European Journal of Pharmaceutics and Biopharmaceutics 80 (2012) 268–273

Contents lists available at SciVerse ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Cationic solid lipid nanoparticles for co-delivery of paclitaxel and siRNA

Yong Hee Yu [a,1], Eunjoong Kim [a,1], Dai Eui Park [b], Gayong Shim [b], Sangbin Lee [b], Young Bong Kim [c], Chan-Wha Kim [b], Yu-Kyoung Oh [a,*]

[a] College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, Seoul, South Korea
[b] School of Life Sciences and Biotechnology, Korea University, Seoul, South Korea
[c] College of Animal Biotechnology, Konkuk University, Seoul, South Korea

ARTICLE INFO

Article history:
Received 28 March 2011
Accepted in revised form 4 November 2011
Available online 13 November 2011

Keywords:
Solid lipid nanoparticles
Co-delivery
Paclitaxel
SiRNA
Combined cancer therapy

ABSTRACT

In this study, we formulated cationic solid lipid nanoparticles (cSLN) for co-delivery of paclitaxel (PTX) and siRNA. 1,2-Dioleoyl-sn-glycero-3-ethylphosphocholine-based cSLN were prepared by emulsification solidification methods. PTX-loaded cSLN (PcSLN) were characterized by zeta potential and gel retardation of complexes with small interfering RNA (siRNA). The sizes of PcSLN did not significantly differ from those of empty cSLN without PTX (EcSLN). The use of cSLN increased the cellular uptake of fluorescent dsRNA in human epithelial carcinoma KB cells, with PcSLN complexed to fluorescence-labeled dsRNA promoting the greatest uptake. For co-delivery of therapeutic siRNA, human MCL1-specific siRNA (siMCL1) was complexed with PcSLN; luciferase-specific siRNA (siGL2) complexed to EcSLN or PcSLN was used as a control. MCL1 mRNA levels were significantly reduced in KB cells treated with siMCL1 complexed to PcSLN, but not in groups treated with siMCL alone or siGL2 complexed to PcSLN. siMCL1 complexed to PcSLN exerted the greatest in vitro anticancer effects in KB cells, followed by siMCL1 complexed to EcSLN, siGL2 complexed to PcSLN, PTX alone, and siMCL1 alone. In KB cell-xenografted mice, intratumoral injection of PcSLN complexed to siMCL1 significantly reduced the growth of tumors. Taken together, our results demonstrate the potential of cSLN for the development of co-delivery systems of various lipophilic anticancer drugs and therapeutic siRNAs.

1. Introduction

Small interfering RNAs (siRNAs) have received considerable recent research attention for their potential therapeutic applications. Notably, a number of studies have explored the use siRNAs to silence the expression of oncogenes and specific targets that promote the proliferation of tumor cells as an anticancer strategy [1]. However, siRNA-based monotherapy offers limited therapeutic efficacy against cancers, which exhibit polygenic and complex pathologies; thus, additional improvements in such approaches are required [2].

Combination therapy with chemotherapeutics and siRNAs has been investigated as an alternative strategy for achieving enhanced anticancer activity. Several groups have designed delivery systems based on liposomes or nanoparticles for co-delivery of anticancer chemotherapeutics and siRNAs. These include cationic liposomes for co-delivery of doxorubicin and siRNA targeting multidrug resistance protein to enhance anticancer efficacy in lung cancer cells [3]; mesoporous silica nanoparticles to deliver doxorubicin and Bcl-2 siRNA for effective treatment of cancer cells [4]; and micellar nanoparticles carrying paclitaxel (PTX) and siRNA specific for polo-like kinase 1 to induce a synergistic tumor-suppressive effect [5].

Solid lipid nanoparticles have been widely used for the delivery of poorly soluble drugs. To date, the application of solid lipid nanoparticles for siRNA delivery has been limited, although recent studies have tested cationic solid lipid nanoparticles (cSLN) as an siRNA delivery system [6]. Solid lipid nanoparticles have been shown to have advantages over other carriers, including high stability in body fluids and tissues, the ability to release drugs for sustained periods, biodegradability, owing to the lipid component of the nanoparticle matrix, ease of manufacture [7], and the capacity to scale up to industrial production levels at relatively low cost [8,9]. However, few studies have addressed the feasibility of using solid lipid nanoparticles for anticancer chemotherapeutics/siRNA co-delivery systems.

In this study, we formulated cSLN encapsulating PTX and complexed them with human MCL1-specific siRNA (siMCL1). We report that the co-delivery of PTX and siMCL1 using cSLN improved anticancer efficacy in vitro and in vivo compared to either agent alone.

* Corresponding author. College of Pharmacy and Research Institute of Pharmaceutical Sciences, Seoul National University, 599 Gwanak-ro, Gwanak-gu, Seoul 151-742, South Korea. Tel.: +82 2 880 2493; fax: +82 2 882 2493.
E-mail address: ohyk@snu.ac.kr (Y.-K. Oh).
[1] Both authors contributed equally to this manuscript.

0939-6411/$ - see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2011.11.002

2. Materials and methods

2.1. Materials

PTX was purchased from LKT Laboratories Inc. (St. Paul, MN, USA). Glyceryl trioleate, retinol, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). The lipids 1,2-diphytanoyl-sn-glycero-3-phosphatidylethanolamine (DPhPE), 3β[N-(N′,N′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol), 1,2-Diol eoyl-sn-glycero-3-ethylphosphocholine (EDOPC), and methoxy-polyethylene glycol 2000-distearoyl phosphatidylethanolamine (mPEG-DSPE) were from Avanti Polar Lipids, Inc. (Alabaster, AL, USA).

2.2. Preparation of cSLN

Empty cSLN (EcSLN) and PTX-loaded cSLN (PcSLN) were prepared using a slight modification of the solvent-emulsification method [6]. Briefly, for the preparation of EcSLN, retinol (3 mg, 31% [w/w]), DC-Chol (3 mg, 31% [w/w]), DPhPE (2 mg, 20.7% [w/w]), EDOPC (1.5 mg, 15.5% [w/w]), mPEG-DSPE (0.1 mg, 1% [w/w]), and glyceryl trioleate (0.08 mg, 0.8% w/w) were dissolved in 1 ml of chloroform/methanol mixture (2:1) in a glass tube. For the preparation of PcSLN, the same composition of EcSLN was used except PTX (0.08 mg, 0.8% [w/w]) that was substituted for glyceryl trioleate. To each lipid mixture, 5 ml of a 5% (w/v) glucose solution was added and vortexed thoroughly, and the resulting emulsion was sonicated for 5 min. The solvent was removed by placing the solution in a rotary evaporator for 10 min at 52 °C. After cooling at room temperature, the resulting EcSLN and PcSLN were stored at 4 °C until use.

2.3. Particle size and zeta potentials

Particle sizes of EcSLN and PcSLN were measured using an ELS-8000 dynamic light-scattering instrument (Photal, Osaka, Japan) after diluting each sample in deionized water. The hydrodynamic diameters of the particles were measured using dynamic He–Ne laser (10 mW) light scattering at an angle of 90° and a temperature of 24.1 °C. Zeta-potential values were also assessed using the ELS-8000 apparatus. Zeta potentials were determined by laser Doppler microelectrophoresis at an angle of 22°. The ELS-8000 software package was used for data analysis.

2.4. Gel retardation

The siRNA complexation capacity of EcSLN and PcSLN, prepared at various nitrogen/phosphate (N/P) ratios, was evaluated by gel retardation assay. Each complex was loaded onto a 1.5% agarose gel containing 0.5 μg/ml ethidium bromide and separated by electrophoresis for 25 min at 100 V in Tris–borate–ethylenediaminetetraacetic acid buffer. After electrophoresis, siRNA in gels were visualized using a Gel Doc System (Bio-Rad Lab., Hercules, CA, USA).

2.5. In vitro release test

The amounts of PTX released from PcSLN were measured using dialysis method. Five milliliter of PcSLN or free PTX (16 μg/ml) was placed in a dialysis bag (Spectrum Laboratories, Inc., Rancho Dominguez, CA, USA). After sealing of both ends, the bag was immersed in 50 ml of PBS (pH 7.4) containing 0.1% Tween 80. For sampling, 1 ml of medium was collected at various time points and replaced with the same volume of fresh medium. The concentrations of released paclitaxel were measured by UV/Vis spectrophotometer (U-3210, Hitachi, Ltd., Tokyo, Japan) at 227 nm.

2.6. siRNA cellular uptake

Human epithelial carcinoma KB cells (American Type Culture Collection, Rockville, MD, USA) were cultured in RPMI 1640 medium containing 10% fetal bovine serum (HyClone, Logan, UT, USA) and 100 units/ml penicillin plus 100 μg/ml streptomycin (Sigma–Aldrich Co.). Cells were seeded onto 24-well plates at a density of 8×10^4 cells/well and incubated at 37 °C for 24 h before treatment. Fluorescent dsRNA (Block-iT; Invitrogen, Carlsbad, CA, USA) was mixed with EcSLN or PcSLN at an N/P ratio of 4:1. The complexes were added to cells and incubated for various periods. The cells were then harvested and washed three times with cold phosphate-buffered solution (PBS) containing 2% fetal bovine serum and analyzed by flow cytometry using a FACSCalibur system and Cell Quest Pro software (BD Bioscience, San Jose, CA, USA).

2.7. In vitro siRNA-mediated gene silencing

siRNA-mediated reduction of target gene expression was studied using human MCL1 as a target gene. KB and MDA-MB-231 cells were seeded onto 24-well plates 1 day before transfection. The cells were incubated with 20 nM human MCL1-specific siRNA (siMCL1; ST Pharm. Co., Seoul, South Korea) or luciferase-specific siRNA (siGL2; ST Pharm. Co.) in complexes with cSLN. After 24 h, total RNA was isolated and the efficacy of cSLN/siRNA complexes in silencing MCL1 expression was evaluated using reverse transcription–polymerase chain reaction (RT-PCR). The sequences of primers used to amplify human MCL1 were 5′-AGCTGCATCGAACCATTAGC-3′ (sense) and 5′-GCTCCTACTCCAGCAACACC-3′ (antisense). MCL1 mRNA levels were normalized to those of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

2.8. In vitro anticancer activity

The in vitro anticancer activity of siMCL1-complexed PcSLN against KB cells was measured by MTT assay. The cells were seeded onto 48-well plates at a density of 2×10^4 cells/well. After 24 h, cells were treated with EcSLN or PcSLN complexed with 20 nM siGL2 or siMcl1 at an N/P ratio of 4:1. For comparison, cells were treated with 20 nM PTX, free or encapsulated in PcSLN. After 24 h, 20 ml of MTT stock solution (5 mg/ml in sterile PBS, pH 7.4) was added to each well. Next, the cells were incubated for 2 h in the presence of MTT. After addition of 200 μl of extraction buffer (0.04 N HCl/isopropanol solution), absorbance was measured at 570 nm using an enzyme-linked immunosorbent assay reader (Sunrise-Basic TECAN, Männedorf, Switzerland). The viability of MDA-MB-231 cells was expressed as a percentage of untreated-cell viability.

2.9. In vivo antitumor efficacy

The in vivo antitumor efficacy of siMCL1 complexed to EcSLN or PcSLN was tested in a KB xenograft model. All animal experiments were approved by the Ethics Committee of Seoul National University and conducted in accordance with Declaration of Helsinki. Six-week-old female BALB/c nu/nu mice (Orient Bio, Inc. Seongnam, Gyeonggi, South Korea) were subcutaneously inoculated in the right flank with 2×10^6 KB cells. On days 7, 9, and 11 after tumor inoculation, mice were intratumorally injected with saline, EcSLN, PcSLN, siMCL1 (0.17 mg/kg) complexed to PcSLN containing paclitaxel at a dose of 0.05 mg/kg, or siMCL1 (0.17 mg/kg)

complexed to EcSLN alone or with free paclitaxel at a dose of 0.05 mg/kg. Tumor volumes were measured using calipers.

2.10. Statistics

For statistical analyses, Student *t*-tests or ANOVAs were performed using SigmaStat software with Student–Newman–Keuls as a post hoc test (version 3.5; Systat Software, San Jose, CA, USA). A *P*-value less than 0.05 was considered significant.

3. Results

3.1. Characterization of cSLN

Entrapment of PTX in the lipid core of cSLN (Fig. 1) did not affect the physicochemical properties of cSLN. EcSLN (Fig. 1A) and PcSLN (Fig. 1B) did not differ with respect to physicochemical properties (i.e., siRNA-complexation ability, zeta potentials, and sizes). Gel retardation assays revealed electrostatic complex formation of cSLN with siRNAs. Both EcSLN and PcSLN showed complete retardation of siRNAs at N/P ratios of 4:1 and above (Fig. 2A). Zeta potential values were 43.2 ± 0.9 mV for EcSLN and 43.9 ± 2.7 mV for PcSLN. The average particle sizes were 151.4 ± 13.4 nm for EcSLN and 140.4 ± 12.9 nm for PcSLN. After complexation with siRNA, the mean diameters of cSLN/siRNA complexes were 180.9 ± 14.9 nm for EcSLN and 183.1 ± 12.0 nm for PcSLN. After complexation with siRNA, the mean diameters of cSLN/siRNA complexes were 180.9 ± 14.9 nm for EcSLN and 183.1 ± 12.0 nm for PcSLN. As shown in Fig. 2B, PTX was continuously released from PcSLN over 12 h. At 1 h after incubation, 5.3% of encapsulated PTX was released from PcSLN. However, at 12 h after incubation, 96.7% of PTX was released into the medium.

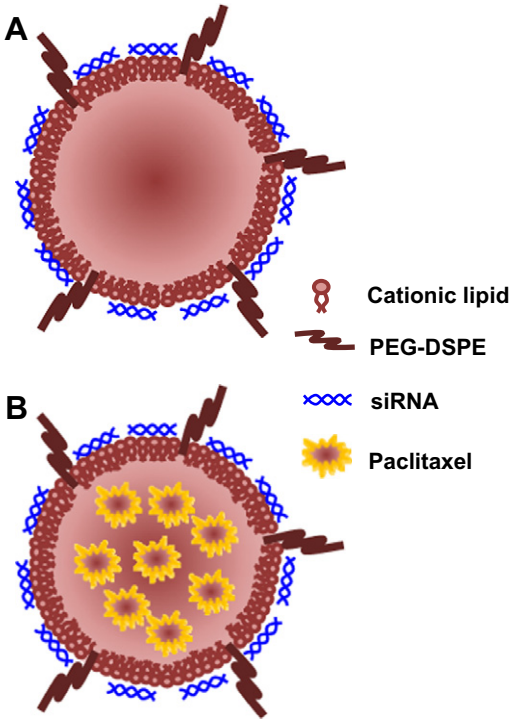

Fig. 1. Schematic illustration of an cSLN complexed with siRNA. EcSLN (A) and PcSLN (B) were prepared using a solidification emulsification method. The resulting cSLN were complexed with siRNA through electrostatic interaction. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

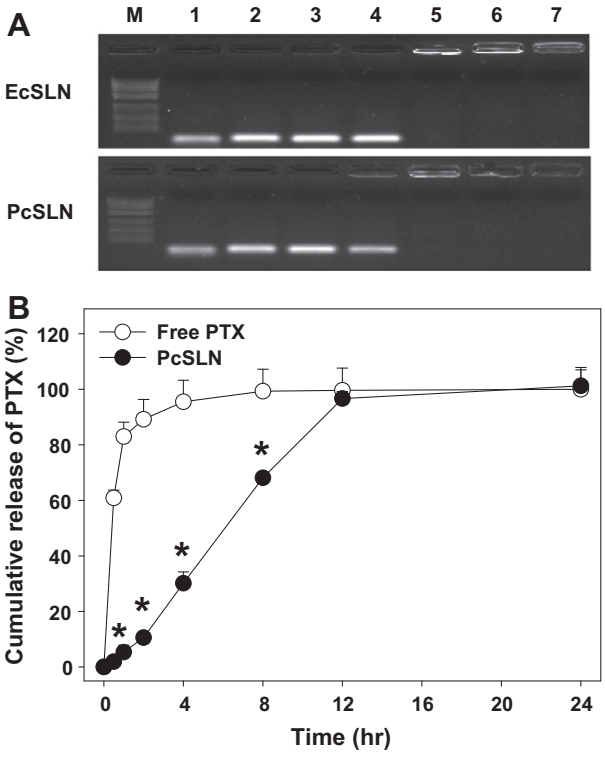

Fig. 2. Characterization of cSLN. (A) EcSLN or PcSLN were complexed with siRNA at various N/P ratios and separated on a 1.5% agarose gel. M, 1-kb-plus ladder; Lane 1, siRNA alone; lane 2, N/P ratio 1:1; lane 3, N/P ratio 2:1; lane 4, N/P ratio 4:1; lane 5, N/P ratio 8:1; lane 6, N/P ratio 16:1. (B) In vitro release of PTX from PcSLN was measured by UV/Vis spectrophotometer at 227 nm.

3.2. Cellular delivery of dsRNA by PcSLN

Complexation with PcSLN or EcSLN significantly enhanced the cellular delivery of fluorescent dsRNA. At 4 h of incubation, cellular delivery of fluorescent dsRNA increased 34.8- and 33.0-fold after treatment with EcSLN and PcSLN, respectively, compared to cells treated with naked fluorescent dsRNA. Specifically, 2.8 ± 0.3% of KB cells were fluorescence-positive after treatment with naked dsRNA, whereas 96.6 ± 1.0% and 91.3 ± 1.2% of cells were fluorescence-positive after 4 h treatment with EcSLN and PcSLN, respectively. Representative flow cytometry data are shown for untreated cells (Fig. 3A) and cells treated with fluorescent dsRNA in naked form (Fig. 3B) or complexed to EcSLN (Fig. 3C) or PcSLN (Fig. 3D). After 24-h incubation, the population of fluorescence-positive cells was significantly lower in the group treated with PcSLN as compared to EcSLN (Fig. 3E).

3.3. In vitro target gene silencing by siRNA complexed to PcSLN

The cellular delivery of siRNA using PcSLN effectively silenced the expression of target-specific proteins. Quantitative RT-PCR revealed that siMCL1 delivered using EcSLN or PcSLN decreased MCL1 mRNA levels to a significantly greater degree than naked siMCL1 (Fig. 4). Moreover, MCL1 mRNA levels in cells treated with siMCL1/EcSLN or siMCL1/PcSLN complexes were significantly lower than those in cells treated with luciferase-specific siGL2 complexed to EcSLN or PcSLN.

3.4. In vitro anticancer activity of siMCL1 co-delivered with PTX using PcSLN

The co-treatment of siMCL1 and PTX using PcSLN revealed the lowest survival of cancer cells among the groups tested in vitro.

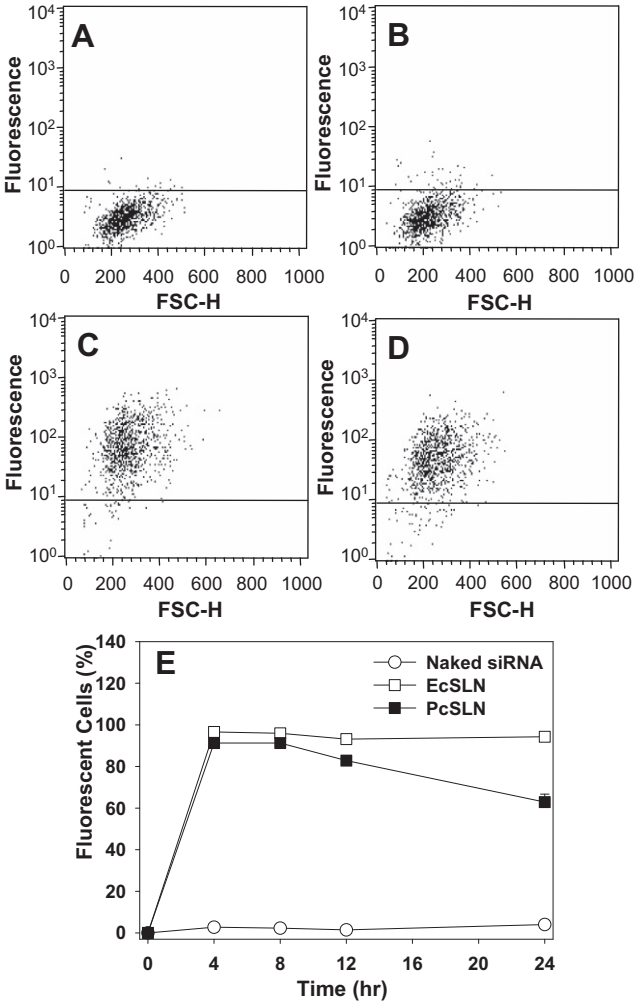

Fig. 3. Cellular uptake of fluorescent dsRNA complexed to cSLN. (A) The fluorescent cell populations were measured after 4 h treatment of KB cells with fluorescein-labeled dsRNA in naked or complexed forms ($n = 4$). Representative flow cytometry data are shown for KB cells incubated for 4 h with fluorescein-labeled dsRNA in naked form (C) or in complex with EcSLN (D) or PcSLN (E). Untreated KB cells (B) were used as a control. (F) The fluorescent cell populations were measured after various times of treatment with fluorescein-labeled dsRNA in naked or complexed forms ($n = 4$).

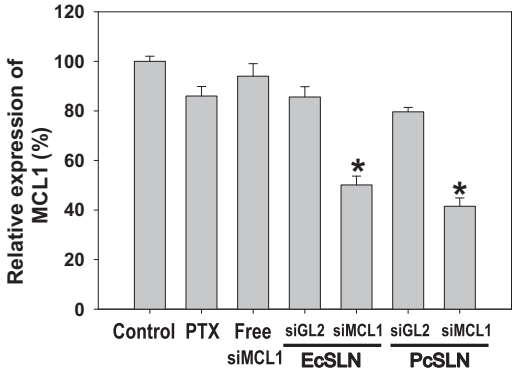

Fig. 4. MCL1 mRNA levels after delivery of siMCL1 using PcSLN. KB cells were treated with PTX, siRNA (siGL2, siMCL1) alone, or in complexes with cSLN. mRNA expression levels in KB cells were determined by RT-PCR. The results are expressed as the mean ± SEM of four independent experiments ($^{*}p < 0.05$ vs. other groups).

After treatment of KB cells with free PTX or naked siMCL1, cell survival was 90.9 ± 2.8% and 91.9 ± 7.6%, respectively (Fig. 5). In contrast, only 57.9 ± 7.8% of cells survived after delivery of siMCL1 complexed to EcSLN. Delivery of siMCL1 complexed to PcSLN was even more effective, decreasing cell survival to 38.8 ± 1.5%. Additional control experiments showed that cell survival was greater after treatment with siGL2 complexed to EcSLN than after treatment with siMCL1 complexed to EcSLN. Similarly, survival was higher in cells treated with siGL2 complexed to PcSLN than in cells treated with siMCL1 complexed to EcSLN.

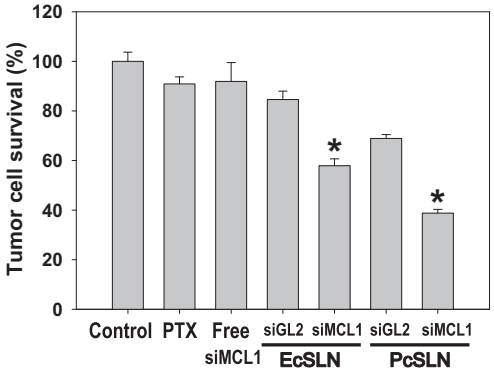

Fig. 5. In vitro antitumor effect of siMCL1 co-delivered with PTX using PcSLN. KB cells were treated with PTX, naked siRNA (siGL2, siMCL1), or complexed with cSLN (EcSLN, PcSLN) at an N/P ratio of 4:1. After treatment for 24 h, the survival of KB cells was measured by MTT assay. The results are expressed as the mean ± SEM of four independent experiments ($^{*}p < 0.05$ vs. siGL2/EcSLN-treated group; $^{**}p < 0.05$ vs. siGL2/PcSLN-treated group; ANOVA and Student–Newman–Keuls).

3.5. In vivo antitumor efficacy of siMCL1 co-delivered with PTX using PcSLN

In vivo co-delivery of siMCL1 with PTX using PcSLN significantly inhibited the growth of tumor tissues as compared to other treatment groups (Fig. 6). On day 16 after inoculation, the average tumor volume of control mice treated with saline was 1373.1 ± 241.0 mm^3. Intratumoral administration of siMCL1 complexed to EcSLN plus free PTX decreased tumor volume to 714.2 ± 230.0 mm^3. The highest inhibition of tumor growth was observed following intratumoral co-delivery of PTX and siMCL1 using PcSLN, which decreased tumor volume to 172.0 ± 73.7 mm^3.

4. Discussion

In this study, we used cSLN to simultaneously administer PTX and siRNA for combination anticancer therapy, demonstrating that

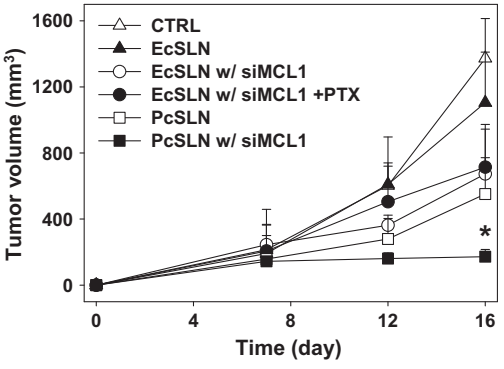

Fig. 6. In vivo antitumor efficacy of siMCL1 co-delivered with PTX using PcSLN. KB tumor-bearing mice were injected intratumorally three times with saline, EcSLN, PcSLN, or siMCL1 complexed to EcSLN or PcSLN on days 7, 9, and 11 after inoculation. Tumor size was measured with calipers ($^{*}p < 0.05$ vs. other groups; ANOVA and Student–Newman–Keuls).

siRNA and PTX co-delivered using EDOPC-based cSLN exerted effective anticancer activity in vitro and in vivo. Given the increasing clinical significance of combined anticancer therapy, cationic EDOPC-based cSLN may have applications in a variety of co-delivery settings, in which chemical anticancer drugs and therapeutic anticancer siRNAs are used.

As shown in Fig. 1A, we formulated cSLN as a carrier of siRNA. cSLN have been previously studied as potential carriers of plasmid DNA and RNA. However, few studies have addressed the co-delivery of siRNA and anticancer chemotherapeutics using cSLN in vitro and in vivo. Protamine-based cSLN have been reported to increase the transfection efficiency of plasmid DNA in the Na1300 cell line [10]. Bondi et al. [11] showed that dimethyldioctadecylammonium bromide–based cSLN promote internalization of plasmid DNA into liver cancer cells. Kim et al. [6] reported target-gene silencing after delivery of pegylated siRNA using cSLN in green fluorescent protein–overexpressing cell lines. In this latter study, 3β-[N-(N′,N′-dimethylamino ethane)carbamoyl]-cholesterol was used as the cationic lipid component to confer cationic charges to solid lipid nanoparticles. To date, most studies have focused on characterizing the physiochemical properties of cSLN/siRNA complexes and confirming the feasibility of cSLN as nucleic acid delivery systems at the in vitro level.

In this study, poorly soluble anticancer drug PTX was entrapped in cSLN (Fig. 1B). Although many polymeric nanoparticles (e.g., dendrimer-based nanostructures) have been designed to deliver anticancer drugs by covalent conjugation [12,13], solid lipid nanoparticles are suitable for physical loading of poorly soluble anticancer agents, such as topotecan [14] and baclofen [15], into the core matrix without altering their chemical structures [16]. Moreover, by varying the lipid compositions of the solid lipid nanoparticles, various charges can be conveniently conferred to the nanoparticles, allowing a broad spectrum of charged bioactive substances to be loaded via electrostatic complexation [7]. In vitro release study of PTX from PcSLN (Fig. 2B) shows that PTX was released from solid lipid nanopartcles over time. We did not observe the burst release of PTX from PcSLN. The gradual increase of PTX release over 12 h supports that PTX was entrapped inside of PcSLN, rather than existing on the surface of nanoparticles.

Here, we observed the enhanced transfection of siRNA using EcSLN (Fig. 3C) and PcSLN (Fig. 3D). For both EcSLN and PcSLN, EDOPC was used as a cationic lipid to confer positive charges to solid lipid nanoparticles. EDOPC-based cationic liposomes were shown to be more effective in delivering plasmid DNA than liposomes containing other cationic lipids, such as 1,2-dioleoyl-sn-glycero-3-hexylphosphocholine and 1,2-dierucoyl-sn-glycero-3-ethylphosph ocholine [17]. The higher transfection efficiency provided by EDOPC-based cationic liposomes has been suggested to reflect increased interaction and mixing of these liposomes with cellular lipids. Although the entrapment of PTX in cSLN did not affect the delivery of fluorescent dsRNA at 4 h after treatment, the lower fluorescence was observed in PcSLN group as compared to EcSLN group at 24 h after treatment (Fig. 3E). Such a decrease in fluorescence at 24 h might be in part due to the death of the cells by PTX of PcSLN used for delivery of dsRNA. Actually, we could not perform 48 h experiment due to the severe death of the cells treated with PcSLN (data not shown).

Although siRNA is considered to represent next-generation therapeutic technology, the therapeutic efficacy of siRNA monotherapy for cancer treatment is limited by the polygenic nature of cancers. The need for improvements in this approach has motivated a number of groups to investigate therapeutic strategies that combine anticancer chemotherapeutics and siRNA. One such study combined PTX and siRNA using micelles. In this study, micellar nanoparticles carrying PTX and siRNA specific for polo-like kinase 1 were reported to induce a synergistic tumor-suppressive effect in an MDA-MB-435s xenograft murine model [5]. Our group recently reported that administration of cationic liposomes loaded with the anticancer agent suberoylanilide hydroxamic acid (a histone deacetylase inhibitor) and complexed with siMCL1 resulted in enhanced anticancer effects in xenografted mice [18]. Co-delivery of doxorubicin-loaded cationic liposomes with siRNAs targeting a multidrug resistance protein was reported to enhance anticancer efficacy in multidrug-resistant lung cancer cells [3]. A similar increase in anticancer efficacy was reported with doxorubicin and Bcl-2 siRNA co-delivered to multidrug-resistant cancer cells using mesoporous silica nanoparticles [4].

Here, we co-delivered PTX with siMCL1 using EDOPC-based cSLN. PTX has poor water solubility properties and was, thus, studied in solid lipid nanoparticle formulations. Previous reports have shown that PTX encapsulated in solid lipid nanoparticles in a solubilized form exerts increased anticancer effects in mice. For combination with PTX, siMCL1 was chosen since siMCL1 has been shown to increase the sensitivity of cancer cells to anticancer chemotherapeutics [19,20], including ovarian carcinomas [21] and mesothelioma cells [22].

We observed the highest anticancer effect in the group treated with siMCL1 and PcSLN (Fig. 5). The highest anticancer effects could be due to the lower expression of MCL1 and the anticancer activity of PTX. It is unlikely that PTX in PcSLN affected the expression levels of MCL1 in the cells. The MCL1 expression data (Fig. 4) indicate that the treatment of cells with siMCL1-complexed PcSLN did not highly reduce the levels of MCL1 as compared to the group treated with siMCL1 complexed to EcSLN.

The in vivo data of Fig. 6 suggest the improved anticancer activity of siMCL1 co-delivered with PTX using PcSLN. Notably, the group treated with siMCL1 complexed to EcSLN plus free PTX exerted significantly higher tumor volume than siMCL1 complexed to PcSLN. Such a higher anticancer effect of siMCL1 with PcSLN could be due to the enhanced delivery of PTX to the same cell transfected with siMCL1.

Although cSLN were used specifically to co-deliver PTX and siMCL1 in this study, cSLN can be applied to deliver other poorly soluble anticancer drugs and siRNA combinations. Recently, several studies have reported the synergistic effects of anticancer drugs and siRNAs as chemosensitizers, showing that siRNA targeting the DNA repair enzyme O-methylguanine-DNA methyltransferase increased the anticancer activity of temozolomide in tumor cells [23], and siRNA-mediated silencing of nuclear factor erythroid-2-related factor 2 increased the sensitivity of small lung cancer cells to carboplatin [24]. Given such combination-mediated synergistic effects, cSLN might be further utilized for co-delivery of anticancer chemotherapeutics and synergy-providing siRNA for effective treatment of cancers.

Acknowledgements

This study was financially supported by Grants from the Ministry of Education, Science and Technology (2010K-001356), from the Bio & Medical Technology Development Program of the National Research Foundation funded by the Korean government (MEST) (No. 20110019640), and from the Korean Health Technology R&D Project (Grant No. A090945), Ministry for Health, Welfare & Family Affairs, Republic of Korea.

References

[1] Y.K. Oh, T.G. Park, siRNA delivery systems for cancer treatment, Adv. Drug Deliv. Rev. 61 (10) (2009) 850–862.
[2] A.S. Azmi, Z. Wang, P.A. Philip, R.M. Mohammad, F.H. Sarkar, Proof of concept: network and systems biology approaches aid in the discovery of potent anticancer drug combinations, Mol. Cancer Ther. 9 (12) (2010) 3137–3144.

[3] M. Saad, O.B. Garbuzenko, T. Minko, Co-delivery of siRNA and an anticancer drug for treatment of multidrug-resistant cancer, Nanomedicine (Lond) 3 (6) (2008) 761–776.
[4] A.M. Chen, M. Zhang, D. Wei, D. Stueber, O. Taratula, T. Minko, H. He, Co-delivery of doxorubicin and Bcl-2 siRNA by mesoporous silica nanoparticles enhances the efficacy of chemotherapy in multidrug-resistant cancer cells, Small 5 (23) (2009) 2673–2677.
[5] T.M. Sun, J.Z. Du, Y.D. Yao, C.Q. Mao, S. Dou, S.Y. Huang, P.Z. Zhang, K.W. Leong, E.W. Song, J. Wang, Simultaneous delivery of siRNA and paclitaxel via a "two-in-one" micelleplex promotes synergistic tumor suppression, ACS Nano 5 (2) (2011) 1483–1494.
[6] H.R. Kim, I.K. Kim, K.H. Bae, S.H. Lee, Y. Lee, T.G. Park, Cationic solid lipid nanoparticles reconstituted from low density lipoprotein components for delivery of siRNA, Mol. Pharm. 5 (4) (2008) 622–631.
[7] M.D. Joshi, R.H. Müller, Lipid nanoparticles for parenteral delivery of actives, Eur. J. Pharm. Biopharm. 71 (2) (2009) 161–172.
[8] S.S. Shidhaye, R. Vaidya, S. Sutar, A. Patwardhan, V.J. Kadam, Solid lipid nanoparticles and nanostructured lipid carriers – innovative generations of solid lipid carriers, Curr. Drug Deliv. 5 (4) (2008) 24–331.
[9] S.A. Wissing, O. Kayser, R.H. Müller, Solid lipid nanoparticles for parenteral drug delivery, Adv. Drug Deliv. Rev. 56 (9) (2004) 1257–1272.
[10] E. Vighi, B. Ruozi, M. Montanari, R. Battini, E. Leo, pDNA condensation capacity and in vitro gene delivery properties of cationic solid lipid nanoparticles, Int. J. Pharm. 389 (1-2) (2010) 254–261.
[11] M.L. Bondi, A. Azzolina, E.F. Craparo, N. Lampiasi, G. Capuano, G. Giammona, M. Cervello, Novel cationic solid–lipid nanoparticles as non-viral vectors for gene delivery, J. Drug Target. 15 (4) (2007) 295–301.
[12] S. Zhu, M. Hong, G. Tang, L. Qian, J. Lin, Y. Jiang, Y. Pei, Partly PEGylated polyamidoamine dendrimer for tumor-selective targeting of doxorubicin: the effects of PEGylation degree and drug conjugation style, Biomaterials 31 (6) (2010) 1360–1371.
[13] D.K. Tosh, L.S. Yoo, M. Chinn, K. Hong, S.M. Kilbey, M.O. Barrett, I.P. Fricks, T.K. Harden, Z.G. Gao, K.A. Jacobson, Polyamidoamine (PAMAM) dendrimer conjugates of "clickable" agonists of the A3 adenosine receptor and coactivation of the P2Y14 receptor by a tethered nucleotide, Bioconjug. Chem. 21 (2) (2010) 372–384.
[14] L.G. Souza, E.J. Silva, A.L. Martins, M.F. Mota, R.C. Braga, E.M. Lima, M.C. Valadares, S.F. Taveira, R.N. Marreto, Development of topotecan loaded lipid nanoparticles for chemical stabilization and prolonged release. Eur. J. Pharm. Biopharm. (2011) [Epub].
[15] L. Priano, G.P. Zara, N. El-Assawy, S. Cattaldo, E. Muntoni, E. Milano, L. Serpe, C. Musicanti, C. Pérot, M.R. Gasco, G. Miscio, A. Mauro, Baclofen-loaded solid lipid nanoparticles: Preparation, electrophysiological assessment of efficacy, pharmacokinetic and tissue distribution in rats after intraperitoneal administration, Eur J Pharm Biopharm. (2011) [Epub].
[16] H. Bunjes, Lipid nanoparticles for the delivery of poorly water-soluble drugs, J. Pharm. Pharmacol. 62 (11) (2010) 637–1645.
[17] R. Koynova, L. Wang, R.C. Macdonald, Cationic phospholipids forming cubic phases: lipoplex structure and transfection efficiency, Mol. Pharm. 5 (5) (2008) 739–744.
[18] G. Shim, S.E. Han, Y.H. Yu, S. Lee, H.Y. Lee, K. Kim, I.C. Kwon, T.G. Park, Y.B. Kim, Y.S. Choi, C.W. Kim, Y.K. Oh, Trilysinoyl oleylamide-based cationic liposomes for systemic co-delivery of siRNA and an anticancer drug, J. Control. Release (2010) [Epub].
[19] C. Denoyelle, P. Gauduchon, L. Poulain, Mcl-1 is an important determinant of the apoptotic response to the BH3-mimetic molecule HA14-1 in cisplatin-resistant ovarian carcinoma cells, Mol. Cancer Ther. 8 (11) (2009) 3162–3170.
[20] L. Song, D. Coppola, S. Livingston, D. Cress, E.B. Haura, Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non-small cell lung cancer cells, Cancer Biol. Ther. 4 (3) (2005) 267–276.
[21] E. Brotin, M. Meryet-Figuière, K. Simonin, R.E. Duval, M. Villedieu, J. Leroy-Dudal, E. Saison-Behmoaras, P. Gauduchon, C. Denoyelle, L. Poulain, Bcl-XL and MCL-1 constitute pertinent targets in ovarian carcinoma and their concomitant inhibition is sufficient to induce apoptosis, Int. J. Cancer 126 (4) (2010) 885–895.
[22] E. Varin, C. Denoyelle, E. Brotin, M. Meryet-Figuière, F. Giffard, E. Abeilard, D. Goux, P. Gauduchon, P. Icard, L. Poulain, Downregulation of Bcl-xL and Mcl-1 is sufficient to induce cell death in mesothelioma cells highly refractory to conventional chemotherapy, Carcinogenesis 31 (6) (2010) 984–993.
[23] T. Kato, A. Natsume, H. Toda, H. Iwamizu, T. Sugita, R. Hachisu, R. Watanabe, K. Yuki, K. Motomura, K. Bankiewicz, T. Wakabayashi, Efficient delivery of liposome-mediated MGMT-siRNA reinforces the cytotoxity of temozolomide in GBM-initiating cells, Gene Ther. 17 (11) (2010) 1363–1371.
[24] A. Singh, S. Boldin-Adamsky, R.K. Thimmulappa, S.K. Rath, H. Ashush, J. Coulter, A. Blackford, S.N. Goodman, F. Bunz, W.H. Watson, E. Gabrielson, E. Feinstein, S. Biswal, RNAi-mediated silencing of nuclear factor erythroid-2-related factor 2 gene expression in non-small cell lung cancer inhibits tumor growth and increases efficacy of chemotherapy, Cancer Res. 68 (19) (2008) 7975–7984.