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Enhanced skin permeation using polyarginine modified nanostructured lipid carriers

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ABSTRACT

The objective of the present study was to investigate the effect of polyarginine chain length on topical delivery of surface modified NLCs. Design of experiments (DOE) was used to optimize number of arginines required to deliver active drug into deeper skin layers. The NLCs were prepared by hot-melt technique and the surface of NLCs was modified with six-histidine tagged cell penetrating peptides (CPPs) or YKA. *In vivo* confocal microscopy and Raman confocal spectroscopy studies were performed using fluorescent dye encapsulated NLCs and NLC-CPPs. Spantide II (SP) and ketoprofen (KP) were used as model drugs for combined delivery. *In vitro* skin permeation and drug release studies were performed using Franz diffusion cells. Inflammatory response corresponding to higher skin permeation was investigated in allergic contact dermatitis (ACD) mouse model. NLCs had a particle size of 140 ± 20 nm with higher encapsulation efficiencies. The negative charge of NLC was reduced from -17.54 to -8.47 mV after surface modification with CPPs. *In vivo* confocal microscopy and Raman confocal spectroscopy studies suggested that a peptide containing 11 arginines (R11) had significant permeation enhancing ability than other polyarginines and TAT peptides. The amount of SP and KP retained in dermis after topical application of NLC-R11 was significantly higher than solution and NLC after 24 h of skin permeation. SP was not found in receiver compartment. However, KP was found in receiver compartment and the amount of KP present in receiver compartment was increased approximately 7.9 and 2.6 times compared to the control solution and NLCs, respectively. In an ACD mouse model, SP + KP-NLC-R11 showed significant reduction ($p<0.05$) in ear thickness compared to SP + KP solution and SP + KP-NLC. Our results strongly suggest that the surface modification of NLC with R11 improved transport of SP and KP across the deeper skin layers and thus results in reduction of inflammation associated with ACD.

1. Introduction

Psoriasis, atopic dermatitis and allergic contact dermatitis are skin disorders that are characterized by chronic inflammation with a significant neurogenic component. Currently, these disorders are principally treated with topical corticosteroids that target a variety of pathways of the inflammatory cascade [1]. However, the corticosteroid therapy is associated with local side effects such as skin atrophy, telangiectasia, acne and secondary infections which may results in contact dermatitis and perioral dermatitis. To overcome unwanted side effects associated with monotherapy, a combination approach is widely used since two disease modifying drugs when used together can act through different pathways and offer possibility for synergistic or additive effects. Spantide II (SP) [2–4] and ketoprofen (KP) [5,6] are well studied for reducing the swelling and inflammation associated with skin disorders. Therefore use of two anti-inflammatory drugs, SP and KP, can modify the inflammation conditions favorably when delivered simultaneously. SP is a peptide that specifically binds to neurokinin-1 receptor (NKR-1) and blocks pro-inflammatory activity associated with Substance P [7]. On the other hand, KP is a potent non-steroidal anti-inflammatory drug (NSAID) which inhibits arachidonic acid metabolism by potent inhibitory actions on cyclooxygenase and lipooxygenase.

In the dermatological treatment, improving clinical efficacy requires high drug levels at specific site of the skin with less systemic absorption. Stratum corneum (SC) is the biggest barrier for skin delivery which reduces the absorption of active drugs in the skin layers [8]. Several approaches have been proposed and are being used to enhance the skin permeation of drugs using either chemical [9,10] and physical enhancement techniques like ultrasound [11], radiofrequency [12], iontophoresis [13,14], magnetophoresis [15], electroporation [16], microneedles [17] but they have their respective limitations in terms of toxicity and therapeutic feasibility. One of the most pursued methods to increase the drug transport across the skin is the use of nanocarriers [18]. The potential of nanosized carriers (liposomes, polymeric and lipid nanoparticles) for topical delivery has been addressed in several studies [19]. Delivery systems such as polymeric nanoparticles or liposomes have limitations in their

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a) industrial applications like physical stability problems with liposomes b) lack of a regulatory acceptance of many polymers and c) high costs for effective large scale production [20]. To overcome these problems, nano lipid carriers (NLCs) have been developed using the blend of both solid lipids and liquid lipids (oils) [21]. NLCs have higher loading capacity and lower drug leaching on storage compared to other lipid nanocarriers. Also, NLCs have demonstrated potential for topical route of application. NLCs are known to improve stability and controlled release of the incorporated drugs [22,23]. In addition, NLCs are well tolerated by skin and easy to scale up [20].

In our efforts to enhance the delivery of lipid nanocarriers into deep epidermis, we have already shown that a well known cell penetrating peptide (CPP), transactivating transcriptional activator (TAT), when linked to NLCs has the potential to carry their payloads across the skin layers [24]. In recent years, CPPs have opened new avenues in the field of drug delivery to deliver various molecules like peptides, proteins, nucleic acids, antibodies and imaging agents across the cellular membranes [25,26]. Several CPPs like polyarginine-7 (R7), polyarginine-9 (R9; NONA), transporter-9, polylysine-9 (K9), penetratin (PEN), and RALA have been studied to enhance the skin delivery of active drugs [19,27,28]. Further the effect of arginine chain length of polyarginines (Rn; $n=4$ to 16) has been widely investigated for improving the permeation enhancing ability and cellular localization of active drugs in cancer cells [29,30]. However, there are no reports available on the use of a polyarginine peptide containing more than 9 arginines to improve skin permeation of active drugs. Therefore a systematic study to investigate the effect of polyarginine chain length containing more than 9 arginines on the skin delivery of cargo molecule needs to be investigated.

The US Food and Drug Administration's Quality by Design (QbD) initiative encourages the use of statistical tools for improving the development of the pharmaceutical formulations with high quality [31]. Design of experiment (DOE), a powerful statistical tool, has been established as a convenient method for developing optimum products precisely and is known to minimize the number of experiments with efficient screening of the process parameters. Further it assures the quality of process or product in terms of mathematical relationships [32]. The results of statistically planned experiments find better acceptance than those of traditional single variable experiments. Therefore, a mixture design was used to optimize number of arginines containing peptide required to deliver a drug into deeper skin layers wherein number of arginines containing peptides and different skin depths were selected as independent variables and percent fluorescence intensity, calculated from confocal images along with Raman intensity were selected as dependent variables. Based on desirability function, the optimum number of arginines containing peptide was selected and used for surface modification of NLCs comprising SP and KP. These surface modified NLCs were then assessed for *in vitro* skin permeation and *in vivo* treatment of inflammatory skin disorder like allergic contact dermatitis (ACD) in mice model.

2. Materials and methods

Miglyol 812 was kindly gifted by Sasol Germany GmbH (Witten, Germany). Precirol, Monosterol and Compritol 888 ATO were gifted by Gattefosse (Saint Priest, France). 1,2-Dioleoylsn-glycero-3-[(N-(5-amino-1-carboxypentyl) imidodiacetic acid) succinyl nickel salt] (DOGS-NTA-Ni), L-phosphatidylinositol (liver bovine sodium salt) (PI), L-phosphatidyl choline (egg-hydrogenated) (PC) were purchased from Avanti Polar lipids (AL, USA). Polyoxyethylene-20 oleyl ether (Volpo-20) was a kind gift from Croda Inc (NJ, USA). The six histidine tagged polyarginine peptides (R8: RRRRRRRR-6 histidine tag), (R11: RRRRRRRRRRR-6 histidine tag), (R15: RRRRRRRRRRRRRRR-6 histidine tag); TAT (YGRKKRRQRRR-6 histidine tag) cell penetrating peptide and YKA peptide (YKALRISRKLAK-6 histidine tag) peptide as a control were custom synthesized by CHI Scientific, Inc (MA, USA). Vivaspin centrifuge filters (Molecular weight Cut-off: 10,000 Da) were procured from Sartorius Ltd, (Stonehouse, UK). Tetrahydrofuran, tween 80, phosphate buffered saline sachets (PBS, pH 7.4), trifluoroacetic acid (TFA), 2,4-dinitrofluorobenzene (DNFB) and dialysis membrane (flat width of 23 mm) were purchased from Sigma-Aldrich Co (MO, USA). HPLC grade of acetonitrile, water and ethanol were purchased from Sigma-Aldrich Co (MO, USA). Ketoprofen was purchased from Spectrum chemical mfg corp. (CA, USA). Spantide II (SP) was purchased from American peptide company Inc, (CA, USA). Topgraf® (tacrolimus ointment 0.1%) was purchased from GlaxoSmithKline Pharmaceuticals Limited (Thane, India). Poloxamer 188 (Lutrol F68) was kindly gifted by BASF Corporation (NJ, USA).

2.1. Animals

Hairless rats (CD®(SD) HrBi, Male) and C57BL/6 mice (6 weeks old, male) (Charles River Laboratories, Wilmington, MA) were grouped and housed ($n=6$ per cage) in cages with bedding. The animals were kept under controlled conditions of 12:12 h light:dark cycle, 22 ± 2 °C and 50 ± 15% RH. The mice were fed (Harlan Teklad) and water ad libitum. The animals were housed at Florida A&M University in accordance with the standards of the Guide for the Care and Use of Laboratory Animals and the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). The animals were acclimatized to laboratory conditions for 1 week prior to experiments. The protocol of animal study was approved by the Institutional Animal Care and Use Committee (IACUC), Florida A&M University, FL.

2.2. Preparation of nanoparticles

NLCs were prepared by modified hot melt homogenization technique [24]. Briefly, PC (0.2% w/w) was dispersed in (0.04% w/w) ethanol. To this miglyol (3% w/w) and DOGS-NTA-Ni (0.02% w/w) were added. Ethanol was evaporated by applying mild heating. To this, compritol (7% w/w) or a mixture of precirol (4% w/w) and monosterol (3% w/w) were added with constant heating in water bath, kept at 50–60 °C to melt the solid lipids. This represented a lipid phase. Water phase was prepared separately by dissolving Lutrol® F68 (poloxamer 188) (1% w/w), followed by dispersing span 80 (0.5% w/w) in water. This water phase was also brought to the same temperature as that of lipid phase. Further the water phase was added to lipid phase under high speed mixing (20,000 rpm for 13 min).

DiO- or DID-NLC were prepared using compritol as a core lipid by dissolving dye into the chloroform. To prepare SP-NLC, SP (0.025% w/w) and 40 μl of 25 mg/ml solution of PI were dissolved in 0.04% w/w ethanol and then mixed with lipid phase. Similarly to prepare KP-NLC, KP (0.05% w/w) was dissolved in ethanol and mixed with lipid phase. To prepare SP + KP-NLC, SP and KP were dissolved in 0.04% w/w ethanol and mixed with lipid phase.

For surface modification, 200 μl of prepared NLCs were mixed with 50 μl of 5 mg/ml 6 his-tagged CPPs solution, prepared in water and was incubated for 2 h at room temperature with constant stirring. Freshly prepared NLCs were used for all experiments and surface modification was performed 2 hr before starting the experiment. For all the experiments NLCs or NLC-CPP (nanoparticle dispersion) prepared in water were used without any change in the composition.

KP solution (KP-Solution; 0.05% w/w), SP solution (SP-Solution; 0.025% w/w) and a combination of SP + KP solution (SP + KP-Solution) were prepared by dissolving drugs in 1% w/w ethanol and then adjusting volume with PEG-400.

2.3. Characterization of nanoparticles

The particle size and zeta potential of NLC or NLC-CPP were measured in distilled water using Nicomp 380 ZLS (Particle Sizing Systems,