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European Journal of Pharmaceutics and Biopharmaceutics 68 (2008) 545–554
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Novel cationic solid lipid nanoparticles enhanced p53 gene transfer to lung cancer cells

Sung Hee Choi [a], Su-Eon Jin [a], Mi-Kyung Lee [b], Soo-Jeong Lim [c], Jeong-Sook Park [d], Byung-Gyu Kim [e], Woong Shick Ahn [f], Chong-Kook Kim [a,*]

[a] College of Pharmacy, Seoul National University, Seoul, Republic of Korea
[b] Department of Pharmaceutical Engineering, Woosuk University, Samrye, Republic of Korea
[c] Department of Bioscience and Biotechnology, Sejong University, Seoul, Republic of Korea
[d] College of Pharmacy, Chungnam National University, Daejeon, Republic of Korea
[e] Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
[f] Department of Obstetrics and Genecology, The Catholic University of Korea, Seoul, Republic of Korea

Received 4 November 2006; accepted in revised form 28 July 2007
Available online 8 August 2007

Abstract

Mutations in the p53 tumor suppressor gene are the most common molecular genetic abnormalities to be described in lung cancer. However, there have been few reports of nonviral vector-mediated p53 gene delivery in lung cancer. A new formulation of cationic solid lipid nanoparticles (SLNs) for gene delivery was produced by the melt homogenization method with slight modification, and the SLNs were formulated by mixing tricaprin (TC) as a core, 3β[*N*-(*N*′, *N*′-dimethylaminoethane) carbamoyl] cholesterol (DC-Chol), dioleoylphosphatidylethanolamine (DOPE) and Tween 80 in various ratios. Plasmid DNA (pp53-EGFP)/SLNs complexes were transfected into human non-small cell lung cancer cells (H1299 cells) and transfection efficiency was determined by FACS analysis. The gene expression was determined by reverse transcription-polymerase chain reaction (RT-PCR) and Western blot analysis. The cellular growth inhibition and apoptosis of treated cells with pp53-EGFP/SLNs complexes were assessed by trypan blue exclusion assay and annexin V staining, respectively. *In vivo* biodistribution of plasmid DNA was investigated by PCR and RT-PCR. The transfection efficiency of SLN1 (TC:DC-Chol:DOPE:Tween 80 = 0.3:0.3:0.3:1), which showed the highest transfection efficiency among the SLN formulations, was higher than that of commercially available Lipofectin®. The SLNs-mediated transfection of the p53 gene resulted in efficient high levels of wild-type p53 mRNA and protein expression levels in H1299 cells. The efficient reestablishment of wild-type p53 function in lung cancer cells restored the apoptotic pathway. Taken together, our results reveal that cationic SLN-mediated p53 gene delivery may have potential for clinical application as a nonviral vector-mediated lung cancer therapy due to its effective induction of apoptosis and tumor growth inhibition.

Keywords: p53; Nonviral vector; Cationic SLN; Lung cancer

1. Introduction

Lung cancer is one of the leading causes of death worldwide [1]. Adenocarcinoma, squamous cell carcinoma, and large-cell carcinoma, which together make up the majority of lung cancers, are referred to as "non-small cell lung cancers" (NSCLCs). Patients with early stage NSCLC are typically treated with surgery; 5-year survival rates range from 25% to 80%, depending on the stage of the disease [2]. Current treatments for lung cancer have shown little success because they cannot cure disseminated tumors with an acceptable level of toxicity. Thus, one alternative strategy that has shown promise in the treatment of cancer is gene therapy.

* Corresponding author. Laboratory of Excellency for Drug and Gene Delivery, College of Pharmacy, Seoul National University, San 56-1, Sillim-dong, Gwanak-gu, Seoul 151-742, Republic of Korea. Tel.: +82 2 880 7867; fax: +82 2 873 7482.
E-mail address: ckkim@plaza.snu.ac.kr (C.-K. Kim).

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doi:10.1016/j.ejpb.2007.07.011

Mutations in the p53 tumor suppressor gene are the most common tumorigenic process in human cancers and have been detected in more than 50% of NSCLCs [3]. Wild-type p53 causes cell cycle arrest at the G1 stage of the cell cycle and leads to programmed cell death, known as apoptosis [4]. Reintroduction or overexpression of the wild-type p53 gene in mutant cells has been known to induce apoptosis and growth arrest in various lung cancer cell lines [5]. Roth et al. successfully employed this strategy in a clinical trial (phase I/II), in which a p53-expressing adenovirus vector was transduced into NSCLC patients; the study confirmed the efficiency and safety of this strategy [6].

Many studies have reported that p53 genes transfected by nonviral cationic vectors were effective in inhibiting the growth of carcinoma [7–9]. Despite the low efficiency of nonviral cationic vector-mediated gene delivery, it is less immunogenic and the quality of this process is easier to control than gene delivery with viral vectors [10]. However, the main problem with the cationic lipid-based gene delivery system is the lack of physical stability of DNA/lipid complexes. Moreover, the gene delivery systems should be manufactured in large batches, shipped, and stored in order to make them marketable pharmaceutical products.

Of these cationic lipid formulations, solid lipid nanoparticles (SLNs) have gained increasing attention as promising colloidal carrier systems [11]. Although there are a large number of publications about cationic liposomes and cationic lipid emulsions for gene therapy, only a few reports about the use of SLNs for gene delivery have been published [12–15]. In terms of application, the production of SLNs is easier to scale up and preserve by freeze-drying. Freeze-dried formulations offer the potential for long-term stability at ambient temperatures [16]. Freeze-drying can be a promising means by which to increase the chemical and physical stability of SLNs over extended periods of time. Large-scale production can be performed in a cost-effective and relatively simple way using high pressure homogenizing, which produces SLNs.

Here, we prepared a stable new cationic SLNs formulation that was composed of positively-charged lipid bilayers and investigated the stability and efficient gene delivery into lung cancer NSCLC cells using p53 as a target gene.

2. Materials and methods

2.1. Reagents

3β [*N*-(*N*′, *N*′-dimethylaminoethane)carbamoyl] cholesterol (DC-Chol) and dioleoylphosphatidylethanolamine (DOPE) were purchased from Avanti Polar Lipids (Birmingham, AL, USA). Tricaprin (TC) and 3-(4,5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Tween 80 was obtained from Aldrich Chemical Co. (St. Louis, MO, USA). RPMI-1640 and Lipofectin® were purchased from Gibco-BRL (Burlington, ON, USA). Trypsin–EDTA was purchased from Life Technologies (Paisley, UK).

2.2. Cell lines

H1299 cells (a human non-small cell lung carcinoma cell line that contains a homozygous deletion of the p53 gene) were obtained from Korean Cell Line Bank (Seoul, Republic of Korea) and cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin. Cell lines were maintained in an incubator at 37 °C with a 5% CO_2 water-saturated atmosphere.

2.3. Plasmid

The pp53-EGFP plasmid DNA (Clontech, Palo Alto, CA) encodes the p53-EGFP Signaling Probe, which is a fusion of enhanced green fluorescent protein (EGFP) and p53. Plasmid DNAs were prepared using the EndoFree Qiagen kit (Qiagen, CA, USA) to remove the bacterial endotoxins.

2.4. Preparation of cationic SLNs

SLNs were prepared by the melt homogenization method with slight modification [17]. In brief, various amounts of TC, DOPE, DC-Chol, and Tween 80 were mixed and dissolved in approximately 1 ml of tertiary butyl alcohol (Table 1). After rapid freezing in a liquid nitrogen tank, mixtures were dried in an Ultra 35EL freeze-dryer (Virtis, USA). Finely-dispersed cakes were obtained after overnight drying and cakes were then put in a water bath at 50 °C. Preheated (50 °C) water for injection was slowly added to the melts (2 g of final total weight) and sonicated in a bath type sonicator for 30 min at 50 °C until crude and milky emulsions were obtained. These crude emulsions were homogenized for seven cycles at 60–70 °C and 100 MPa using a high pressure homogenizer (Emulsiflex EF-B3, Avestin Inc., Canada) wired with heating tape (Thermolyne, Barnstead International, USA). SLNs were produced by subsequent cooling of

Table 1
The mean diameters and compositions of the various lipid

	DC-Chol (mg/ml)	DOPE (mg/ml)	Tween 80 (mg/ml)	TC (mg/ml)	Mean diameter (nm)	Zeta potential (mV)
SLN1	0.3	0.3	0.3	1	69	8
SLN2	0.3	0.3	0.3	0.15	64	13
SLN3	0.3	0.3	0.15	0.3	80	7
SLN4	0.9	0.3	0.3	0.3	75	15