Skin permeating nanogel for the cutaneous co-delivery of two anti-inflammatory drugs

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ABSTRACT

The aim of this study was to develop an effective drug delivery system for the simultaneous topical delivery of two anti-inflammatory drugs, spantide II (SP) and ketoprofen (KP). To achieve this primary goal, we have developed a skin permeating nanogel system (SPN) containing surface modified polymeric bilayered nanoparticles along with a gelling agent. Poly-(lactide-co-glycolic acid) and chitosan were used to prepare bilayered nanoparticles (NPS) and the surface was modified with oleic acid (NPSO). Hydroxypropyl methyl cellulose (HPMC) and Carbopol with the desired viscosity were utilized to prepare the nanogels. The nanogel system was further investigated for *in vitro* skin permeation, drug release and stability studies. Allergic contact dermatitis (ACD) and psoriatic plaque like model were used to assess the effectiveness of SPN. Dispersion of NPSO in HPMC (SPN) produced a stable and uniform dispersion. *In vitro* permeation studies revealed increase in deposition of SP for the SP-SPN or SP+KP-SPN in the epidermis and dermis by 8.5 and 9.5 folds, respectively than SP-gel. Further, the deposition of KP for KP-SPN or SP+KP-SPN in epidermis and dermis was 9.75 and 11.55 folds higher, respectively than KP-gel. Similarly the amount of KP permeated for KP-SPN or SP+KP-SPN was increased by 9.92 folds than KP-gel. The ear thickness in ACD model and the expression of IL-17 and IL-23; PASI score and TEWL values in psoriatic plaque like model were significantly less ($p < 0.001$) for SPN compared to control gel. Our results suggest that SP+KP-SPN have significant potential for the percutaneous delivery of SP and KP to the deeper skin layers for treatment of various skin inflammatory disorders.

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1. Introduction

In the last decade, an increasing number of investigations concerning the use of nanoscale structures for drug and gene delivery purpose have been reported. Nano-carriers have been investigated for delivery of drugs to the specific anatomical sites such as brain [1], eyes [2], lungs [3], intestine [4], nose [5] and skin [6] etc. To improve the notoriously low drug absorption from the skin surface, nanoparticulate carriers have proven to be efficient and advantageous. Development of successful topical/transdermal drug delivery systems has been limited in scope due to the significant penetration barrier provided by the stratum corneum (SC) whose composition limits the application of number of suitable drugs for topical and transdermal delivery. However, there is a growing interest in the development of efficient targeted drug delivery systems to physiological sites in the skin [7]. Several attempts have been made and are still under investigation to develop topical

formulation of macromolecules for the treatment of various skin diseases. Currently, these diseases are principally treated with topical corticosteroids that target a variety of pathways of the inflammation cascade [8]. However, clinical use of corticosteroid therapy is limited due to associated local side effects such as skin atrophy, telangiectasia, acne and secondary infections as well as contact dermatitis and perioral dermatitis. Spantide II (SP), a neurokinin-1 (NK1) receptor antagonist, is a neuropeptide with known anti-inflammatory activity [9,10]. Combination of a new neuropeptide, SP, along with ketoprofen (KP), a well known potent non-steroidal anti-inflammatory drug (NSAID) [11] in a topical formulation will have great impact for the treatment of skin disorders with minimal adverse effects.

In the field of dermatology and cosmetology, micro and nano-sized particles have been thoroughly investigated and some formulations are already commercially available. Recently solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC) and lipid nanocapsules have shown improved drug permeation through the skin. However due to their limited drug loading and phase stability issue, their application for clinical use is restricted. Therefore, increased attention has been given to polymeric

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nanoparticles. Various non-toxic and biodegradable synthetic or semi-synthetic polymers, including polylactic acid (PLA) [12], poly(lactic-co-glycolic acid) (PLGA) [13], poly(ε-caprolactone) [14], chitosan [15] have shown promising results for topical drug delivery. These polymeric nanoparticles offer advantages of controlled and sustained release via modification of polymer composition and reducing irritation associated with direct contact of drug with skin.

PLGA based nanoparticles have been extensively studied since they offer number of advantages for skin delivery including non-toxicity and biodegradability, by hydrolysis leading to formation of water and carbon dioxide and entrapment of various therapeutic moieties [16]. Drug delivery into the skin by PLGA nanoparticles can be enhanced by modifying the particle surface with a cationic polymer such as chitosan. Chitosan is a cationic polysaccharide with many interesting biopharmaceutical properties, such as non-toxicity, biodegradability, bioadhesion and has been extensively used as a penetration enhancer in topical formulations [17]. The surface modification of the PLGA nanoparticles with chitosan can be advantageous for topical delivery in many aspects, such as (a) ability to incorporate two drugs in inner and outer layers of the nanoparticles, (b) increased stability of the macromolecules like SP which can be encapsulated in the PLGA inner core, (c) reversal of zeta potential promoting skin adhesion and thus enhancing skin delivery and (d) ability to conjugate with other molecules such as penetration enhancer through the free amino groups of chitosan. When polymeric nanoparticles were used for topical drug delivery, it was observed that the drug permeation was enhanced by gradual drug release from the nanoparticles on the skin surface but the intact nanoparticles were unable to permeate in deeper skin layers [18,19]. Other attempts to verify the penetration of nanoparticles across the skin were met with little success, where only few of the researchers were able to show permeation of nanoparticles into the skin passively through the hair follicles while most of the nanoparticles were primarily restricted to the uppermost layers of the SC and unable to permeate the skin.

The most extensively investigated enhancement strategy for the skin delivery involves the use of chemicals that can reversibly compromise the skin's barrier function and consequently allow the entry of poorly penetrating molecules into the skin. One of the widely investigated penetration enhancers is oleic acid (OA), a monostructured fatty acid and a membrane fluidizing agent. OA is an FDA approved potent chemical permeation enhancer and is widely used in commercial formulations. OA extracts a fraction of the endogenous SC membrane compounds, promoting phase separation in the SC membrane system. Reducing the proportion of crystalline lipids and creating more permeable OA-rich domains can lead to enhancement effect of the drugs into the skin [20]. Electron microscopic studies have suggested that lipid domain is stimulated within the SC bilayer lipids upon exposure to OA [21]. The formation of such pools provides permeability defects within the lipid bilayers and thus facilitates the permeation of macromolecules into the deeper epidermal and dermal layers. Therefore, surface modification of PLGA-chitosan bilayered nanoparticles with OA (NPSO) can open the channels in the SC, which can enhance the delivery of incorporated active drugs into deeper skin layers where the site of action is present for the inflammatory skin diseases.

One of the limitations of topical dosage forms is the relatively short lasting time of the active drugs at the application site. In order to obtain prolonged skin retention and controlled release for the desired therapeutic effect, it is appropriate to incorporate NPSO into a proper gel matrix (Nanogel system). Hydrophilic polymers are considered most suitable for topical applications, but type and concentration of the polymer forming gel matrix can influence the stability and release rate of the drugs [22]. Nanogels are nano-sized network of chemically or physically cross linked polymer particles. The gel can aid in creating a uniform dispersion of the nano-carriers in the matrix and increases the contact time which results in enhanced skin penetration of the drug payload [23]. In the present study, we hypothesize that incorporating the surface modified bilayered PLGA-Chitosan nanoparticles with OA into a skin penetrating nanogel system (SPN) can further increase the efficiency by maximizing skin contact time and preventing the 'run-off' effect associated with aqueous nanoparticle dispersion.

The specific aims of this research were to: (1) prepare SPN for SP and KP loaded bilayered nanoparticles, where SP was loaded into PLGA inner core and KP was incorporated into the chitosan outer layer; (2) formulate SPN using hydroxypropyl methyl cellulose (HPMC) or Carbopol and characterize for rheological behavior to get optimum viscosity without affecting particle size of nanoparticles; (3) study the effect of NPSO and SPN for *in vitro* human skin permeation and (4) compare the therapeutic efficacy of the SPN in an *in vivo* allergic contact dermatitis (ACD) and a psoriatic plaque like model with a marketed formulation of tacrolimus, Topgraf® (GlaxoSmithKline Pharmaceuticals Limited, Thane, India).

2. Materials and methods

Poly(lactic-co-glycolic acid) (PLGA) was purchased from PURAC biomaterials (Lincolnshire, IL). Polyvinyl alcohol (PVA), chitosan, dichloromethane, tween 80, sodium tripolyphosphate (TPP), polyethylene glycol 400 (PEG-400), phosphate buffer saline sachets (PBS, pH 7.4), trifluoroacetic acid (TFA) and 2,4-dinitrofluorobenzene (DNFB) were purchased from Sigma–Aldrich Co (St Louis, MO). HPLC grade of acetonitrile, water and ethanol were purchased from Sigma–Aldrich Co (St Louis, MO). Oleic acid-PEG-succinimidyl glutarate ester (OA) was custom synthesized from Nanocs Inc (New York, NY). Ketoprofen (KP) was purchased from Spectrum chemical mfg corp. (Gardena, CA). Spantide II (SP) was purchased from American peptide company Inc, (Sunnyvale, CA). Hydroxypropyl methyl cellulose (HPMC; METHOCEL™ K4M Premium CR Grade) was generously gifted by DOW Chemical Company (Midland, MI). Carbopol (Carbopol 981® NF) was generously gifted by Lubrizol Advanced Materials, Inc. (Cleveland, OH). Imiquimod (IMQ) was purchased from VWR International (Suwanee, GA). Topgraf® (tacrolimus ointment 0.1%) was purchased from GlaxoSmithKline Pharmaceuticals Limited (Thane, India). IL-17 and IL-23 antibodies along with ABC staining immunohistochemistry kit were purchased from Santa Cruz Biotechnology Inc (Santa Cruz, CA).

2.1. Preparation of surface modified bilayered nanoparticles (NPSO)

Bilayered nanoparticles (NPS) were prepared by modified emulsion solvent evaporation method [24]. Briefly, 10 mg of PLGA was dissolved in 1.5 ml of dichloromethane. The organic phase was added to 20 ml of 0.1% w/v PVA solution comprising 4 ml of 0.5% w/v chitosan and 1.5 ml of tween 80 with constant stirring to form a coarse emulsion. This emulsion was broken down into nanodroplets by high speed homogenization for 15 min at 30,000 rpm. The nanoparticles were stirred for 30 min to evaporate the organic phase. Five milliliters of the nanoparticles dispersion was transferred to a scintillation vial. The chitosan, present on the outer layer of nanoparticles was then cross linked with 100 μl of 1% w/v TPP to prepare NPS. This prepared NPS dispersion was stirred at 300 rpm for 2 h to ensure complete cross-linking of chitosan.

The SP nanoparticles (SP-NPS) were prepared by dissolving spantide II and phosphatidylinositol (PI) in ethanol and then mixed with organic phase containing PLGA. NPS were prepared by homogenization as described above. To this nanoparticulate dispersion, TPP was added for cross-linking of chitosan coat. To prepare SP and KP nanoparticles (SP+KP-NPS), ketoprofen was dispersed in the 1% w/v TPP and added drop-wise to SP-NPS. To prepare KP nanoparticles (KP-NPS), SP was excluded from the NPS preparation technique.

For surface modification, NPS was suspended in phosphate buffer, pH 8.0 and incubated for 2 h with OA (mole ratio of chitosan to OA in 1:6), previously dissolved in 10 μl of DMSO. The surface modified NPS were represented as KP-NPSO, SP-NPSO and SP+KP-NPSO for ketoprofen, spantide II, and a combination of spantide II and ketoprofen, respectively.

NPS and NPSO were characterized for particle size and zeta potential using Nicomp 380 ZLS (Particle Sizing Systems, Port Richey, FL). Further the drug content and entrapment efficiency was characterized as reported by Patlolla et al. [25]. TNBS method was performed to estimate the percent of surface accessible amino groups by colorimetric reaction [26].