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Preparation and characterization of magnetic cationic liposome in gene delivery

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ABSTRACT

Low transfection efficiency in vivo and failure to deliver therapeutic nucleic acids to the target organs limit the use of cationic liposomes (CLs) in gene therapy. Magnetic drug targeting (MDT) was applied in this study to improve the transfection efficiency and overcome the limitations. Magnetic cationic liposomes (MCLs) were prepared by incorporating MAG-T (magnetite) into CLs. The inclusion of relatively high concentration of MAG-T significantly increased the size of liposomes/lipoplexes, reduced the zeta potential, and decreased the cell viability. The transfection efficiency of MCLs in gene delivery was evaluated by using plasmid DNA (pDNA) containing a luciferase reporter gene in THLE-3 cells. Results suggested that the transfection efficiency of MCLs/pDNA complexes with a relatively lower content of MAG-T (0.75 mg/ml) was the same as that of CLs/pDNA complexes without a magnetic field but was higher (about 2.6-fold) with magnetic induction. Finally, using MCLs/pDNA complexes and a static magnetic field placed over the liver of rats, luciferase reporter gene expression in the liver increased as compared to MCLs/pDNA complexes and CLs/pDNA complexes in the absence of an external magnetic field.

1. Introduction

Cationic liposomes (CLs) are usually employed as a gene delivery system due to their low toxicity and immunogenicity, potential for oncogenicity, size independent delivery of nucleic acids (from oligonucleotides to artificial chromosomes), ease of preparation, and quality control (Kostarelos and Miller, 2005). However, cationic liposomes result in relatively poor transfection efficiency in vivo and are a failure to deliver therapeutic nucleic acids to their desired site of action in cells of the target organs of choice. To improve tissue selectivity or introduce foreign plasmid DNA (pDNA) into specific cell, some recent studies have been focused on the engineering surface proteins or coupling targeting ligands such as asialoglycoprotein, lactose, transferrin, epidermal growth factor, and antibodies (Watanabe et al., 2007; Shigeta et al., 2007; Lu et al., 2007). These surface modifications usually require specialized devices and time-consuming processes to fabricate the substrate. Hence in practice, these modalities of targeting are often insufficient for achieving the sustained expression levels required by many applications (Scherer et al., 2002).

Magnetic drug targeting (MDT) uses paramagnetic particles as drug carriers, guides their accumulation in target tissues with strong local magnetic fields, and has been used in the treatment of cancer patients with some success (Fortin-Ripoche et al., 2006; Lübbe et al., 2001). Some researchers have applied MDT to gene vectors and have demonstrated its applicability to gene delivery (Lübbe and Bergemann, 1998; Scherer et al., 2002). Hirao et al. (2003) developed magnetic cationic liposomes (MCLs) by incorporating magnetite particles into small cationic liposomes and a gene delivery system combining MCLs and magnetic induction. This gene delivery system was found to achieve enhanced transfection efficiency in human osteosarcoma cells.

For magnetic targeting system, the most important parameters that should be considered are (Dandamudi and Campbell, 2007a; Alexiou et al., 2000): (a) the concentration and type of ferro fluid employed, (b) the magnetic strength of the external magnetic field, and (c) the length of time the target tissue is exposed to the external magnet. All the three parameters were carefully selected and optimized for targeted purpose.

In the present study, in order to develop a gene delivery vehicle that is (a) capable of improving the transfection efficiency of conventional cationic liposomes in human normal cells, (b) capable of responding to an external magnetic field, and (c) relatively specific to liver as the target organ in vivo experiment, MCLs were developed by incorporating MAG-T (magnetite fluid, tartaric acid matrix) into CLs and their cytotoxic activities were measured using MTT assay. MCLs/pDNA complexes with different concentration of ferro fluid were prepared. The influences of MCLs/pDNA mixing ratio, incu-

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bation time, and magnetic induction time on in vitro transfection efficiency of MCLs/pDNA complexes were evaluated by measuring the luciferase activity. In addition, the in vivo transfection efficiency of MCLs/pDNA complexes was investigated.

2. Materials and methods

2.1. Materials

Dioleoylphosphatidylethanolamine (DOPE) and 3β-[*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Fluid MAG-T (aqueous dispersion of magnetite Fe_2O_3; tartaric acid matrix; mean diameter, 20 nm) was kindly provided by Dr. Zhang Yong (Southeast University, Jiangsu, China). Permanent magnets were provided by Shanghai institute of microsystem and information technology (Shanghai, China). For in vitro and in vivo experiments, 0.3 T (Tesla) and 0.45 T neodymium magnets were used, respectively. pCMV-Luc was constructed by subcloning the Hind III/Xba I firefly luciferase cDNA fragment from pGL3-control vector (Promega, WI, USA) into the polylinker of pcDNA3.0. pDNA was amplified in the *Escherichia coli* strain DH5α, isolated, and purified using a QIAGEN Plasmid Giga Kit (QIAGEN, Hilden, Germany). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from GIBCO (Invitrogen Co., CA, USA). All other reagents were of analytical grade.

2.2. Cell culture and media

The THLE-3 cell line (the human normal liver cells) was purchased from ATCC (American Type Culture Collection, VA, USA). THLE-3 cells were maintained in DMEM supplemented with 10% FBS, 25 mM HEPES buffer, 100 U/ml penicillin and 100 μg/ml streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C.

2.3. Preparation of MCLs and MCLs/pDNA complexes

Liposomes composed of DC-Chol together with DOPE have been classified as one of the most efficient vectors for the transfection of pDNA into cells and in clinical trials. In the present study, DC-Chol and DOPE (1:1 molar ratio) were chosen as the liposome composition and MAG-T was used as the core, and the MCLs were prepared using reverse phase evaporation. In brief, DC-Chol and DOPE (around 60 μmol) were dissolved in chloroform/ether (9:1, v/v). Various concentrations of MAG-T (0.75, 1.5, and 3 mg/ml) in 2.5 ml 5% dextrose solution were added into the lipid solution and the resulting mixture was emulsified by sonication. The organic phase was evaporated at 25 °C using a rotary evaporator under reduced pressure (Buchi R 200/205, Tokyo, Japan), and 3 ml 5% dextrose solution was added into the mixture of lipid and MAG-T colloid. The suspension was sonicated for 15 min and then centrifuged at 1000 × *g* for 15 min, which precipitated unincorporated MAG-T and retained the MCLs in the supernatant (Nobuto et al., 2004). Thereafter, MCLs were passed through a 0.22 μm pore size filter (MILLEX HV filter unit, Durapore PVDF membrane, Millipore Corporation, MA) to sterilize and then stored at 4 °C. All MCLs concentration values were expressed as the net magnetite concentration. CLs composed of DC-Chol and DOPE (1:1 molar ratio) were prepared with the same method except that the MAG-T suspension was replaced by 5% dextrose solution.

The MCLs/DNA complexes were prepared by mixing pDNA and MCLs in various ratios (typically corresponding to weight ratio of lipid:pDNA from 1:1 to 10:1) in serum-free DMEM, and the mixture was incubated at room temperature for 30 min (Salvati et al., 2006). The CLs/pDNA complexes were prepared with the same procedure.

2.4. Zeta potential and particle size analyses

The zeta potential and particle size of CLs, MCLs, MCLs/DNA complexes, and CLs/pDNA complexes were measured using a Zetasizer Nano S (Malvern Instruments, UK) after the samples were diluted with water. The mean particle sizes of samples were determined using the dynamic light scattering, and the zeta potential of them was automatically calculated from the electrophoretic mobility.

2.5. Transmission electron microscopy (TEM) and energy dispersive spectrometer (EDS)

TEM studies were carried out on JEM-2010 instrument (JEOL Ltd., Japan). The MCLs/DNA complexes were drop cast onto a copper grid (mesh size-200) with Formvar copper support film. After deposition the dispersion was blotted away with a strip of filter paper, and air-dried at room temperature before observing under the microscope. Characteristics of complexes were measured by TEM computer magic image analysis system and energy dispersive spectrometer (EDS). Detecting conditions for EDS were as follows: accelerating voltage 200 keV; take off angle 3.99°; live time 198.9 s; dead time 69.88 s.

2.6. Cytotoxicity studies

THLE-3 cell suspensions (100 μl) were seeded at 1×10^5 cells/ml in each well of a 96 well plate. Cells were allowed a sufficient amount of time to adhere to well plate and cells were treated with different amounts of liposomes (typically from 0.4 to 4 μg per well, corresponding to weight ratio of lipid:pDNA from 1:1 to 10:1). After incubation for 24 h, plates containing cells were washed with phosphate buffer saline (PBS) to remove unbound liposomes and cellular debris. Cell viability was assayed using MTT [3-(4,5-s-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] as described by Mosmann with minor modifications (Suzuki et al., 2007). Briefly, MTT (5 mg/ml, 10 μl) was added to each well and the cells were incubated at 37 °C for 4 h. The formazan product was dissolved in 100 μl of 10% sodium dodecyl sulfate (SDS) containing 15 mM HCl. Color intensity was measured using a microplate reader (ELx800, Biotek, USA) at test and reference wavelengths of 595 and 655 nm, respectively.

2.7. In vitro transfection experiment

Prior to transfection experiment, THLE-3 cells were seeded in 24 well sterile culture plates at a density of 2×10^5 cells/well and grown overnight to approximately 80% confluency. At the time of transfection, the medium in each well was removed and the cells were washed twice with preheated PBS. The complexes with various weight ratios (1:1–1:10 for pDNA: lipid) in serum-free DMEM were added to the wells (1.6 μg pDNA per well) and cells were incubated for various periods of time (1, 4, 6, 8, and 12 h). After transfection, the incubation medium was replaced with DMEM supplemented with 10% FBS and incubated for another 24 h prior to evaluation of transfection efficiency using luciferase assay.

For magnetic induction of MCLs/DNA complexes and CLs/pDNA complexes, a permanent magnet of strength 0.3 T was placed under the cell culture plate. The magnet was removed 10, 30, and 60 min after addition of transfection suspension and the incubation continued for a total of 8 h. The following procedures were the same as previously described.

For the analysis of luciferase activity, the transfected cells in each well were carefully washed twice with 3 ml of PBS and the cells were lysed with 200 μl of cell culture lysis buffer (Promega, WI, USA). The luciferase activity in a 20 μl aliquot of cell lysate was measured with a Minilumat LB9506 luminometer (EG&G Berthold, BadWilbad, Germany) and a luciferase assay kit (Promega, WI, USA). The protein concentration of cell lysate was determined with a modified Lowry method using BSA as a standard (Wang and Smith, 1975). The luciferase activity in each sample was indicated as the relative light unit (RLU) per milligram of protein.

2.8. In vivo gene expression

Male Wistar rats (120–160 g) were randomly divided into 3 groups (six rats per group) and intravenously injected with 50 μg pDNA (pCMV-Luc) per rat. In group 1, the rats were intravenously injected with CLs/pDNA complexes; in group 2, the rats were intravenously injected with MCLs/DNA complexes (MCLs, 1.5 mg/ml) in the absence of an external magnet; in group 3, the rats were intravenously injected with MCLs/DNA complexes (MCLs, 1.5 mg/ml) in the presence of an external magnet (0.45 T) that was placed over the liver for 4 h to recruit complexes to the liver (Babincová et al., 2000; Plassat et al., 2007). Twenty-four hours later, rats were sacrificed, and the major organs were removed. The organs were washed twice with cold saline, and then homogenized with lysis buffer (0.1 M Tris–HCl, 2 mM EDTA and 0.1% Triton X-100, pH 7.8) using a homogenizer. The homogenates were centrifuged at 10,000 × *g* for 10 min at 4 °C. Sixty microliters of the supernatant was subjected to measurement of luciferase activity as described in Section 2.7. The protein content of each supernatant was also determined.

2.9. Statistical analysis

The statistical significance of the data was evaluated with Student's *t*-test or ANOVA test. A P value < 0.05 was considered statistically significant.

3. Results and discussion

3.1. Physicochemical characterization

It has been shown that the transfection efficiency of CLs/pDNA complexes is strongly affected by some physicochemical parameters of the complexes such as the charge of cationic liposomes, the size of the complexes (Kennedy et al., 2000; Rejman et al., 2004; Higuchi et al., 2006), and the total lipid/pDNA ratio (Kennedy et al., 2000; Koltover et al., 1999). These parameters can monitor liposome or lipoplexes stability and reproducibility, and reflect their performance in vivo (Heyes et al., 2002). For MCLs/DNA complexes, whether MAG-T is incorporated into the aqueous core or liposome bilayer may influence the extent to which pDNA is incorporated. Moreover, the amount of MAG-T incorporated into liposomes must be high enough for responding to an external magnetic field (Dandamudi and Campbell, 2007b). For these reasons, the mean particle sizes and zeta potential (surface charge potential) of MCLs with three different concentrations of MAG-T (0.75, 1.5, and 3 mg/ml) and CLs were measured. Complexes prepared by mixing these MCLs or CLs with pDNA at a weight ratio of 8.0 (lipid:pDNA) were also examined.

As shown in Table 1, the means of particle sizes of both MCLs and MCLs/pDNA complexes were higher than those of CLs and CLs/pDNA complexes, respectively. Lower concentrations (0.75 mg/ml) of MAG-T had no effect on mean diameter of CLs and CLs/pDNA complexes; whereas higher concentrations of MAG-T (1.5 and 3 mg/ml) significantly increased the mean diameter. The means of particle sizes of both MCLs/pDNA complexes and CLs/pDNA complexes were higher than those of MCLs and CLs, respectively. These might be due to the coherence and fusion of pDNA with the liposomes (Salvati et al., 2006).

The zeta potential of MCLs and MCLs/pDNA complexes was found to be significantly decreased as compared to that of the CLs and CLs/pDNA complexes, respectively (Table 1). The inclusion of MAG-T at 0.75 mg/ml resulted in the most significant decrease in zeta potential for MCLs and MCLs/pDNA complexes compared to those without MAG-T (CLs and CLs/pDNA complexes). The zeta potential of MCLs and MCLs/pDNA complexes was decreased when the MAG-T content was increased. However, the zeta potential for liposomes and complexes containing 0.75 mg/ml MAG-T was not significantly different from those containing 1.5 mg/ml ($P > 0.05$). These findings suggested that MAG-T interacted with the (cationic) polar head group region of the bilayer in the CLs and neutralized some of the positive surface charge characteristics due to charge shielding (Dandamudi and Campbell, 2007b).

3.2. TEM image and energy dispersive spectrometer of MCLs/pDNA complexes

The TEM photograph of MCLs and MCLs/pDNA complexes was shown in Fig. 1. MCLs and MCLs/pDNA complexes appeared to be spherical. TEM image and the corresponding energy dispersive spectrometer analysis of two parts in the image confirmed incorporation of MAG-T into the CLs and association of MAG-T and pDNA with the CLs.

3.3. Viability studies

The effects of MCLs (with three different concentrations of MAG-T) and CLs on THLE-3 cell viability at various amounts of MCLs or CLs (corresponding to the different weight ratios of lipid:pDNA) were evaluated. No toxic effects were observed on cell growth against THLE-3 cells for MCLs (0.75 mg/ml) and CLs at any amount tested here. For MCLs (3 and 1.5 mg/ml), no toxic effects on cell growth were observed at the amount ≤1 μg/well (corresponding to weight ratio 2.5) (Fig. 2), but decreased cell viabilities were observed as the amount increased to 2 μg/well (corresponding to weight ratio 5.0) ($P < 0.05$). At the amount of 1 μg/well (corresponding to weight ratio of 2.5), the percentages of viable cells after exposure to MCLs (3 mg/ml), MCLs (1.5 mg/ml), MCLs (0.75 mg/ml) and CLs were 87, 86, 97, and 87%, respectively, compared to 59, 61, 78, and 80%, respectively, at the amount of 4 μg/well (corresponding to weight ratio of 10) (Fig. 2).

This result indicated that relatively higher amount of MAG-T (≥1.5 mg/ml) incorporated into CLs could result in higher cell toxicity to THLE-3 cells while this toxicity could be reduced when the amount of MAG-T incorporated was 0.75 mg/ml.

3.4. In vitro transfection experiment

Many experimental factors affected the transfection efficiency of cationic liposomes-mediated gene delivery, including type of cell transfected, pDNA to CLs ratios, liposome concentration, and incubation time (Caplen et al., 1995). Among these factors, the cationic liposome charge primarily determined by the pDNA to liposome ratio should be the most variable for optimizing in vitro transfection protocols (Caplen et al., 1995). The MCLs/pDNA complexes structure was more suitable for cellular uptake, and the surrounding lipids might protect the pDNA from intracellular degradation (Sternberg et al., 1994). In addition, for magnetic target system, the magnetic induction time could be an important parameter for the optimization of the magnetic induction. All of these variables should be

Table 1
The mean particle size and zeta potential values of liposomal preparations.

Group	Sample	Size (nm)	Zeta potential (mv)	Group comparisons	*P* value	
					Sizes	Zeta potential
1	CLs	122.3 ± 1.60	83.8 ± 0.45	1 and 2	NS	$P<0.01$
2	MCLs (0.75 mg/ml)	128.4 ± 4.27	78.6 ± 0.34	2 and 3	NS	NS
3	MCLs (1.5 mg/ml)	133.3 ± 1.19	78.0 ± 0.34	3 and 4	$P<0.05$	$P<0.05$
4	MCLs (3 mg/ml)	139.7 ± 2.66	75.7 ± 0.87	1 and 3	$P<0.01$	$P<0.01$
5	CLs/pDNA complexes	201.1 ± 4.88	51.3 ± 0.31	5 and 6	NS	$P<0.01$
6	MCLs/pDNA complexes (0.75 mg/ml)	207.3 ± 4.40	38.0 ± 0.45	6 and 7	NS	NS
7	MCLs/pDNA complexes (1.5 mg/ml)	215.4 ± 3.93	37.5 ± 0.50	7 and 8	$P<0.01$	$P<0.05$
8	MCLs/pDNA complexes (3 mg/ml)	238.3 ± 2.98	35.5 ± 0.87	5 and 7	$P<0.05$	$P<0.01$

taken into account and adjusted to their optimal value for efficient pDNA delivery in different cell lines (Hirao et al., 2003; Peters et al., 1999).

3.4.1. Optimization of MCLs/pDNA mixing ratio

pDNA was mixed with MCLs in different mixing ratios (w/w) to form MCLs/pDNA complexes, and the effect of mixing ratio on transfection efficiency was investigated at MCLs concentrations of 0.75, 1.5, and 3 mg/ml. The incubation time for transfection was 8 h. At the MCLs concentration of 0.75 mg/ml, MCLs/pDNA complexes showed greater luciferase activity at a mixing ratio of 5:1 whereas for MCLs/pDNA complexes (MCLs, 1.5 and 3 mg/ml) and CLs/pDNA complexes, the maximum luciferase activities were observed at a mixing ratio of 2.5:1 (Fig. 3).

3.4.2. Optimization of incubation time

Using MCLs/pDNA complexes with the optimal mixing ratio, the effect of incubation time on transgenic efficiency was investigated. For MCLs/pDNA complexes (MCLs, 3, 1.5, and 0.75 mg/ml) and CLs/pDNA complexes, maximal luciferase activity was observed at 4, 6, and 8 h incubation, respectively (Fig. 4). The optimal incubation time reduced as the content of MAG-T increased, which might be due to the incorporation of MAG-T resulting in an increased accumulation of lipoplexes on cell surface by gravity.

3.4.3. Comparison of transfection efficiency under optimal transfection condition in THLE-3 cells

Transfection efficiencies of MCLs/pDNA complexes (with three different concentrations of MAG-T) and CLs/pDNA complexes were compared by luciferase assay using the optimal mixing ratio and incubation time as in previous studies. As shown in Fig. 5, the luciferase activities in the cells transfected with MCLs/pDNA complexes (MCLs, 0.75 mg/ml) and CLs/pDNA complexes were comparable while with MCLs/pDNA complexes (MCLs, 1.5 and 3 mg/ml), the luciferase activities were relatively lower compared to the former two lipoplexes. This indicated that the behavior of MCLs/pDNA complexes with a relatively lower content of MAG-T was the same as that of CLs/pDNA complexes in the absence of a magnetic field, which was consistent with the result of cell viability. In the cytotoxicity studies, MCLs or CLs were added into the cells without pDNA, but cell toxicity was increased when MCLs/pDNA complexes or CLs/pDNA complexes were added into the cells (data not shown). These findings were consistent with the reports that the delivery of pDNA could cause extra cell toxicity (Tanswell et al., 1998). Therefore, the relatively lower gene transfection efficiency of MCLs/pDNA complexes with higher MAG-T content (1.5 and 3 mg/ml) might be due to the extra cellular toxicity as discussed in the cell viability (Caplen et al., 1995). Another reason for this result might be that complexes with higher MAG-T content

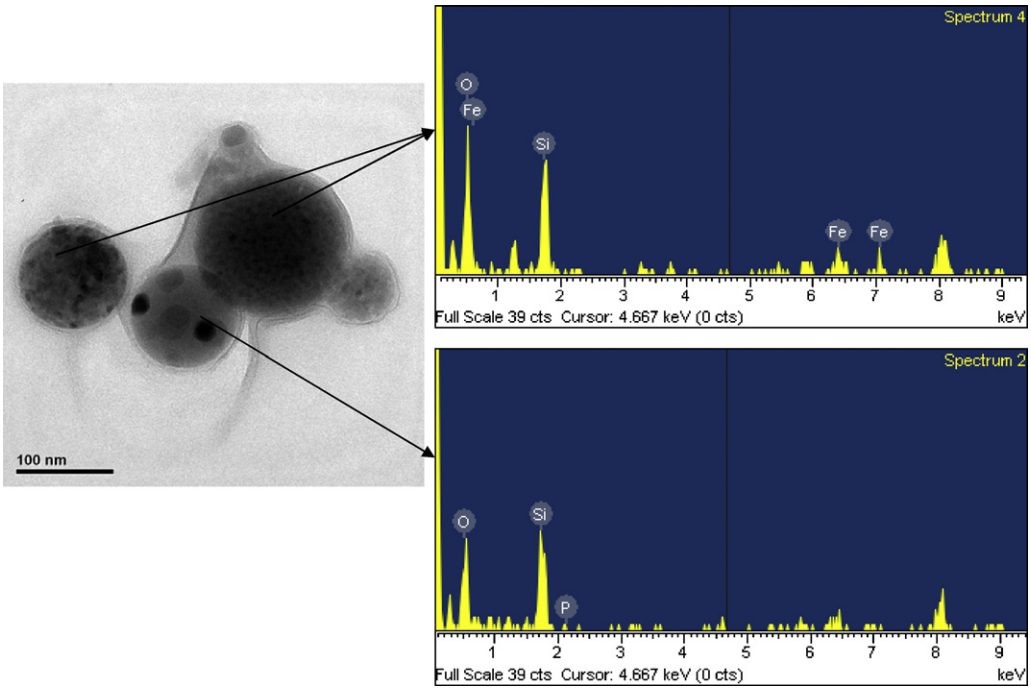

Fig. 1. Transmission electron micrograph and energy dispersive spectrometer analysis of MCLs/pDNA complexes. The concentration of MAG-T in the MCLs was 1.5 mg/ml and the weight ratio between MCLs and pDNA was 8.0.

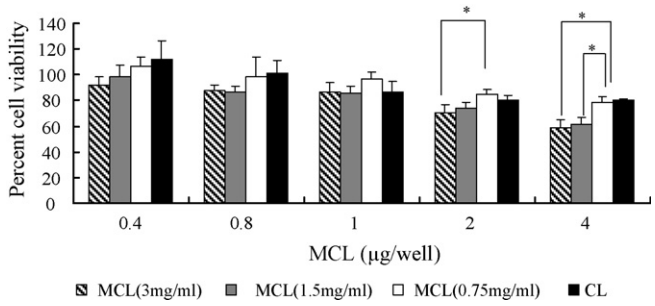

Fig. 2. Percent cell viability of THLE-3 cells treated with MCLs or CLs: Cells were seeded at 1×10^5 cells/ml in a 96 well plate and incubated at 37 °C. Percent of cell viability was determined following 24 h of exposure to various amounts of MCLs (0.4, 0.8, 1, 2, and 4 μg per well, corresponding to weight ratio of lipid:pDNA 1:1, 2:1, 2.5:1, 5:1, and 10:1, respectively). Data represents the percent of cell viability compared with untreated cells. Each value represents the mean ± S.D. of 3 different experimental determinations ($^*P < 0.05$).

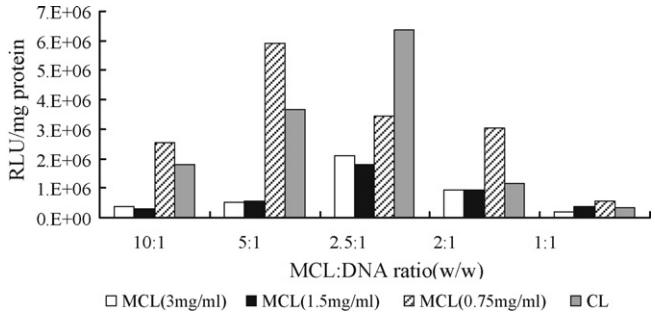

Fig. 3. Optimization of MCL/pDNA mixing ratio evaluated by luciferase assay in THLE-3 cells. Each result represents the mean ($n = 2$).

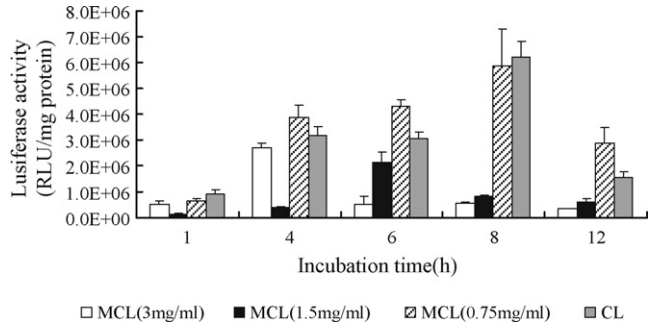

Fig. 4. Optimization of incubation time evaluated by luciferase assay in THLE-3 cells. Each value represents the mean ± S.D. ($n = 3$).

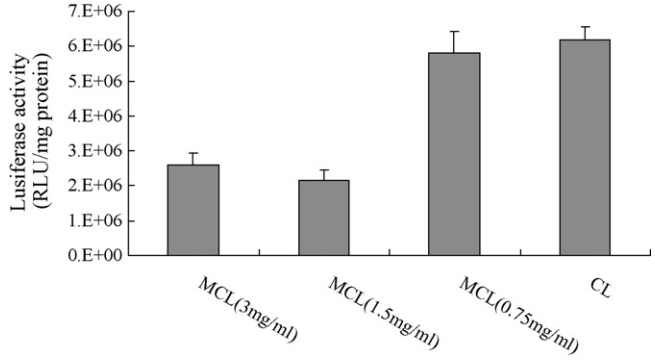

Fig. 5. Comparison of transfection efficiency of each lipoplexes under the optimal transfection conditions in THLE-3 cells. Each value represents the mean ± S.D. ($n = 3$).

showed relatively larger size and lower zeta potential (Table 1). The magnetic particles were probably entrapped in the liposomes, so larger size and lower zeta potential resulted in relatively lower membrane charge density (sigmaM, average charge/unit area of membrane). The complexes with low sigmaM remained trapped in the endosome and exhibited a reduced level of transfection efficiency (Ahmad et al., 2005).

3.4.4. Enhanced transfection efficiency of MCLs/pDNA complexes by magnetic induction

Having established optimal conditions that gave efficient transfection, we next examined the effect of a magnetic field on transfection efficiency. The effect of a magnetic field on transfection efficiency was evaluated by measuring the luciferase activity under magnetic induction. The time of magnetic induction was varied from 10 to 60 min and the luciferase activities without magnetic induction of each preparation were measured at the same time. MCLs/pDNA complexes with different concentrations of MAG-T exhibited different magnetic induction time to get an enhanced gene expression. For MCLs/pDNA complexes (MCLs, 3 and 1.5 mg/ml), magnetic induction for 10 min produced maximal luciferase activities, and the activities were 2.8 and 1.9-fold higher than that without magnetic induction, respectively (Fig. 6A and B). In the case of MCLs/pDNA complexes (MCLs, 0.75 mg/ml) the time for effective magnetic induction was extended to 30 min, and the activity was 2.6-fold higher than that without magnetic induction (Fig. 6C). This result was in agreement with that of Hirao et al. (2003). Compared to magnetofection in previous work, the transfection activity in this study was low due to the difference in magnetic nanoparticle type and preparation of complexes. However, the additional toxicity of the cationic polymers during magnetofection should be considered.

The mechanism of MCLs/DNA complexes uptake into cells was probably the same as that of CLs/DNA complexes, which was consistent with the comparable transfection activity of CLs/DNA complexes and MCLs/DNA complexes without magnetic induction. Magnetic nanoparticles are co-internalized with vectors into cells (Mykhaylyk et al., 2007). Magnetic interaction between applied magnetic fields and magnetic nanoparticles could accelerate accumulation of the complexes on the surface of the cells. Then during the intracellular processing nanoparticles at a certain concentration may probably interact with the cell membrane, which could result in the nonspecific changes of membrane properties (such as ion transport potential and possibly fluidity) or destabilization of the endosomal environment (Bhattarai et al., 2008). These could contribute to the rapid and effective gene delivery under a magnetic field. However, longer application of magnetic induction (>10 min) for MCLs/pDNA complexes (MCLs, 3 and 1.5 mg/ml) resulted in lower activities as compared to that without magnetic induction, probably due to increased cellular toxicity.

3.5. In vivo gene expression

As gene expression could be much different between in vitro and in vivo experiments. Agarose gel electrophoresis, mean particle sizes, zeta potential, and entrapment efficiency of DNA in MCLs/pDNA complexes were analyzed to determine the weight ratio between MCLs and pDNA. Results showed that MCLs/DNA complexes prepared with the ratio of 8.0 between MCLs and pDNA represented good properties on the four above mentioned aspects (data not shown). Therefore, in the studies of in vivo gene experiments, MCLs/DNA complexes were used with the ratio of 8.0 between MCLs and pDNA.

The luciferase activities in liver, kidney, spleen, lung, and heart following intravenous administration with CLs/pDNA complexes

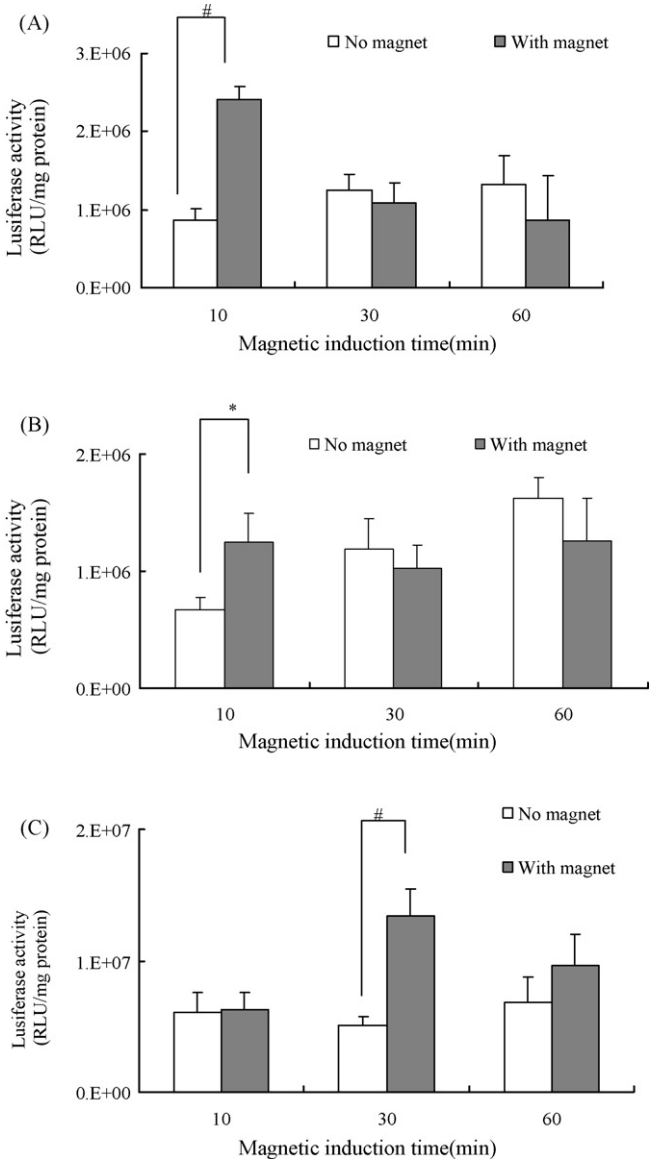

Fig. 6. Effects of a magnetic field and magnetic induction time on transfection efficiency: (A) MCLs/pDNA complexes (MCLs, 3 mg/ml); (B) MCLs/pDNA complexes (MCLs, 1.5 mg/ml); and (C) MCLs/pDNA complexes (MCLs, 0.75 mg/ml). These experiments were carried out under the optimal transfection conditions and magnetic induction time was varied from 10 to 60 min. Each value represented the mean ± S.D. ($n = 3$). $^{*}P < 0.05$, $^{\#}P < 0.01$.

and MCLs/pDNA complexes were shown in Fig. 7. In the case of CLs/pDNA complexes, the highest gene expression was observed in the lung while the gene expression in the kidney was much lower in all the formulations tested. As far as MCLs/pDNA complexes under the magnetic induction were concerned, significantly increased (approximately 1.5-fold higher, $P < 0.01$) gene expression in the liver while significantly decreased gene expression ($P < 0.05$) in the lung were observed when compared to that without a magnetic field. This result was consistent with that of in vitro transfection experiments, which demonstrated that the applicability of the magnetic drug targeting was applicable in gene delivery. For in vivo gene transfection, the MCLs/pDNA complexes without magnetic induction showed higher gene expression than CLs/pDNA complexes in the tested organs except for the lung, but for in vitro experiments the transfection efficiency of MCLs/pDNA complexes was the same as that of CLs/pDNA complexes in the absence of a magnetic field

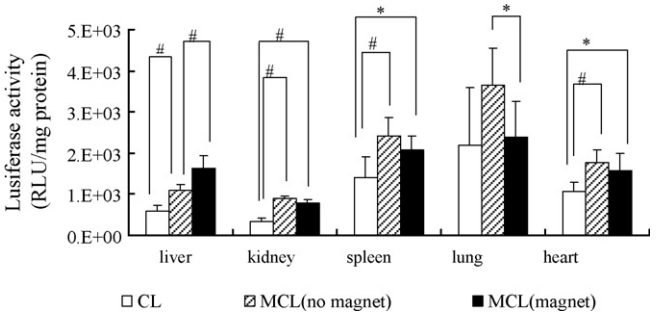

Fig. 7. Effect of external magnetic field on the transfection activity of MCLs/pDNA complexes in vivo. The concentration of MAG-T in the MCLs was 1.5 mg/ml and the weight ratio between MCLs and pDNA was 8.0. Luciferase activity was determined 24 h post-injection in the liver, kidney, spleen, lung, and heart, respectively. Each value represented mean ± S.D. ($n = 6$). $^{*}P < 0.05$, $^{\#}P < 0.01$.

(Figs. 3 and 4). This difference might be due to increased particle size resulting in the enhanced transfection efficiency (Higuchi et al., 2006). Another possible explanation for these findings was the decreased zeta potential, resulting in reduced association between hydrophobic, negatively charged proteins and the lipoplexes, which inhibits direct cellular uptake of the lipoplexes (Kostarelos and Miller, 2005).

In conclusion, as the amount of MAG-T incorporated into the liposomes and complexes increased, the particle size of MCLs and MCLs/pDNA complexes increased and the zeta potential of them decreased but maintained a cationic charge potential. MCLs/pDNA complexes can respond to an external magnet in vitro and in vivo under certain conditions. An approach combining MCLs/pDNA complexes and magnetic induction was found to achieve rapid and enhanced gene delivery in cultured THLE-3 cells. Moreover, enhanced in vivo luciferase reporter gene expression in the liver was obtained using MCLs/pDNA complexes with an external magnetic field.

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