Colloids and Surfaces B: Biointerfaces 135 (2015) 458–464

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Combination of elastic liposomes and low frequency ultrasound for skin permeation enhancement of hyaluronic acid

Chawankorn Kasetvatin[a], Soravoot Rujivipat[a], Waree Tiyaboonchai[a,b,c,*]

[a] Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand
[b] The Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on High Education, Ministry of Education, Thailand
[c] The Center of Excellence in Medical Biotechnology, Naresuan University, Phitsanulok 65000, Thailand

ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form 5 July 2015
Accepted 28 July 2015
Available online 31 July 2015

Keywords:
Elastic liposomes
Hyaluronic acid
Low frequency ultrasound
Transepidermal delivery

ABSTRACT

Hypothesis: The synergistic approach of using elastic liposomes (ELs) and low frequency ultrasound (LFU) was developed to enhance transepidermal delivery of hydrophilic macromolecules, hyaluronic acid (HA).
Experiment: HA loaded ELs were prepared with varying cholesterol contents by reverse phase evaporation technique. Their mean sizes were evaluated using dynamic light scattering. Entrapment efficacy (%EE) was determined by UV–vis spectrophotometry. *In vitro* permeation studies using porcine ear epidermis were investigated. In addition, skin barrier disruption was assessed by transepidermal water loss and histology.
Findings: The HA loaded ELs showed mostly elliptical shaped with a mean size of ~700 nm and a zeta potential of ~−40 mV. Up to 77% drug entrapment efficiency was achieved. As ELs cholesterol content decreased, vesicle size, elasticity of liposomes and HA permeation profile increased. The *in vitro* permeation studies demonstrated that HA solution cannot permeate through the porcine epidermis. The combination of ELs/LFU showed greater HA permeation than ELs and HA/LFU, 2.1 times and 6.4 times, respectively. Increased LFU exposure times augmented HA permeation, but greater skin disruption was observed. Nevertheless, no skin damage was observed at the optimized 1 min exposure time. This ELs/LFU combination provides an efficacious protocol for transcutaneous drug delivery.

1. Introduction

Transdermal drug delivery offers many advantages such as ease of delivery and non-invasive administration, avoidance of first pass metabolism, minimization of pain and control of drug release. The skin barrier is considered to be the greatest obstacle to efficient drug penetration. To increase drug penetration through the skin, several methods have been proposed, such as the use of physical penetration techniques (e.g., ultrasound), chemical enhancers (e.g., surfactants) and delivery vesicles (e.g., liposomes, elastic vesicles). Even though each method has shown to improve transdermal drug transport, their combined use has been proposed as a superior means of enhancing drug penetration [1]. For the best synergistic effect, a different mechanism of enhancing drug penetration is needed.

Owing to the skin barrier, transdermal drug delivery is generally restricted to hydrophobic drugs with molecular weight of less than 500 Da [2]. However, there is an increasing number of hydrophilic macromolecules developed for medical and cosmetic applications. Therefore, the challenge is to find a means to enhance the transdermal delivery of these hydrophilic macromolecules.

To enhance the transdermal delivery of hydrophilic macromolecules we propose a combination of elastic liposomes (ELs) and low frequency ultrasound (LFU). LFU is an alternative and interesting method to enhance transdermal delivery. Prior studies reported that LFU has been efficiently improved transdermal delivery of large molecules such as insulin, γ-interferon, and tetanus toxoid [3,4]. This technique improves skin permeability by a cavitation phenomenon of disordering bilayers and generating 'channel-like regions' within intercellular lipids [5,6].

Conventional liposomes (CL), commonly known as rigid liposomes, are interesting delivery vesicles for hydrophilic molecules that are entrapped in the core of the vesicle. Several researchers studied the combination use of ELs/LFU. Unfortunately, this combination decreases transdermal drug delivery due to the repair of the disrupted skin by the liposomal phospholipid when the liposomes

* Corresponding author at: Department of Pharmaceutical Technology, Faculty of Pharmaceutical Sciences, Naresuan University, Phitsanulok 65000, Thailand. Fax: +66 55 963731.
E-mail address: wareet@nu.ac.th (W. Tiyaboonchai).

http://dx.doi.org/10.1016/j.colsurfb.2015.07.078
0927-7765/

are broken during the ultrasound treatment [7]. On the contrary, ELs exhibit lipid bilayers which imitate CL, but with the incorporation of an edge activator (single chain surfactant) to increase deformability. Thus, they offer a promising approach due to their excellent biocompatibility and deformable competency that lead to enhanced skin permeability of various drugs. ELs can infiltrate the skin integrity, while conserving their intact vesicles, via the lipid lamellar regions of the skin and shunt pathways [8,9]. Therefore, a combination of ELs/LFU is considered as a potential means to enhance transdermal delivery of hydrophilic macromolecules. To the best of our knowledge, this is the first time this combination has been reported.

In this study, we demonstrate that the simultaneous treatment of ELs/LFU on porcine ear epidermis improves transdermal delivery of the hydrophilic macromolecule, hyaluronic acid (HA). HA, a natural glycosaminoglycan, is ubiquitous in many tissues of the human body, especially in the dermis, soft connective tissue of the skin and cartilage and joint fluids. It plays a significant role in the water holding capacity to conserve skin hydration and tissue healing. However, as skin ages, the HA in the skin decreases which accounts for the loss of skin moisture, ultimately resulting in loss of elasticity and development of wrinkles [10]. Thus, HA is considered as a promising dermal filler agent. In addition, HA is also widely used in osteoarthritis to ease pain and lubricate the joints [11]. However, owing to its high molecular weight and charge nature, it is difficult to deliver HA into the skin. Thus, HA was chosen as the model drug. The purposes of this study were to develop and characterize HA loaded ELs. The synergistic effect of ELs and LFU on skin permeation enhancement of HA was tested using porcine ear epidermis. In addition, for safety considerations, the effect of LFU exposure time on skin disruption was evaluated.

2. Materials and methods

2.1. Materials

HA (MW 1500 kDa), cholesterol (CH), and stain-all (SA) were purchased from Sigma Chemical (Steinheim, Germany). Chloroform and methanol were purchased from RCI Labscan (Bangkok, Thailand). Lipoid S100-3 (Hydrogenated phosphatidylcholine, HPC) was received as a gift from Lipoid GmbH Co., Ltd. (Ludwigshafen, Germany). All other chemicals and solvents were analytical grade. Polycarbonate membranes, diameter 19 mm, with 0.4 and 0.1 μm pore size (Nuclepore™, Whatman®) were purchased from Whatman international Ltd. (New York, USA). De-ionized (DI) water was used in the preparation of solutions and dispersion of HA loaded ELs.

In this study, three formulations, EL1, EL2 and EL3, have been prepared with different HPC:CH:Tween 80 mM ratio of 9:7:2, 9:9:2 and 9:11:2, respectively. The conventional liposome, CL, was prepared with HPC:CH mM ratio of 9:9.

2.2. Determination of HA by colorimetric assay

SA, a cationic dye, is known to bind glycosaminoglycan and form complexes with different optical properties compared to the free dye. Thus, the amount of HA was determined by complexing with SA as reported by Fagnola et al. [12]. Briefly, HA aqueous solutions were prepared with a concentration ranging from 0.3 to 5 mg/ml. A dye solution (0.1 mg/ml) was prepared by dissolving SA in a mixture of water and methanol (22:3, v/v). Then, HA solution was mixed with SA solution in a ratio of 7:1. The HA/SA complex was detected using a UV–vis spectrophotometer at 640 nm (Genesys 10 Series, Thermo Fisher Scientific Inc., USA).

2.3. Preparation of HA loaded elastic liposomes (ELs)

HA loaded ELs were prepared by the reverse phase evaporation technique (REV). Briefly, the organic phase containing HPC and CH was dissolved in a mixture of ethanol and chloroform (4:1, v/v). The aqueous phase containing HA and Tween 80 was added to the organic phase and mixed. This mixture system was sonicated in a sonicator bath (Transsonic 829/H, Elma®, Lebanon) at 50 °C for 10 min. ELs dispersion was obtained by removing the organic solvents using a rotary evaporator (R153, Buchi, Switzerland).

2.4. Physico-chemical characterization of HA loaded ELs

2.4.1. Morphology

Twenty μl of HA loaded ELs was deposited on a carbon-coated 300 mesh copper grid. Then, the sample was negatively staining with 10 μl of 2% w/v uranyl acetate. Excessive solvent was removed with a Whatman no.1 filter paper and allowed to air–dry. Finally, the sample was kept in a desiccator for further observation by a transmission electron microscope (TEM, Tecnai 12, Philips, OR, USA).

2.4.2. Mean vesicle size, size distribution and zeta potential

The mean size (MS) and polydispersity index (PI) of all samples were characterized by dynamic light scattering (DLS) employing the ZataPALS® analyzer (Brookhaven Instrument Corporation, Holtsville, USA). This instrument was equipped with a 35 mW HeNe laser diode operating at 632.8 nm and a BI-200SM Goniometer connected to a BI-9010AT digital correlator. The MS and PI values were obtained by the auto measuring mode at a fixed angle of 90°. Samples were dispersed in DI water and run for 6 measurement cycles. The MS was analyzed using hydrodynamic diameter.

The zeta potential was determined using phase analysis light scattering with a ZetaPALS®. Measurement was then carried out at 25 °C and an angle of 14.8° to the incident light. Samples were dispersed with DI water and run for 10 measurement cycles. The zeta potential was calculated by the electrophoresis mobility based on the Smoluchowski equation [13]. The measurement was performed in triplicate.

2.4.3. Determination of entrapment efficiency (EE)

The content of the HA incorporated in the ELs was determined by the centrifugation method. One hundred mg of the HA loaded ELs was accurately weighed and dispersed in 2 ml of DI water. The dispersion was then centrifuged at 18,000 rpm for 30 min. After discarding the supernatant, the pellets were re-dispersed in 10 ml of DI water and further sonicated for 2 min by ultrasonic probe (VCX130, Vibra cell™, Sonic & materials Inc., USA). Then, 1 ml of supernatant was centrifuged at 18,000 rpm for 30 min. Finally, the amount of HA in the supernatant was determined using a UV–vis spectrophotometer at 640 nm as described above. The percentage of HA entrapped was then calculated from:

$$\text{HA entrapment}(\%) = \frac{\text{Amount of HA detected}}{\text{Initial amount of HA}} \times 100 \quad (1)$$

2.4.4. Deformability measurement

The elasticity of the liposomal membranes was assessed utilizing an extruder device as mentioned by Aggarwal and Goindi [14]. The principle of this experiment is based on the alteration of the vesicle size while passing through the polycarbonate filter which had a pore size of 100 nm. Briefly, the different liposome formulations were extruded through the filter membrane by a manual mini-extruder (Avanti Polar Lipid Inc., Alabaster, AL). Each of the samples was performed in triplicate. The MS was determined by DLS. The elasticity of the vesicles was computed by the following