Innovative Natural Active Ingredient with Anti-Inflammatory Properties

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Homeostasis belongs to the main principles of biological process as it deals with maintaining our organism functions and constants (temperature, hydrous balance). In the skin, homeostasis occurs in the epidermis and corresponds to the keratinocytes’ proliferation and differentiation equilibrium: the continuous renewal of the basal keratinocytes layers is offset by the loss of corneocytes in the stratum corneum and the intermediate differentiation effects on cohesion of cells in epidermis. As a result, homeostasis helps to preserve skin barrier function, either for external aggressions such as penetration of antigens or for protection of the inner equilibrium by regulating the transepidermal water loss (TEWL).

Many molecules (growth factors, hormones and trace elements such as zinc or copper) are involved in regulating homeostasis. Disorders can happen biologically (aging, physiological dysfunction such as psoriasis) or can be induced by contact with irritant products. These conditions lead to a modification of the multiplication speed of the basal keratinocytes and can influence the differentiation process to corneocytes and affect the stratum corneum thickness. These effects come from the inflammation induction and the associate immunological disorders. This state is very difficult to treat, because it is an auto-induced phenomenon that follows an amplifying cycle. For cosmetic applications, inflammation is a very important concern because it can be initialized by many causes, including can provoke its initialization (aging, UV or pollution).

A new extract of Centella asiatica shows very interesting anti-inflammatory properties: keratinocytes physiological growth is maintained, but their abnormal development in an inflammatory state is regulated. Tests were performed to evaluate the efficiency of this active ingredient in the reduction of inflammation, the normalization of epidermal homeostasis and the restructuring of the extra-cellular matrix.

Centella asiatica

Centella asiatica is a small herbaceous plant of the Umbelliferae family, which grows wild in tropical moist and shady regions, at an ideal altitude of 800 to 1,200 meters. The plant has long been used in American, Asian, African and Madagascan traditional medicine, mainly in the treatment of skin and mucosal diseases.

Therapeutic properties are mainly due to the presence of molecules that belong to the triterpene series, which participate in the natural defense of the plant. The parasitic attacks allow the plant to develop its natural defenses and the biosynthesis of saponins.

In its natural state, the plant converts saponins in triterpenic acids in order to acidify its cytoplasmic pH and exert the antimicrobial properties (principally gram-positive) of free aglycones to protect itself against yeasts and molds, among other aggressors. In order to ensure that the cell tolerates these substances, sufficient concentrations of substances providing anti-inflammatory cellular protection are required. These substances are heterosides, which include madecassoside.

To date, three of the four known triterpenes (asiaticoside, asiatic acid and madecassic acid) have been widely used in pharmacy and cosmetics, especially for their capacity to stimulate collagen synthesis and their dermis and venous wall-restructuring properties.

The study presented here concerns our experiments with the fourth known triterpene – madecassoside – which is, in reality, composed of two isomer molecules: madecassoside and terminoloside (Figure 1).
Inflammation State

The skin faces daily urban attacks (from solar radiation, pollution, artificial light, air conditioning and other harmful effects) that, in the long term, progressively induce an insidious irritative condition that is marked by a series of visible imbalances within the skin:

- A generally weak, but constant activation of a pro-inflammatory condition marked by an almost permanent production of pro-inflammatory ligands (such as cytokines and prostaglandins) by the keratinocytes in response to these repeated attacks.
- An acceleration of the keratinocytes’ renewal process, whose functionality is affected, thus seriously depleting their natural youth reserves.
- Keratinization deficiencies resulting from cellular hyper-proliferation result in an acceleration of the differentiation of keratinocytes into corneocytes. The latter have less time to organize properly, leading to irregularities in the horny layer, which in turn increase the risk of penetration of external aggressors into the skin.

In general, this activation of the epidermis results in a generalized disorder of the auto-regulation capacities. The skin is then incapable of keeping intact the physiological tissue barrier (homeostasis), or maintaining its natural defense capacities. The skin, thus weakened, loses the capacity to protect itself against internal disorders and external pathogen attacks.

Inflammatory Process

Daily aggression by environmental conditions helps the penetration of antigens into the dermis, which is the origin of the inflammation cycle (Figure 2). After having captured antigens in the epidermis, the dendritic cells (Langherans cells in particular) migrate into the dermis and activate the T lymphocytes and defensive chain reactions in the skin. IFN gamma (released by activated T lymphocytes) stimulates the pro-inflammatory neuromediators by keratinocytes. This leads to a hyperproliferation of T lymphocytes and to the invasion of the tissues by polynuclears. These events provoke the increase of the interferon gamma (IFN gamma) and protease levels. The high level of cytokines produced by keratinocytes causes the synthesis of mediators (autocrine effect): interleukins 1 and 8 (IL1, IL8), prostaglandine 2 (PGE2) and Skin AntiLeicoProteases (SKALP).

These molecules are at the origin of the induction but they also participate in maintaining the inflammation cycle. As the multiplication of T lymphocytes increases (IL1 participate in the activation) and the inflammation-involved cells (T lymphocytes, polynuclear cells) migrate by chemotactism into the epidermis (PGE2, IL8), and the concentration of ligands produced by keratinocytes rises continually. These conditions lead to an inflamed, thick and dull skin.

Prevention and Correction of Immune Disorders

**Material and methods:** All the tests were performed on normal human cultured keratinocytes. The assay is based on...
the evaluation of soluble pro-inflammatory cytokines production and secretion by keratinocytes in response to a stress induced by a non-sensitizing contact irritant (Phorbol-12-myristate 13-acetate (PMA)) or by the stimulation of a neuromediator (IFN gamma) biologically released by T lymphocytes in the irritation cycle.

IL1 alpha production and PGE2 release by keratinocytes after stimulation by PMA are evaluated on control and treated cultures. IL8 production was evaluated after the addition of IFN gamma to the medium on control and treated cultures.

Results: Madecassoside prevents and corrects immune disorders and irritative attacks by attenuating the release of the main pro-inflammatory epidermal ligands (such as cytokines, chemokines, prostaglandins, interferon gamma). This mixture of isomers modulates, in a dose-dependent manner, the expression and release of interleukins (including IL1 alpha and IL8) by human culture keratinocytes, as well as the expression of arachidonic acid derivatives (such as PGE2) during irritative stress induced either by phorbol ester or IFN gamma (Figure 3).

By continuously modulating the strong inflammatory response at various levels of the immune disorder previously described, madecassoside succeeds in normalizing renewal of keratinocytes.

Protection of the Cells from Inflammatory Stress

Method: The assay seeks to compare the SKALP expression by keratinocytes in a normal medium or in a hyperproliferative medium. The normal medium is a keratinocytes growth medium (KGM); the hyperproliferative medium is obtained by addition of 5% of fetal calf serum (FCS) to KGM. The SKALP production is evaluated after treatment by madecassoside at different concentrations.

Results: The impact of a strong proteolytic environment constituted by FCS, whose high concentration normally leads to a defensive secretion of SKALP by the skin, is decreased when madecassoside is added to the medium. Madecassoside protects the cells from inflammatory stress by reducing the keratinocytes’ response to stress and also by decreasing the protease sensitivity of keratinocytes.

The protective and/or desensitizing activity by madecassoside on keratinocytes is proven by a 40% decrease in the hyperproliferative medium (Figure 4).

Preservation of Renewal Potential of Keratinocytes

Method: The aim is to make sure that the action of madecassoside doesn’t play a role on the cells’ normal metabolism. A test is done by stimulating keratinocytes either by KGF or by human leukocytic elastase (HLE) with or without different concentrations of madecassoside. KGF represents a normal proliferation medium whereas HLE corresponds to a pro-inflammatory medium, which induces hyperproliferation. The cellular density is then measured after 2 and 5 days.

Results: The keratinocytes’ renewal potential is totally preserved, as shown by the normal growth of keratinocytes under the effect of KGF in the presence of madecassoside. On the other hand, the abnormal growth caused by EGF is regulated by this active ingredient. The proteins and ligands synthesis capacity of the cells remains intact. The action of madecassoside does not have any effect on the normal metabolism (growth and multiplication) and immune function of the cell itself (biosynthesis and release of ligands) (Figure 5).

Other tests show that madecassoside preserves all the nucleus functionalities.
For example, there is no inhibition of the NFkappaB – involved in nuclear transcription – and no significant decrease of TNF alpha (tumor necrosis factor, which acts in cellular vigilance against anomalies).

**Restructuring of the Extra-Cellular Matrix**

**Method:** This test was performed on normal human cultured fibroblasts. The fibroblasts were grown in a medium supplemented with trypsin and EDTA solutions. The collagen synthesis – especially for collagen types I and III – was measured by the ELISA system.

**Results:** Madecassoside assists in maintaining the structure and quality of the extra-cellular matrix to a very high degree, and contributes to the improvement of the link between the epidermis and dermis by assuring an increased expression of type I and type III collagens (Figure 6).

**Pre-clinical study**

Efficiency of madecassoside was confirmed in vivo in a pre-clinical study on psoriasis, a severe chronic inflammatory disease. For six weeks, patients used daily an oil-in-water emulsion containing 3% madecassoside. The results show a great improvement of the skin appearance (Figures 7 and 8), validating the activity of this active ingredient on the inflammation state and supporting its interest for cosmetic applications.

**Formulation**

As previously mentioned, inflammation and its consequences can appear in many skin problems and can be treated in a preventive or a curative way by madecassoside. Environmental conditions, especially pollution, can rupture immunological equilibrium because these conditions bring antigens that activate T lymphocytes and the inflammation process.

In aging skin or for physiological evolutions, immunological disorders are biological and the break of the self-induced...
inflammation cycle can avoid homeostasis deregulation and hypersensitivity; moreover the activity on collagen reduces wrinkles and line formation.

Another environmental condition is UV radiation. It causes proteinic degradation and chromosomic alteration in cells, generating inflammation that must be balanced to reduce photo-induced aging. Formula 1 shows an anti-aging composition containing madecassoside.

Conclusion
The patented mixture of madecassoside and termino-loside is positioned as a true ally of the skin, which is under environmental attack and, as a result, is becoming prematurely aged or simply weakened and incapable of self-defense. It allows regulation at three levels:

• Modulation of skin’s chronic inflammatory condition by controlling and regulating the overproduction of inflammation mediators in keratinocytes linked to immuno-stimulations
• Normalization of keratinocytes hyper-proliferation by re-balancing their natural renewal with the attenuation of the auto-amplification loop of the inflammatory process. Thus the natural epidermal homeostasis, characterized by a better organization of the horny layer, is re-established, restructuring the natural protection of the skin.
• Restructuring of the extra-cellular matrix of the dermis through activating collagen expression by fibroblasts, in order to preserve the functional and physical qualities of the skin, during chronological aging (anti-aging).

The immuno-regulating activity is demonstrated here. Madecassoside doesn’t play a role on the cells’ essential physiological functions and allows its the use of madecassoside in cosmetic products. The efficiency has also been confirmed by a clinical study carried out on an auto-immunological disease. The improvement of the state of psoriatic patients offers promising perspectives for the treatment of this illness. Other studies will deal with extending the clinical study and with improving our knowledge of the activity mechanism.

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References
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