Journal of Controlled Release 199 (2015) 168–178

Contents lists available at ScienceDirect

Journal of Controlled Release

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

Peptides as skin penetration enhancers: Mechanisms of action

Sunny Kumar [a,b], Michael Zakrewsky [a,b,1], Ming Chen [a,b,1], Stefano Menegatti [a,b], John A. Muraski [c,*], Samir Mitragotri [a,b,*]

[a] Center for Bioengineering, University of California, Santa Barbara, CA, United States
[b] Department of Chemical Engineering, University of California, Santa Barbara, CA, United States
[c] Convoy Therapeutics, 405 W Cool Drive, Suite 107, Oro Valley, AZ 85704, United States

ARTICLE INFO

Article history:
Received 5 October 2014
Accepted 8 December 2014
Available online 9 December 2014

Keywords:
Cell penetrating peptide
Cyclosporine
Keratin
Transporter
Topical
Transcellular

ABSTRACT

Skin penetrating peptides (SPPs) have garnered wide attention in recent years and emerged as a simple and effective noninvasive strategy for macromolecule delivery into the skin. Although SPPs have demonstrated their potential in enhancing skin delivery, they are still evolving as a new class of skin penetration enhancers. Detailed studies elucidating their mechanisms of action are still lacking. Using five SPPs (SPACE peptide, TD-1, polyarginine, a dermis-localizing peptide and a skin penetrating linear peptide) and a model hydrophobic macromolecule (Cyclosporine A, CsA), herein we provide a mechanistic understanding of SPPs. To evaluate the mechanism and safety of SPPs, their effects on skin lipids, proteins and keratinocyte cells were evaluated. Three SPPs (SPACE, Polyarginine and TD-1) significantly enhanced CsA penetration into the skin. SPPs did not alter the skin lipid barrier as measured by skin resistance, transepidermal water loss (TEWL) and Fourier transform infrared (FTIR) spectroscopic analysis. In contrast, SPPs interacted with skin proteins and induced changes in skin protein secondary structures (α-helices, β-sheet, random coils and turns), as evaluated by FTIR analysis and confirmed by in-silico docking. SPPs enhanced CsA skin penetration, via a transcellular pathway, enhancing its partitioning into keratin-rich corneocytes through concurrent binding of SPP with keratin and CsA. Interaction between SPP and keratin best correlated with measured CsA skin transport. Many SPPs appeared to be safe as shown by negligible effect on skin integrity, nominal skin irritation potential and cytotoxicity. Among the peptides tested, SPACE peptide was found to be least toxic to keratinocytes, and among the most effective at delivering CsA into the skin.

1. Introduction

The skin is the largest and most easily accessible organ of the human body for drug delivery [1]. Drug delivery through the skin offers numerous advantages and has great implications for pharmaceutical and cosmetic products. However, drug penetration into and across the skin is a serious challenge [1–3]. The skin serves as a natural protective barrier from the external environment and its low permeability severely limits the transport of most pathogens, toxins and drug molecules [4]. The main transport barrier resides in the outermost layer of the skin, the stratum corneum (SC), which is comprised of keratin-rich cells embedded in multiple lipid bilayers [4]. To penetrate through the SC, drugs must navigate through the tortuous lipid pathways surrounding the keratin-rich cells, or repeatedly partition between the aqueous, keratin-rich phase and the lipid phase [5]. Therefore, only potent drugs with optimal physicochemical properties (molecular weight <500 Da, high hydrophobicity, and adequate solubility in aqueous and non-aqueous solvents) can be passively transported through the SC [4,6].

Numerous skin penetration enhancement strategies have been evolved to promote drug delivery across the SC including active and passive methods [7]. Active skin penetration enhancers generally include devices, which are effective but may be difficult to use on large skin areas [7]. In contrast, passive methods such as chemical permeation enhancers (CPEs) are simpler to use and can be applied to large skin areas. Most CPEs enhance skin permeation by affecting the lipid region of the SC either through lipid extraction, fluidization or introduction of other modes of structural reorganization [2,8]. Although CPEs offer high potential in overcoming the skin barrier to enhance transport of drug molecules, their safety as skin penetration enhancers is a potential concern; a balance must often be sought between transport

Abbreviations: SPP, skin penetrating peptide; SPACE, skin penetrating and cell entering; SDS, sodium dodecyl sulfate; Poly-R, poly-arginine; CsA, Cyclosporine A; SC, stratum corneum; FTIR, Fourier transform infrared; Da, Dalton; CPE, chemical penetration enhancer; PBS, phosphate buffer saline; FDC, Franz diffusion cells; TEWL, transepidermal water loss; HEKa, human epidermal keratinocytes; LP-12, Linear Peptide-12mer; DLP, dermis localizing peptide

* Corresponding authors.
E-mail addresses: jmuraski@convoytx.com (J.A. Muraski), samir@engineering.ucsb.edu (S. Mitragotri).

[1] Equal contribution.

http://dx.doi.org/10.1016/j.jconrel.2014.12.006
0168-3659/

enhancement and skin irritation [2]. A major reason behind CPE-associated skin toxicity is that a large number of CPEs (such as azone derivatives, fatty acids, alcohols, esters, sulphoxides, pyrrolidones, glycols, surfactants and terpenes) are small molecules (<500 Da) that can penetrate the skin in significant quantities and can cause skin irritation, cytotoxicity or irreversibly alter the skin barrier [8,9].

To realize the benefits of transdermal/dermal delivery in the clinic, skin penetration enhancers must not only overcome the barrier properties of the skin but also meet safety and patient-compliance requirements. Hence, the search continues for novel skin penetration enhancers that are effective, non-invasive, non-toxic and non-irritating to the skin. Recently, small peptides (1000–1500 Da) have been identified as safer alternatives to enhance the delivery of small and large molecules into and across the skin [2–11]. Introduced just a decade ago, skin-penetrating peptides (SPPs) are still evolving as a class of skin penetration enhancers [4]. The use of SPPs for transdermal drug delivery is particularly intriguing because the SC presents a formidable, non-specific barrier to the penetration of peptides (>500 Da) [10–14]; hence, the ability of peptides to act as penetration enhancers is unexpected. Several studies have reported the use of SPPs [10–23], and a few have hypothesized potential mechanisms ranging from transient pore formation in appendages [10] to interactions with skin lipids or keratin in the skin [11–13]; however, a comprehensive investigation of the primary underlying mechanisms responsible for SPP-mediated skin penetration enhancement has yet to be undertaken for several reported SPPs in a single concerted study.

Herein, we report the first study aimed at understanding the mechanism by which SPPs, as a group, mediate skin penetration enhancement of macromolecules. To this end, we evaluated, using five different peptides (Fig. 1, Table 1), skin penetration enhancement of a model macromolecule (Cyclosporine A, CsA), structural changes in the microscopic domains of the skin SC barrier, and cellular toxicity. Of the five peptides studied here, three have been previously reported in the literature and shown to enhance skin delivery (SPACE peptide [11,17,18], polyarginine (Poly-R) [14,20,22,23] and TD-1 [10,15,16,19,21]). One peptide has been reported before but not well-studied (dermis localizing peptide, DLP [11]) and one peptide is reported here for the first time (Linear Peptide-12 mer, LP-12). Molecular properties of all five peptides are reported in Table 1. The results of this study suggest that SPPs, as a class, enhance transport of CsA through a single mechanism: transcellular partitioning of drug through binding of SPPs to keratin. Moreover, SPPs appeared to interact with keratin without causing irritation. Taken together, this study suggests that transport of hydrophobic macromolecules may be enhanced by pairing with a keratin-binding peptide and further establishes that SPPs are an advantageous alternative delivery strategy for topical and transdermal products.

2. Materials and methods

2.1. Chemicals

The peptides were chemically synthesized by Genscript Inc. (Piscataway, NJ, USA). Cyclosporine A (CsA) was purchased from Abcam (Cambridge, MA, USA). ^{3}H-Cyclosporine A was purchased from PerkinElmer (Waltham, MA, USA). All other common chemicals required for the experiments were obtained from Fisher Scientific (Fair Lawn, NJ, USA).

2.2. In-vitro skin penetration study

Full thickness porcine skin was procured (Lampire Biological Laboratories, Pipersville, PA, USA) and processed as reported in our earlier studies [17]. The skin integrity was determined by measuring the skin conductivity [24]. In-vitro skin penetration studies were performed using Franz diffusion cells (FDCs) under occlusive condition at 37 ± 1 °C. The effective penetration area and receptor cell volume were 1.77 cm^2

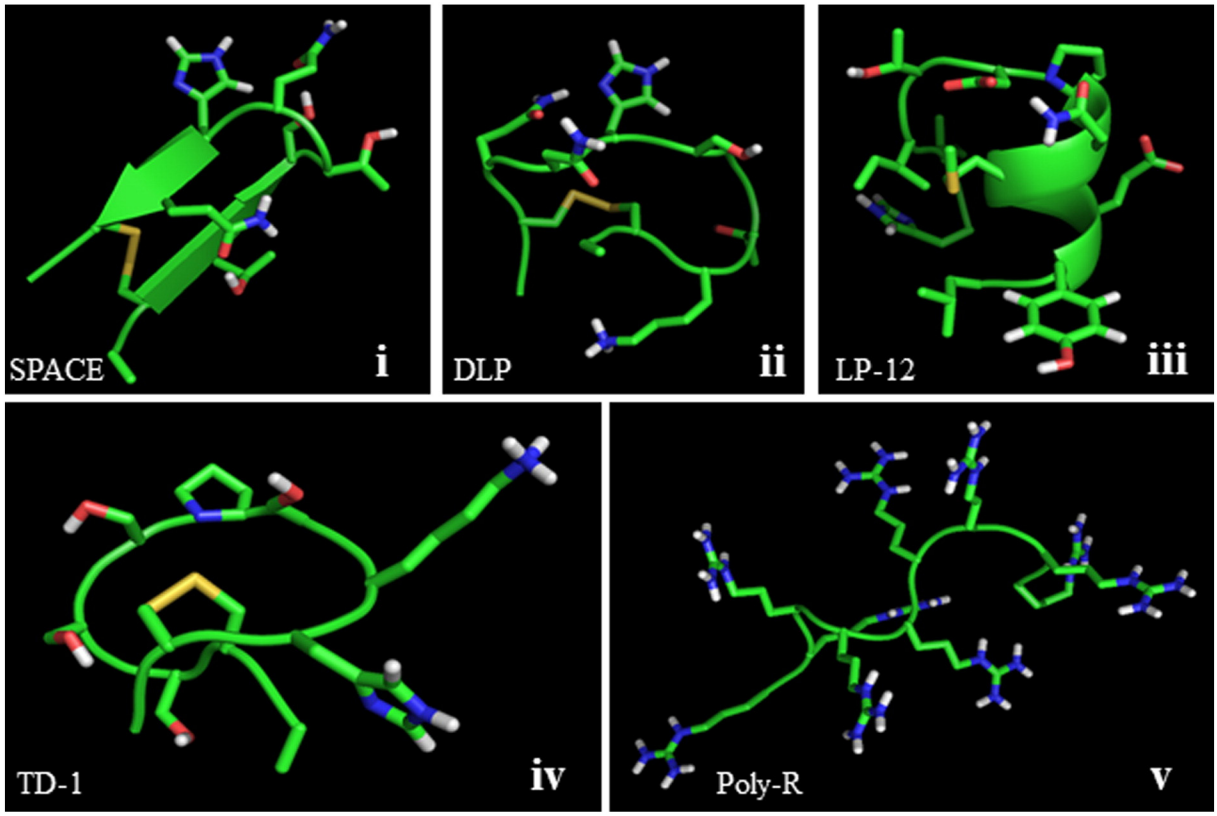

Fig. 1. Structures of skin penetrating peptides (SPPs). 3D conformations of SPPs predicted using Pep-Fold 1.5: (i) SPACE, (ii) DLP, (iii) LP-12, (iv) TD-1, and (v) Poly-R.