International Journal of Pharmaceutics 543 (2018) 311–317

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Effects of cationic liposomes with stearylamine against virus infection

Kohei Tahara[a], Manami Kobayashi[a], Satoko Yoshida[a], Risako Onodera[a], Naoki Inoue[b], Hirofumi Takeuchi[a,*]

[a] Laboratory of Pharmaceutical Engineering, Gifu Pharmaceutical University, Gifu, Japan
[b] Laboratory of Microbiology and Immunology, Gifu Pharmaceutical University, Gifu, Japan

ARTICLE INFO

Keywords:
Liposome
Stearylamine
Baculovirus
HSV-1
A549

ABSTRACT

In this study, we demonstrated that cationic liposomes with incorporated stearylamine (SA) inhibit viral infectivity without preloaded active pharmaceutical ingredients. Specifically, we correlated physiochemical properties of liposomes, such as zeta potentials and particle sizes, with virus infectivity using the BacMam™ reagent, which is based on recombinant baculovirus (BV). Compared with neutral or negatively-charged liposomes, SA liposomes suppressed BV infectivity in several mammalian cell lines, including A549 cells. SA liposomes inhibited BV infection over 80% by optimizing the liposomal concentration and exposure time with cells. Moreover, these antiviral SA liposomes were not cytotoxic, and reducing the embedded cholesterol contents intensified the antiviral effects and simultaneously increased the binding of SA liposomes to the cell membranes. These data indicate that binding of SA liposomes to cell membranes may block virus entry. Finally, we also demonstrated the antiviral effects of SA liposomes on herpes simplex virus type 1 in A549 cells, and showed comparable efficacy to that of the antiviral drug acyclovir.

1. Introduction

Several antiviral drugs have been formally approved for the treatment of human infectious diseases (De Clercq and Li, 2016). However, antiviral drug resistance is induced by prolonged drug exposure and remains an increasing concern (Strasfeld and Chou, 2010). Drug resistance can be controlled by optimizing host and drug delivery factors, by implementing alternative therapies based on known mechanisms of resistance, and by developing novel antiviral drugs. However, novel and potent antiviral drugs with low toxicity are eagerly awaited to target host factors or viral replication mechanisms and to reduce the risk of drug resistance.

Liposomes comprising biocompatible lipids are promising nanoparticulate delivery systems. Previous studies reported conventional applications for liposomal drug delivery systems as drug carriers that encapsulate antiviral drugs to improve bioavailability and pharmacological effects. Accordingly, liposome encapsulated acyclovir (ACV) and distamycin A formulations for ocular delivery showed increased interactions with ocular tissue and improved drug absorption (Chetoni et al., 2015; Law et al., 2000). Liposomes have also been investigated for delivery of interferon-α and nucleic acid medicines such as siRNA (Thitinan and McConville, 2009; Zatsepin et al., 2016), and as model membranes in analyses of virus–membrane fusion (Larsen et al., 1993).

Konopka et al. (1990) showed that 1,2-dioleyloxy-3-trimethylammonium propane chloride (DOTMA) liposomes enhance infectivity of human immunodeficiency virus (HIV), whereas cardiolipin (CL) liposomes decrease infectivity and 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) liposomes were ineffective. These experiments indicated potential antiviral effects of liposomal lipids. Recently, Malavia et al. (2011) demonstrated inhibition of HIV infectivity by CL liposomes that were optimized for topical (intravaginal) administration in vivo (Malavia et al., 2011). Similarly, Hendricks et al. (2015) generated decoy liposomes that present heparan sulfate octasaccharide, which binds to pathogens and inhibits respiratory syncytial virus, human parainfluenza virus 3, and herpes simplex virus (HSV) infections. Maitani et al. (2013) investigated a novel application and developed liposomes that lacked antiviral drugs but contained branched polyethylenimine. After topical application, these particles inhibited HSV type 2 (HSV-2) via a unique mechanism. These previous publications showed that functionalized liposomes have the potential to prevent viral infections, with broad-spectrum activities and antiviral mechanisms that differ from those of existing antiviral drugs.

Thus, we evaluated the influence of liposomal characteristics on viral infectivity using a commercially available virus-based reagent (BacMam™ technology). This reagent is based on recombinant baculovirus (BV) and can be used as a model virus because it delivers and expresses a fluorescent protein in host mammalian cells and required minimal handling and manipulation. In our experiments, we compared

* Corresponding author at: Gifu Pharmaceutical University, 1-25-4 Daigaku-nishi, Gifu 501-1196, Japan.
E-mail address: takeuchi@gifu-pu.ac.jp (H. Takeuchi).

https://doi.org/10.1016/j.ijpharm.2018.04.001
Received 15 November 2017; Received in revised form 26 March 2018; Accepted 2 April 2018
Available online 03 April 2018
0378-5173/ © 2018 Published by Elsevier B.V.

the effects of liposomes with varying surface charges and particle sizes on virus infectivity. Subsequently, we investigated the inhibitory activities of cationic liposomes with incorporated stearylamine (SA) on BV infectivity. The simple experiments using BV (not pathogenic for humans) helped to clarify the antiviral mechanism, which was necessary to demonstrate the antivirus effect of liposomes against pathogenic viruses. Finally, we determined the anti-HSV-1 activities of these liposomes.

The liposomal formulations without loading antiviral drugs evaluated in this study are very simple, easy to produce, and relatively less expensive compared with several expensive prescription medicines for virus infectivity (McConachie et al., 2016; Mikkelsen and Andersen, 2016). This anti-virus liposome is potentially useful in infection therapy because incorporating existing drugs or a molecular species such as a specific virus receptor into the liposome has the potential to enhance the inhibition of virus infectivity.

2. Material and methods

2.1. Materials

The liposome component 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC; COATSOME MC-8080) was purchased from Nippon Oil and Fats Company, Ltd. (Tokyo, Japan). SA was purchased from Tokyo Kasei Company (Tokyo, Japan). Dicetyl phosphate (DCP) and cholesterol (Chol) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trypan blue solution (0.4 w/v%) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). CellLight Nucleus-GFP/RFP BacMam 2.0 reagents were obtained from Thermo Fisher Scientific (Waltham, MA, USA), and 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) was purchased from Lambda Probes & Diagnostics (Graz, Austria). All other chemicals were of commercial reagent grade.

2.2. Cell lines

Human A549 lung epithelial cells (RCB0098; RIKEN BRC, Tsukuba, Japan) were maintained as adherent monolayer cultures in Dulbecco's Modified Eagle's Medium (DMEM; Thermo Fisher Scientific) supplemented with 10% (v/v) fetal bovine serum (FBS), 100 IU/mL penicillin, and 100 IU/mL streptomycin. A human conjunctival cell line (Wong-Kilbourne derivative of Chang conjunctiva, clone 1-5c-41, CCL-20.2™, American Type Culture Collection [ATCC], Manassas, VA, USA) was cultured in medium-199 with Hank's salts (MP Biomedicals, LLC, France) supplemented with 10% FBS, 0.22% $NaHCO_3$, 100 µg/mL streptomycin, and 100 µg/mL penicillin. The B16-BL6 mouse melanoma cell line (RCB2638; RIKEN BRC) was cultured in DMEM containing 10% FBS and 0.1% penicillin/streptomycin solution.

2.3. Preparation of liposomes

Liposomes were prepared using a hydration method followed by extrusion (LipoFast™-Pneumatic; Avestin, Inc., Ottawa, Canada) through size-controlled polycarbonate membranes (Whatman® Nuclepore™ Track-Etched Membrane). Briefly, lipid mixtures were dissolved in small volumes of chloroform in round-bottomed flasks and were dried in a rotary evaporator under reduced pressure at 40 °C to form thin lipid films. DiI was also dissolved in lipid solution in chloroform when fluorescence labeling to liposome. The final DiI concentration in the resultant liposomal suspension was 1.3 µM. Films were then dried in a vacuum oven overnight to ensure complete removal of the solvent. Phosphate buffered saline (PBS) was then added and lipid films were incubated in a water bath at 70 °C for 30 s and were finally vortexed for approximately 30 s. This cycle was repeated five times and the resulting suspension of multilamellar vesicles (MLVs) was incubated at 10 °C for 30 min and was then extruded 41 times under nitrogen pressure (200 psi). Finally, particle sizes and zeta potentials of the liposomes were measured using a Zetasizer Nano ZS instrument (Malvern, Worcestershire, UK).

2.4. Cytotoxicity

A549 cells were seeded into 96 well plates at an initial density of 2×10^4 cells/well and were cultured for 24 h. The medium was then replaced with fresh medium containing various concentrations of compounds, and the plates were incubated for another 4 h. Cytotoxicity was assessed using CellTiter 96® Aqueous One Solution Assays (Promega, Madison, WI, USA). After incubation for 1 h in the respective reagents, absorbance was determined at a test wavelength of 490 nm and a reference wavelength of 660 nm using a microplate reader (MTP-100; Corona Electric, Tokyo, Japan). Specifically, the formation of formazan was determined at 490 nm and was assumed to be proportional to the number of viable cells. Cell viability (%) was calculated relative to the control wells that contained the cell culture medium without the test sample.

2.5. In vitro antiviral assays of the BV model

The CellLight Nucleus BacMam 2.0 system comprises BV vectors for delivery of genes encoding nuclear localized GFP or RFP, and was used as a model virus according to the manufacturer's protocol. In these experiments, cells were seeded in 24-well plates at a density of 0.5×10^5 cells/well. After 24 h, the cells were washed three times in PBS and were then treated with liposomes and/or virus using one of the two methods shown in Fig. 1. Using the first method (Method 1), liposomal preparations were added to cells in serum-free DMEM, which were then incubated for 2 h at 37 °C. Cells were then rinsed three times in PBS, and the culture medium containing virus (10 particles per cell) was added and the cells were cultured for 22 h. Using the second method (Method 2), liposomes were added to the cells with the virus and were then incubated in serum-free DMEM for 22 h at 37 °C. After the treatments according to either Methods 1 or 2, the cells were washed twice with PBS and trypsinized to prepare cell suspensions in the culture medium. GFP-expressing cells, indicating BV infection, were counted using a FACSVerse flow cytometer (BD Biosciences), and the data were analyzed using BD FACSuite software.

2.6. Microscope studies

A549 cells were added to Lab-Tek® II Chamber Slides (Nalgen Nunc International, Rochester, NY, USA) at a density of 0.5×10^5 cells/well and were cultured for 24 h. Cells were then washed, and liposomes and CellLight™ Nucleus-RFP were added as a model virus according to Methods 1 or 2. After infection, the cells were washed three times in ice-cold PBS and cell monolayers were fixed with 0.5 mL of 4% paraformaldehyde solution for 1 h. After another wash with PBS, the fixed cells were observed using a fluorescence microscope (model BZ-9000; Keyence, Osaka, Japan).

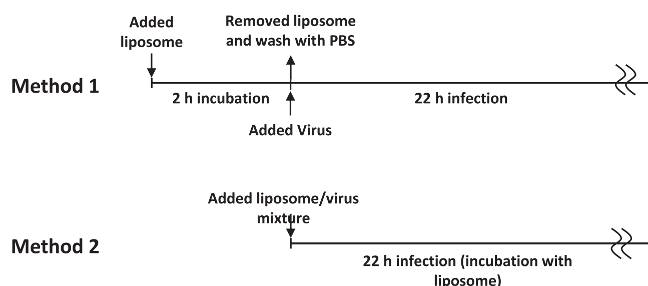

Fig. 1. Experimental schedule for determinations of BV infectivity in cells.

Download English Version:

https://daneshyari.com/en/article/8520014

Download Persian Version:

https://daneshyari.com/article/8520014

Daneshyari.com