Antimicrobial Peptides as a Solution for Atopic Dermatitis

Subjects: Dermatology

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Atopic dermatitis (AD) is a chronic inflammatory skin disorder that is the result of various environmental, bacterial and genetic stimuli, which culminate in the disruption of the skin's barrier function. Characterized by highly pruritic skin lesions, xerosis and an array of comorbidities among which skin infections are the most common, this condition results in both a significant loss of quality of life and in the need for life-long treatments (e.g., corticosteroids, monoclonal antibodies and regular antibiotic intake), all of which may have harmful secondary effects. This, in conjunction with AD's rising prevalence, made the development of alternative treatment strategies the focus of both the scientific community and the pharmaceutical industry. Given their potential to both manage the skin microbiome, fight infections and even modulate the local immune response, the use of antimicrobial peptides (AMPs) from more diverse origins has become one of the most promising alternative solutions for AD management, with some being already used with some success towards this end.

AMPs atopic dermatitis chronic inflammatory disease skin infection barrier disruption skin microbiome

1. Introduction

To understand the dos and don'ts of peptide applications in human skin one must first understand not only the skin's role and characteristics, but also what makes skin diseases, such as atopic dermatitis, so hard to treat and manage.

Considered by most to be the largest organ in the human body (as it possesses a surface area of approximately 2 m^2) the skin's main function is to be a physical barrier, which protects us from the external surrounding environment [1]. While this barrier function is mostly physical, there is also a "gatekeeper" aspect to it, as the combination of the cells and matrix elements that constitute the skin acts as a "custom agent" that permits or denies microorganism colonization of the skin surface and determines which compounds can migrate through the various layers and either reach the bloodstream or leave the body [2].

From a structural perspective, the skin is constituted of three major layers, which are, from inside out, the hypodermis, the dermis and the epidermis (**Figure 1**). As can be seen from **Table 1**, each layer comprises different cellular constituents having different functions and is home to different structures, such as blood vessels in the

hypodermis and dermis, mechanoreceptors in the dermis, and a stratified keratinized epithelium in the epidermis [1][2].

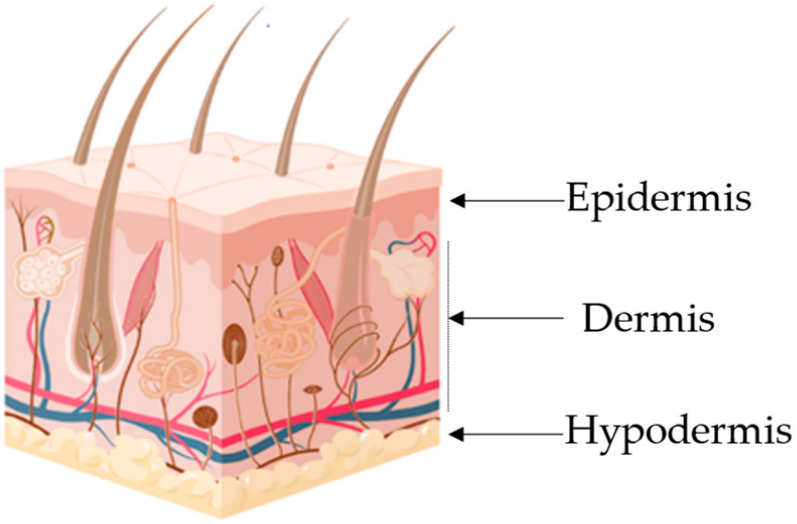

Figure 1. Schematic 3D representation of the human skin. Image produced using Biorender.

Table 1. Major constituents and functions of the different skin layers.

Layer	Major cellular Constituents	Major Functions	References
Hypodermis	Adipocytes, fibroblasts, endothelial and muscle cells	Insulation, mechanical integrity, support, conductance of vascular and neural signals	[1][2]
Dermis	Endothelial cells, fibroblasts, Langerhans and muscle cells	Mechanical integrity, support, thermal barrier, energy storage, protection from physical injury	[2][3]
Epidermis	Keratinocytes, melanocytes, Langerhans and Markel cells	Outermost barrier, immune function, protection from oxidative and mechanical stress	[4][5]

2. Atopic Dermatitis

Atopic dermatitis is one of the most common and recurrent chronic non-infectious skin inflammatory diseases, which is characterized by a persistent itching sensation in the skin. It is a skin disorder that usually appears in early childhood (about 80% of the cases) and is reported to affect 15–20% of children. Although ca. 70% of paediatric patients outgrow the disease, the prevalence in adults remains around 1–3%, although the figures vary greatly from country to country [6][7][8][9]. AD's worldwide prevalence is rising, with two- to three-fold increases in incidence

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in industrialized countries being reported for the last decades, having reached a plateau only in countries where its prevalence is highest [7][10]. In contrast to most allergic diseases, AD has a high social and economic impact. The chronic skin inflammation with continuous itching leads to skin thickening, lichenification and overall discomfort. This will lead to a compromise in sleep patterns, which have social consequences and create economic burdens. All of these social and economic impacts are what makes AD a disease with a high toll on patients and their families [11][12].

From a clinical standpoint, AD belongs in the spectrum of atopic disorders, such as allergic asthma or food allergies. Coincidently, these are companion diseases of AD patients due to the commonly denominated "atopic march", a curious denomination given to the range of allergic disorders that, in later years, manifest in AD patients [13][14][15]. Atopic dermatitis clinical diagnostics are characterized by eczema-like eruptions, papules, exudative lesions, and various degrees of skin dryness. In addition, there are several comorbidities, such as skin infections and cardiovascular and neuropsychiatric disorders, which have also recently been associated with AD, despite the mechanism behind these associations being still unknown [11][16][17][18][19][20]. As with most atopic diseases, there is a strong environmental link with immunoglobulin E (IgE)-related environmental responses, with this factor being one of the primary drivers of AD outbreaks [21]. However, AD is a complex multifactorial disease that cannot be attributed to one single cause.

From a pathophysiologic standpoint, AD results from several genetic defects that potentiate the immune response and disrupt the skin barrier. The most reported mutations are associated with filaggrin (FLG) production, which have been recognized as some of the most relevant, with various authors reporting that up to 60% of Europeans with AD exhibit alterations in FLG expression [11]. Another defect which plays a key role in AD are tight junctions (TJs) mutations and alterations. These are structures that have a connection role between cells, exist in every human epithelium, and have various roles depending on the tissue they are located in (e.g., homeostasis control in the central nervous system or impeding the penetration of pathogens in the intestine) [22][23]. In healthy skin, TJs are part of the mechanism for managing cellular differentiation, proliferation and cascading processes involved in maintaining and managing skin homeostasis and permeability [24][25]. On the other hand, in AD patients, TJs are normally dysfunctional and contribute to the irregular barrier function of the skin. They appear to impede stratum corneum (SC, the outermost layer of the epidermis) formation due to increased pH values and, among other effects, affect the processing of polar lipids and profillagrin, both of which are critical for SC formation. All these alterations lead to increased permeability to exogenous material and bacteria, which results in an increased inflammatory condition and a vicious circle, where the barrier dysfunction potentiates the skin's inflammatory response. Interestingly, the TJ dysfunction is not directly affected by the FLG mutation, with both mechanisms appearing to be independent [26][27].

In addition to these two main alterations, several other structural proteins have been described as downregulated in AD, such as desmogleins, desmocolins, involucrin and keratins [28][29][30][31][32]. These alterations to expression and mutations again result in compromise of the skin's shield function, increased transepidermal water loss (i.e., higher dehydration), and an increased exposure to external toxins and allergens that translates into activation of the local immune system.

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Of all the identified changes, the FLG mutation is a particularly interesting one when seeking to understand the cascade of potential problems that arise from a single mutation: (1) it disrupts the production of moisturizing factors in the skin's stratum granulosum; (2) it disrupts lamellar body secretion and, therefore, alters the composition of the SC; (3) it arrests the metabolic pathway part-way through resulting in the absence of acidic metabolites. This translates into an upswing of the SC pH and allows for an increase in proteolytic enzyme activity (increased desquamation) and the proliferation of *Staphylococcus aureus* (*S. aureus*), a bacterial species whose predominance in skin flora has been strongly associated with AD flares [17][33][34][35].

Considering the pathophysiology of AD, it would be natural to expect that its treatment would involve a multifaceted approach capable of mitigating or ameliorating several facets of the disease, with a multitude of approaches and options for patients. However, this could not be further from reality. As AD is considered as being only a skin disorder, the treatments follow the paradigm "one-size-fits-all", with solutions being very limited, and an almost recipe-like approach being followed by physicians all over the world [21]. In fact, most AD patients' treatment has, as a first line of approach, anti-inflammatory treatment, more particularly, topical corticosteroids or topical calcineurin inhibitors, in conjunction with an antimicrobial ointment to help with *S. aureus* control [36][37]. For more severe cases, this is normally followed by the use of ultraviolet light and potent cocktails of immunosuppressant drugs, such as ciclosporin A, methotrexate and mycophenolate mofetil [21][36][38]. These regimens, while mostly effective, are not without costs, as several side-effects related to cutaneous application of immunosuppressants are well-described in the literature [39][40]. Thus, there has been an increasing demand for alternative solutions to AD treatment.

Currently, there are already some therapeutic alternatives being tested, such as Janus kinase inhibitors (e.g., Delgocitinib and Ruxolitinib), phosphodiesterase-4 inhibitors (e.g., Crisaborole and Difamilast) and aryl hydrocarbon receptor agonists (e.g., Tapinarof), all of which, through topical application, target the inhibition of enzymes, receptors or transcriptional factors involved in AD [41][42][43]. Another option is the use of human monoclonal antibodies (mAbs), which are also already being applied in AD patients via systemic application [44][45]. However, both of these alternatives are used mainly for moderate to severe cases and, thus, are not suitable for everyday applications [46]. Thus, alternatives are still required for the daily management of AD. Among the natural alternatives currently being studied, antimicrobial peptides (AMPs) are some of the most promising.

3. Antimicrobial Peptides

By definition, AMPs are small molecules that are widely present in nature and are part of the immune response of most human inflammatory responses. They are generally constituted by 100 amino acid residues or less and have a positive net charge and amphiphilic structure, which provides these molecules enhanced biological potential due to their natural strong interaction with hydrophobic surfaces and membranes [47]. In terms of their characterization, AMPs can be divided according to their structure (α-helical, β-sheets, extended peptides and loop peptides) and covalent bonds (class I to IV) [48].

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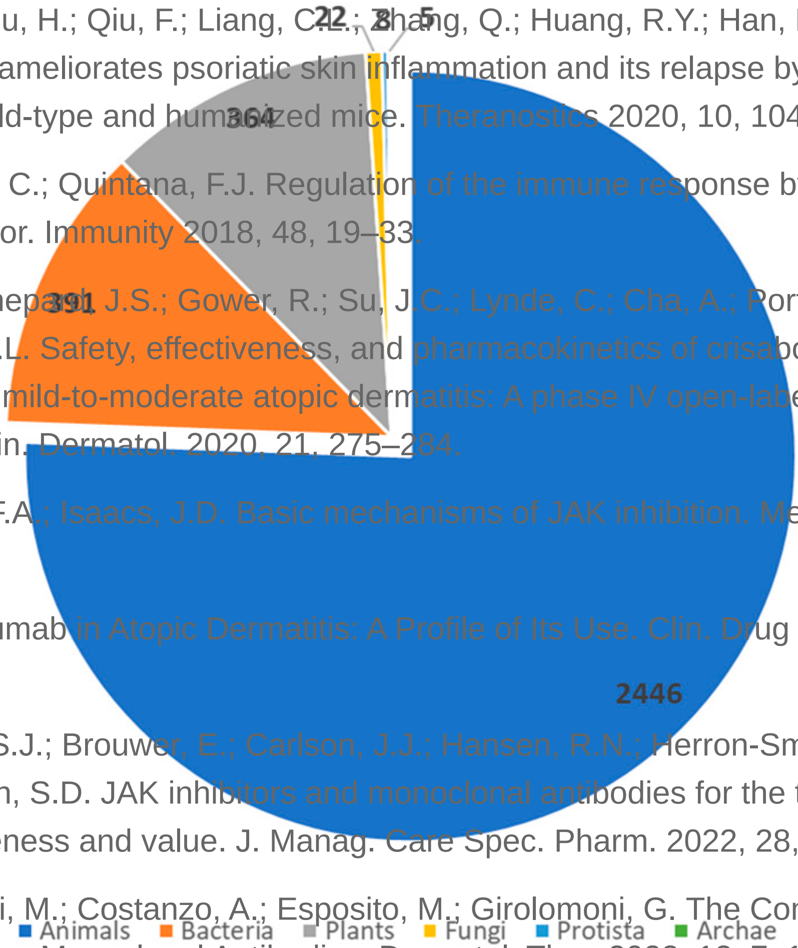

Figure 2. Distribution of known AMPs across the different sources. Data accessed from the Antimicrobial Peptide Database https://aps.unmc.edu (accessed on 15 August 2023).

From a mechanistic standpoint, the application of AMPs as pharmacodynamic agents is dependent on a variety of factors (such as peptide concentration, tissue location, the local environment and the target pathogen) that one must consider when evaluating AMP potential application in skin and AD in particular [53]. While there are some disadvantages regarding the clinical application of AMPs, like their rapid degradation by esterases or aminopeptidases and their low stability in vivo, there are also advantages to their use. These include their capability for penetrating deeper skin layers, as shown by their presence in viable skin even 24 h after application, and the fact that AMP-based treatment provides opportunities for interventions that closely resemble natural pathways and represent more of a replacement therapy than conventional treatment. In this regard, they supplement patients with AMPs in locations where their endogenous levels are low or absent and, consequently, are less likely to promote adverse responses [52][54]. Furthermore, even the previously mentioned disadvantages do not represent a limitation to AMP application in AD management, as, nowadays, there are several strategies to enhance their bioavailability and overcome their limitations. These include peptide immobilization, encapsulation, or even chemical modification to increase AMP permeability through the skin [55].

4. AMPs in Clinical Trials

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As with all pharmaceutical-related applications, clinical trials are the last and crucial step that a molecule must pass before the European Medicines Agency (EMA) or Food and Drug Agency (FDA) approved for human application [56]. The purpose of these trials is twofold—first, to validate in vivo the biological activity verified in vitro; second, to establish a safety profile of the formulated peptides so correct dosages and side-effects can be correctly ascertained [57].

As can be seen in **Table 2**, there are numerous examples of AMPs under consideration with a vast array of applications being considered, with their status ranging from phase I to III. The success rate of these trials and the posterior application of AMPs as therapeutic agents is variable as, while there are currently several AMPs approved for clinical use, such as ghrelin, nisin, gramicidin or daptomycin, there are others, such as CZEN-002 or NVB-302, which have been discontinued as they failed in their clinical trials [56][57][58][59]. The causes for these failures are usually unknown and unexpected as all AMPs are validated by various in vitro, in silico and in vivo models before reaching this stage [58]. One example of such failures is the Friulimicin AMP, which was terminated due to unfavorable kinetics in healthy volunteers and another is the Murepavadin AMP as, in a stage III trial of patients with nosocomial pneumonia, it caused higher than expected kidney injuries [58].

Table 2. Examples of AMPs which underwent and are undergoing clinical trials.

AMP Name	Clinical Trial ID	Phase	Target	Reference
AP-214	NCT00903604	II [a]	Post-surgical organ failure	[60]
C16G2	NCT03004365	II [c]	*Streptococcus mutans*	[61]
CZEN-002	NCT03145220	II [a]	Antifungal	[62]
Daptomycin	NCT01922011; NCT00093067; NCT01104662; NCT02972983	III/IV [c]	Skin infection/bacteremia	[63]
Delmitide (RDP58)	ISRCTN84220089	II [c]	Inflammatory bowel disease	[64]
DPK-060	NCT01447017; NCT01522391	II [c]	Acute external otitis, topical treatment of microbial infections	[65]
EA-230	NCT03145220	II [d]	Sepsis/renal failure	[62]

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AMP Name	Clinical Trial ID	Phase	Target	Reference
Friulimicin	NCT00492271	I [a]	MRSA/pneumonia	[66]
Ghrelin	NCT00763477	II [c]	Chronic respiratory infection	[67][68]
Gramicidin	NCT00534391	III [d]	Infected wounds and ulcers	[69]
GSK1322322	NCT01209078	II [c]	Bacterial skin infection	[70]
hLF1-11	NCT00430469	I/II [a]	Bacterial/fungal infections	[71][72]
Iseganan (IB-367)	NCT00118781; NCT00022373	III [a]	Pneumonia/oral mucositis	[73]
LFF571	NCT01232595	II [c]	*C. difficile*	[74]
LL-37	EUCTR2012-002100-41	II [a]	Leg ulcers	[75]
LTX-109	NCT01803035; NCT01158235	I/II [c]	MRSA/impetigo, antiviral	[76]
Mel4	ACTRN1261500072556	II/III [c]	Contact lenses antimicrobial	[77]
Melittin	NCT02364349, NCT01526031	I/II [c]	Inflammation	[78]
Murepavadin	EUCTR2017-003933-27-EE	II [b]	*P. aeruginosa, K. pneumoniae*	[79]
Nal-P-113	ChiCTR-OIC-16010250	III [c]	Periodontal disease	[80]
Neuprex®	NCT00462904	III [a]	Pediatric meningococcemia	[57]

Subjects with COVID-19 Infection; WHO: Geneva, Switzerland, 2021.

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AMP Name	Clinical Trial ID	Phase	Target	Reference
Nisin	NCT02928042; NCT02467972	n.a. [c]	Gram-positive bacteria	[81]
Novexatin (NP213)	NCT02933879	II [a]	Fungal nail infection	[82]
NVB-302	ISRCTN40071144	I [a]	*C. difficile*	[57]
Omiganan	NCT00231153; NCT02456480	II/III [c]	Antisepsis/catheter, Atopic dermatitis	[83]
OP-145	ISRCTN84220089	I/II [c]	Chronic middle ear infection	[84]
PAC113	NCT00659971	II [c]	Oral candidiasis	[85][86]
P60.4Ac	ISRCTN12149720	II [c]	Chronic ear infections	[87]
Pexiganan (MSI-78)	NCT00563394; NCT00563433; NCT01590758; NCT01594762	III [a]	Diabetic foot ulcers	[88]
PMX-30063	NCT01211470; NCT02052388	II [c]	Acute bacterial skin infection	[89]
Polymyxin B	NCT00490477; NCT00534391	III [d]	Gram-negative bacteria	[90]
Polymyxin E (Colistin)	NCT01292031; NCT02573064	III [c]	*A. baumannii*/pneumonia	[91]
PXL01	NCT01022242	II/III [c]	Postsurgical adhesions	[92][93]
SGX942(Dusquetide)	NCT03237325	III [c]	Oral mucositis	[94][95]

[a] [b] [c] [d]

Of the examples presented in **Table 2**, it is interesting to see that only Omiganan targets AD-related factors directly. In fact, a cursory analysis of the table shows that most AMPs target bacterial growth, a clear reflection of their namesake, and which has directed research efforts over the past years. When one considers this, the scope for

AMP Name	Clinical Trial ID	Phase	Target	Reference
Surotomycin (CB-315)	NCT01597505	III [a]	*C. difficile*	[96]
XF-73(Exeporfinium chloride)	NCT03915470	II [c]	*Staphylococcal* infection	[97]

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