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Skin as a Psychoneuroendocrine Immunology Microcosm: The New Frontier of Low Dose Therapy With Cytokines and Growth Factors in the Systemic Treatment of Chronic Autoimmune Inflammatory Diseases in Dermatology

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Abstract

In recent years, the central role of signaling molecules, such as hormones, cytokines and growth factors, has become evident in both physiological and pathological processes; these signaling molecules are the main regulating effectors of whole body biological functions, in accordance with the guiding principle of psychoneuroendocrine immunology (PNEI). Low dose medicine (LDM) represents an innovative medical approach in which the latest evidence in the fields of molecular biology, PNEI and nano-concentration pharmacology are merged. LDM suggests the use of low-doses of activated biological molecules in order to manage PNEI homeostasis, and this approach represents a new opportunity for the development of therapeutic strategies based on immune balancing interventions. Scientific evidence regarding the efficacy and safety of the LDM approach in the treatment of psoriasis vulgaris, and positive preliminary data regarding the oral administration of low dose activated cytokines for vitiligo and atopic dermatitis treatment, support the LDM-based therapeutic approach for many dermatological diseases.

Keywords: Psycho-Neuro-Endocrine-Immunology, Low Dose Medicine, Signaling Molecules, Sequential Kinetic Activation, Psoriasis Vulgaris, Dermatologic Diseases

1. Context

In recent years, a unified vision of the biological functions of the body has been theorized, in accordance with the guiding principles of psychoneuroendocrine immunology (PNEI) (1-4). The PNEI approach represents a paradigm shift from a strictly biomedical view of health and disease to an interdisciplinary view. The main unifying PNEI element is identified in the bi-directional cross-talk (5) between the psychoneuroendocrine systems and the immune system, which is mediated by a complex network of signaling (messenger) molecules (cytokines, hormones, growth factors, neuropeptides) that are the vehicles for the biological information necessary for the complex and efficient regulation of cellular responses to stimuli.

The signaling molecules play a decisive role in determining a person's state of health or disease, and it is compelling to consider diseases as expressions of the change in concentrations (both in excess or in defect) of these substances. In order to test the hypothesis that messenger molecules can be used for therapeutic purposes, research in the medical world is increasingly directed toward the study of the messenger molecules, which determine the fate of many pathological conditions in a positive (healing) or negative (disease) way.

The multi-system axes, such as the gut-brain-axis and the skin-gut-axis, are controlled and managed by a continuous information exchange driven by cytokines, neuropeptides, neuro-hormones and other signaling molecules, in order to maintain PNEI homeostasis.

In summary, altered cross-talk caused by the imbalance between specific signaling molecules is a fundamental requisite for the onset of inflammatory, allergic and autoimmune diseases (6, 7). Preserving and/or restoring the physiological signaling, based on messenger molecule trafficking, represents an effective strategy for the management of homeostatic equilibrium.

2. Evidence Acquisition

2.1. Alteration of PNEI Homeostasis and Dermatologic Disease Onset

Focusing our attention on the skin, we can appreciate that the skin's defense system is composed of three main levels: the mechanical barrier, the innate immunity and the acquired immunity (8, 9). These levels have specific roles used to protect the body against external and internal inflammatory triggers and infectious agents. A disorder in a specific level can reverberate to the others levels, and the loss of skin immune homeostasis contributes to the pathogenesis of inflammatory skin diseases. Clearly, it appears that skin is not an isolated system; conversely, it is perfectly integrated within the PNEI network and represents itself as a sort of PNEI microcosm. In fact, the relations between the skin layers, the external environment and the internal microenvironment are granted by typical homeostatic cross-talk.

An example of intercellular cross-talk at the cutaneous level is exemplified by the signaling pathways within the epidermal melanin morpho-functional unit the complex of keratinocytes and melanocytes that controls the skin pigmentation phenomena. Keratinocytes are known to produce growth factors and other active molecules, which can promote migration, differentiation and melanization of melanocytes. Keratinocyte-melanocyte cross-talk represents the smallest PNEI unit at the epidermal level.

The following observations highlight the pivotal role of PNEI homeostatic mechanisms in the maintenance of healthy skin conditions:

In 1947, Mary Rawles (10) discovered that melanocytes and some nervous cell subsets have the same embryologic origin, specifically from the neural crest (the psycho-neuro component of the PNEI network).

The intercellular signaling between keratinocytes and melanocytes is managed and maintained in a homeostatic condition by growth factors and cytokines, which represent the endocrine component of the PNEI network.

The immune component is represented by the involvement of melanocytes in anti-oxidative stress protective processes mediated by keratinocyte-derived basic-fibroblast growth factor (b-FGF); the breakdown of these defense mechanisms may cause an immune response mediated by inflammatory triggers (this mechanism is involved, for example, in vitiligo onset).

Particularly crucial is the role played by the immune system in the context of the PNEI network within the "skin system".

Numerous dermatologic diseases are characterized by the presence of a shift in the immunological balance, which is mainly reflected in an imbalance between the cytokines expressed by the Th1/Th17 and Treg/Th2 lymphocyte subpopulations. The so-called Th1/Th2 paradigm is based on the evidence that Th1 cytokine hyper-production is strictly linked with organ-specific autoimmune diseases; skin diseases such as psoriasis, vitiligo and alopecia areata fully fit with this pathological immune picture, characterized by an important inflammatory component.

2.2. Low dose Medicine: Theoretical, Physiological and Biochemical Basis

The latest knowledge in molecular biology and psychoneuroendocrine immunology is represented by the theoretical milestones of low dose medicine (LDM), a new therapeutic approach developed from the results of research in the field of low dose pharmacology.

LDM is grounded on the fundamental principle represented by the centrality of the human being as a whole mind-body entity. The individuality of each patient is considered during the design of a specific therapy for a particular disease, and the inner disease etiology (instead of the symptoms) represents the real target of low dose therapy.

The restoration and/or preservation of starting physiological (homeostatic) conditions is the original idea that supports the LDM approach. This goal is reached through the use of the same biological signaling (messenger) molecules (neuropeptides, hormones, cytokines and growth factors) that are the signaling carriers within the PNEI network. The use of biological molecules to control and drive homeostatic functions in order to restore the physiological homeostasis is the innovative core of low dose therapy.

The distinctive characteristics of the LDM therapeutic approach are the oral administration of the signaling molecules and their systemic activity. Indeed, they exert a fine-tuning of specific cellular signaling pathways. The efficacy of oral administration of cytokines and other molecules, in particular their ability to modulate the immune response, is validated by data reported in the scientific literature. The interaction between orally delivered signaling molecules and M cells at the intestinal epithelium level is the key event for the comprehension of the mechanism that underlies the effectiveness of this method of administration (11-13).

The modulation of the immune response after oral intake of messenger molecules is due to the activation of T cells at the Peyer's patch lymph node level, where M cells act as carriers of signaling molecules between the intestinal lumen and T cells (14).

The major pitfall of this refined signaling pathway is represented by the low bioavailability (1% - 2%) of orally administered messenger molecules (and peptides in general)

(15); to overcome this critical point, an effective drug delivery system is required.

Sequential kinetic activation (SKA) technology, codified and standardized by GUNA S.p.a. (Italy), is a drug delivery system that allows nano-concentrations to be active even below the minimum dose classically considered as effective. SKA technology also allows low dose molecules to be effective as classic recombinant peptides administered at high concentrations. The mechanism of action of SKA low dose cytokines, hormones, neuropeptides and growth factors consists of sensitization or activation of some units of cellular (or plasmatic) receptors, by virtue of their low concentration. Indeed, SKA low dose signaling molecules are administered with respect to their physiological working ranges [between 10-6 (micromolar) for hormones (16) and 10-12 (picomolar) for the other messenger molecules] (17).

The particular ligand/receptor interaction exerted by low dose SKA molecules allows the reactivation and fine modulation of a great number of intercellular signaling pathways, contributing to the restoration and/or protection of the biological function of the whole PNEI network. SKA low dose molecules are able to activate auto-regulation mechanisms and represent an innovative and effective instrument of LDM.

2.3. LDM: A New Instrument for Treatment of Skin Diseases

Skin autoimmune diseases have a very complex etiology, with the participation of both innate and adaptive immune responses. From a PNEI point of view, a great number of homeostatic intercellular signaling pathways are compromised, and a fundamental distinctive tract is represented by an enhanced expression of Th1 proinflammatory cytokines such as IFNs, IL-1 and TNF-a.

Since the 1970s, the growing evidence regarding the fundamental roles of the signaling molecules for the maintenance of physiological efficiency of the whole body, has allowed researchers and physicians to hypothesize regarding the use of so-called "anti-cytokine therapy" for the treatment of autoimmune and inflammatory diseases. The core of this strategy is represented by the therapeutic use of Th2 cytokines and specific antibodies to counteract the excessive production of Th1/Th17-derived proinflammatory cytokines.

The anti-cytokine therapy approach obviously considers the use of high doses of recombinant (human or chimerical) proteins (both cytokines and specific antibodies) that can be detected and evaluated by performing dose-response assays; however, the side effects linked with the high dosages have slowed the development of possible new drugs. The most important and limiting pitfalls connected with the use of high dose cytokines and other signaling molecules are:

1) The need for high doses of active molecules with possible onset of acute and chronic adverse effects.

2) The low compliance of systemic administration, which is usually performed by injective routes; this is a strong limiting factor for chronic therapies.

There is a question about how to solve the apparently irreconcilable conflict between dosage, efficacy and compliance. A possible answer can be found in the LDM approach. The availability of low dose SKA signaling molecules (cytokines, growth factors, hormones, and neuropeptides) makes it possible to use lower doses of activated molecules (active range between picomoles and femtomeoles) (18-22) with therapeutic outcomes comparable to those induced by high doses but without the side effects.

Modern biotechnology used in the production of human recombinant proteinsm, and the availability of the aforementioned pharmaceutical technique called SKA, have allowed researchers to formulate new therapeutic strategies based on the use of lower doses of hormones, neuropeptides, cytokines and growth factors with therapeutic results similar to those induced by high concentrations but without the side effects of the latter.

3. Results

In the field of dermatology the first studies on low dose SKA activated cytokines are beginning to be published. A multicenter double-blind placebo-controlled clinical study performed by Roberti et al. (22) described the efficacy of specific low dose SKA cytokines (IL-4, IL-10 and IL-11 at the concentration of 10 fg/mL) for the treatment of psoriasis vulgaris. The two outcomes chosen for the evaluation of the treatment with low dose SKA cytokines were:

1) The presence and extension of psoriatic lesions

2) The improvement of quality of life

These two parameters were evaluated using the rating scales psoriasis area severity index (PASI) and dermatology life quality index (DLQI), respectively. Roberti et al. clarified some aspects of the action of low dose SKA cytokines against psoriasis vulgaris, and the results of the study allowed the researchers to confirm that low dose SKA cytokine administration is effective, safe and long-lasting, which are crucial aspects for the hypothetical treatment of other chronic diseases such as vitiligo.

Another study, regarding atopic dermatitis in a pediatric population, has stimulated intense interest in the scientific community. Preliminary data (presented at the XXVI SIPPS Congress [Societa Italiana di Medicina Preventiva e Sociale], November 27 - 29th, 2014) have shown the ef-

ficacy of low dose SKA interleukin-12 and IFN-γ (both at 10 fg/mL) as reported by the physicians involved in the study.

Recently, researchers and clinicians operating in the field of LDM have also investigated the possibility of treatment of vitiligo with low dose cytokines, growth factors and neuropeptides. The research group of Barygina et al. evaluated the effects of low dose SKA signaling molecules, in terms of reduction of oxidative stress cellular damages and cell proliferation maintenance, by performing a panel of preliminary in vitro assays on an immortalized human keratinocyte cell line (HaCaT) treated with a stress-inducing agent. Severe oxidative stress was induced by incubation of HaCaT cells with 2, 2'-Azobis (2-amidinopropane) dihydrochloride (AAPH). Thereafter, the same cells were treated with low dose SKA IL-4, IL-10, b-FGF, anti-IL-1 or beta-endorphin (10 fg/mL) for 24 hours. At the end of the treatment the proliferation rate and the intracellular/extracellular oxidative status were assessed by fluorometric assay and by flow cytometry, respectively. The obtained (unpublished) results showed that incubation of HaCaT with AAPH brought a significant and persistent (for 48 hours) increase of both intracellular and extracellular oxidative stress phenomena. The intracellular oxidative stress was significantly reduced in cell samples treated with low dose SKA IL-4, IL-10, b-FGF, and the extracellular oxidative stress was reduced, especially after cell treatment with low dose SKA IL-4 and b-FGF. The incubation with low dose SKA anti-IL-1 and b-FGF led to a mild increase in the cell proliferation rate.

Starting from these preliminary data, Barygina et al. (23) planned and conducted the previously synthetically described basic research preclinical study, in order to evaluate the effects of low dose SKA IL-4, IL-10, b-FGF, and β-endorphin in the modulation of intracellular and extracellular oxidative stress and on the proliferation of human perilesional keratinocytes collected from skin samples obtained from skin lesion biopsies of vitiligo patients.

After examining Barygina's study in depth, we affirm that the obtained results confirmed the preliminary data and showed a significant reduction of intracellular oxidative stress in perilesional keratinocyte samples, in particular with low dose SKA IL-4, IL-10 and b-FGF. The extracellular oxidative stress levels were also reduced with low dose SKA IL-4 and b-FGF, and there was an increase in cell viability with low dose SKA IL-10, b-FGF and β-endorphin, when compared to control perilesional keratinocytes samples.

Further, the recently published retrospective spontaneous clinical study conducted by Lotti et al. (24) requires a deeper analysis. In Lotti's retrospective study, heterogeneous groups of patients were evaluated. The clinical results were obtained from two groups of patients, one treated with orally administered low dose SKA IL-4, IL-10 and anti-IL-1 antibodies, and the other with low dose SKA b-FGF. The results were compared with the results obtained in other groups of patients treated with topical dexamethasone cream (alone and in combination with both groups of low dose SKA molecules) and narrow-band UVB radiation (alone and in combination with both groups of low dose SKA molecules). Two additional groups of patients were only treated with natural sunlight exposure and systemic oral intake of ginkgo biloba titrated extract. These groups were evaluated and the collected results were used as control parameters.

Only patients with vitiligo lesions on the skin surface that did not exceed 15% of the total were evaluated. The results showed the ability of the low dose SKA treatment to reduce significantly the depigmented skin surfaces and to block the spread of the lesions in a significant number of subjects.

Low dose SKA b-FGF and the co-administration of low dose SKA IL-4, IL-10 and anti-IL-1 of antibodies exerted positive effects in a significant number of evaluated cases. A further increase in the positive results was registered in patients treated with the combination of low dose SKA and topical UVB radiation, assessing the effectiveness of the use of the two approaches.

The discussed preclinical and clinical results validate the new LDM therapeutic approach aimed to reduce/slow the spread of vitiligo and counteracting the LGCI with oral administration of low dose SKA IL-4, IL-10 and anti-IL-1 antibodies, inducing skin repigmentation by direct stimulation of melanocytes (with low dose SKA b-FGF) and restoring homeostasis of keratinocyte-melanocyte intercellular cross-talk.

These results represent the attainment of a fundamental goal for low dose pharmacology, paving the way for innovative strategies for vitiligo treatment and for the development of specific treatments of dermatological diseases characterized by the presence of chronic inflammation, loss of immune homeostasis and intercellular cross-talk breakdown.

4. Conclusions

A large number of dermatological diseases are characterized by the presence of an altered immune response caused by the imbalance between Th1/Th17 and Th2-driven responses, which represent a fundamental etiological component. Counteracting the immunological imbalance due to this altered cytokine profile could represent an innovative strategy for the treatment of these diseases.

A great number of both basic and clinical trials have underlined the efficacy of the administration of high doses

of recombinant signaling molecules, such as cytokines, antibodies, neuropeptides and growth factors, against some immune aspects of skin autoimmune diseases. Unfortunately, mild to severe dose-dependent and time-dependent side effects are contextually described.

The possibility of a fine adjustment of the immune response through the use of suitably identified signaling molecules is still considered a good therapeutic opportunity for chronic inflammatory autoimmune diseases of the skin.

The availability of low dose SKA-activated cytokines and the LDM approach, validated by an increasing number of scientific studies, in terms of efficacy and safety, induces the researchers to postulate a new therapeutic approach based on systemic oral administration of low doses of activated cytokines and growth factors. This represents an innovative strategy for the treatment of dermatological diseases that are characterized by an immune Th1/Th2 imbalance such as atopic dermatitis, psoriasis vulgaris and vitiligo.

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