Carbohydrate Polymers 94 (2013) 634–641

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

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

Construction of hyaluronic acid noisome as functional transdermal nanocarrier for tumor therapy

Ming Kong[a,*,1], Hyunjin Park[b,1], Chao Feng[a], Lin Hou[a], Xiaojie Cheng[a], Xiguang Chen[a,*]

[a] College of Marine Life Science, Ocean University of China, Yushan Road, Qingdao 266003, Shandong Province, China
[b] Graduate School Biotechnology, Korea University, 1,5-Ka, Anam-Dong, Sungbuk-Ku, Seoul 136-701, South Korea

ARTICLE INFO

Article history:
Received 19 November 2012
Received in revised form 9 January 2013
Accepted 24 January 2013
Available online 8 February 2013

Keywords:
Hyaluronic acid
Niosome
Transdermal
Tumor therapy

ABSTRACT

To develop a functional nanosized transdermal drug delivery system for tumor therapy, amphiphilic hyaluronic acid (HA) based niosome was constructed combining transdermal and tumor targeting ability in one entity. HA esterified with monostearin, the conjugate labeled as HA–GMS self-assembled onto niosome surface and formed HA–niosome. The multilayer vesicle had small size (around 40 nm), good stability and desirable drug encapsulating efficacy, and well compatible with blood. It exhibited better endocytosis to mouse breast tumor cell (4T1) than the control chitosan nanoparticle, which was verified qualitatively and quantitatively. Skin permeation of HA–niosome was proven to be efficient using in vitro stratum corneum model and in vivo fluorescence observation. Histological section study confirmed the security and efficiency of transdermal permeation. The results evidence HA–niosome to be exciting and promising for tumor therapy through trandermal administration.

1. Introduction

Carrier-mediated drug delivery has long been the interest to improve the efficacy of existing therapeutics. By virtue of small size, peculiar surface characteristics, unique physico-chemical and biological effects, numerous nanocarrier based drug delivery and drug targeting systems are currently developed or under development (Bang, Yu, Hwang, & Park, 2009; Liu & Park, 2009; Sonneville-Aubrun, Simonnet, & L'Alloret, 2004; Xia, Li, & Nel, 2009). They are tailored to enhance the in vivo efficiency of many drugs, especially anti-cancer drugs, with target to minimize drug degradation upon administration, prevent undesirable side effects, and increase drug bioavailability and the fraction of the drug accumulated in the target site (Torchilin, 2007). Conventional drug administration using nanocarrier, i.e. oral and intravenous injection (iv), offer good performance on efficacy though, its high administration frequency, low patients compliance and hepatic toxicity still need to be settled in practice.

Transdermal drug delivery (TDD) is a route of administration that drugs are delivered across the skin for systemic distribution. It is a manner to achieve drug input over extending periods of time at essentially sustained and controlled rates (Guy, 2007). TDD represents an attractive alternative to oral delivery and hypodermal injection (Prausnitz & Langer, 2008), which has been proven beneficial in reducing dose frequency, achieving target delivery and avoiding hepatic first pass metabolism (Mitragotri, 2004). The administration is easier and able to be suspended if necessary. Furthermore, steady absorption of drug over hours or days is usually preferable to the blood level spikes and troughs produced by oral dosage forms (Subedi, Oh, Chun, & Choi, 2010). Potential nanocarriers for TDD involve microemulsions (MEs), vesicles and nanoparticles (Neubert, 2011). Since non-compressible and rigid structure, nanoparticles are unable to cross pores comparable to or smaller than their own diameter (Cevc & Vierl, 2010). Even though MEs have substantial penetration enhancing effects for extremely lipophilic drugs in colloidal phase, the large content of surfactants causes severe irritation to skin (Santos, 2008). Owing to bilayer elasticity and transformable peculiarity, vesicular (liposomal) carriers turn out to be the most popular (Cevc & Vierl, 2010). Major advantage ascribed to their amphipathic nature, which allows the incorporation of both hydrophilic and hydrophobic drugs (Pham, Jaafar-Maalej, Charcosset, & Fessi, 2012).

Niosomes and liposomes are two representative vesicles made of non-ionic surfactants and phospholipids, respectively. In spite of liposomes could be of great interest for drug delivery, there are shortcomings associated with their physico-chemical stability (phospholipid hydrolysis or oxidation), high cost in production and variable purity of natural phospholipids. Besides, drug entrapment efficiency of liposomes is less than niosomes due to cholesterol content (Kazi et al., 2010). Noisome are non-ionic surfactant vesicles obtained on hydration of synthetic nonionic surfactants, with

* Corresponding authors at: 5# Yushan Road, Ocean University of China, Qingdao 266003, China. Tel.: +86 532 82032586; fax: +86 532 82032586.
E-mail addresses: kongming@ouc.edu.cn (M. Kong), xgchen@ouc.edu.cn (X. Chen).
[1] The author contributed equally to this study with the first author.

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.01.091

Table 1
Characteristics of HA–GMS conjugates and HA–N.

HA–GMS	HA (kDa)	DS (%)[b]	Size (nm)	PdI	ξ (mV)[c]	E.E. (%)[d]
H6.5[a]	110	7	47.2 ± 0.2	0.46	−23.77 ± 2.21	56.5 ± 3.6
H14	110	14	46.0 ± 0.3	0.44	−22.51 ± 1.67	–
H23	110	23	44.2 ± 0.3	0.45	−20.39 ± 2.43	72.4 ± 2.5
L6	10	6	38.2 ± 0.4	0.37	−20.24 ± 1.98	94.3 ± 1.2
L22	10	22	36.3 ± 0.2	0.39	−17.83 ± 1.83	–

[a] Capitals denoted M_w property of HA and numbers were DS of HA–GMS.
[b] DS, defined as the number of GMS molecules per 100 sugar residues of HA.
[c] The ξ potential of HA–N (1 mg/mL) in PBS (7.4).
[d] E.E. was calculated as the percentage of ratio of encapsulated VE mass to total VE mass (1 mg/mL).

or without incorporation of cholesterol or other lipids, which are biodegradable, relatively nontoxic, more stable and inexpensive, an alternative to liposomes (Patel, 2007). Slow penetration of drug through skin is the major drawback of transdermal route of delivery (Jayaraman, Ramachandran, & Weiner, 1996). Transdermal delivery of drug incorporated in niosomes results in enhanced delivery of drugs through the stratum corneum, and the delivery would be specifically enhanced if in combination of hydrophilic surfactants, which generate flexible bilayer flexible for "elastic" vesicles (Torchilin, 2007).

Hyaluronic acid (HA) is a naturally occurring polymer. Owing to its biocompatibility and biodegradability, HA has been extensively investigated for biomedical applications. HA derivatives acquire additional physicochemical characteristics besides their inherently superior properties. Amphiphathic derivatives endow HA with ability to be active in surface arrangement and colloidal edge adjustment (Kong, Chen, & Park, 2011). Moreover, HA can specifically bind to various cancer cells that over-express CD44, offering targeting ability to tumor for anti-cancer therapeutics (Choi et al., 2010). In addition, stabilin 2, HA receptor for endocytosis protein, promotes the uptake by tumor cells (Harris & Weigel, 2008). In this study, a novel drug nanocarrier HA niosome was developed, which combined transdermal delivery and tumor targeting together. Concerning physicochemical characteristics and biocompatibility were studied. In vitro and in vivo transdermal activities were evaluated qualitatively and quantitatively. 4T1 cell line was used to assess the endocytosis of HA niosome labeled by FITC.

2. Materials and methods

2.1. Materials

Sodium form of HA (M_w, 110 and 10 kDa) was a gift of Kolon Life Science, Korea. EDC·HCl (99%) and N-hydroxy succinimide (NHS, 99%) were purchased from Shanghai Medeep Co., Ltd. Monostearin (glycerol α-monostearate, GMS), ethylenediamine (EDA) were purchased from Tianjin Chemical Reagent. Methylene chloride, sodium bromide, Triton X-100 and acetonitrile (HPLC grade) were purchased from Huasheng Chemical. α-tocopherol (97%) (Vitamin E, VE), fluorescein isothiocyanate (FITC), POE (20) sorbitan monooleate (Tween 80) and sorbitan monolaurate (Span 20) were purchased from Sigma. Tissue-Tek® O.C.T™ Compound was ordered from Sakura Finetek USA, Inc. Water, used for synthesis and characterization was purified by distillation, deionized and subjected to reverse osmosis using a Milli-Q Plus apparatus (Millipore, USA). All the chemicals were analytical grade and were used as received.

2.2. Preparation of HA–GMS and FITC labeled HA–GMS

Different batches of amphiphilic HA–GMS were synthesized by a previous method (Kong, Chen, & Park, 2011), as shown in Table 1. Briefly, GMS was conjugated to HA by the formation of ester linkages through an EDC-mediated reaction. HA (190 mg) and EDC/NHS were dissolved in PBS at 1:1:1 mole ratio and maintained for 2 h. The solution was then added to GMS (60–540 mg) acetone solution dropwise with magnetic stirring (IKA RO10, Germany). The resultant mixture was centrifuged (15,000 rpm, 20 min) and the apparent solution was extensively dialyzed and lyophilized. The degree of substitution (DS) of GMS was determined by ^{1}H NMR, and was calculated from the ratio of the relative peak integrations of the GMS ending methyl protons (peaks at around approximately 1.0 ppm) and methyl protons in HA acetamide (approximately 2.0 ppm) (Kong, Chen, & Park, 2011). The obtained HA–GMS conjugate was dissolved in formamide along with EDC/NHS at molar ratio of 1:12:12 allowing for 2 h reaction. The solution was added dropwise into EDA, the free mole ratio of HA–GMS and EDA was about 1:200. After reaction for 6 h, the EDA–HA–GMS was dialyzed against water for 48 h. Ethanol solution of FITC was slowly added into EDA–HA–GMS. The reaction was ended 4 h later and dialyzed for 48 h before lyophilization.

2.3. Preparation of HA–niosome (HA–N)

HA–N was prepared by emulsion–evaporation method. HA–GMS emulsion was formed firstly based on modified protocol (Kong, Chen, & Park, 2011). Nonionic surfactants Tween 80/Span 20 (molar ratio 5:4) were dissolved in methylene chloride at proportion of 60%. The mixture (1 mL) was dispersed in 1 mg/mL HA–GMS PBS (7.4) solution with final volume of 20 mL, which was then sonicated twice (pulse on, 10.0 s; pulse off, 2.0 s), using a Sonics Vibra-Cell CV33 ultrasonic probe (Sonics & Materials, USA) at 225 W in an ice bath for 3 min. The methylene chloride was removed by a rotary vacuum evaporator at 35 °C, the suspension obtained was heated on a water bath at 60 °C for 10 min to yield HA–N. VE loading niosome was generated by dissolving VE in methylene chloride in preparation.

2.4. Characteristics of HA–N

Size analysis and zeta potential were determined on a Zetasizer ZEN 3600 Nano Series apparatus (ZEN, UK).

The morphology of HA–N was observed via a transmission electron microscope (TEM, JEM-1200EX JEOL Ltd., Japan).

VE loading niosomes were firstly purified by Sephadex G25 fine column. The amount of loaded VE was measured by HPLC at 280 nm using a reversed phase C18 column (Nova-Pak® C18, 3.9 mm × 150 mm, Waters Associates) operated at room temperature, a Waters 2690 Separation Module (Waters Associates) and a Waters 996 photodiode Array Detector (Waters Associates). Acetonitrile (HPLC grade) was used as eluent at a flow rate of 1 mL/min. Encapsulation efficiency (E.E.) was calculated as the percentage of ratio of encapsulated VE mass to total VE mass.