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Passive targeting of phosphatiosomes increases rolipram delivery to the lungs for treatment of acute lung injury: An animal study

Chia-Lang Fang [a,b], Chih-Jen Wen [c,d], Ibrahim A. Aljuffali [e], Calvin T. Sung [f], Chun-Lin Huang [g], Jia-You Fang [g,h,i,*]

[a] Department of Pathology, College of Medicine, Taipei Medical University, Taipei, Taiwan
[b] Department of Pathology, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan
[c] School of Medicine, Chang Gung University, Kweishan, Taoyuan, Taiwan
[d] Center for Vascularized Composite Allotransplantation, Chang Gung Memorial Hospital, Kweishan, Taoyuan, Taiwan
[e] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[f] Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, CA, USA
[g] Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan
[h] Chinese Herbal Medicine Research Team, Healthy Aging Research Center, Chang Gung University, Kweishan, Taoyuan, Taiwan
[i] Research Center for Industry of Human Ecology, Chang Gung University of Science and Technology, Kweishan, Taoyuan, Taiwan

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ABSTRACT

A novel nanovesicle carrier, phosphatiosomes, was developed to enhance the targeting efficiency of phosphodiesterase 4 (PDE4) inhibitor to the lungs for treating acute lung injury (ALI) by intravenous administration. Phosphatiosomes were the basis of a niosomal system containing phosphatidylcholine (PC) and distearoylphosphatidylethanolamine polyethylene glycol (DSPE-PEG). Rolipram was used as the model drug loaded in the phosphatiosomes. Bioimaging, biodistribution, activated neutrophil inhibition, and ALI treatment were performed to evaluate the feasibility of phosphatiosomes as the lung-targeting carriers. An encapsulation percentage of >90% was achieved for rolipram-loaded nanovesicles. The vesicle size and zeta potential of the phosphatiosomes were 154 nm and −34 mV, respectively. Real-time imaging in rats showed a delayed and lower uptake of phosphatiosomes by the liver and spleen. Ex vivo bioimaging demonstrated a high accumulation of phosphatiosomes in the lungs. In vivo biodistribution exhibited increased lung accumulation and reduced brain penetration of rolipram in phosphatiosomes relative to the control solution. Phosphatiosomes improved the lungs/brain ratio of the drug by more than 7-fold. Interaction with pulmonary lipoprotein surfactants and the subsequent aggregation may be the mechanisms for facilitating lung targeting by phosphatiosomes. Rolipram could continue to inhibit active neutrophils after inclusion in the nanovesicles by suppressing $O_2^{\bullet-}$ generation and elevating cAMP. Phosphatiosomes significantly alleviated ALI in mice as revealed by examining their pulmonary appearance, edema, myeloperoxidase (MPO) activity, and histopathology. This study highlights the potential of nanovesicles to deliver the drug for targeting the lungs and attenuating nervous system side effects.

1. Introduction

The activation of neutrophils is affected by intracellular levels of cyclic adenosine monophosphate (cAMP), which can be regulated by phosphodiesterase (PDE) isoenzymes. PDE4 inhibitors have been recognized as anti-inflammatory drugs for the treatment of respiratory disorders such as chronic obstructive pulmonary disease (COPD) and asthma [1]. The adverse effects of emesis and nausea as well as the low therapeutic indices have limited the wide use of PDE4 inhibitors. Until now, only one PDE4 inhibitor, roflumilast, had been approved by the USFDA for the treatment of COPD [2]. The emesis caused by PDE4 inhibitors is produced in the postrema of the brain [3]. PDE4 inhibitors can be a powerful anti-inflammatory drug for lung treatment if the brain penetration is reduced. An efficient way to achieve this is the employment of nanocarriers. Niosomes are nanovesicles containing nonionic surfactants and cholesterol for forming bilayer systems. They act as potential nanosystems for controlled drug delivery with nontoxicity, excellent stability, and ease of production [4].

After parenteral injection, niosomes are always filtered by the lungs first, and then cleared mainly by a mononuclear phagocyte system (MPS) such as the liver and spleen [5]. This may lead to the limitation of the dose available in the diseased site. A strategy to resolve this problem is targeting the region of interest. Antibody conjugation to nanocarriers is a paradigm for active targeting. However, the antibody-ligand systems are usually accompanied by toxicity concern [6,7]. The cost of the antibody is also high. Passive targeting by modulation of nanoparticulate composition and structure is another choice for efficient delivery.

* Corresponding author at: Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, 259 Wen-Hwa 1st Road, Kweishan, Taoyuan 333, Taiwan.
E-mail address: fajy@mail.cgu.edu.tw (J.-Y. Fang).

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Pulmonary surfactants are a complex mixture of lipoproteins, which can stabilize the alveolar air sacs during respiration. Phosphatidylcholine (PC) is the predominant lipid component of the surfactants [8]. Previous studies have shown that exogenous PC can strongly interact with and adsorb pulmonary surfactants [9,10]. The increased lung uptake thus can be attained. The addition of phospholipids in nanosystems elevates the interface rigidity and biocompatibility [11,12]. It is hypothesized that incorporation of PC would target the niosomes to the lungs and minimize the propensity for liver and spleen uptake, making the nanovesicles more attractive for drug delivery. Here, we introduced "phosphatiosomes" for accomplishing this purpose. Rolipram is used as the model drug in this report because it is the prototypic PDE4 inhibitor [13].

Acute lung injury (ALI) is a life-threatening disease with a high mortality rate of 30%–50% [14]. It remains a challenging problem for intensive care medicine. The ALI patients are usually in an emergency condition. Inhibition of activated neutrophils in the lung tissues and vessels plays an essential role for treating ALI. Intravenous administration provides an efficient route to alleviated ALI [15,16]. We tried to ameliorate ALI by intravenously administering rolipram in phosphatiosome form by using mice as an animal model. In vivo bioimaging was utilized to monitor tissue distribution of phosphatiosomes in rats by employing 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indotricarbocyanine iodide (DiR) as the dye. The biodistribution of rolipram in niosomes and phosphatiosomes was compared. In addition to the lung targeting, whether the rolipram-loaded phosphatiosomes could interact with activated neutrophils or inhibit oxidative stress was also studied.

2. Materials and methods

2.1. Preparation of nanovesicles

A thin-film method was utilized to compose niosomes and phosphatiosomes. Span 60 (0.35% of the final concentration, Sigma-Aldrich, St. Louis, MO, USA), cholesterol (0.3%, Sigma-Aldrich), and distearoylphosphatidylethanolamine polyethylene glycol (DSPE-PEG) with a molecular weight of 5000 (0.2%, Nippon Oil, Tokyo, Japan) were dissolved in a mixture of chloroform and ethanol (1:1). Soybean PC (American Lecithin, Oxford, CT, USA) at a concentration of 0.2% was incorporated into the mixture to produce phosphatiosomes. The organic solvent was evaporated in a rotary evaporator at 60 °C. The residual solvent was removed under a vacuum overnight. Water was added to hydrate the film by homogenizer (Pro250, Pro Scientific, Monroe, CT, USA) at 12000 rpm and probe-type sonicator (VCX600, Sonics and Materials, Danbury, CT, USA) at 35 W for 10 min, respectively. DiR (0.05%, AAT Bioquest, Sunnyvale, CA, USA) and rolipram (0.1%, Cayman, Ann Arbor, MI, USA) were added as the dye and drug if necessary.

2.2. Average diameter and zeta potential

The mean diameter (z-average) and zeta potential of the vesicles were measured using a laser-scattering method (Nano ZS90, Malvern, Worcestershire, UK). The dispersion was diluted 100-fold with water before testing. The vesicle number of the nanocarriers was detected using a qNano particle counter (Izon, Christchurch, New Zealand).

2.3. Encapsulation efficiency of rolipram

The percentage of rolipram loading in nanovesicles was measured using an ultracentrifugation method (Optima MAX®, Beckman Coulter, Fullerton, CA, USA). The dispersion was centrifuged at 48000 ×*g* at 4 °C for 30 min. The supernatant and precipitate were withdrawn and analyzed by high-performance liquid chromatography (HPLC) to calculate the encapsulation percentage of the initial amount of rolipram added.

2.4. Transmission electron microscopy (TEM)

The morphology of the nanovesicles was monitored by H-7500 electron microscopy (Hitachi, Tokyo, Japan). One drop of the dispersion was pipetted onto a carbon-film-coated copper grid to form a thin-film specimen and stained with 1% phosphotungstic acid. The prepared samples were photographed by TEM.

2.5. In vitro rolipram release

A cellulose membrane (Cellu-Sep® T1, molecular weight cutoff = 3500 Da) was mounted between the donor and receptor compartments of Franz cell. The donor medium consisted of 0.5 ml of control solution or vesicle systems. The receptor medium consisted of 5.5 ml of 30% ethanol in pH 7.4 buffer. The available area for diffusion was 0.785 cm^2. The stirring rate and temperature were maintained at 600 rpm and 37 °C, respectively. The receptor medium was withdrawn (0.3 ml) and immediately replaced with an equal volume of fresh medium at determined intervals. The amount of rolipram in receptor was quantified by HPLC.

2.6. Animals

Male Sprague–Dawley rats (200–300 g) and C57BL/6 J mice (22–25 g) were acquired from Lasco (Taipei, Taiwan). All animal procedures were performed in accordance with protocols approved prospectively by the Institute of Animal Care and the Use Committee of Chang Gung University. Alfalfa-free food and water were given ad libitum. Animals fasted for 8 h before the experiments.

2.7. In vivo and ex vivo bioimaging

The rats were anesthetized using Zoletil® 50 (60 mg/kg). Control solution or nanovesicles with DiR was injected into the femoral vein at a volume of 0.8 ml/kg. Real-time imaging was achieved using Pearl® Impulse (Li-Cor, Lincoln, NE, USA) at near-infrared wavelength. Isoflurane/oxygen was employed to maintain the anesthetized condition. The rats were sacrificed 4 h post-injection. The organs were harvested and washed with normal saline. The fluorescence of the organs was then examined using Pearl® Impulse. This bioimaging was repeated on at least three animals, and representative images were exhibited.

2.8. Imaging of organ sections

After ex vivo imaging, the lungs and brain were cryosectioned at a thickness of 20 μm and photographed under a Zeiss AxioImager fluorescence microscope (Oberkochen, Germany) at a near-infrared channel. Photos from each field of vision were integrated as a panorama.

2.9. Biodistribution of rolipram

Rats were sacrificed 4 h after intravenous dosing of the control solution or nanovesicles at 0.8 ml/kg (0.8 mg/kg for rolipram). The lungs, liver, brain, and spleen were removed. The blood was withdrawn from tail vein at determined intervals. The plasma was obtained by centrifugation of the blood at 2000 ×*g* for 20 min. Acetonitrile (0.6 ml) was mixed with plasma (0.2 ml) for 10 s for deproteinization. The supernatant was analyzed using HPLC after a centrifugation at 8000 ×*g* for 10 min. The organs were weighed and homogenized in methanol for 5 min. After a centrifugation at 10,000 ×*g* for 10 min, the supernatant (0.2 ml) was withdrawn and then mixed with acetonitrile (0.6 ml). Following a centrifugation (8000 ×*g* for 10 min), the supernatant was analyzed using HPLC to measure the rolipram concentration.