European Journal of Pharmaceutics and Biopharmaceutics xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

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

Research Paper

Cutaneous penetration of soft nanoparticles via photodamaged skin: Lipid-based and polymer-based nanocarriers for drug delivery

Chi-Feng Hung [a,1], Wei-Yu Chen [b,c,1], Ching-Yun Hsu [d], Ibrahim A. Aljuffali [e], Hui-Chi Shih [f,g], Jia-You Fang [f,h,i,*]

[a] School of Medicine, Fu Jen Catholic University, Hsinchuang, New Taipei City, Taiwan
[b] Department of Pathology, College of Medicine, Taipei Medical University, Taipei, Taiwan
[c] Department of Pathology, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan
[d] Department of Nutrition and Health Sciences, Chang Gung University of Science and Technology, Kweishan, Taoyuan, Taiwan
[e] Department of Pharmaceutics, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia
[f] Pharmaceutics Laboratory, Graduate Institute of Natural Products, Chang Gung University, Kweishan, Taoyuan, Taiwan
[g] Chang Gung Memorial Hospital, 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

ARTICLE INFO

Article history:
Received 16 February 2015
Revised 7 May 2015
Accepted in revised form 11 May 2015
Available online xxxx

Keywords:
Photodamage
Skin
Cutaneous penetration
Lipid nanoparticle
Polymer nanoparticle

ABSTRACT

Photoaging is recognized as the factor damaging skin-barrier function. The aim of this study was to examine the impact of ultraviolet (UV) irradiation on the cutaneous penetration of soft nanoparticles, including nanostructured lipid carriers (NLCs) and poly(lactic-co-glycolic acid) polymer nanoparticles (PNs). In vitro cutaneous permeation of retinoic acid (RA) carried by nanoparticles was evaluated. In vivo nude mouse skin distribution of topically applied nanoparticles was observed by fluorescence and confocal microscopies. The association of nanoparticles with cultured keratinocytes was measured by flow cytometry and fluorescence microscopy. The average diameter and surface charge were 236 nm and −32 mV for NLCs, and 207 nm and −12 mV for PNs. The ultrastructural images of skin demonstrated that the application of UV produced a loss of Odland bodies and desmosomes, the organelles regulating skin-barrier function. UVA exposure increased skin deposition of RA regardless of nanoparticle formulation. UVB did not alter RA deposition from nanoparticles as compared to the non-treated group. Exposure to UVA promoted RA delivery into hair follicles from NLCs and PNs by 4.2- and 4.9-fold, respectively. The in vivo skin distribution also showed a large accumulation of Nile red-loaded nanoparticles in follicles after UVA treatment. The soft nanoparticles were observed deep in the dermis. PNs with higher lipophilicity showed a greater association with keratinocytes compared to NLCs. The cell association of PNs was increased by UVA application, whereas the association between NLCs and keratinocytes was reduced two times by UVA. It was concluded that both follicles and intercellular spaces were the main pathways for nanoparticle diffusion into photodamaged skin.

1. Introduction

Nanomedicine provides potential benefits in the fields of drug delivery, medical diagnosis, cancer therapy, and tissue engineering [1]. The growth of nanomedicine has led to the translational development of industrial techniques and consumer products. Functional nanoparticles have gained much attention in targeted and controlled drug delivery. Within this field, the skin is the predominant target organ for nanoparticle exposure. The drug-loaded nanocarriers are an effective approach to target skin regions and cell populations of interest [2]. The impairment of skin integrity is becoming significant due to today's lifestyle and severe climate change. The barrier function of skin exposed under occupational or environmental conditions is not ideal. Ambient particulate matters, air pollution, and solar radiation all pose the risk of skin-barrier damage [3]. It is anticipated that nanoparticle delivery via damaged skin is quite different from that via intact skin. A

* 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).

[1] Equal contribution.

http://dx.doi.org/10.1016/j.ejpb.2015.05.005
0939-6411/

comprehensive elucidation of nanoparticle-skin interaction for damaged skin is highly relevant.

The thinning of the ozone layer has produced increased ultraviolet (UV) irradiation of the skin and the subsequent syndromes of photodamage in the past few decades. The impact of photoaging on nanoparticle penetration into the skin has already been studied by using quantum dots (QDs), titanium dioxide nanoparticles, and fullerenes [4–7]. These nanoparticles can be categorized as rigid nanoparticles according to the definition by Papakostas et al. [8]. Rigid nanoparticles are generally made of inorganic materials with crystalline and solid properties, while soft nanoparticles are made of organic materials (e.g. lipids, polymers, and proteins) with deformable characters when undergoing stress or contacting with surface. However, there is a lack of study of soft nanoparticle penetration of photodamaged skin with respect to lipid-based and polymer-based systems. The goal of this study was to compare the permeation of soft nanoparticles via healthy and photodamaged murine skin. If the nanoparticles can deliver into the skin tissues, they interact with the local cellular environment. In the present work, we evaluated the interaction between soft nanoparticles and skin with regard to skin permeation and keratinocyte uptake.

Nanostructured lipid carriers (NLCs) consisting of liquid and solid lipids as the inner matrix were employed as a model of lipid nanoparticles in this study. NLCs are extensively used for medical and cosmetic products with good drug targeting, improved drug stability, and easy scale-up [9]. We selected poly(lactic-co-glycolic acid) (PLGA) as the material to prepare polymer nanoparticles (PNs) due to its high biocompatibility and the approval by the USFDA and the European Medicine Agency for application in drug delivery systems [10]. The model drug used was all-*trans* retinoic acid (RA, tretinoin) because of its wide use for inclusion in topically applied nanoparticles [11]. Topical RA is the first drug therapy approved by the USFDA to treat photoaging [12]. It is also utilized for topical treatment of wrinkling, psoriasis, acne, and skin tumors [13]. The topical RA administration can cause skin irritation and peeling. The photostability of RA is also poor. RA incorporation into nanoparticles can resolve these shortcomings. We examined the in vitro and in vivo RA absorption in the skin under the influence of photoaging. The fluorescent dye was also included in the nanoparticles in order to see the distribution within the skin. Finally, the association of nanoparticles with the cultured keratinocytes was evaluated in the presence and absence of UV irradiation.

2. Materials and methods

2.1. Materials

RA, squalene, Pluronic F68, PLGA (50:50, molecular weight 30–60 kDa), poly(vinyl alcohol) (PVA), Nile red, and rhodamine 800 were purchased from Sigma–Aldrich (St. Louis, MO, USA). Precirol® ATO 5 was provided by Gattefossé (Gennevilliers, France). Soy phosphatidylcholine (SPC, Phospholipon® 80H) was obtained from American Lecithin (Oxford, CT, USA).

2.2. Fabrication of NLCs

Squalene (900 mg), Precirol® (300 mg), and SPC (20 mg) were mixed as the lipid phase, while the aqueous phase consisted of Pluronic F68 (240 mg) and double-distilled water. RA was added in the lipid phase as a 40 mg dose. The total volume of the nanodispersion was 10 ml. Both phases were heated separately to 85 °C for 15 min. The aqueous phase was added to the lipid phase, and then mixed with a high-shear homogenizer (Pro 250, Pro Scientific, Monroe, CT, USA) at 12,000 rpm for 10 min. A probe-type sonicator (VCX 600, Sonics and Materials, Newtown, CT, USA) was used to further treat the nanodispersion for 10 min to fabricate the final product.

2.3. Fabrication of PNs

PLGA (100 mg) and RA (40 mg) were dissolved in acetone (3 ml) as the organic phase. The aqueous phase consisted of PVA (240 mg) in double-distilled water (12 ml). The aqueous phase was heated at 85 °C for 15 min, then mixed by the probe-type sonicator for 5 min. After a cooling time of 15 min, the organic phase was added by drops to the aqueous phase. Subsequently, the mixture was mixed with a magnetic stirrer at 300 rpm overnight. After the evaporation of the organic solvent, the mixture was centrifuged in an Amicon® Ultra-15 tube at 900 rpm for 30 min. After the removal of the supernatant, the nanodispersion was reconstituted using double-distilled water up to 10 ml.

2.4. Size and zeta potential

The mean diameter (*z*-average) and zeta potential of the nanoparticles were measured by a laser-scattering technique (Nano ZS90, Malvern, Worcestershire, UK). The nanodispersion was diluted 100-fold with water before the measurement.

2.5. Encapsulation efficiency of RA

The percentage of RA loading in the nanoparticle matrix was measured using an ultracentrifugation method. The nanodispersion was centrifuged at 48,000*g* at 4 °C for 30 min. The supernatant and precipitate were separated and analyzed by high-performance liquid chromatography (HPLC) described previously [14].

2.6. Molecular environment

The solvatochromism of Nile red in nanoparticles can act as an indicator for demonstrating the polarity of the nanodispersion. The nanosystem with Nile red (1 ppm) was prepared as described earlier. The emission fluorescence spectrum was examined with a fluorescence spectrometer. The λ_{ex} and λ_{em} were set at 546 and 550–700 nm, respectively. The scanning speed was 300 nm/min.

2.7. Animals

Eight-week-old female nude mice (JCR-Foxn1nu) were supplied by the National Laboratory Animal Center (Taipei, Taiwan). The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Chang Gung University. Ethical issues with animal experiments complied with Directive 86/109/EEC from the European Commission.

2.8. UV exposure

A Bio-Sun Illuminator (Vilber Lourmat, Marne-la-Vallée, France) was employed to produce the radiation of UVA (365 nm) or UVB (312 nm). The distance between the dorsal skin of nude mice and the UV lamp was 10 cm. The spectral irradiance was 9 J/cm^2 and 190 mJ/cm^2 for UVA and UVB, respectively. The dorsal area was exposed with UVA every other day for five days. UVB treatment was performed once a day for five days.

2.9. Physiological examination

Transepidermal water loss (TEWL) and skin lightness (L*) of the dorsal skin were measured 1 h after the completion of the UV

Please cite this article in press as: C.-F. Hung et al., Cutaneous penetration of soft nanoparticles via photodamaged skin: Lipid-based and polymer-based nanocarriers for drug delivery, Eur. J. Pharm. Biopharm. (2015), http://dx.doi.org/10.1016/j.ejpb.2015.05.005