at a 0.8/1 or 1.5/1 weight ratio. After complex formation, PAM-Dexa/pCMV-Luc complex was incubated with DNase I for 30 or 60 min. Naked DNA was completely degraded by DNase I after 30 min of incubation (Fig. 3, lane 2). However, PAM-Dexa protected plasmid DNA for over 60 min (Fig. 3, lanes 4–9).

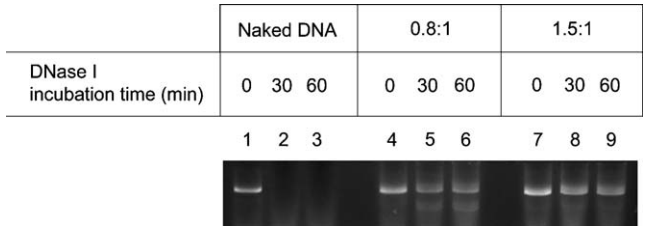

Fig. 3. DNase I protection assay PAM-Dexa/pCMV-Luc complex was prepared as described in materials and methods. The complex solution was incubated with DNase I for 30 or 60 min. After incubation, DNA was analyzed by 1% agarose gel electrophoresis.

Table 1
Zeta-potential and size measurements of PAM-Dexa/plasmid DNA complexes at various weight ratios

	Weight ratio (polymer:DNA)		
	0.6:1	0.8:1	1:1
Size (nm)	ND	487.5	365.1
Zeta-potential (mV)	-23.5 ± 8.5	-10.1 ± 3.4	7.1 ± 0.4

3.3. Zeta-potential and size measurements of the complexes with plasmid DNA

PAM-Dexa/pCMV-Luc complexes were prepared at 0.6/1, 0.8/1, and 1/1 weight ratios and the zeta-potentials and particle sizes of the complexes were measured (Table 1). Gel retardation assay showed complete retardation of plasmid DNA at a 1/1 weight ratio. As expected from the gel retardation assay, a positive zeta-potential was obtained at a 1/1 weight ratio. At a 0.8/1 weight ratio, the complex showed a slight negative zeta-potential.

3.4. Transfection experiments on cell lines

To evaluate the transfection efficiency, in vitro transfection assay was performed with PAM-Dexa. First, the transfection condition of PAMAM to 293 cells was optimized. PAMAM/pCMV-Luc complexes were prepared at various weight ratios and transfected to 293 cells. In Fig. 4(A), PAMAM had the highest transfection efficiency at a 4/1 weight ratio. Therefore, PAMAM/pCMV-Luc complex was prepared at a 4/1 weight ratios in the following experiments. To optimize the transfection condition of PAM-Dexa, PAM-Dexa/pCMV-Luc complexes were prepared at various weight ratios and transfected to 293 cells. In Fig. 4(B), the highest transfection efficiency of PAM-Dexa was obtained at a 0.8/1 weight ratio, suggesting that the conjugation of dexamethasone to PAMAM reduced the required amount of the polymeric carrier for optimum transfection. In the following experiments, PAM-Dexa/pCMV-Luc was prepared at a 0.8/1 weight ratio.

PAM-Dexa was compared to PEI (25 kD) and PAMAM in terms of transfection efficiency. In the absence of serum during the transfection step, PAM-Dexa showed approximately two-fold higher transfection efficiency to 293 cells than PAMAM (Fig. 5(A)). The transfection efficiency of PAM-Dexa to 293 cells was lower than that of PEI in the absence of serum (Fig. 5(A)). However, in the presence of serum during the transfection step, PAM-Dexa had higher transfection efficiency than PEI or PAMAM in the presence of serum (Fig. 5(A)). The transfection efficiencies of PEI and PAMAM decreased in the presence of serum. It may be due to the interaction between serum proteins and positively charged polymer/plasmid DNA complexes. As shown in gel retardation assay and zeta-potential measurement, PAM-Dexa/plasmid DNA complex at a 0.8/1 weight ratio had a slightly negative zeta-potential. Therefore, the serum effect was minimized and the transfection efficiency of

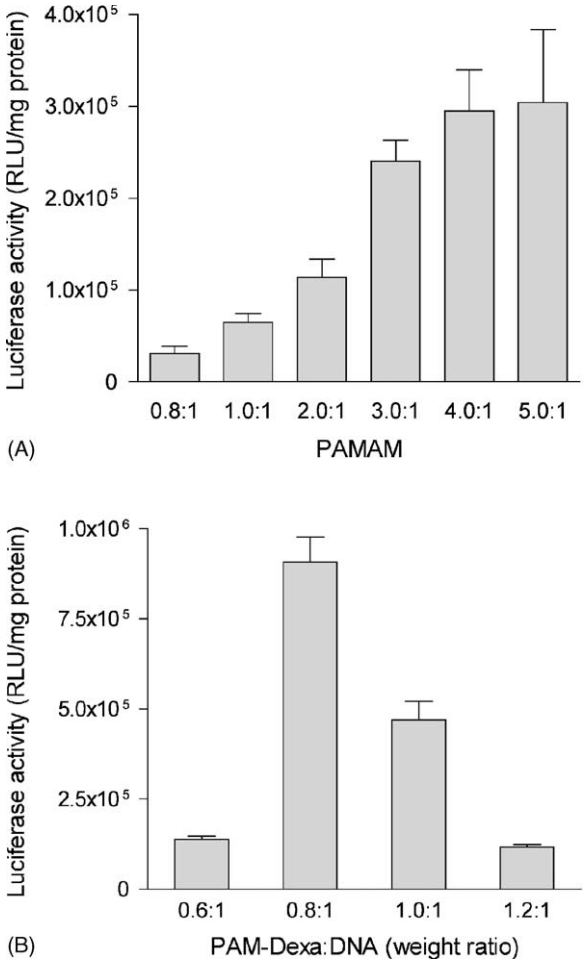

Fig. 4. Transfection PAMAM (A) and PAM-Dexa (B) into 293 cells. PAMAM/pCMV-Luc (A) and PAM-Dexa/pCMV-Luc (B) complexes were prepared at various weight ratios and transfected to 293 cells. Transfection efficiency was measured by luciferase assay. The data is expressed as mean values (±standard deviation) of four experiments.

PAM-Dexa was not changed as much as that of PEI or PAMAM in the presence of serum (Fig. 5(A)).

The transfection efficiency of PAM-Dexa to Nuero2A cells was higher than PEI or PAMAM irrespective of the presence of serum (Fig. 5(B)). In the absence of serum, PAM-Dexa showed over two-fold increase in transfection efficiency compared to PEI and over six-fold increase compared to PAMAM. In addition, PAM-Dexa displayed one order of magnitude higher transfection efficiency compared to PEI and PAMAM in the presence of 10% FBS (Fig. 5(B)).

It was previously reported that the dexamethasone-induced translocation of the glucocorticoid receptor was inhibited by BAPTA-AM (Strubing and Clapham, 1999). The cells were pre-treated with 10 μM BAPTA-AM before transfection to inhibit the translocation of the glucocorticoid receptor. The pre-treatment with BAPTA-AM decreased the transfection efficiency of PAM-Dexa to 37% of the untreated control (Fig. 6). However, the efficiency of PAMAM was not significantly decreased after the treatment with BAPTA-AM. This result suggests that the enhanced transfection efficiency of PAM-Dexa

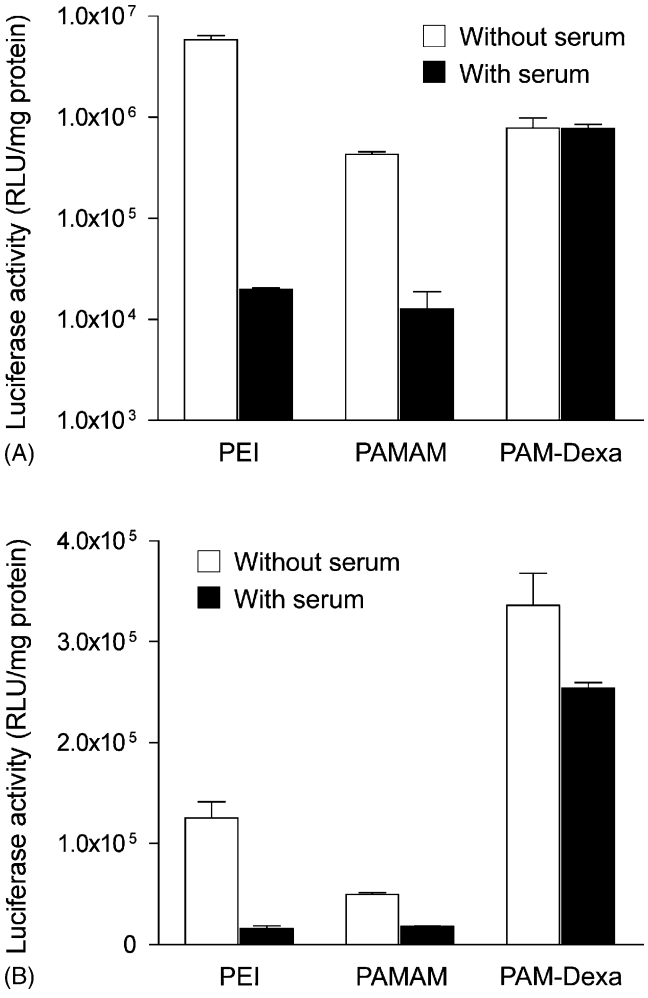

Fig. 5. Transfection efficiency of PEI, PAMAM, or PAM-Dexa into 293 cells (A) and Neuro2A cells (B). Polymer/pCMV-Luc complexes were prepared as described in Section 2 and transfected to 293 cells (A) and Neuro2A cells (B). Transfection efficiency of each complex was measured by luciferase assay. The data is expressed as mean values (±standard deviation) of four experiments.

may be due to the dexamethasone-induced translocation of the glucocorticoid receptor.

3.5. Cytotoxicity assay

The cytotoxicity of PAM-Dexa was compared with those of PEI and PAMAM at their respective optimum transfection condition. As shown in Fig. 7(A), for 293 cells, PEI and PAMAM showed approximately 70% cell viability compared to control, whereas PAM-Dexa showed no toxicity at 0.6/1, 0.8/1, or 1/1 weight ratios. For Neuro2A cells, PEI showed higher toxicity in comparison with PAMAM and PAM-Dexa (Fig. 7(B)).

3.6. Confocal microscopy studies

The plasmid DNA was labeled with RITC to construct fluorescent complexes for localization in the cultured cells. Each complex was prepared at the condition displaying optimal transfection efficiency. The complex was incubated with 293 cells for 4 h in the presence of serum. After further incubation for

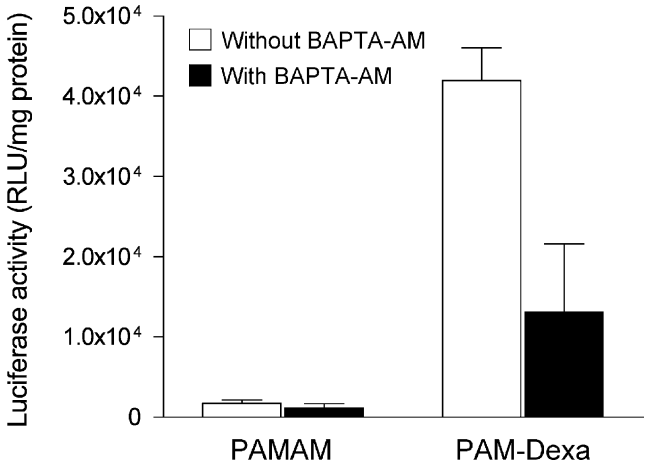

Fig. 6. Effect of the pre-treatment with BAPTA-AM, The cells were pre-treated with BAPTA-AM before transfection to inhibit the translocation of the dexamethasone induced glucocorticoid receptor into nucleus. PAMAM/pCMV-Luc and PAM-Dexa/pCMV-Luc complexes were prepared at various weight ratios and transfected to 293 cells. The transfection efficiency was measured by luciferase assay. The data is expressed as mean values (±standard deviation) of three experiments.

24 h, the confocal images were obtained for PAMAM/plasmid DNA (Fig. 8(A)) and PAM-Dexa/plasmid DNA complexes (Fig. 8(B)). The nucleus was stained in blue and the location of polymer/DNA complex was observed in red. In general, as shown in Fig. 8, more PAM-Dexa/DNA complexes were observed in the nucleus region compared to PAMAM/DNA complexes. The results suggest that the higher level of gene transfection efficiency of PAM-Dexa compared to that of PAMAM (Fig. 5(A)) might be due to the nuclear localizing effect of dexamethasone.

4. Discussion

Low efficiency of non-viral carriers-mediated gene transfection has limited their clinical applications. As well as the problems in physicochemical properties of the polymeric or liposomal gene carriers, the outer and inner membrane cell structures are the main barriers for gene delivery using non-viral carriers (Nishikawa and Huang, 2001). Thus, diverse approaches are undertaken by researchers to overcome the huddles towards efficient gene delivery carriers by making use of cellular ligands/receptors, membrane-disrupting peptides, or nuclear targeting signals.

The conjugation of glucocorticoid to polymeric gene carriers may have two favorable effects. First, glucocorticoid receptor exists in cytoplasm in the absence of its ligand. When the receptor binds to its ligand, the receptor–ligand complex is translocated from cytoplasm to nucleus. Therefore, it can be used as a nuclear translocation signal. The receptor may facilitate the translocation of dexamethasone-decorated complex into nucleus (Adcock and Caramori, 2001). Recently, this approach was demonstrated by the direct conjugation of dexamethasone to DNA (Rebuffat et al., 2001). They showed that the dexamethasone conjugated DNA was effectively accumulated in the nucleus. In addition, more recently, the dexamethasone-

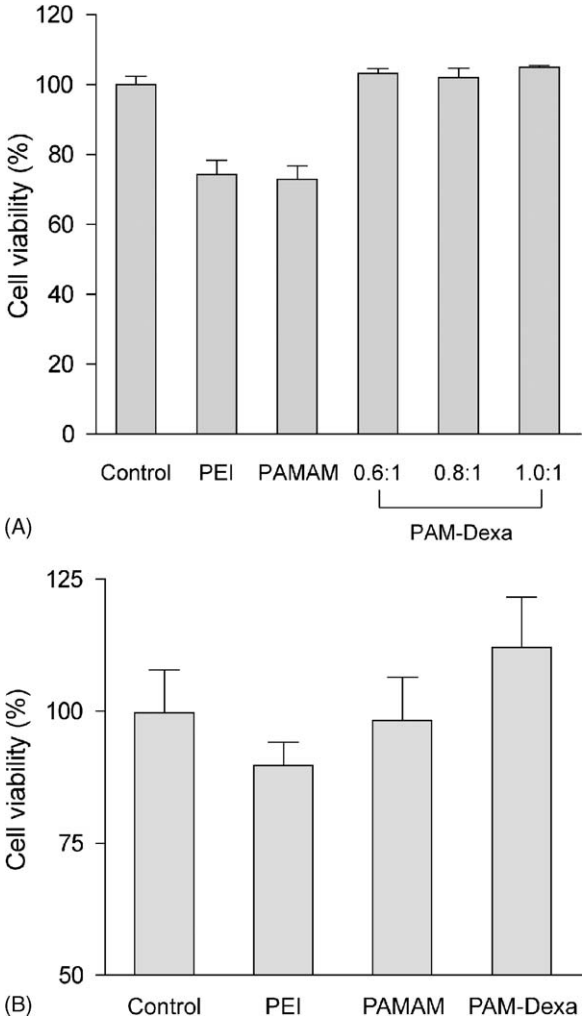

Fig. 7. Cytotoxicity of PEI, PAMAM, or PAM-Dexa to 293 cells (A) and Neuro2A cells (B). Polymer/pCMV-Luc complexes were prepared as described in Section 2 and transfected to 293 cells (A) and Neuro2A cells (B). After transfection, the cell viability was measured by MTT assay. The data is expressed as mean values (±standard deviation) of six experiments.

containing cationic liposome showed the enhanced gene delivery capability through the effective translocation of cationic liposome/DNA complexes into the nucleus. Second, the glucocorticoid receptor dilates the nuclear pore up to 60 nm in the process of translocation into the nucleus (Shahin et al., 2005). The dilation of nuclear pore by glucocorticoid receptor may facilitate the nuclear translocation of polymer/DNA complex.

In this study, dexamethasone was conjugated to PAMAM. PAM-Dexa formed complexes with plasmid DNA efficiently and retarded plasmid DNA completely at a 1/1 weight ratio in gel retardation assay. The highest transfection efficiency of PAM-Dexa was obtained at a 0.8/1 weight ratio, which showed a slightly negative zeta-potential. It is not clear why PAM-Dexa/plasmid DNA complex showed the highest transfection efficiency at this ratio. We speculate that the amount of dexamethasone on the surface of the complex may be an optimum at this ratio. At a higher ratio, more dexamethasone would be present on the surface and more glucocorticoid receptor would bind to the polymer/DNA complex, which is not efficient in nuclear translocation. The previous report showed that DNA linked to more than one NLS peptides decreased the nuclear translocation compared with the DNA linked to a NLS peptide. This suggests that the amount of dexamethasone on the surface of the complex should be optimized and therefore, the conjugation ratio of dexamethasone to a polymeric carrier should also be optimized.

PAM-Dexa had lower cytotoxicity than PEI and PAMAM. To 293 cells, PAM-Dexa had higher transfection efficiency than PEI and PAMAM in the presence of serum (Fig. 5(A)). Especially, PAM-Dexa showed higher transfection efficiency to Neuro2A cells than PEI and PAMAM irrespective of the presence of serum (Fig. 5(B)). In addition, the pre-treatment of the cells with BAPTA-AM decreased the transfection efficiency of PAM-Dexa significantly, suggesting that the enhanced transfection efficiency might be due to the dexamethasone-induced translocation of the glucocorticoid receptor (Fig. 6).

From the confocal microscopy studies, it was observed that more PAM-Dexa/DNA complexes were located in the nucleus region than PAMAM/DNA complexes in the presence of serum,

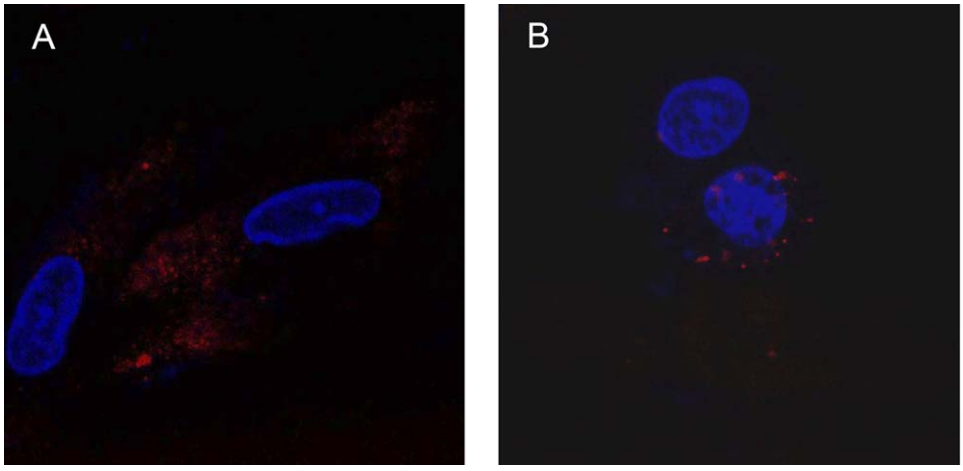

Fig. 8. Confocal images of 293 cells incubated with PAMAM/DNA complexes (A) and PAM-Dexa/DNA complexes (B). Each complex was prepared as described in Section 2. DAPI was used to stain nucleus (blue) and RITC-pCMV-Luc was used to localize the polymer/DNA complexes (red).

although the five times more amount of PAMAM (4 μg) was used in the transfection than PAM-Dexa (0.8 μg). This suggests that dexamethasone in the conjugate might have contributed to the efficient nuclear localization effect of the complexes.

Dexamethasone is a well-known pharmacological drug as an effective anti-inflammatory reagent (Adcock and Caramori, 2001). When dexamethasone binds to its receptor, the receptor increases or decreases the expressions of the responsive genes. The alteration of gene expression may be beneficial to some inflammatory disease gene therapy. For this kind of disease, PAM-Dexa may have synergistic effect such as enhanced transfection and anti-inflammatory effect.

In summary, the conjugation of dexamethasone to PAMAM enhanced transfection efficiency. In addition, PAM-Dexa had lower cytotoxicity than PEI and PAMAM. These results indicated that PAM-Dexa might be useful for non-viral gene delivery system.

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