Chapter 1 Introduction

Dermal Delivery of Protein/Peptide Based Antimicrobial to Treat Secondary Infection in Psoriasis and Eczema

1.1 Introduction

Eczema and psoriasis are the most challenging skin conditions encountered worldwide by skincare professionals. Atopic dermatitis (AD) (atopic eczema) is a chronic inflammatory disease characterized by dry eczematous skin lesions with intense pruritus and itching [1]. The prevalence of AD in adults is around 2.1-4.9%, and in children, it varies between 2.7-20.1%, presenting a significant influence on the quality life of patients [1, 2]. Psoriasis is a long-lasting, disfiguring, immune-mediated, painful, non-communicable, and disabling incurable disease having a great negative impact on quality of life patients [3]. The reported occurrence of psoriasis in developed countries varies from 0.09% to 11.4% [4-6], making psoriasis a severe disease with at least 100 million people affected globally.

Various risk factors influencing psoriasis and its recurrence are genetic changes, infections, allergens, sunlight exposure, alcohol intake, smoking, and endocrine factors [7]. Moreover, psoriasis can triggered by internal and external triggers, together with infections, mild trauma, stress, and systemic drugs [8]. Randomized clinical trial results have shown that the condition may worsen by skin/gut colonization with microorganisms, i.e., *S.aureus*, *Propionibacterial species*, *candida albicans*, and some other species [9-11]. Specifically, *S.aureus* may induce purulent superinfection and increase inflammation by superantigen-mediated T-cell activation [12]. Also, the recent clinical survey demonstrated that people with psoriasis had a higher frequency of getting serious infections (20/1000 person) [13]. Whereas in AD, various environment and genetic factors were involved in the progression of AD [14, 15]. The patients with atopic eczema are mostly colonized with *S. aureus*. A recent meta-analysis demonstrated the 70 % pooled prevalence of *S. aureus* colonization in lesional skin compared to non-lesional skin 39% [16, 17]. Additionally, decreased expressions of antimicrobial peptides (AMPs) for example, cathelicidins LL-37, human beta-defensin 2 and 3 (hBD-2 and hBD-3) were often associated with eczema [18-20].

1.2 Selection of Peptide

AMPs are multi-functional immunomodulatory peptides with broad-spectrum antimicrobial activity against a number of micro-organisms, are recently developing as

novel therapeutics. More than 3000 AMPs have been identified either from the living organisms or by synthetic derivatization [17, 18]. In this work, Omiganan and DPK-060 were selected as AMPs.

Omiganan:

Omiganan is a novel cationic peptide (12 amino acid) and an analog of indolicidin. Omiganan has antimicrobial activity against several gram negative and gram positive micro-organism including fungi [23, 24]. Apart from its antimicrobial effect, Omiganan also has anti-inflammatory activity [25]. These antimicrobial and anti-inflammatory properties make Omiganan a promising agent for treating eczema/atopic dermatitis and psoriasis. Furthermore, the positive phase II clinical results were obtained in patients with AD (mild to moderate) with Omiganan 1% gel [26].

- Omiganan was synthesized by S-Biochem, Kerala, India. The detailed sequence of Omiganan is mentioned below:
    - 12- amino acid sequence (ILRWPWWPWRRK-NH2)
    - Molecular weight: 1779.2 gm/mole ($C_{90}H_{127}N_{27}O_{12}$)

DPK-060:

DPK-060 is a synthetic 17 amino acid peptide, structural derivative from the human protein kininogen [25]. DPK-060 mainly acts by membrane disruption mechanism along with immunomodulation, thus demonstrating strong broad-spectrum antimicrobial activity against both gram +ve and -ve bacteria, including *methicillin-resistant S. aureus (MRSA) in-vitro* and *in-vivo* [26]. Moreover, the safety and effectiveness of 1% DPK-060 in a polyethylene glycol-based ointment has been evaluated in phase II clinical trial (NCT01522391) to treat AD patients. Additionally, the positive results were obtained in these clinical trials but found not statistically conclusive due to the instability of DPK-060 as a drug substance in the formulation [27]. Further, nanotechnology-based formulations of DPK-060 have been developed to improve the functionality, stability, and release profile of DPK-060 [28-31].

- DPK-060 was synthesized from S-Biochem, Kerala, India. The detailed sequence of DPK-060 is:
    - 17- amino acid sequence (GKHKNKGKKNGKHNGWKWWW)
    - Molecular weight: 2505 gm/mole

1.2.1 Current treatment approach and limitations

Treatment of psoriasis and AD is still based on controlling the symptoms. Different topical/systemic treatments, along with phototherapy, are existing. Actually, a combination of these treatments is frequently used. The treatment is typically lifetime and is meant at remission. Up to now, no therapy would cure eczema and psoriasis. The topical treatment of eczema involves emollients, topical corticosteroids (hydrocortisone, clobetasone, and mometasone). In contrast, topical treatment of psoriasis requires vitamin D_3 analogs, corticosteroids, i.e., betamethasone and hydrocortisone, topical retinoids in the form of a gel, ointments, creams, lotions, and foams. At the same time, phototherapy (UV light therapy) and the use of systemic treatments of methotrexate, cyclosporine, and biological agents (Alefacept, infliximab, adalimumab, ustekinumab) are other treatment approaches. In treating bacterial infections associated with psoriasis and eczema, fusidic acid and some antibacterials were used that had a chance of resistance when applied on a long-term basis [31]. The current treatments though manage inflammatory symptoms but have several limitations, i.e., corticosteroids can cause thinning of the skin, vitamin D_3 analogs irritate the skin, retinoids do not act as quickly as topical corticosteroids. In contrast, phototherapy can cause severe and long-lasting burns. Systemic and biological treatments also have limitations, i.e., methotrexate can cause liver damage and decrease the production of RBCs, cyclosporine may offer fast relief from symptoms, but the improvement stops when therapy is discontinued, Alefacept can increase the risk of infection, possibly including cancer [31].

1.3 Dermal route for drug delivery

Protein and peptides based therapeutics currently investigated for various disorders such as cancer, diabetes, immune diseases, brain disorders, etc. The potential benefits of protein and peptide antimicrobials for dermal use are their no or reduced chances of development of resistance, the broad spectrum of antimicrobial activity with almost no side

effects observed in conventional therapy, and may also possess anti-inflammatory activity [32, 33]. Delivery of these classes of drugs through the dermal route remains challenging due to conformational instability, low permeability across stratum corneum, partitioning in a different stage of the subdermal region, enzymatic degradation, etc. [34]. Dermal route for delivery of therapeutics has been continuously investigated to supplement the limitation of oral and other routes of drug delivery for skin disorders. There are limitations of delivering protein and peptide antimicrobial-based therapeutics orally due to the harsh environment of the gastrointestinal tract. Delivery via the intravenous route, intramuscular, intra-peritoneal routes display higher bioavailability, are invasive ones and limit patient compliance, and are of little benefit in treating skin disorders. The dermal route of drug delivery is non-invasive and offers the advantage of ease of application. [34].

1.3.2 Carriers for protein/peptide delivery

Carriers of protein and peptide therapeutics may involve lipidic and polymeric vectors that are a diverse class of delivery systems with biocompatibility. Delivery systems investigated for dermal delivery includes nano-structured lipid carriers [35], liposomes [36], cubosomes [37], solid-lipid nanoparticles (SLN) [38], lipid nano-capsules [39, 40], micelles/reverse micelles [41, 42]. Polymeric vectors such as nanoparticles composed of natural (chitosan, [43]) and synthetic polymers [polyalkylcyanoacylates, poly-glycolic acid (PGA), poly-ε-caprolactone (PCL), poly-lactic acid (PLA)] have been investigated [44, 45]. Thus, various delivery systems have been investigated and can further be explored for delivering the peptide therapeutics successfully via the dermal route.

Lipid-based nano-carriers such as liposomes, Nano-structure lipid carriers (NLCs), and SLN have a natural affinity to the skin lipids and facilitate the drug passage across the skin by enhancing its partitioning from the vehicle to the skin. Moreover, they offer enhanced permeation of drug(s) due to improved contact with the skin, sustained-release properties, and an occlusive effect [46]. Also, in water-soluble drugs, the high lipidic content can lead to an occlusion and development of the film on the skin, increasing skin hydration due to decreased trans-epidermal water loss, helping the diffusion of active ingredients into the SC [46, 47].

1.3.2.1 Nano-structure lipid carriers (NLCs)

NLCs are considered second-generation SLN, which comprise a blend of solid and liquid lipids and thus have high drug loading capability and stability. NLCs also offer an innovative formulation approach for proteins and peptides with poor water solubility and are ideal for dermal delivery [48, 49].

1.3.2.2 Liposomes

Liposomes are the most extensively studied nano-carrier based drug delivery systems [50, 51], mainly due to their ability to improve the bioavailability, safety and biocompatibility profiles of encapsulated drugs [52]. Liposomes are lipid vesicles having single or multiple lipidic bilayers having blends of long/short hydrocarbon chain phosphatidylcholines. They can be designed to suit the need to achieve various morphological states based on the processing conditions such as hydration, temperature, and composition [53, 54].

1.3.2.3 Lotion

The lotion-based formulation approach has several advantages: bypassing first-pass metabolism, ease of application, circumvention of gastrointestinal incompatibility, and enhanced patient compliance [55]. Lotions are typically suspensions of solids in an aqueous media and has low to medium viscous formulation and thicker and more emollient than solution [55].

1.4 Aims and Objective

The prime objective of the study is to formulate efficient delivery systems to deliver therapeutic protein/peptide to combat bacterial infections and the screening of anti-inflammatory activity of selected peptides in eczema and psoriasis, offering improved efficacy and a better treatment approach.

The proposed research aims to:

- *Formulation of antimicrobial peptide-loaded nano-carrier(s) (liposomes and nano-lipid constructs)*
- *Characterization and evaluation of the designed peptide(s) delivery system for their physicochemical parameters and in vitro release characteristics.*
- *Evaluation of cell uptake studies and toxicity of the designed delivery system in cell lines.*
- *Study in vivo performance of the designed protein/peptide(s) delivery system for their efficacy using the suitable animal model.*

1.5 Plan of Work

- ❖ Review of related literature
- ❖ Synthesis of peptides and procurement of excipients
- ❖ Analytical method development and pre-formulation studies
- ❖ Formulation, Optimization, and Characterization of Omiganan loaded nano-lipid constructs, liposomes, and lotion
- ❖ Formulation, Optimization, and Characterization of DPK-060 loaded nano-lipid constructs and lotion
- ❖ *In-vitro* cytotoxicity studies and cellular uptake
- ❖ Stability study as per ICH guidelines
- ❖ *In-vivo* pharmacodynamic studies
- ❖ Summary and conclusion

1.6 References

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