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Review article

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Rationale and utility of sub-therapeutic/low dose cytokines and growth factors in dermatology: an overview

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The review presents data on a new low-dose medicine approach in several dermatoses such as psoriasis, atopic dermatitis and vitiligo based on the signaling molecules, which are responsible for the cross-talk between the psychoneuroendocrine and immune system and regulate the cellular responses to internal and external stimuli. An imbalance of specific signal molecules leads to inflammatory, allergic and autoimmune disorders. The mechanisms of signal molecules' action and aspects of Psycho-neuro-endocrine-immunology are presented. Recent studies on efficacy of low-dose medicine along with recommended strategies in psoriasis vulgaris (IL-4, IL-10, IL-11), atopic dermatitis (IL-12, IFNγ), and vitiligo (IL-10, IL-4, anti-IL-1, b-FGF) are observed.

Keywords: *psoriasis vulgaris; atopic dermatitis; vitiligo; low dose medicine; cytokines; growth factors; overview*

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1. Brief introduction to sub-therapeutic/Low Dose Medicine

The Sub-therapeutic/Low Dose Medicine (LDM) was born from the encounter between Molecular Biology with Psycho-Neuro-Endocrine-Immunology (PNEI), spearheaded by research in the field of nanopharmacology. Its guiding principles are given below:

1. Treating the host, and not only disease.
2. To address the cause of disease and not focus on symptomatic cure alone.
3. To consider the human mind-body as a single system.

The concept of sub-therapeutic/LDM is grounded on an innovative point of view in medical field: to restore the healthy physiological conditions in a sick individual by utilizing identical biological molecules (signaling molecules), as synthesized by human body under optimum homeostatic conditions, to maintain all body functions.

Most of these molecules, including hormones, cytokines, and growth factors are fundamental regulatory molecules for cellular and tissue functions. They have been collectively defined in molecular biology as signaling (or messenger) molecules, substances mainly of protein nature which can lead to different cells in the body the "right signals" for their proper operation.

Signaling molecules and the PNEI network

Since the 1970s researchers in the fields of physiology and molecular biology have highlighted the indispensable role of signaling molecules in both healthy and diseased conditions.

They recognized the corner-stone role of these substances, their altered expression (hypo or hyper) resulting in diseases. Thus, medical research is looking with increasing interest to the study of signaling molecules and to the possibility of their use for therapeutic purposes.

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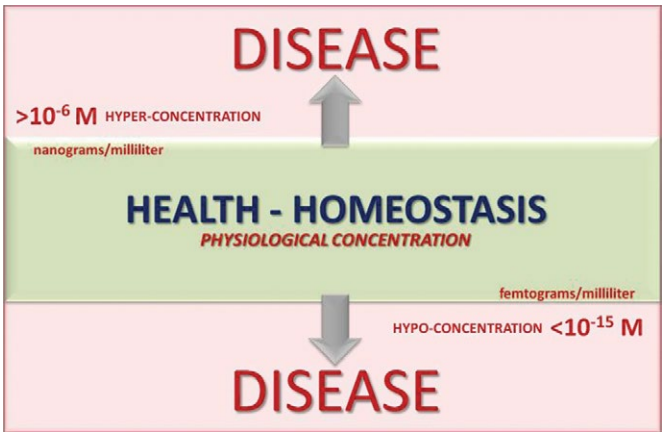

Figure 1. Range of physiological concentration of signaling molecules at the level of the extracellular matrix.

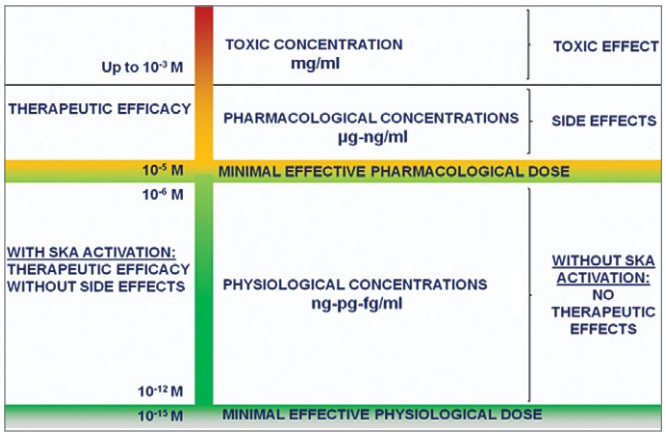

Figure 3. Relationship between signaling molecules concentrations and their effects.

Over the recent years, researchers have accepted a more unified concept of the biological functions of our body, as suggested by PNEI principles, overriding the classic separatist design of human body functioning [1–4].

PNEI approach signals an innovative and fundamental paradigm shift in medicine: from an individualistic and mono-systemic concept of health and disease to a multi-system interdisciplinary one. The primary unifying PNEI concept is embedded in the cross-talk between the psychoneuroendocrine and immune system.

A network of signaling molecules is responsible for this cross-talk, which in-turn regulates the cellular responses to internal and external stimuli. An imbalance of specific signal molecules leads to impaired (or altered) cross-talk, resulting in inflammatory, allergic and autoimmune disorders [5–7]. Thus, the recent therapeutic goal is aimed at preserving and restoring the serum and tissue levels of these messenger molecules to obtain homeostatic equilibrium.

In homeostatic (healthy) conditions, these molecules fluctuate in a minute range (nanograms/ml to femtograms/ml) in the extra-cellular matrix (ECM) [8, 9], however, the range of this fluctuation widens in pathologic conditions (increased or decreased levels) [10–14] (**Figure 1**).

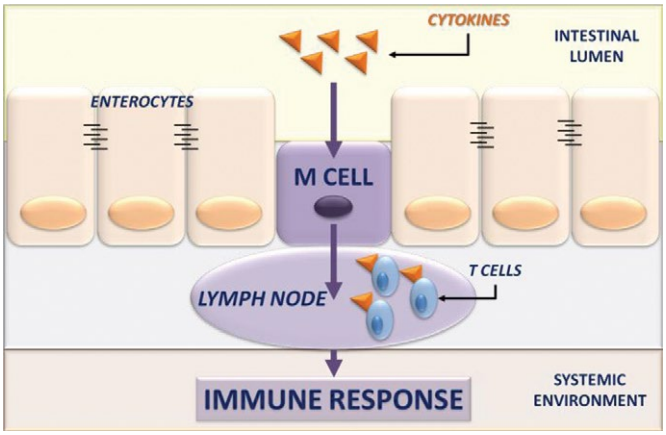

Figure 2. The role of M cells in the processes of absorption of signaling molecules at the level of the intestinal lumen.

LDM refers to the use of biological molecules which regulate cellular functions in order to restore normal homeostasis.

Thus, it is essential to understand some aspects of PNEI network management, which are necessary for a proper understanding of LDM concept:

- The PNEI cross-talk is bi-directional, as are the effects of its alteration [15–17].
- Cellular signaling occurs by diffusion of messenger molecules in ECM, any aberration leads to altered communication between cells, organs and systems leading to disease [18, 19].
- Signal transduction occurs following ligands-receptors interaction, concentration of substrate, receptor affinity and saturation kinetics substrate key parameters [20, 21].

Recently, biomedical researchers are trying to balance specific alterations of the immune system by using cytokines or endocrine disorders with hormones. However, the clinical utilization of this concept is restricted by severe dose-dependent side effects at usual and common pharmacological (not low dose) concentration.

LDM therapeutic tools, represented by physiological low doses of signaling molecules, are orally administered to achieve systemic results. Recent literature also supports effectiveness of oral cytokines in regulating immune response [22–24].

Orally administered peptides possibly act via M cells at intestinal epithelium level. M cells directly recognize signaling molecules in the intestinal lumen and present them to immune T cells within Peyer's patches lymph nodes [25] to initiate a targeted immune response (**Figure 2**).

However, reduced bioavailability (typically less than 1–2%) of low-dose oral molecules (and peptides

in general) is a recognized limitation. This problem may be overcome by newer, effective drug delivery systems. Therapeutic efficacy of oral low-doses (nanograms-femtograms) of signaling molecules may be ensured by using Sequential Kinetic Activation (SKA) technology (**Figure 3**). It is an innovative drug delivery system (codified and standardized by GUNA S.p.a, Milan, Italy), guided by principles of Quantum Physics [26]. This enables the activity of substances in nano-concentration, much below the standard minimum effective dose, with comparable therapeutic efficacy to higher concentrations.

Low-dose SKA cytokines, hormones, neuropeptides and growth factors act by sensitization (or activation) of cellular and plasmatic receptors due to their high dilution, practically in their physiological working range between 10^{-6} (microgram) for hormones [8] and 10^{-15} (femtogram) for the other messenger molecules [9, 10] (**Figure 3**). The sensitized receptors, in turn, trigger specific intracellular chain reactions to activate the PNEI network auto-regulation mechanisms. LDM may have the following therapeutic role:

• Restoration of PNEI homeostasis by addressing a pathological cellular pathway by using physiological, low-dose (SKA) cytokine, hormone, neuropeptides or growth factors the same;

• Attaining the physiological concentration of up-regulated molecules by using antagonistic molecules (low dose SKA), by negative feedback inhibition.

2. The Low Dose Research

Basic and clinical research has tested and validated the theoretical aspects of LDM. In 2009 the journal "Pulmonary Pharmacology & Therapeutics" published the index paper highlighting the effects of low dose SKA cytokines in an animal model of allergic asthma [27]. Several other publication have followed suit [28–44].

The authors have documented the experimental and clinical utility of LDM approach on immunological diseases. All authors have agreed on the ability of SKA low dose signaling molecules to selectively modulate immunological responses. Thus, SKA low dose molecules were found to be effective despite their low/sub-therapeutic dose. This addressed the problem of discontinuing cytokines and other signaling molecules, when used in higher pharmacological doses due to adverse effects.

Minimum effective dose refers to a concentration between lowest pharmacologicalone (10^{-5}) and highest physiological (10^{-6}) levels (**Figure 3**); low dose Pharmacology employs the physiological concentration of signaling molecules below the cited concentrations; the effectiveness of SKA low doses signaling molecules being largely dependent on the the ligand-receptor interactions.

Receptor affinity of specific ligands is the key factor in activating postreceptorial downstream actions [45, 46]; ligand saturation usually causes receptor freezing and/or its down-regulation. Low dose signaling molecules induce a direct physiologic receptor-mediated stimulation and response of immune cells in their homeostatic range; LDM satisfies a key principle of PNEI disease approach-restoring the physiological equilibrium panel of signaling molecules.

3. Dermatology and Low Dose Medicine

Skin diseases are characterized by a complex etiology, frequently characterized by dysregulation of both innate and adaptive immune pathways, involving a wide variety of signaling networks. The Low Grade Chronic Inflammation (LGCI), characterized by over-expression of Th1 proinflammatory cytokines like IFNs, IL-1, IL-6 and TNFα represents a common etiological pathway for many dermatologic diseases.

During the last decades, growing evidence has suggested the cardinal role of signaling molecules in maintaining physiological homeostasis of whole body, including the skin. This has encouraged both researchers and physicians to study the possibilities of therapeutic use of interleukins and other signaling molecule in order to counteract the autoimmune and inflammatory etiological components of many dermatologic diseases.

The role of Th2/T-reg-derived cytokines and specific antibodies in modulating over-expressed Th1/Th17-derived signaling molecules form the theoretical background of the anti-cytokine therapy.

However, the two major limitations are high doses of active molecules and the low compliance of their parenteral administration (most common route), thus increasing the risk of severe adverse effects. These limitations may be overcome by using low dose SKA signaling molecules, thus achieving maximum therapeutic efficacy with minimum adverse effects.

LDM has been tried in the treatment of following dermatological disorders:

Psoriasis

Few studies have been conducted to assess the role of low dose SKA signaling molecules in psoriasis. In 2014, M. Roberti, et al. [31] published their results of the first clinical study on a dermatologic disease (Psoriasis vulgaris) with oral low-dose cytokines (SKA technology).

The researchers assessed the efficacy of orally administered low dose SKA IL-4, IL-10 and IL-11 (at the concentration of 10 fg/ml) in psoriasis vulgaris, in a multicenter double-blind placebo-controlled study design.

Theirprimary and secondary outcomes were reduction of Psoriasis Area Severity Index (PASI) score and improvement of Dermatology Life Quality Index (DLQI) respectively. The authors also highlighted the safety of low dose SKA interleukins along their long-lasting benefits. Thus, a new treatment protocol

Table 1

LDM treatment protocol for Psoriasis Vulgaris

Medicament	Treatment regimen
GUNA IL-4	20 drops twice daily, for 3 consequtive months.
GUNA IL-10	20 drops twice daily, for consequtive 3 months.
GUNA IL-11	20 drops twice daily for 3 months continuously. The cycles may be repeated according to individual clinical severity and response to treatment. All medicines can be administered together, dissolved in a little water, preferably in empty stomach. In children below 6 years, the dosage is 10 drops (vis a vis 20 drops in adults).

was formulated for ssoriasis, and subsequently, for other chronic dermatoses involving LGCI (**Table 1**).

Atopic dermatitis

Atopic Dermatitis (AD) is the most common chronic, inflammatory skin disorder in children, where LDM has been tried. Genetic, immunologic, and environmental factors play important roles in its pathogenesis, while skin barrier dysfunction is the hallmark defect.

From the therapeutic pint of view, it is important to highlight that no therapies are currently curative for this condition. Recently, growing evidences allowed the researchers to test the possible direct modulating action on Th1/Th2 switch through the administration of specific signaling molecules (cytokines). However, this approach is limited by severe dose-dependent adverse of cytokines. LDM may provide a better alternative treatment option to address this disorder with minimum adverse effects.

D. Carello, et al. [39] evaluated the efficacy of low dose SKA cytokines (IL-12 and IFNγ at a concentration of 10 fg/ml) administered *per orally*, in addition to a low-dose multicomponent natural medication (exerting connective drainage action), in a pediatric cohort.

The researchers conducted a randomized, double-blind controlled trial to evaluate the effects of a long-term treatment with the cited low dose SKA cytokines. The clinical trial included children with low to mild AD in acute phase evaluated through Scoring Atopic Dermatitis (SCORAD) index (minimum score: 6; maximum score: 40) with ≥4 episodes per year and skin lesions persisting for at least six months following enrollment.

The reduction in the severity of Atopic Dermatitis was evaluated as primary outcome using the SCORAD index and the "disease-free interval" span was assumed as secondary outcome. The authors also assessed the safety, tolerability and compliance of treatment regimen. The results showed that the LDM reduced the SCORAD score by 54% and maintained across follow-up period, end-of-treatment SCORAD reduced by 64%. The requirement of other medications also reduced. LDM regimen also improved the quality of life and sleep (**Table 2**).

Vitiligo

Vitiligo is an acquired de-pigmenting skin disorders associated with significant psychological morbidity [47–49]. The central event is melanocyte destruction. Although, exact pathogenesis is unclear, cellular immunity plays a key role.

Authors have noted altered cytokine expression from lesional skin.

The immune response shows a shift from normal Tregs/Th2-related pathway to Th1/Th17 axis, thus releasing pro-inflammatory cytokines to promote this inflammatory autoimmune disease [50–53]. TNFα, an important proinflammatory mediator, plays an instrumental role to initiate oxidative stress-enhanced cytotoxicity against both melanocytes and keratinocytes [54, 55].

The chronic inflammatory status clearly appears fundamental in Vitiligo etiopathology; the disruption of the cross-talk between the two most relevant skin cellular subsets (keratinocytes and melanocytes) caused by an excessive oxidative stress and an altered immune response in vitiliginous areas are simultaneously induced and maintained by the altered levels of involved specific cytokines and growth factors. The comprehension of the Vitiligo pathological processes is tightly linked with the study of the intra- and intercellular signaling pathways at skin level, distinctive element to better understand and to study new, safe and effective therapeutic approaches.

Table 2

LDM therapeutic strategy for Atopic dermatitis

Medicament	Treatment regimen
GUNA IL-12	15 drops twice] daily for 8 consequtive months.
GUNA IFNγ	15 drops twice daily for 8 months continuosly.
GALIUM-HEEL®	15 drops twice daily for consequtive 8 months consequently. The therapy cycles can be repeated according to the clinical history of every single patient, the severity of the disease and the individual response of each patient. All the medicines can also be administered all together, dissolved in a little water, preferably far from the meals. In children below 5 years, the dosage is 8 drops (instead of 15 drops such as in adults).

Physiopathology of the epidermal unit of melanization and Vitiligo onset

Keratinocytes and melanocytes represent the main cellular subsets at the cutaneous level, forming the so-called epidermal unit of melanization which controls the skin physiologic pigmentation [56–59].

Melanocytes are responsible of melanosomes production, a unique intracellular organelle which contains melanin and specific enzymes such as tyrosinases, crucial enzyme for melanin maturation (it is a specific target for antibodies-mediated autoimmune response, a cardinal point of Vitiligo onset) and lytic enzymes such as acid-dependent hydrolases, involved in melanin trafficking from melanocytes to keratinocytes [60, 61].

The melanosomes are moved to keratinocytes by an unclear process; a proposed mechanism is grounded on the presence of a synapse-like structure between melanocytes and keratinocytes. Keratinocytes express a specific receptor, Proteinase-Activated Receptor 2 (PAR-2), which is involved in phagocytosis processes that mediate melanosomes transfer [62, 63]. Melanin is transferred in keratinocytes in order to forms a cap-structure around cells nuclei and, consequently, to protects the DNA from UV radiations; a well-known physiologic effect of this process is skin hyper-pigmentation in response to prolonged sunlight exposure. Ethnical differences in PAR-2 expression are reported, PAR-2 levels are increased in dark skin compared to light one clarifying its pivotal role in cutaneous pigmentation, in agreement with the above described mechanism [64].

The homeostatic interaction between keratinocytes and melanocytes is ensured by a specific panel of growth factors and cytokines and the breakdown of keratinocytes-melanocytes signaling pathways is linked with skin degenerative phenomena and immune/autoimmune responses mediated by inflammatory phenomena [65, 66].

An alteration of the immunological balance is characteristic of Vitiligo; manifested by an imbalance between the cytokines expressed by Th1/Th17 (TNFα, INFγ, IL-1, IL-17, IL-2, IL-6, IL-8) and by Treg/Th2 pathways (IL-4). Abnormally high levels of Th1-related cytokines are linked with autoimmune diseases; the same also holds true for vitiligo [50, 51, 67–69].

The exposed remarks clarify the role of immune homeostatic mechanisms breakdown in Vitiligo onset: a Low Grade Chronic Inflammation (LGCI) status (which is reflected in an altered cytokines chronobiology) and autoimmunity are present; abnormally high oxidative stress impairs the epidermal unit of melanization disrupting the keratinocytes-melanocytes cross-talk and enhancing cellular toxicity; in particular the breakdown of basic-Fibroblast Growth Factor (b-FGF) stimulating pathway on melanocytes is answerable for the depigmentation phenomena.

In Vitiligo, the destabilization of this intercellular cross-talk caused by inflammatory and autoimmune phenomena results in melanocytes numerical reduction and in loss of function of the melanization unit; these dysfunctions at skin level generates not only to aesthetic (and obviously psychological) troubles but also a severe diseases such as Squamous Cell Carcinoma (SCC) linked with loss of melanin anti UV-radiation protective barrier.

Keratinocytes-melanocytes cross-talk management

Keratinocytes synthesize specific signaling molecules such as Endothelins (ET), Stem Cell Factors (SCFs) and b-FGF which are involved in melanocytes growth and differentiation and in melanin synthesis [70–75] (**Figure 4**). ET and SCFs bind their specific receptors EDNRB (endothelin receptor type B) and c-Kit starting a signaling pathway [76–81] that finally stimulates ERK1/2 (Extracellular

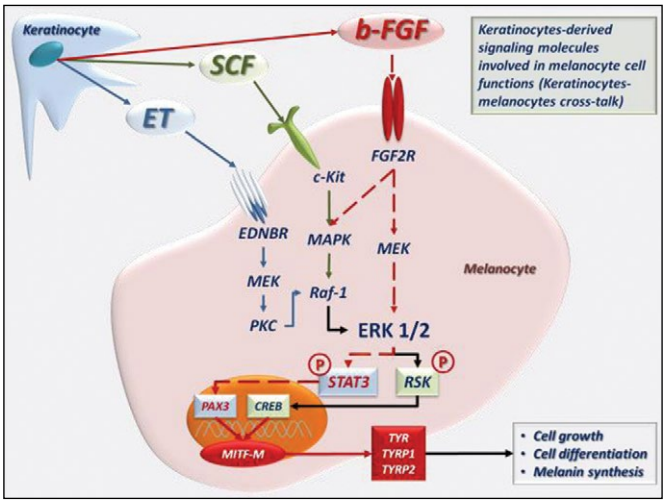

Figure 4. Schematic representation of the main intercellular pathways involved in melanocytes growth and differentiation and melanin synthesis under stimulation of keratinocytes-derived signaling molecules (ET, SCF, b-FGF).

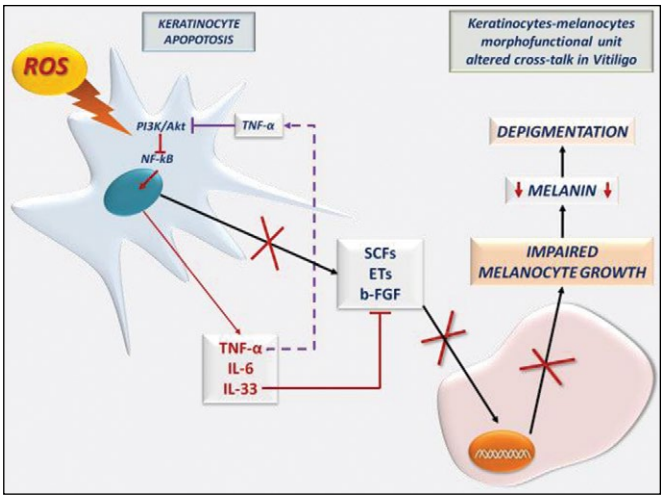

Figure 5. Keratinocytes-melanocytes cross-talk. Melanocytes growth and melanin production are impaired in presence of a ROS-mediated inflammatory response mainly driven by the proinflammatory cytokines TNFα, IL-6 and IL-33 (IL-1).

signal-regulated kinases 1/2)-mediated RSK (ribosomal s6 kinase) nuclear translocation; the nuclear transcription factor cAMP Response Element-Binding (CREB) protein is activated by RSK, thus promoting the expression of specific genes that encode for tyrosinases (TYR; TYRP1/2) *via* microphthalmia-associated transcription factor (MIT-F) activation [82, 83].

Instead, the b-FGF-mediated signaling downstream involves the b-FGF specific receptor FGF2R which activates the secondary intermediates Mitogen-Activated Protein Kinases (MAPK/MEK) resulting in Extracellular-Signal-Regulated Kinases 1/2 (ERK1/2) nuclear transcription factors activation; consequently, the Signal Transducer And Activator of Transcription 3 (STAT3) nuclear translocation activates Paired Box 3 (PAX3) nuclear transcription factor which promotes the transcription of specific genes encoding for tyrosinases [84]. Keratinocytes secrete b-FGF (growth factor) after being stimulated by of Forkhead box N1 (Foxn1) transcription factor activation [85, 86]; Foxn1 is involved in the regulation of keratinocytes growth and differentiation and in the recruitment of chemotactic factor for melanocytes. Through b-FGF, Foxn1 induces (in collaboration with the cited ET/SCFs and other mediators such as beta-endorphin and ACTH) skin pigmentation promoting the melanosome transfer [87].

The homeostatic management of these signaling pathways guarantees the correct tuning of melanocytes cellular functions and leads to optimal skin pigmentation. b-FGF also exerts a paracrine signal to keratinocytes and melanocytes, b-FGF is also involved in redox detoxifying processes to inhibit oxidative cellular damages (activation of PI3K/Akt and inhibition of NF-kB nuclear translocation), in addition to being a pro-mitotic and pro-migrating factor [58, 88].

Keratinocytes apoptosis and impaired activity of epidermal unit of melanization are due to excessive oxidative stress phenomena

Elevated serum levels of reactive oxygen species (ROS) are detected in Vitiligo and contribute to its onset. Pathologically enhanced ROS activity is linked with elevated concentration of H_2O_2 (increased levels from 10^{-6} M to 10^{-3} M in the epidermis) and concomitant impaired catalase activity. This altered redox environment exerts deleterious effects at cellular signaling and metabolism level, in particular affecting lipid peroxidation [87, 88]. Malondialdehyde (MDA) is an final metabolite of lipid peroxidation and it is a useful diagnostic hallmark of oxidative stress; serum levels of MDA are elevated in Vitiligo, showing the central role of oxidative stress in Vitiligo onset [89].

Increased levels of proinflammatory cytokines, catecholamines and Nitric Oxide (NO) [70, 91] impaired growth factors activity [92] down-regulated expression of scavenger and antioxidant molecules [93–95] are the main detectable pro-oxidative key factors in Vitiligo.

Increased oxidative stress is responsible of the excessive production of free radicals, key trigger for keratinocytes and melanocytes apoptosis; it causes the disruption of epidermal unit of melanization unit and the consequent skin depigmentation. In homeostatic physiological conditions, melanocytes express a complete enzyme panel like hemeoxygenase-1 (HO-1), superoxide dismutase (SOD) and catalase to counter ROS over-expression [96]. Nuclear translocation of NF-E2-related factor (Nrf2) along with specific genes regulate the generation of scavenger enzymes for free radicals.

In Vitiligo, this protective mechanism is disturbed, particularly affecting the Nrf2-ARE-HO-1 axis [97, 98], thus leading to oxidative damage of malanocytes and keratinocytes.

Melanin uptake and secretion of anti-oxidant enzymes (e.g.: quinone oxidase and NQO-1) by keratinocytes are regulated by PAR-2 activation, to neutralize ROS mediated oxidative stress [77, 96]. The disruption of PAR-2/Nrf2 cross-talk is involved in Vitiligo onset: at skin depigmented lesions level, PAR-2 expression is reduced with subsequent impaired anti-oxidant response [99–101].

In summary, in active Vitiligo the increased oxidative stress-mediated cytotoxicity is not effectively counteracted by the antioxidant enzymes activity; possibly due to disturbed Nrf2 pathway in both melanocytes and keratinocytes.

Chronic inflammation status and altered immune response both induce keratinocytes apoptosis. High levels of TNFα reduce PI3K/Akt activation with consequent down-regulation of NF-kB blocking mechanism. Enhanced NF-kB-mediated proinflammatory response results in over-expression of Th1-related cytokines like IL-6, IL-33 (belonging to IL-1 superfamily) and TNFα, which in-turn establishes a negative feedback loop [79, 102] (**Figure 5**).

Keratinocytes apoptosis drives to an impaired expression of ET, SCF and b-FGF [51, 53] with consequent reduced melanocytes stimulation; IL-33 also down-regulates ET, SCF and b-FGF contributing to cross-talk breakdown [102]. In Vitiligo the reduced melanin levels are induced by the impaired melanocytes activity; the importance of IL-1/IL-33 over-expression in Vitiligo onset and maintenance is highlighted by the observation of high levels of these interleukins within the active lesions. The chronic pro-inflammatory microenvironment established by IL-1/IL-33 and TNFα is a key factor for keratinocytes-melanocytes cross-talk breakdown resulting in impaired b-FGF expression and action.

The complexity of the dysregulated cellular mechanisms which participate to Vitiligo onset is a critical point for the comprehension of its etiopathogenetic aspects but, on the other hand, they also represent a source of "theoretical handholds" for the design of newer treatment options for this disorder.

Low Dose Medicine for Vitiligo treatment

The reduction of melanocytes' number and their impaired viability at vitiliginous lesions level originate skin depigmentation, the classic visible expression of Vitiligo.

The growing scientific evidences regarding the involvement of cytokines and growth factors in keratinocytes-melanocytes cross-talk allowed the scientific community to carefully evaluate the possible therapeutic role of these signaling molecules in Vitiligo treatment [103, 104].

Regrettably, the therapeutic goal of an effective modulation of cell signaling through the use of specific cytokines, antibodies and growth factors is affected by enormous problems linked with severe adverse effects which greatly reduce the safety and effectiveness of this approach.

The LDM approach described in chapter 1 can represent the "joining link" between the necessity of new specific therapeutic tools for Vitiligo treatment and a feasible, effective and safe signaling molecules-based therapeutic approach.

The LDM approach for vitiligo treatment aims to counteract the inflammatory phenomena rebalancing pro-and anti-inflammatory response with selected low dose SKA cytokines and antibodies (IL-10, IL-4 and anti-IL-1) and, concomitantly, using SKA low dose b-FGF to stimulate melanogenesis by upregulating transmembrane receptors, represents.

Chronic inflammation is a key component of Vitiligo pathogenesis. An original and innovative treatment is conceivable for vitiligo although it is a systemic chronic autoimmune inflammatory disease, resulting from dysregulation in oxidative and inflammatory pathways.

Low dose SKA IL-4, IL-10 and anti-IL-1 antibodies address this etiologic axis along with additional benefits [105–108].

- To restore the Th1-Th17/Th2-Tregequilibrium and reduce inflammation and autoimmune hyperactivation.
- To minimize oxidative stress.

In particular, low dose SKA IL-4 and IL-10 are critical for suppressing hyper-responsive Th1/Th17 axis, to reduce inflammation in autoimmune disorders like vitiligo and Psoriasis Vulgaris [109]. IL-4, the key cytokine of Th2 pathway is responsible for down-regulating both chronic and acute inflammatory responses, by enhancing IL-10 and subsequent NO (anti-oxidant) synthesis.

Low dose SKA IL-10 exerts a direct anti-inflammatory action by inhibiting pro-inflammatory mediators' over-expression and promoting the production of anti-inflammatory molecules like soluble TNFα receptors and the interleukin-1 receptor antagonist (IL-1RA). Low dose anti-IL-1 antibodies synergistically act with IL-4 and IL-10 to exert a potent anti-inflammatory action by reducing circulating IL-1β and IL-1RA levels. Reduced serum levels of pro-inflammatory cytokines minimize oxidative stress, by enhancing ROS-scavenger cellular activity and also correct immune dysfunction.

Keratinocytes express specific signaling molecules like b-FGF for normal epidermal pigmentation; these molecules regulate the proliferation and survival of melanocytes stimulating melanin production in response to skin stress conditions. Low dose SKA b-FGF also exerts a pro-mitotic and pro-migrating action on melanocytes, consequently improving keratinocyte-melanocytesinteraction; low dose SKA b-FGF further corrects oxidative stress, thus reducing chronic inflammation.

Efficacy and safety of low dose SKA signaling molecules for Vitiligo treatment

V. Barygina, et al. [33] evaluated the effects of low dose SKA signaling molecules by assessing their effect on oxidative stress cellular damages and cell proliferation maintenance in-vitro on stressed immortalized human keratinocyte cell line (HaCaT).

Severe oxidative stress was induced by incubating of HaCaT cells with 2,2'-Azobis (2-amidinopropane) dihydrochloride (AAPH). Subsequently, they were treated with low dose SKA IL-4, IL-10, b-FGF, anti-IL-1 or beta-endorphin (10 fg/ml) for 24 h; and their proliferation rate and the intracellular/extracellular oxidative status were analyzed at end-of-treatment.

Unpublished results demonstrated persistent stress (48 hours) following incubation of HaCaT with AAPH. Treatment with low dose SKA IL-4, IL-10, b-FGF significantly reduced intra-cellular oxidative stress (18 ± 4%, 31 ± 3% and 26 ± 2 % respectively), while low dose SKA IL-4 and b-FGF also reduced extra-cellular oxidative stress (28 ± 4% and 37 ± 5% respectively) (*in-vitro*).

Low dose SKA anti-IL-1 and b-FGF additionally increased the cell proliferation rate (23 ± 4% and 22 ± 3% vs control, respectively).

Thus, V. Barygina, et al. [38] preformed the preclinical study to evaluate the effects of low dose SKA IL-4, IL-10, b-FGF, and β-endorphin in the modulation of intra- and extra-cellular oxidative stress and proliferation of human perilesional keratinocytes vitiligo patients.

Thus, low dose SKA IL-4, IL-10 and b-FGF significantly reduced intra-cellular oxidative stress in perilesional keratinocytes (-18.1 ± 0.5%, -19.2 ± 15% and -21 ± 6%, respectively), consistent with in-vitro results. particular with Low dose SKA IL-4 and b-FGF also significantly reduced extra-cellular oxidative stress (-26 ± 5.6% and -36.2 ± 11.5% respectively). Cell viability was enhanced by low dose SKA IL-10, b-FGF and β-endorphin, (+9.2 ± 1%, +15.7 ± 3.26% and +13.5 ± 2.7% respectively vs controls).

Another recent study by T. Lotti, et al. [33] also focuses on the role of low dose SKA IL-4, IL-10, anti-IL-1 antibodies and low dose SKA b-FGF in Vitiligo (BSA involvement <15%). They included following groups-one group received these LDM orally, while other groups received topical dexamethasone cream (alone

and in associations with both groups of low dose SKA molecules) and narrow-band UVB radiations (alone and in associations with both groups of low dose SKA molecules). Two other groups were treated only with natural sunlight exposure and systemic oral intake of *Ginkgo biloba* titrated extract, and served as controls.

Study results highlighted the effectiveness of SKA low dose treatment in significantly reducing the area of cutaneous depigmentation and preventing the spread of disease in a large number of patients.

Low dose SKA b-FGF improved the disease in 74% of patients, while 77% improved on co-administration of low dose SKA IL-4, IL-10 and anti-IL-1 of antibodies. Improvement was considered when patients reported moderate (reduction of depigmentation in 25–50% of the affected area) to excellent (reduction of depigmentation in > 75% of the affected area) outcome. Low dose SKA treatments with topical NB UVB obtained maximum benefit in 93% patients.

Thus, these studies highlight the substantial role of oral LDM (low dose SKA IL-4, IL-10, b-FGF and anti-IL-1-antibodies) in achieving pigmentation and arresting the spread of vitiligo. The possible mechanism seems to be the correction of keratinocyte-melanocyte dysregulation. Thus, a new avenue has been generated to explore the beneficial effects of low dose pharmacology in vitiligo and other chronic dermatoses [110].

Low Dose Cytokines for Vitiligo

There is evidence regarding the use of 4 orally administered low dose medicines (available as oral drops in 30 ml containers) in vitiligo acting.

Conclusions

To conclude, LDM is an exciting treatment option for several chronic skin disorders like psoriasis vulgaris, atopic dermatitis and vitiligo, not responding to conventional therapies. LDM acts by correcting the immunological dysfunction (imbalance between Th1/Th17 and Th2/T-reg axis), thereby inhibiting Low Grade Chronic Inflammation, a key causative factor for many dermatological disorders. Effectiveness and safety are the most important features of LDM, which involves oral administration of Low Dose SKA signaling molecules. However, further large scale trials are needed to validate these results and explore newer dermatological conditions to increase the use of LDM.

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