Asian Journal of Pharmaceutical Sciences 000 (2018) 1–13

Available online at www.sciencedirect.com

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

Original Research Paper

Vesicular carriers containing phenylethyl resorcinol for topical delivery system; liposomes, transfersomes and invasomes

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ARTICLE INFO

Article history:
Received 6 November 2017
Revised 12 February 2018
Accepted 26 February 2018
Available online xxx

Keywords:
Phenylethyl resorcinol
Transfersomes
Invasomes
Tyrosinase inhibition

ABSTRACT

Topical administration of phenylethyl resorcinol (PR) has attracted much attention as skin lightening agent with potent anti-tyrosinase activity. Two novel types of elastic carriers were developed to overcome the limitation of PR as topical delivery by increasing the solubility, stability and decreasing skin irritation compared to conventional liposomes. In addition, it also promotes skin penetration of PR to reach deep skin layer at the target site. The lead formulations were obtained from the invasomes containing 1% (w/v) D-limonene mixed with 10% (v/v) absolute ethanol as the skin enhancer, and transfersomes containing 15% (w/w) sodium deoxycholate (SDC) as edge activator. All formulations gave a vesicle size < 500 nm, polydispersity index (PDI) < 0.3, high zeta potential, entrapment efficiency $> 50\%$, and good stability on storage at 30 °C at 75% RH for 4 months. Transfersomes have a lower degree of deformability (6.63%) than invasomes (25.26%). In contrast, the liposomes as rigid vesicles do not show a deformable property. This characteristic affects the skin permeation, and thus, transfersomes with high elastic property provided a significantly higher cumulative amount, steady state flux (J_{ss}) and permeability coefficient (K_p) compared to other formulations. However, *in vitro* PR accumulation in full-thickness newborn pig skin demonstrated that the application of elastic carrier formulations gave significantly higher accumulation than liposomes, and gave anti-tyrosinase activity up to 80%. These results are straightforwardly related to the results of cellular level study. Transfersomes and invasomes showed higher tyrosinase inhibition activity and melanin content reduction when compared to liposomes in B16 melanoma cells. In addition, acute irritation test in rabbits confirmed that these formulations are safe for skin application. Therefore, elastic vesicle carriers have the

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https://doi.org/10.1016/j.ajps.2018.02.004

efficiency to deliver PR into the deep skin in both quantity and effectiveness which are better than conventional liposomes and appropriate for a skin lightening product.

1. Introduction

Melanin is a complex polymer derived from the amino acid tyrosine and produced by the melanogenesis pathway in melanocytes which is located in the basal layer of the epidermis. The light absorption of melanin in skin plays an important role for photo-protection from the ultraviolet (UV) radiation [1]. However, overproduction of melanin causes skin darkening and abnormal distribution of melanin in different and specific parts of the skin which in fact causes hyperpigmentation disorders including melasma, freckles, lentigines, etc. [2]. Tyrosinase inhibition is an effective strategy for treating hyperpigmentation because tyrosinase controls the rate-limiting steps of melanin synthesis.

Phenylethyl resorcinol (4-(1-phenylethyl) 1, 3-benzenediol, PR) is phenolic compound developed by Symrise (Holzminden, Germany) under trade name as SymWhite®377. PR, which is a new skin lightening agent, inhibits the tyrosinase activity by obstruction of the conversion of tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) [3]. It reduced tyrosinase activity approximately 22 folds more effectively than kojic acid *in vitro* mushroom tyrosinase and *in vivo* epidermal model (MelanoDerma™), respectively [4]. It can increase the fairness of Asian human skin *in vivo* at a concentration of 0.5% [5]. In addition, PR has antioxidant property and improved UV protection activity when incorporated with inorganic titanium dioxide (TiO_2) hybrid composites rather than bare TiO_2 [6]. Recently, PR showed potential in antifungal activity. It was effective against the nine dermatomycoses: *Microsporum gypseum*, *Microsporum canis*, *Trichophyton violaceum*, *Arthroderma cajetani*, *Trichophyton mentagrophytes*, *Epidermophyton floccosum*, *Nannizzia gypsea*, *Trichophyton rubrum* and *Trichophyton tonsurans* which is highly active than antifungal agent fluconazole [7]. However, the poor aqueous solubility and low stability in light are the limitations of PR for the formulation as cosmetic and pharmaceutical dermal products. The color changes from white to pink tone when the formulation is exposed to light which may cause the application of PR to be ineffective whereas the poor aqueous solubility may reduce its absorption when used [8]. In addition, it has been reported that, a 1% PR in the formulation, causes skin irritation, and may lead to consumer rejection of the product [9].

Nanoencapsulation technique was used in liposomes [10], transfersomes [11], invasomes [12] and ethosomes [13] to increase the solubility and photo-stability, and decrease skin irritation of PR by protecting the encapsulated actives from external environment and from directly contact to the skin. Flexible or elastic liposomes are the type of phospholipid vesicles which were modified for improving skin drug delivery systems. Although it is similar to conventional liposomes in morphology, the permeability across skin is different. Transfersomes or ultra-flexible vesicles are the first generation of elastic liposomes. They were achieved by incorporating edge activators (EA) to the lipid bilayers. An edge activator is often a single-chain surfactant such as sodium cholate, sodium deoxycholate, dipotassium glycyrrhizinate, Spans and Tweens. A single-chain surfactant with a high radius of curvature and mobility, are able to destabilize the lipid bilayers of vesicles, and increase the elasticity and flexibility of the lipid bilayer which makes the vesicles have ultra-deformable property [14]. Several studies reported that transfersomes are efficient for transdermal and topical drug delivery [11]. Ethosomes are the fluid vesicles which contain ethanol at relatively high concentration (20%–45%) as skin enhancer. The elastic vesicle of ethosomes is an important feature related to skin permeability enhancement, which may be due to the synergistic mechanism between high concentration of ethanol, phospholipid vesicles, and lipid bilayers in stratum corneum. Therefore, they can penetrate into the skin and allow enhanced delivery of various drugs to deeper skin strata [15]. Invasomes are soft flexible liposome vesicles with very high membrane fluidity. They contain ethanol and terpenes which play the role of penetration enhancer. The mechanism of skin permeation of invasomes is similar to ethosomes [12].

Traditional or conventional liposome vesicles are of large size, more than 400 nm in diameter and have a rigid structure. Cholesterol is added in the formulation to stabilize the structure and generate more rigid liposomes. These are too large to fit within the intercellular lipid domains of the stratum corneum, and the liposomes are generally reported to accumulate in the stratum corneum, upper skin layers and in the appendages, with minimal penetration to deeper tissues or the systemic circulation [16]. Conventional liposomes remain near the skin surface, dehydrate and fuse with the skin lipids, whereas deformable transfersomes squeeze through stratum corneum lipid lamellar regions penetrating deeper by following the osmotic gradient to permeate to the deeper layers of the epidermis. Due to the flexibility conferred on the vesicles by the other vesicle compositions molecules such as ethanol and surfactant, elastic vesicle compositions have been investigated to develop systems that are capable of carrying drugs and macro- molecules to deeper tissues.

These vesicles have a unique skin permeation mechanism. Transfersomes can pass through the intact skin and can retain their shape, because of the presence of edge activators in the formulation, whereas the ethosomes have ethanol, and invasomes have combination of ethanol and terpenes in the formulation which play the role of penetration enhancer by disturbing the organization of the stratum corneum lipid bi-

Please cite this article as: Thanaporn Amnuaikit et al., Vesicular carriers containing phenylethyl resorcinol for topical delivery system; liposomes, transfersomes and invasomes, Asian Journal of Pharmaceutical Sciences (2018), https://doi.org/10.1016/j.ajps.2018.02.004