Journal of Controlled Release 158 (2012) 413–423

Contents lists available at SciVerse ScienceDirect

Journal of Controlled Release

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

Monitoring the transport of polymeric micelles across MDCK cell monolayer and exploring related mechanisms

Shanshan Zhao [a,b], Wenbing Dai [a], Bing He [a], Jiancheng Wang [a], Zhonggui He [b], Xuan Zhang [a,*], Qiang Zhang [a,**]

[a] State Key Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing, 100191, PR China
[b] Department of Pharmaceutics, School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, 110016, PR China

ARTICLE INFO

Article history:
Received 6 June 2011
Accepted 19 December 2011
Available online 29 December 2011

Keywords:
Polymeric micelles
MDCK
Endocytosis
Transport
Transcytosis

ABSTRACT

Although some formulations based on nanotechnology are already available, the transport of nanoscale particles including polymeric micelles (PMs) across epithelial cell monolayer was barely studied. To prove the transport of PMs across the epithelial barrier and explain the mechanisms related, a typical PM system containing a fluorescent probe Coumarin 6 (C6) was prepared and the Madin–Darby canine kidney (MDCK) cell line was used as an epithelial cell model. Four different approaches were applied to monitor the transport of PMs prepared, including the real-time and *in situ* imaging by a novel approach developed by our group. And the solid evidence of PMs' transport was obtained. The mechanism related was explored by different techniques. With the absence of caveolae mediated endocytosis and macropinocytosis, the clathrin mediated pathway might play a great role here, and a fraction of PMs bypassed the degradative lysosome pathway, probably due to the clathrin and caveolae independent mechanisms. Interestingly, some inhibitor was found to inhibit transcytosis of PMs significantly but not their endocytosis, suggesting different mechanisms involved in endocytosis and exocytosis. The Forster resonance energy transfer (FRET) phenomenon still existed after FRET PMs were internalized by cells. Anyhow, a multiple process with active transcellular pathway was indicated.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Biological barriers are the main hindrance against uncontrolled uptake of a variety of substances, and they are typically represented by epithelial tissues [1]. Epithelial cells are throughout the body and are commonly found in the kidney, small intestine, corneal tissues and nasal mucosa etc. [2], and play important roles in host defense, nutrient absorption and ion transport. Epithelial cells are highly polarized, with apical surfaces exposed to the outside environment and basolateral surfaces contacting the underlying basal lamina. Tight junctions divide plasma membrane into apical and basolateral parts that possess different functions based on their differential compositions of proteins and lipids.

However, many new chemical entities and therapeutic agents are limited by their inability to reach the systemic circulation because of these biological barriers. This has always been a great challenge for pharmaceutical sciences and pharmaceutical industry. In recent years, the nanotechnology based drug delivery system as nanomedicines have attracted much interest. Nanomedicines offer an enormous potential for their ability to cross biological barrier and deliver active substances into the circulation or organism. Great progress has been made, and specifically, FDA has approved several formulations of nanomedicines in a rather short period of time [3], signaling the ushering in of a new era.

Among all the nanoscale drug delivery systems, polymeric micelles (PMs) have caused more attention. PMs are colloidal particles, generally less than 100 nm, composed of the amphiphilic block copolymer which could be self-assembled to form micelles in aqueous solution [4]. Their hydrophobic core serves as a reservoir for poorly soluble drugs and they can greatly increase the drug solubility [5]. Besides, PMs have a very high thermodynamic stability, with a 1000 fold lower critical micelle concentration than that of surfactant micelles [6]. Additionally, the micelles made of polyester or pegylated polyester such as PEG-PLA in this study are biodegradable *in vivo*. So PMs can be used for solubilization, stabilization and controlled release of drugs through a facile way. Especially, the studies with PMs proved its ability to improve the drug absorption greatly [7,8]. Currently, PMs are recognized as one of the most promising modalities of drug delivery systems.

Nevertheless, there were only limited reports on the transport mechanism of PMs across the epithelial cell barrier [9]. More studies were focused on the nanoparticles' cellular uptake [10–12], and there was some on intracellular trafficking recently [13–16]. The most frequently tested systems were some kinds of chitosan nanoparticles [17–19]. While the transport investigation was mostly limited in the study of apparent permeability coefficient (P_{app}) of the drug

* Corresponding author. Tel./fax: +86 10 82802683.
** Corresponding author. Tel./fax: +86 10 82802791.
E-mail addresses: xuanzhang@bjmu.edu.cn (X. Zhang), zqdodo@bjmu.edu.cn (Q. Zhang).

0168-3659/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jconrel.2011.12.018

loaded in the nanocarriers [20–22] or the nanocarrier itself [9], the direct evidence of the nanoparticles themselves transported across the epithelial cell layers as well as the methods to monitor the nanoparticles transport were deficient. In other words, it is really necessary to prove the transport of nanoscale particles across the epithelial barrier and understand more about the related rules before nanotechnology formulations can be considered as a viable concept for the drug with low permeability against biomembranes.

In order to prove the transport of PMs across the epithelial cell monolayer and explain the possible mechanisms related, here we constructed a typical PM system containing a lipophilic fluorescent probe Coumarin 6 (C6). Madin–Darby canine kidney (MDCK) cell line was used as an epithelial cell model in this study, because it is widely adopted to simulate gastrointestinal tract, blood brain barrier and so on, and it is able to differentiate into columnar epithelium with tight junctions when cultured on semipermeable filters. On the other hand, MDCK cells harbor a relatively thin mucus layer [23], and this enabled us to study the specific interaction between the PMs and the transport mechanism independent of the mucus layer. Some nice approaches, including a real-time and *in situ* imaging approach developed by our group, transmission electron microscopy (TEM), Forster resonance energy transfer (FRET), fluorescence co-localization, three-dimensional reconstruction of cell monolayer, etc., were used to monitor and study the transport of PMs prepared.

2. Materials and methods

2.1. Materials

PEG_{3000}-PLA_{3000} (Mw/Mn = 1.19) were purchased from Advanced Polymeric Materials Inc. (Montreal, QC, Canada). Fluorescent probes including rhodamine–phalloidin (RP), DiO, DiI, and Hoechst 33258 were the products of Molecular Probes Inc. (Eugene, OR, USA). Coumarin 6 (C6), rhodamine–dextran (MW: 10K) and inhibitors such as chlorpromazine (CPZ), methyl-β-cyclodextrin (MβCD), monensin, amiloride, and nystatin were all purchased from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin–streptomycin and fetal bovine serum (FBS) were from GIBCO, Invitrogen Corp. (Carlsbad, CA, USA). Transwells (12 wells, pore diameter of 3 μm, polycarbonate) were supplied from Corning Costar (Cambridge, MA). Bicinchoninic acid (BCA) protein assay kit was from Applygen Technologies Inc. (Beijing, China). All the other reagents were of analytical grade and came from commercial source.

2.2. Preparation and characterization of Coumarin 6-loaded polymeric micelles (C6-PMs)

C6-PMs were prepared according to solvent evaporation method [24]. Briefly, C6 and PEG_{3000}-PLA_{3000} were co-dissolved in acetonitrile, and evaporated under vacuum at 60 °C for 30 min. After addition of HEPES buffered saline (HBS, pH 7.4) at 60 °C and ultra-sonication for 2 min, a clear micelles solution was obtained. Finally, the solution was filtered through a 0.22 μm membrane filter. The C6-PMs were characterized by TEM (JEOL, JEM-200CX, Japan), dynamic light scattering analysis (Malvern Zetasizer Nano ZS, Malvern, UK) at 25 °C, and the *in vitro* release test in a dialysis bag (MW cutoff = 14,000 Da) dialyzed against Hank's balanced salt solution (HBSS: 137 mM NaCl, 5.36 mM KCl, 0.44 mM KH_2PO_4, 0.34 mM Na_2HPO_4, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 4.17 mM $NaHCO_3$, 5.6 mM glucose) under continuous gentle stirring at 37 °C.

2.3. MDCK cell culture

MDCK cells (Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing) were maintained in high glucose DMEM, supplemented with 10% FBS and 1% penicillin–streptomycin, in a humidified atmosphere containing 5% CO_2 at 37 °C in 75 cm^2 plastic flasks. The medium was refreshed every 2 days. Cells were passaged at 70%–90% confluency using 0.25% (w/v) trypsin–0.02% (w/v) ethylene diamine tetraacetic acid (EDTA) solution.

For the transport experiments, cells were seeded onto Transwell filters at a density of 1×10^6 cells per well and were allowed to grow and differentiate for 7 days. Cell monolayers were used when the net transepithelial electrical resistance (TEER) exceeded 250 Ω cm^2. The net value was calculated by subtracting the background value, which was measured on cell free chamber (blank), from the value of cell-seeded chamber. For the uptake experiments, MDCK cells were seeded in flat bottom 12-well plates and grown to confluency within 5 days prior to use.

2.4. Demonstration of the transport of micelles across the MDCK cell monolayer

2.4.1. Transport of C6-PMs across the cell monolayer

Prior to the transport experiment, culture media (see Section 2.3) were removed from the mature MDCK monolayers and the monolayers were rinsed twice with 37 °C HBSS. After 30 min incubation of both sides of the monolayers with HBSS, dilutions of C6-PMs were added to the apical chamber for the given time. For the evaluation of P_{app} of C6, at 30, 60, 90, 120 and 150 min, 200 μl sample was removed from the basolateral chamber for analysis (see Section 2.5), and the basolateral chamber was immediately replenished with 200 μl of fresh and preheated HBSS.

2.4.2. Confocal image series of MDCK cell monolayer along the Z-axis

For imaging of the Transwell filter grown cell monolayer, after 1 h transport experiment with C6-PMs, the polycarbonate membrane with cell monolayer was cut carefully to be sandwiched between a glass coverslip and a slide after fixation and staining. The sample was put into the microscope stage to be viewed under Leica TCS SP5 confocal laser scanning microscope (CLSM, Heidelberg, Germany) directly. Serial sections along the *Z*-axis, from the apical side to the basolateral side of cell monolayer at 0.5 μm step size, were used to localize the intracellular C6-PMs. The processing and visualization of three-dimensional reconstruction images was performed using the Leica software.

2.4.3. Real-time imaging of transport process of micelles

For the real-time imaging of the C6-PMs transport across the cell monolayer, we developed a novel approach in which the Transwell insert with integrated cell monolayer was put on a glass-bottomed culture dish and free MDCK cell suspension was added to the basolateral medium. Additionally, we took advantage of the reflection property of the Transwell insert polycarbonate membrane to imaging the non-fluorescence membrane, which was the foundation and support for cell growth, under CLSM (Leica TCS SP2). In the images, the membrane with its 3 μm pores could be clearly seen and depicted. Images were taken as soon as C6-PMs were added after positioning the whole device on the microscope stage.

2.4.4. The direct imaging of the micelles in apical and basolateral chamber by TEM

For the direct imaging of the micelles in apical and basolateral chamber, which was another evidence of transported micelles, both the apical and basolateral medium were collected after the transport test with C6-PMs at 37 °C for 2 h. Then the medium were treated with uranyl acetate and viewed under TEM.

2.5. The transport and uptake of C6 loaded in micelles

2.5.1. Apparent permeability coefficient of C6

The P_{app} is expressed as cm/s. The P_{app} value was obtained by the formula $P_{app} = dQ/dt \times 1/AC_0$, where A is the surface area of the cell monolayers (1.13 cm^2 in this study), C_0 is the initial concentration

Download English Version:

https://daneshyari.com/en/article/10613193

Download Persian Version:

https://daneshyari.com/article/10613193

Daneshyari.com