International Journal of Pharmaceutics 503 (2016) 1–7

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical Nanotechnology

In vitro skin permeation of artemisone and its nano-vesicular formulations

Anupma Dwivedi, Anisha Mazumder, Lizelle T. Fox, Alicia Brümmer, Minja Gerber, Jan L. du Preez, Richard K. Haynes, Jeanetta du Plessis*

Centre of Excellence for Pharmaceutical Sciences, North-West University, Private Bag X6001, Potchefstroom 2520, South Africa

ARTICLE INFO

Article history:
Received 21 January 2016
Received in revised form 23 February 2016
Accepted 25 February 2016
Available online 27 February 2016

Keywords:
Artemisone
Melanoma
Nano-vesicles
Solid lipid nanoparticles
Niosomes
Skin permeation

ABSTRACT

The artemisinin derivative artemisone has antitumor activity. In particular when encapsulated in solid lipid nanoparticles (SLNs) and niosomes, it is active against human melanoma A-375 cells, although such formulations have a negligible effect on human keratinocyte cells. The aim here was to determine whether these formulations could enhance the topical delivery and skin permeation of artemisone as a prelude to evaluating use of artemisone and related compounds for melanoma treatment. *In vitro* skin permeation studies were conducted to determine the concentration of artemisone delivered into the stratum corneum-epidermis and epidermis-dermis. Artemisone-SLNs delivered artemisone into the stratum corneum-epidermis at significantly higher concentration (62.632 μg/mL) than the artemisone-niosomes (12.792 μg/mL). Neither of the controls delivered artemisone into the stratum corneum-epidermis. In the epidermis-dermis, artemisone (13.404 μg/mL) was only detected after application of the SLN formulation. Overall, the excellent topical delivery of artemisone with the SLN formulation coupled with the intrinsic activity of formulated artemisone confirms potential for use in treatment of melanoma.

1. Introduction

The skin represents an important route for drug delivery. Dermal delivery involves application of the drug to the skin for optimizing local therapeutic effects (Higo et al., 1993; Williams, 2013), while transdermal drug delivery is required when systemic effects are desired (Honeywell-Nguyen and Bouwstra, 2005). However, dermal and transdermal delivery are challenging because the skin acts as a natural defense barrier, and many drugs cannot breach this barrier in quantities sufficient for regional and systemic treatment of disease (Okoro et al., 2014). The structure of the stratum corneum, the outermost layer of the skin, is often compared to that of a brick wall because of its highly organized lipid lamellae which impart the barrier properties (Elias, 1983; Wertz, 2000). These lipids are largely responsible for regulating drug flux through the skin (El Maghraby et al., 2008; Williams, 2003).

During this study, we focused on nanovesicular delivery systems for effective permeation of a drug through the skin. In general, therapeutically active pharmaceutical ingredients (APIs) can be encapsulated into nano-vesicles in order to improve selective tissue distribution, increases circulation time in blood, minimize toxic side effects, reduce the total required dose and circumvent the reticuloendothelial system (Dwivedi et al., 2015; Rigon et al., 2015). These drug delivery systems are known for their high stability, controlled drug release characteristics, and enhancement of bioavailability of the encapsulated drug (Pellet et al., 1997). Nano-vesicles offer advantages for delivery of a drug through the skin because they possess physical and chemical properties that may be easily modulated for optimal dermal delivery. The two types of nano-vesicles that were used are niosomes and solid lipid nanoparticles (SLNs). Niosomes comprise a non-ionic surfactant and have structure and function similar to those of phospholipid vesicles. They are useful for dermal delivery involving both hydrophilic and lipophilic substances for drug and cosmetic uses. For example, the hydrophilic compound estradiol encapsulated in niosomes can be delivered through the stratum corneum (Van Hal et al., 1996). Niosomes are superior to conventional formulations for improving the permeation of the polar diuretic furosemide through mouse skin (Azeem, 2008). SLNs are made up of lipids which are in the solid state at room temperature. SLNs are widely used for topical drug delivery due to their ability to inhibit *trans*-epidermal water loss and to control drug release. Many SLN-based cosmetic formulations have been

* Corresponding author.
E-mail address: Jeanetta.DuPlessis@nwu.ac.za (J. du Plessis).

http://dx.doi.org/10.1016/j.ijpharm.2016.02.041
0378-5173/

examined in recent years for enhancing topical applications of sunscreen and moisturizers (Chen et al., 2012; Khameneh et al., 2015).

The compound we focus on here is artemisone which is derived from the Chinese peroxidic antimalarial drug artemisinin (Fig. 1). Artemisinin was isolated in crystalline form in 1973 from the Chinese traditional herb qinghaosu (Artemisia annua), and is potently active against the blood stages of the malaria parasite. Currently, artemisinin and its derivatives dihydroartemisinin, artemether and artesunate, known collectively as artemisinins, are used in combination with other antimalarial drugs for first-line treatment of uncomplicated Plasmodium falciparum malaria (World Health Organisation (WHO), 2014). Artemisinins are active against other parasites (Krishna et al., 2008) and possess antitumor activity (Crespo-Ortiz and Wei, 2012; Efferth et al., 2003; Reungpatthanaphong and Mankhetkorn, 2002; Woerdenbag et al., 1993). However, artemisinin itself and the current clinical derivatives have short half-lives, possess neurotoxicity *in vitro* and *in vivo* and have relatively low bioavailabilities (Golenser et al., 2006; Nontprasert et al., 2002; Wesch et al., 1994). Due to these concerns, new derivatives bearing an alkylamino group at C-10 (cf. Fig. 1) were prepared (Haynes et al., 2006), and of these, artemisone **1** proceeded to Phase II clinical trials against malaria. Artemisone is a highly crystalline compound that is non-neurotoxic, and has considerably greater therapeutic potential, improved bioavailability and stability compared to the current clinical artemisinins (Vivas et al., 2007). Importantly artemisone is cytotoxic towards different cancer cell lines (Gravett et al., 2011; van Huijsduijnen et al., 2013), and displays activity in combination with known anticancer drugs that is consistently better than the corresponding artemisinin combinations. Encapsulation of artemisone into niosomes and SLNs enhances cytotoxic activity against human melanoma A-375 cells compared to the free drug itself whereas there is minimal toxicity towards normal skin cells (Dwivedi et al., 2015).

The potential for a new treatment of melanoma thus becomes apparent. Melanoma develops from the melanocytes (Tran et al., 2009) present in the epidermis (Yamaguchi and Hearing, 2014). The altered melanocytes proliferate above the epidermal basement membrane (*in situ* radial growth phase), and invade the papillary dermis (invasive radial growth phase). They thereby present as an enlarging papule, growing in three dimensions to form a nodule (vertical growth phase) (Clark et al., 1984; Elder, 1999; Guerry et al., 1993). Clearly, the target delivery site for a drug to treat melanoma is in the skin layers. Given the toxicity of artemisone towards melanoma cells, the effectiveness of topical therapy needs to be evaluated. As part of this objective, we have now evaluated the ability of the nano-vesicular formulations of artemisone in niosomes and SLNs to deliver artemisone into the skin layers.

2. Materials and methods

2.1. Materials

Artemisone was purified and supplied by Cipla Ltd. L.B.S. Marg, Vikhroli (W), Mumbai India. Sorbitan monostearate (Span® 60), cholesterol, polyvinyl alcohol (PVA), 1-stearoyl-rac-glycerol and L-α-phosphatidylcholine (from soya bean) and phosphate buffer solution (PBS) were purchased from Sigma-Aldrich (USA). Solvents including chloroform and ethanol (HPLC grade) were procured from ACE Chemical Company (South Africa). Potassium dihydrogen phosphate (KH_2PO_4) and sodium hydroxide (NaOH) were obtained from Merck (USA).

2.2. Methods

2.2.1. Nano-vesicles preparations

SLNs and niosomes were prepared according to the previously described method (Dwivedi et al., 2015). For encapsulation of artemisone, a 2:1 mixture of 1-stearoyl-rac-glycerol and L-α-phosphatidylcholine (w/w) was added to the mixture of artemisone in ethanol (2:1, w/v) and this organic phase was magnetically stirred with heating at 70 ± 2 °C. PVA (0.25%) was dissolved in water to form aqueous phase which was heated to the same temperature as the organic phase. The organic phase was slowly added to the pre-heated aqueous phase by means of a hypodermic needle with continuous stirring. The mixture was then sonicated to obtain the SLNs. For the niosomes, Span® 60, cholesterol and artemisone (3:1:4, w/w/w) were dissolved in chloroform. The solvent was then allowed to completely evaporate at room temperature and the thin film obtained was hydrated with water while stirring until it was dissolved. The niosomes were obtained after sonication for about 30 s (Hielscher UP 200ST ultrasonic device, South Africa). These nano-vesicles were characterized with respect to average size, zeta-potential, entrapment efficiency and release profiles as discussed in our recent study (Dwivedi et al., 2015).

2.2.2. Electron microscopy

The structures of the nano-vesicles (niosomes and SLNs) were examined by using electron microscopy. For transmission electron microscopy (TEM), the samples were diluted with water and a drop of each samples were placed on a copper grid, a drop of 1% osmium tetroxide (in cacodylate buffer) was added and the excess sample was wiped off. The grid was then air dried thoroughly for 10–15 min, after which the samples were examined under TEM (FEI Tecnai™ G2, USA) at 120 kV, whilst micrographs were taken with a bottom mount camera (GATAN). Sample preparation for scanning electron microscopy (SEM) entailed placing a drop of

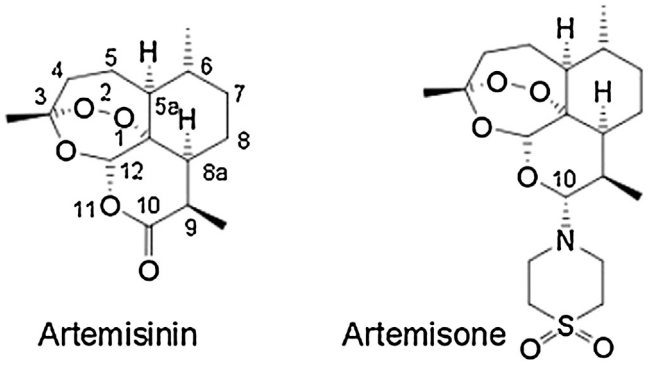

Fig. 1. Structures of artemisinin and the 10-aminoartemisinin artemisone.