Arch Pharm Res Vol 28, No 11, 1302-1310, 2005

Archives of Pharmacal Research
http://apr.psk.or.kr

Glucosylated Polyethylenimine as a Tumor-Targeting Gene Carrier

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(Received June 16, 2005)

Glucosylated polyethylenimine (GPEI) was synthesized as a tumor-targeting gene carrier through facilitative glucose metabolism by tumor glucose transporter. Particle sizes of GPEI/DNA complex increased in proportion to glucose content of GPEI, whereas surface charge of the complex was not dependent on glucosylation, partially due to inefficient shielding of the short hydrophilic group introduced. GPEI with higher glucosylation (36 mol-%) had no cytotoxic effect on cells even at polymer concentrations higher than 200 μg/mL. Compared to unglucosylated PEI, glucosylation induced less than one-order decrease of transfection efficiency. Transfection of GPEI/DNA complex into tumor cells possibly occurred through specific interaction between glucose-related cell receptors and glucose moiety of GPEI. Gamma imaging technique revealed GPEI/DNA complex was distributed in liver, spleen, and tumors.

Key words: Tumor-targeting, Glucose transporter, Glucosylation, Polyethylenimine

INTRODUCTION

Cancer is a complex acquired disease that affects millions of individuals each year. Underlying the pathogenesis of cancer is a variety of molecular genetic abnormalities, which can be inherited or environmentally induced, resulting in unregulated cell proliferation. Conventional therapeutic strategies used to treat cancer, such as surgery, chemotherapy, and radiotherapy, have been only partially successful; thus, new treatment options are critically needed.

Gene therapy is currently explored experimentally as an alternative or addition to the established treatment options for, among others, malignant melanoma, leukemia, and glioma. The aim of this therapy is to introduce a gene or cells, which would either indirectly or directly eliminate tumor cells (Ogris *et al.*, 2002). The receptor-mediated endocytosis systems of various cell types could be useful for tissue-specific delivery, and several gene delivery systems have been developed to introduce foreign DNAs into specific cells through this receptor-mediated endocytosis. Among the currently investigated targeting ligands attached covalently or non-covalently to polycations, including galactose (Park *et al.*, 2000, 2001, 2003a, 2003b, 2003c), mannose (Ferkol *et al.*, 1996; Erbacher *et al.*, 1996), transferrin (Wagner *et al.*, 1990; Kursa *et al.*, 2003), epidermal growth factor (EGF) (Chen *et al.*, 1994), and antibodies (Ferkol *et al.*, 1993), tumor-specific uptake ligands such as EGF, folate (Leamon *et al.*, 1999), and transferrin are conjugated to the cationic polymers.

Polyethylenimine (PEI) has been shown to mediate effective *in vitro* and *in vivo* gene transfers (Boussif *et al.*, 1995). It is an ideal candidate molecule for the design of more sophisticated gene delivery systems, because PEI/DNA complexes are able to escape from endosomes. Extensive applications of PEI for gene transfer *in vivo* have been reported using numerous routes of administration including intravenous (Kircheis *et al.*, 2001), intratumoral, intramuscular (Wang *et al.*, 2001), intraperitoneal (Aoki *et al.*, 2001), intratracheal (Rudolph *et al.*, 2000), and aerosolized (Densmore *et al.*, 2000) administrations. However, *in vivo* studies revealed PEI/DNA complexes interact with blood components and non-target cells, thereby strongly reducing their tumor-targeting efficiency

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(Ogris *et al.*, 1999). Masking the net positive charge to reduce non-specific interactions of the complexed DNA with blood components can be accomplished by shielding the complex surface with hydrophilic polymers, such as dextran, poly(ethylene glycol) or poly(vinyl pyrrolidone) (Oupicky *et al.*, 2002). In addition, glycosylation of PEI has been reported to increase the hydrophilicity and enhance the gene transfer efficiency of PEI/DNA complexes (Bettinger *et al.*, 1999), and, in human carcinoma cells, PEI conjugated with tetraglucose at low substitution degree yielded higher and longer gene transfer efficiency than unsubstituted one (Merlin *et al.*, 2001; Dolivet *et al.*, 2002).

One of the most common and profound phenotypes of highly malignant tumors, as known for more than seven decades, is their ability to metabolize glucose at high rates (Pedersen *et al.*, 2002), even though all mammalian cell membranes contain proteins involved in the glucose transport. This is a characteristic of highly malignant animal and human tumors, including those derived from brain, colon, liver, lung, pancreas, stomach, and retina. For each, a close correlation exists among the degree of differentiation, growth rate, and glucose metabolism, where the most poorly differentiated tumors exhibit the fastest growth and the highest glycolytic rate (Wheeler *et al.*, 1985; Dang *et al.*, 1999). Luciani *et al.* reported that combination of PEG and glucose conjugates on the surface of paramagnetic nonionic vesicles (niosomes) significantly improved tumor-targeting of an encapsulated paramagnetic agent as assessed with magnetic resonance imaging using a human carcinoma xenograft model (Luciani *et al.*, 2004). Recently, our group investigated the aerosol delivery of glucosylated PEI (GPEI)/phosphatase and tensin homolog deleted on chromosome 10 (PTEN) complex into K-*ras* null mice and showed that GPEI/PTEN complex could suppress Akt downstream pathways in the mice lung (Kim *et al.*, 2004).

In this study, GPEI was synthesized with varying substitution degrees to clarify the effect of glucose ligand on the transfection of GPEI/DNA complex. Glucosylation of the primary amino group of PEI at high substitution degree is expected to confer tumor-targeting property through facilitative glucose metabolism by the glucose transporters in tumors, and decreased cellular toxicity. Additionally, using the *in vivo* imaging technique, attempts were made to confirm the successful delivery of the above-mentioned gene complex to the tumor.

MATERIALS AND METHODS

Reagents

Branched PEI with an average molecular weight of 25 kDa (PEI 25K), D-(+)-cellobiose, and sodium cyanoborohydride were obtained from Sigma-Aldrich Korea. A 5.3-kilobase pair expression vector, pGL3-control (Promega, Madison, WI, U.S.A.), contains luciferase gene driven by SV40 promoter and enhancer. The plasmids were propagated in *Escherichia coli* and purified by chromatography (Megaprep Kits, Qiagen, Chatsworth, CA, U.S.A.).

Glucosylation of PEI

One hundred milligrams of PEI 25K and predetermined amount of cellobiose were dissolved in 10 mL of sodium borate buffer (pH 8.2, 200 mM), into which fivefold sodium cyanoborohydride was added based on the mole of cellobiose, and the reaction mixture was stirred for 24 h. The resulting glucosylated PEI (GPEI) was purified using a dialysis membrane (MWCO 12,000~14,000) to remove unreacted cellobiose and the catalyst. The synthesis of GPEI was confirmed by ^{1}H NMR and the yield was over than 90%. Three different kinds of GPEI were prepared according to the glucose substitution degree, and the reaction scheme is shown in Fig. 1.

Determination of substitution degree

Copper chelate of PEI was quantified by spectrophotometry at 690 nm as described earlier (Kunath *et al.*, 2003). Briefly, 100 μL of GPEI solution was mixed with 100 μL of 20 mM copper acetate solution in a 96-well microtiter plate, and the resulting complexes were measured at 690 nm using an ELISA plate reader (GLR 1000, Genelabs Diagnostics, Singapore). The concentration of

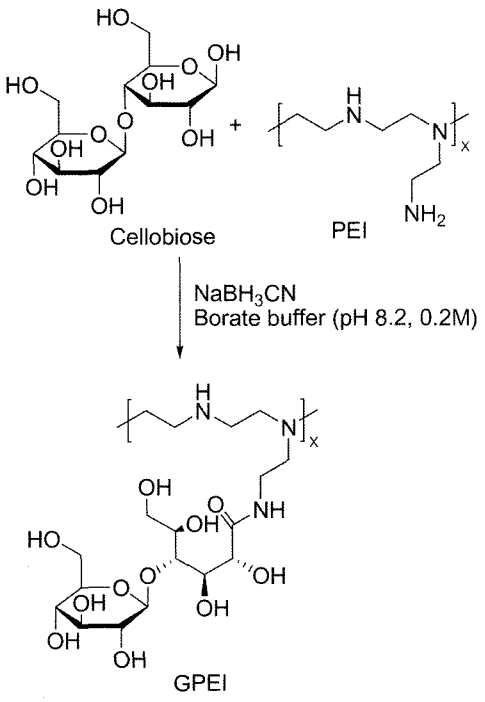

Fig. 1. Reaction scheme of PEI glucosylation

PEI in GPEI was calculated based on the calibration curves using PEI standards ranging between 0.1 and 2 mg/mL.

Glucose substitution was determined by the sulfuric acid micro-method (Monsigny *et al.*, 1988). GPEI was dissolved in PBS buffer, and 20 μL GPEI solution was placed in each well of a 96-well plate, to which 20 μL resorcinol (6 mg/mL in PBS), 100 μL of 75% sulfuric acid, and 50 μL of 2,6,10,14-tetramethyl-pentadecane (Pristane, Sigma, MO, U.S.A.) were added. The 96-well plate was incubated in a 90°C oven for 30 min, followed by cooling at room temperature for 30 min in the dark. Absorbance was measured at 492 nm using an ELISA reader, and the concentration was calculated based on the calibration curve with sugar as the standard. The substitution degree was calculated based on the glucose and nitrogen concentrations and expressed as % of total nitrogen present. GPEIs at substitution degrees of 12, 24, and 36 mol-%, namely GPEI-12, GPEI-24, and GPEI-36, respectively, were synthesized.

Formation of GPEI/DNA complexes

Complex formation was confirmed by electrophoresis on a 1.0% agarose gel using Tris-acetate (TAE) running buffer at 100 V for 45 min. For calculation of the charge ratios, an average mass per charge of 330 daltons was used for plasmid pGL3-Control, which was diluted to 100 mg /mL in 100 μL of phosphate-buffered saline (PBS, pH 7.4) containing 150 mM NaCl. GPEI was diluted at varying concentrations in 100 μL of the same buffer and added to the DNA solution while vortexing gently. The complexes were allowed to stand at room temperature for 30 min prior to further manipulation. DNA complexes containing 300 ng of plasmid were loaded into each well.

Measurements of dynamic light scattering (DLS) and zeta potential

Particle sizes and surface charges of GPEI/DNA complexes were assessed using an electrophoretic light scattering spectrophotometer (ELS 8000, Otsuka Electronics, Osaka, Japan). For the production of complexes, a predetermined amount of GPEI stock solution based on the required charge ratio was diluted with distilled water to prepare 1 mL of GPEI solution, which was then mixed with 1 mL of DNA (40 μg/mL, salmon testes, sodium salt) solution. The resulting solution was left at room temperature for 30 min for further experiments. Hydrodynamic diameter of the particles was obtained by determining the fluctuation in the intensity of the scattered light from the particles undergoing Brownian movement in the solution. Scattering light was detected at a 90° angle through a 400 μm pinhole. For data analysis, the viscosity (0.88 mPas) and the refractive index (1.33) of distilled water at 25°C were applied. The sample compartment was connected to a glass capillary cell. Under a constant voltage, the complexes migrated across the capillary, on which a beam of 10 mW helium-neon laser (633 nm) was focused at a constant angle of 15°. The zeta potential was automatically calculated based on the electrophoretic mobility. Experimental samples (4 mL) contained a final DNA concentration of 20 μg/mL.

Cell lines and cell culture

A549 human lung epithelial carcinoma cell line (Korean Cell Line Bank, Seoul, Korea) was incubated in RPMI 1640 containing 10% fetal calf serum (FCS, Hyclone, U.S.A.), 100 units/mL penicillin, and 100 μg/mL streptomycin at 37°C and 5% CO_2 atmosphere.

Evaluation of cytotoxicity

Cytotoxicity of uncomplexed GPEI was evaluated. A549 cells were seeded in triplicates at 5×10^3 cells/well into 96-well microtitre plates. Cells were incubated for 24 h prior to the addition of GPEI. GPEI-untreated cells in the media were used as positive references. The cell viability was measured through CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega, Madison, WI, U.S.A.). The absorbance was read at 540 nm using a micro-plate reader. A reference wavelength (620 nm) was used to reduce the background contributed by excess cell debris and other non-specific absorbances.

Transfection protocol

Transfection experiments were performed in triplicates. A549 cells were seeded at 5×10^4 cells/mL per well on a 24-well plate and grown to 60-70% confluency. GPEI/DNA complexes diluted with FCS and glucose-free RPMI 1640 were added to the culture plate, which was then incubated at 37°C for 6 h under a 5% CO_2 atmosphere. Subsequently, the transfection medium was replaced with a fresh serum-containing medium, and the plate was incubated at 37°C for additional 24 h under a 5% CO_2 atmosphere before the gene expression was determined.

Tumor-targeting gene delivery of GPEI/DNA complexes was confirmed by the assay of enzyme-dependent light production using a luciferase assay kit (Promega, Madison, WI, U.S.A.). Glucose (10 and 21.1 μM)-added GPEI/DNA complexes were transfected into A549 cells. The cells were then washed twice with PBS, added with 100 μL of lysis buffer (Promega), incubated at room temperature for 10 min, and centrifuged at 12000 × g. Twenty microliters of each sample was placed in a 5 mL polystyrene test tube, and the tubes were loaded into an automated luminometer Autolumat LB953 (EG & G Derthold, Germany). Luciferase substrate (100 μL) was automatically injected into each sample, and total luminescence

was measured for 10 s. Output was quantitated as relative light units (RLU). Protein concentration in the supernatant was determined using the BCA protein assay reagent (Pierce, Rockford, IL, U.S.A.).

RPMI 1640 containing GPEI/DNA complexes was mixed with 0, 20, and 40% FCS. After cell seeding on a 24-well plate, the medium was replaced with the GPEI/DNA complex-containing medium. After additional 24 h incubation, luciferase activity was measured to investigate the serum dependency of the transfection of GPEI/DNA complex into the A549 cell line.

Radiolabeling with ^{99m}Tc

Stannous chloride was dissolved in 0.2 N HCl (0.2 μg/μL, w/v). GPEI/DNA complex was then mixed with 10 μL stannous chloride solution in a vial at room temperature, followed by the addition of 111 MBq ^{99m}Tc-sodium pertechnetate (0.3 mL saline) for labeling. The mixture was allowed to further react for 30 min with occasional shaking. Labeling efficiency was determined using ITLC-SG chromatographic strips, with saline and acetone as the mobile phases 15 and 60 min post-reaction.

Imaging studies

Animal experiments were performed in accordance with the guidelines set by Wonkwang University School of Medicine Committee. Six-week-old female Balb/C mice (Harlan-Asia, Daehanbiolink, Co. Ltd., Seoul, Korea), each weighing 16-18 g, were housed in plastic cages under standard conditions and provided with water and food *ad libitum*. The animals were inoculated with mouse colon cancer CT-26 cells. Using a 30-gauge needle, approximately 2×10^6 trypsinized cells (100 μL) were injected subcutaneously into the left upper arm and left thigh of each mouse. Within 3 weeks, tumor volume reached approximately 1 cm^3. Mouse was sedated with intraperitoneally administered ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture. Static images were acquired 15, 30, and 60 min after the injection of 18.5 MBq ^{99m}Tc-DNA complex *via* the mouse tail vein using a gamma camera (Vertex; ADAC laboratories, Milpitas, CA, U.S.A.) set as follows: pinhole collimator, 5 mm; window setting, 140 keV; width, 20%; acquisition time, 120 s. The images were stored in a 512×512 matrix.

RESULTS AND DISCUSSION

All mammalian cell membranes contain proteins involved in the glucose transport, even though cells of different tissues use different transporting systems, as appears to be the case with cancer cells. Furthermore, an increased glucose uptake has been suggested in tumors, the characteristics of which have been used in the tumor-targeting drug delivery and imaging system (Hatanaka, 1974). One of the major differences between normal and cancer cells is the acquisition of facilitative glucose transporter (GLUT) genes through malignant transformation. Of the seven GLUT isoforms, the basic GLUT has been reported to play a major role, not only in the glucose uptake by tumor cells, but also in the tumor growth (Nogudhi *et al.*, 2000). Therefore, GPEI was synthesized by conjugating cellobiose with PEI to increase the tumor-targeting ability by incorporating glucose moiety into PEI. Through this process we attempted to decrease the cytotoxicity of PEI used as a gene carrier by reducing the ratio of toxic primary amino group without changing the number of overall amino groups of PEI. In actuality, glucose-conjugated niosomes showed higher tumor-to-muscle contrast-to-noise ratio compared to the non-conjugated ones, suggesting that this accumulation of tumor in the glucose-conjugated niosomes could be the result of interaction between glucose moieties in the glucose-conjugated niosomes and GLUT receptors (Luciani *et al.*, 2004). Furthermore, Kim *et al.* reported that GPEI clearly enhanced the gene delivery activity, as demonstrated by GFP expression, as well as increased the PTEN protein expression in the lung after aerosol delivery of GPEI/DNA complex (Kim *et al.*, 2004).

Particle sizes and distributions of GPEI/DNA complexes were measured through dynamic light scattering (Table I), and results revealed that the particle sizes of GPEI/DNA complex were largely dependent on the degree of glucosylation, increasing with increasing glucose content of GPEI. It is suggested that increase in the glucose content of GPEI hindered the compact complexation between GPEI and DNA or sugar moieties interfered with electrostatic condensation between DNA and GPEI. In addition, the particle sizes of all GPEI/DNA complexes decreased with the increase in the charge ratio, which indicates that strong positive charge induced the compaction of the complex and thus prevented unnecessary interactions between the neighboring complexes, resulting in the aggregation of the complex. This slightly increased particle sizes of the GPEI/DNA complex, in certain pathological sites of the body, such as tumors, infarcts, and inflammations, might be beneficial to the delivery of gene into the interstitium through leaky vasculatures (Baban *et al.*,

Table I. Particle sizes (nm) and distribution of GPEI/DNA complex

Mixing Charge Ratio (N/P)	PEI	GPEI 12 (mol-%)	GPEI 24	GPEI 36
2	280.5 ± 175.4	-	-	-
3	97.1 ± 26.0	286.2 ± 58.8	970.8 ± 520.2	359.8 ± 68.0
6	103.2 ± 22.3	197.7 ± 75.6	249.8 ± 70.4	262.2 ± 99.7
9	78.6 ± 17.2	135.5 ± 29.6	256.8 ± 80.1	278.5 ± 105.8

1995). It has also been speculated that large therapeutic agents (approximately 100-300 nm in diameter) such as gene carriers and drug-encapsulated carriers could be selectively delivered to the tumor (Hobbs *et al.*, 1998).

Similar to the PEI/DNA complex, TEM revealed GPEI/DNA complex was dense and compact, with small, uniform particle sizes (data not shown). This result indicates that glucosylation (approximately 36%) of the primary amino group of PEI did not hinder the ionic interaction between GPEI and DNA. Therefore, we suggest that glucosylation of PEI transformed the primary amino group into the secondary amino group with lower pKa value, which also might be protonable due to the Donnan effect caused by the phosphate groups of DNA within the micro-environment around the complex. This suggestion is supported by the finding that zeta potential profiles of GPEI/DNA complexes were not different from that of PEI/DNA complex, irrespective of the glucosylation degree (Fig. 2).

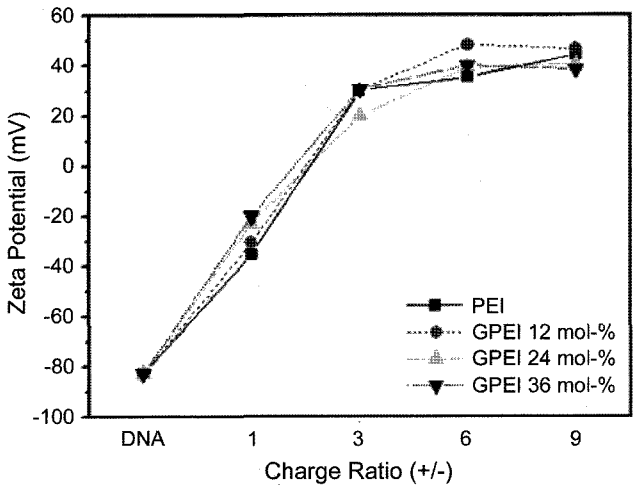

Fig. 2. Zeta potential profiles of GPEI/DNA complex according to the glucosylation degree

The influence of charge ratio on DNA condensation by GPEI was determined by agarose gel electrophoresis (Fig. 3). Similar to the zeta potential data, complexes were formed between DNA and GPEI, irrespective of the glucosylation degree of GPEI. The fact that glucosylation of PEI did not interfere with the complex formation implies that, as with the primary amino group of PEI in the complex, the secondary amine of GPEI, produced through the reductive amination reaction between PEI and cellobiose, could be protonable, a tendency similar to the zeta potential data.

Cytotoxicity of GPEI was compared to that of the unmodified PEI through MTT assay over a wide range of uncomplexed polymer concentrations (Fig. 4). Results

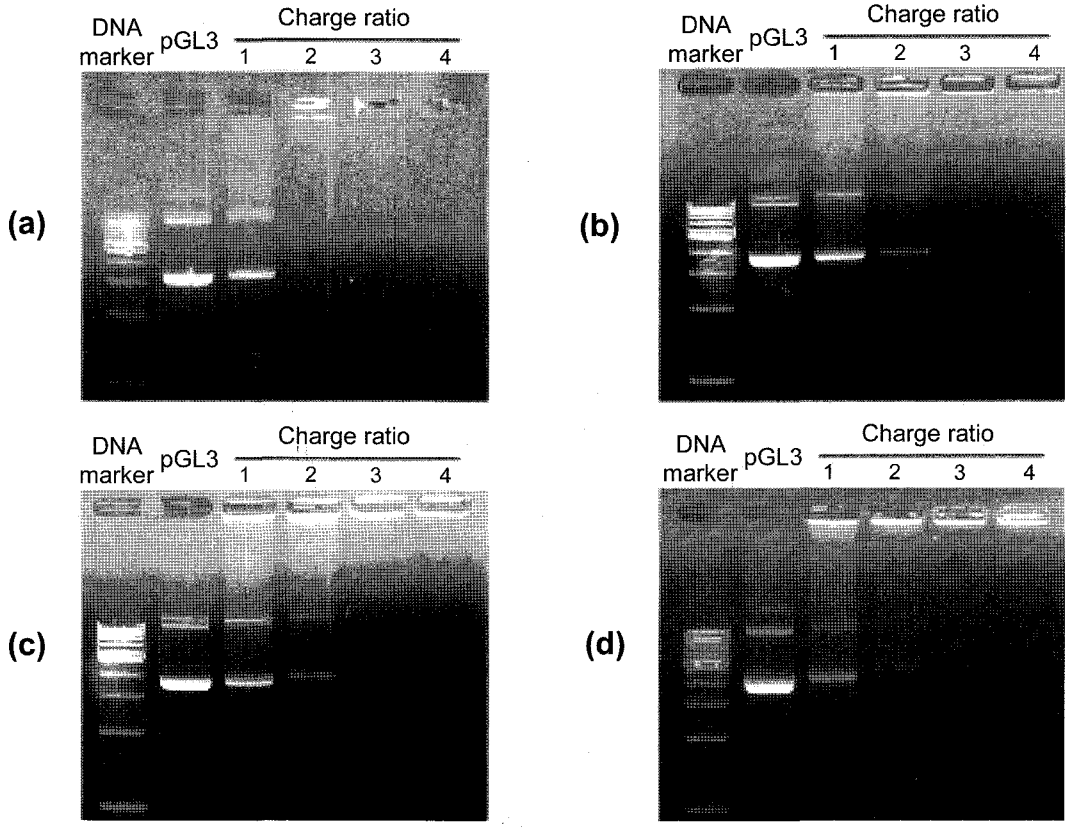

Fig. 3. Electrophoresis of GPEI/DNA (pGL3-control) complexes at various charge ratios: (a) PEI/DNA complex; (b) GPEI-12/DNA complex; (c) GPEI-24/DNA complex; (d) GPEI-36/DNA complex

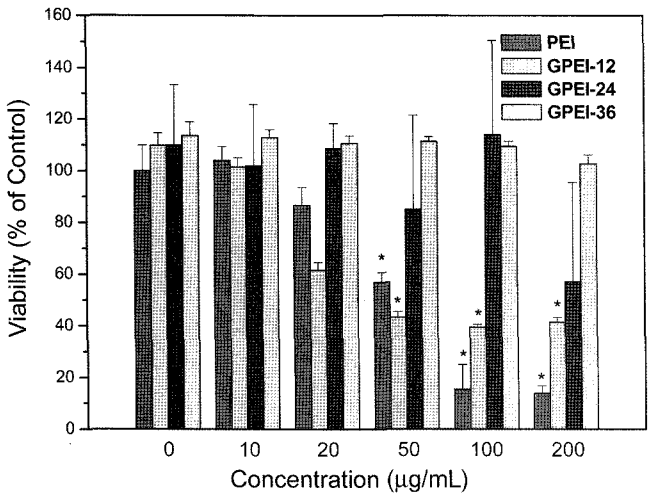

Fig. 4. Effect of GPEI concentration on the cytotoxicity of A549 cell line (n=3; * denotes $p < 0.001$ vs. control)

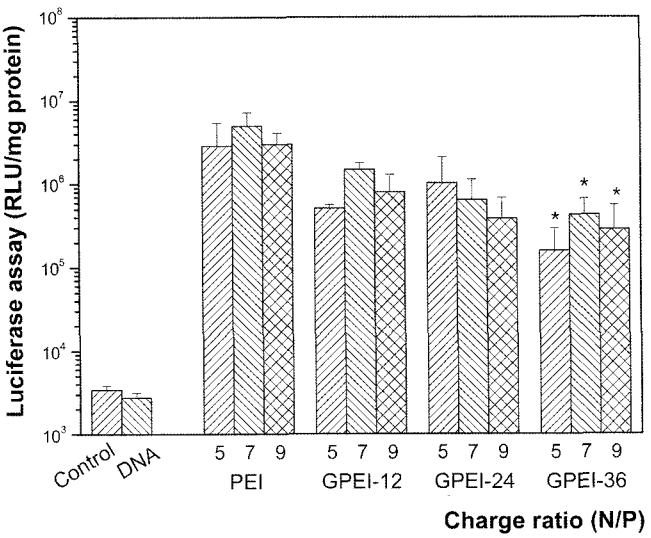

Fig. 5. Effect of glucosylation degree on the transfection efficiency of A549 lung cancer cell line (n=3; * denotes $p < 0.05$ vs. PEI control at the same charge ratio).

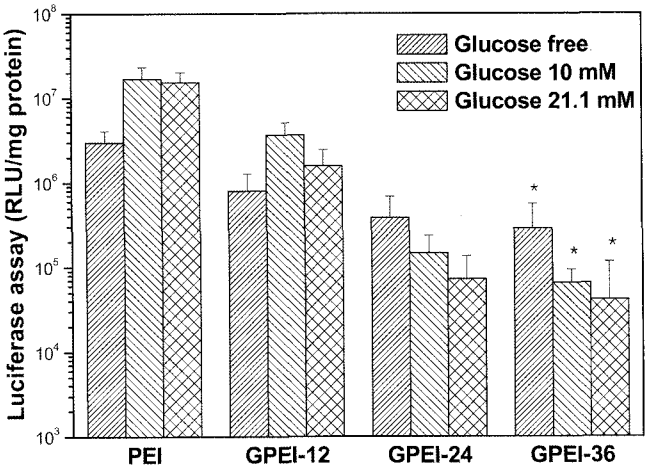

Fig. 6. Luciferase gene delivery of GPEI/DNA complex into A549 cells at various glucose concentrations. Data are presented as means ± S.D. (n=3) of values measured (n=3; * denotes $p < 0.05$ vs. PEI control).

showed that cytotoxicity was strongly dependent on the substitution degree. Viability was drastically decreased at over 50 μg/mL for PEI, whereas GPEI-36 had no effect on the cells even at polymer concentrations higher than 200 μg/mL. GPEI-12 with lower substitution degree exerted higher cytotoxicity than GPEI-24. Furthermore, glucosylation of PEI did not change the surface charge profiles of GPEI as with PEI, which shows that GPEI glucose does not possess charge-shielding effect, possibly due to the insufficiently short hydrophilic glucose moiety grafted onto PEI. The abrupt reduction in the cytotoxicity of GPEI is thought to have been caused by the conversion of the primary amino group, which had a positive charge under physiological conditions, into the secondary amino group, which is protonable only by the phosphate group of DNA. These data also suggest that the primary amino group is closely related to the cytotoxicity (Ahn *et al.*, 2004) ; GPEI and galactosylated PEI with higher substitution degree had the same cytotoxic tendencies (Kunath *et al.*, 2003).

Luciferase reporter gene expression of A549 human lung epithelial carcinoma cell lines, whose glucose is facilitatively metabolized by a GLUT receptor, is shown in Fig. 5. Glucosylation induced less than one-order decrease of the transfection efficiency than without glucosylation. Also, as a whole, the transfection efficiency of the complexes was decreased with an increase of glucosylation degree in GPEI owing to the increase of hydrophilicity on the surface of the complex and reduction in non-specific ionic interaction between the positively charged complex and the negatively charged cell surface membrane. Another possibility of the lower transfection efficiency of GPEI-36 may be due to the larger particle sizes of the complex. Competition assay was performed to investigate the role of GLUT-mediated gene delivery in the transfection of GPEI/DNA complex into A549 cell lines (Fig. 6). In general, transfections in GPEI-12 with lower glucosylation as well as in PEI were not influenced by the glucose concentration of the medium; however, at 10 mM glucose, transfection increased due to the facilitated proliferation of the cell line, while PEI-24 and PEI-36 with higher glucosylation showed decrease of transfection with increasing glucose concentration. Gene expression was strongly dependent on the cell cycle stage, during which non-viral-based gene transfer occurs. Furthermore, non-viral vectors were more easily transfected into the actively dividing cancer cell lines than into non-proliferating cells (Ogris *et al.*, 2002; Pelisek *et al.*, 2002). However, even at high-glucose state required for proliferation, transfection of

GPEI/DNA complex with higher glucose composition into the A549 cell line decreased. Particularly in the case of GPEI-36, 10 mM glucose induced a drastic decrease (about 20%) in transfection, which indicates that GLUT-mediated gene delivery was partly involved in the transfection of GPEI/DNA complex. Although transfection of GPEI/DNA complex was maintained to some degree (about 10% compared to the transfection under glucose-free condition) even at higher glucose concentration (21.1 mM), indicating that two mechanisms are involved in the transfection of GPEI/DNA complex into the cells, one being GLUT-glucose ligand interaction and the other non-specific ionic interaction. However, further experiments should be required to elucidate these mechanisms.

Transfection of non-viral gene therapy *in vivo* was mainly inactivated through the interaction with anionic components in the serum and complement activation, resulting in the RES uptake (Nomura *et al.*, 1997). Therefore, transfection of GPEI/DNA complex in the presence of serum was examined (Fig. 7). Transfections of GPEI/DNA and PEI/DNA complexes drastically decreased in the presence of serum, except for GPEI-36/DNA complex, which showed slightly slower drop in transfection than the others. These data suggest that the grafting of disaccharide into PEI had little preventive effect on the interaction with the serum due to the highly positively charged surface even after glucosylation. Therefore, additional grafting of a longer hydrophilic chain with minimum substitution degree should prevent this interaction.

Up to now, information on the *in vivo* distribution of gene complexes have mostly been derived from sacrificed animals. Recently, however, molecular imaging techniques have been applied to gene therapy, molecular medicine, and biotechnology to non-invasively validate the *in vivo* distribution of delivered complexes in living animals. To

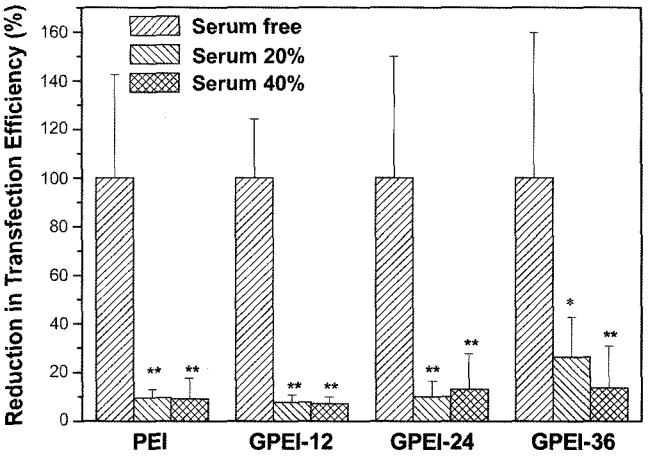

Fig. 7. Serum-dependent luciferase gene delivery of GPEI/DNA complex (charge ratio = 9) n=3; * and ** denote $p = 0.17$ against PEI at serum 20% and $p < 0.05$ vs. serum free control, respectively.

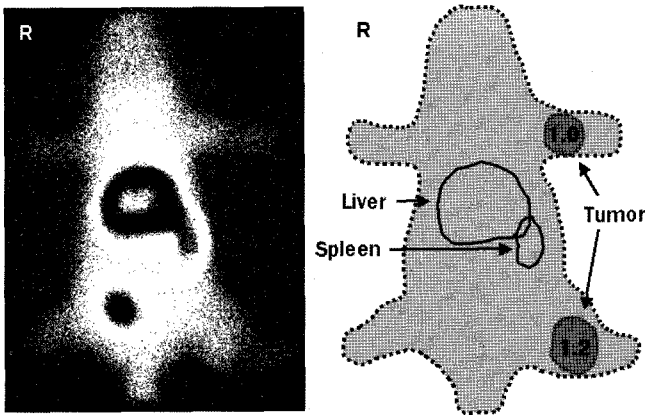

Fig. 8. Gamma image (left) of ^{99m}Tc-GPEI/DNA complexes in CT26 colon cancer-bearing Balb/C mouse and an explanation diagram of gamma image (right). R, right; Arabic number within the tumor in the right diagram, size (cm).

verify the *in vivo* biodistribution based on imaging system of GPEI/DNA complex, we selected the nuclear technique using ^{99m}Tc for its sensitive and easily acquired images. Because GPEI/DNA complex has secondary amines, and these secondary amines can donate lone pairs of electrons to form coordinate covalent bonds with ^{99m}Tc, we were able to acquire gamma images with ^{99m}Tc, through which the distribution of GPEI/DNA complexes in CT26 colon cancer-bearing Balb/C mice was ascertained. The ^{99m}Tc-GPEI/DNA complexes were mainly detected in the liver and spleen after the injection and, to some degree, also in the tumor site (Fig. 8) whereas the ^{99m}Tc-PEI/DNA complexes were almost detected in the liver and spleen (data not shown). Furthermore, compared to the activity in the contralateral side, tumoral activity was higher, and the intensity of the uptake increased as the tumor size increased.

CONCLUSIONS

Evaluation of the potential of glucosylated PEI as a tumor-targeting gene carrier suggests that the transfection of GPEI/DNA complex into tumor cells partially occurred through a specific interaction between glucose transporters of the cells and glucose ligand of GPEI.

ACKNOWLEDGEMENTS

This research was supported by a grant from the Nuclear Energy R & D program, Ministry of Science and Technology, Korea (M20203200028-02A0702-00411). Y.K. Kim was also supported by Brain Korea 21 Program.

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