Efficacy of an Antireactive Red Marine Algae Extract to Protect against the Exposome

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abstract

The research objective: Reactive skin induces cutaneous manifestations such as stinging, redness, dryness, and burning sensation in connection with the exposome. Its pathogenesis is mainly related to the dysfunction of neurosensory and also immune activity.

Experimental methods: We evaluated the effect of polysaccharides extracted from *Halymenia durvillei* (Hd) on reactive skin, using an *ex vivo* model on inflammation and neurosensory discomfort. An *in vivo* study was also conducted on 25 selected volunteers with reactive skin. Clinical grading of facial skin including dryness, roughness, and erythema was assessed (SS-scale). Subject self-assessment questionnaires, photography, erythrosis were also included.

Main observations: *Hd extract* was able to reduce the expression of TRPV-1 and NK1-R as well as to decrease TNF-α, and VEGF suggesting that this active extract may provide protection for sensitive and reactive skin from daily environmental agressions, named exposome. The *in vivo* study shows that *Hd extract* calms feelings of discomfort and redness, and controls the microvascularization. Volunteer's questionnaire revealed self-perceived benefits consistent with expert visual grading.

Conclusion: we show the efficacy of a new antireactive red marine algae rich in polysaccharides extract to protect the skin against the exposome. It will be interesting to evaluate whether this *Hd extract* can modulate skin microbiota, the relationship between skin microbiota and reactive skin having been recently reported.

Introduction

The skin is constantly exposed to various endogenous, exogenous and life style factors that may affect the skin barrier function at the physical, mechanical and microbial levels. The resulting impact can potentially lead to inflammatory skin conditions involving sensitive or irritated skin as well as chronic inflammatory skin diseases, allergies or autoimmune diseases. These endogenous and exogenous factors are included in the exposome concept, which was proposed by Wild [1] to encompass "the totality of human environmental exposures from conception onwards, complementing the genome". More recently, Miller and Jones [2] proposed an alternative definition of the exposome, which explicitly incorporates behavioural risk factors, the body's response to environmental influences, and the endogenous metabolic processes that can alter or process the chemicals to which humans are exposed. Increasing attention is being paid to the exposome of human skin. Mirroring the cutaneous responses to environmental stress [3], the major environmental factors that contribute to skin alterations have been recently regrouped in the so- called "skin aging exposome" which includes (i) sun radiations, i.e. ultraviolet, visible light and infrared wavelengths, (ii) air pollution, (iii) tobacco smoke, (iv) nutrition, and (v) other factors such as temperature, stress and lack of sleep that can alter skin condition.

It has become obvious that the exposome needs to be considered in relation to sensitive skin. The etiology of sensitive skin is multifactorial, involving an underlying genetic susceptibility combined with exogenous and endogenous factors that can trigger or aggravate the clinical expression of the condition [4]. Potential mechanisms of sensitive skin involve skin neurosensory dysfunction, neurogenic inflammation, epidermal barrier disruption, immune cells activity (transient receptor potential channels), and hyperreaction of the blood vessels of the skin [5]. Sensitive skin is also defined by the self-reported presence of different sensory perceptions, including tightness, stinging, burning, tingling, pain and pruritus in response to stimuli that normally should not provoke such sensations [6].

Sensitive and very sensitive skins show high incidence in France [7]. In response to this prevalence, the effects of exposome and the effective treatment of sensitive skin represent excellent targets for active ingredients in cosmetics. Various naturally derived complex mixtures such as botanical extracts have been used for a long time. The application of algae in cosmetic products has recently received more attention in the treatment of skin problems. Algae are rich sources of structurally novel and biologically active metabolites, with great industrial potential and accessibility. A wide range of metabolites such as alginates, polysaccharides, ca-

rotenoids with biological activities like antioxidants has been investigated for cosmeceutical preparations. Algae are thus a source of raw materials for one of the most promising and profitable sectors of the biotechnology industry [8]. Among them, *Halymenia durvillei* (Hd) is a red algae belonging to the Rhodophyceae family, abundant in a vast area of the Indian Ocean. Red algae are often small and can live in different depth scales including great depth for organisms containing chlorophyll. *Halymenia durvillei* contains phycocolloids, which are the constituent polysaccharides of cell membranes. Today, the use of this algae for its phycocolloids is one of the most important industrial opportunities and a source of innovation and valorisation. The current interest in these polysaccharides is due to their known bioactivities, conferred by their antiallergic, neuroprotective, gastroprotective, cardioprotective, cytotoxic, anticoagulant/antithrombotic, antiviral, antilipidemic, antinociceptive, and immunomodulatory properties, making them promising bioactive products and biomaterials.

Based on this data, the aim of this study was to evaluate the effect of *Hd extract* on reactive skin, a skin which is aggressed by the exposome. We therefore investigated the effects of Hd on inflammation (TNF-α) and neurosensory discomfort (TRPV-1, NKR-1) using an *ex vivo* model. An *in vivo* study was also performed in a panel of 25 volunteers using clinical and instrumental evaluations.

Materials and Methods

Ex vivo Study

Twenty-one skin explants with a diameter of about 11 mm (± 1 mm) were prepared from an arm plasty of a woman of the Negroid type aged of 31 years (reference P1822-BN31). The explants were kept alive in BEM (BIO-EC's Explants Medium) at 37°C in a humid atmosphere, enriched with 5% CO_2. The explants were divided into 4 batches as follows: Product, Placebo, Witness plasty, and Witness (T0). At Day 0, Day 2 and Day 5, the products were applied to the surface of the explant at the rate^{-2} of 2 µl.cm^{-2} and spread with a spatula. The control group received no treatment, except for the renewal of the medium (2 mL) on Day 0 and Day 2. At D0, the explants of batch T0 were removed and cut in half. One half was fixed in buffered formalin and one half was frozen at -80°C. On Day 2 and Day 6, 3 explants of each lot were taken and treated in the same way as on Day 0.Culture media from all lots on D2 (2mL) and D6 (2mL) were removed and frozen at -80°C for assays.

We first evaluated the effect of *HD extract* (5%) on TNF-α secretion. The assay was performed with the human Elisa TNF-α kit (ref 589201, Cayman). The culture medium and the TNF-α standard were incubated with acetylcholinesterase (AChE): TNF-α-binding Fab'conjugate in the wells containing the im-

mobilized TNF-α anti-body, overnight at 4°C. According to the manufacturer's instructions, after the washings of the plate, the reaction was revealed for 80 min by a solution containing the AChE substrate. Absorbance at 412 nm was measured with the Tecan M200Pro microplate reader and Magellan7 software.
Then, we evaluated the effect of *Hd extract* on TRPV-1. During the protocol explained above, histological treatments were realized. After 24h in the buffered formalin, the samples were dehydrated and impregnated in paraffin using a Leica PEARL dehydration automaton. They were packaged using a Leica EG 1160 coating station. Sections of 5 µm were made using a Minot microtome, Leica RM2125 and mounted on Superfrost® histological glass slides. Microscopic observations were made by optical microscopy, using a Leica microscope type DMLB or Olympus BX43. The shots were taken with an Olympus DP72 camera and the Cell D software. TRPV-1 is highlighted thanks to a specific anti-body.
Thirdly, NK1-R was scored on paraffin sections with anti-NK1-R polyclonal anti-body (Santa-Cruz, ref sc-365091), diluted 1/50 in PBS-BSA 0.3% – Tween20 0.05% for 1h at room temperature with a Universal VECTASTain RTU VECTOR streptavidin/peroxidase system and revealed in VIP, a purple peroxidase substrate (Vector, SK-4600). Immunostaining was performed using an immunostaining automaton (Dako, AutostainerPlus) and evaluated by microscopic observation and image analysis.
Finally, VEGF was scored on paraffin sections with anti-VEGF polyclonal anti-body (Santa-Cruz, ref sc-7269). Immunostaining was performed using an immunostaining automaton (Dako, AutostainerPlus) and evaluated by microscopic observation and image analysis.

In vivo Study

Twenty-five female volunteers aged 41 ± 10 years old with skin phototypes I-IV and dry and sensitive skin participated in this study after having given their written informed consent. The study was performed by comparing before and after hemi-face application of *Hd extract* 3% vs placebo. All of them presented a reactive skin as defined from the stinging test (total score ≥ 3) and also presented erythrosis as defined from clinical scoring. Exclusion criteria were the presence of any skin-related pathologies and abnormalities (eczema, psoriasis, etc.), allergies, hypersensitivity to the tested product, acute and/or chronic inflammation or infection of facial skin, sunlight or artificial UV rays exposure within 15 days, pregnancy and nursing. Subjects were advised to avoid the application of any other similar product during the whole study.
Evaluations were performed at baseline (D0) and 28 days (D28) after twice-daily application. Subjects were evaluated in the standard skin situation (last face washing the night before the visit, without any cosmetic, water, and makeup application until the measures). Clinical scoring of erythrosis (Measurement and analysis of blood micro-circulation by videocapillaroscopy: Moritex MS500 with a x50 magnification: exploration area = 13.6 mm^2), assessment of functional signs using the Sensitive Scale [9] and soothing effect were evaluated. We also took pictures, using a VISIA from CANFIELD® imaging system. Women were also asked to report their overall opinion about the product, with 3 sentences maximum for remarks after the 28 days of application.

Statistical Analysis

Descriptive statistics are provided for each parameter. The significance threshold was fixed at 5% in two-sided test, and the tendency between 5 and 10%. Statistical comparison was carried out with the Student-t Test or Wilcoxon test (depending on the normality of the distribution). The analyses were carried out with Statistica Version 12 and Graphpad Instat Version 3.06.

Results

Ex vivo Study

We noted that *Hd extract* induces a non significant decrease of TNF-α (-21%) compared to control after 6 days of treatment (**Fig. 1**). At the same time, the expression of TRPV-1 in the epidermis significantly decreases. A significant decrease of NK1-R (-43%, p< 0.01: **Fig. 2**) and a decrease of VEGF were also reported when compared to the control (data not shown).

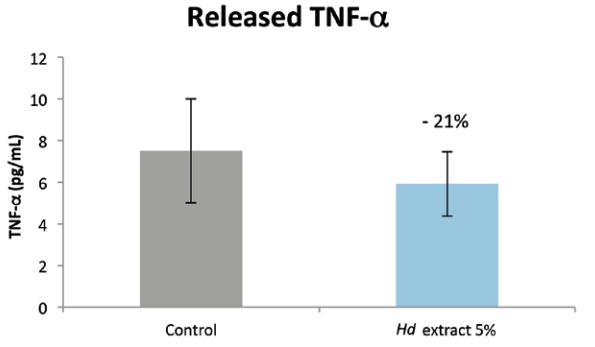

Fig. 1 TNF-α secretion after 6 days of *Hd extract* treatment (expressed in $pg.ml^{-1}$).

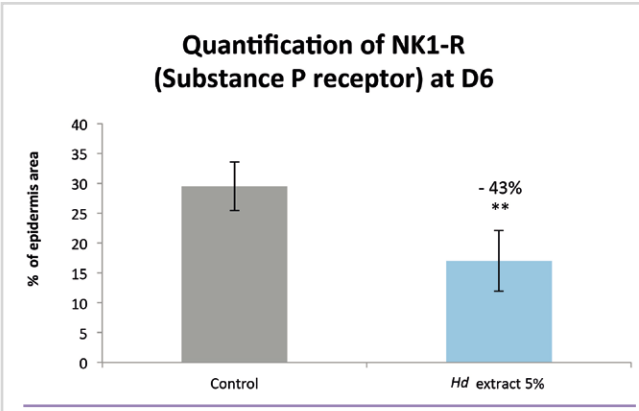

Fig. 2 Percentage of area occupied by the substance P receptor in the epidermis at Day 6. ** p< 0.01 vs control.

	D28 vs D0
Skin sensitivity	-19%*
Skin irritability	-34%*
Heating sensations	-40%*
Pain	-33%*
Flushes	-37%*
Redness	-24%***

Tab. 1 Evolution of the skin irritability, sensitivity, heating, pain, flushes, redness, and hydration state from D0 to D28 using the SS-10 scale [10]. * p<0.05, *** p<0.001 vs D0.

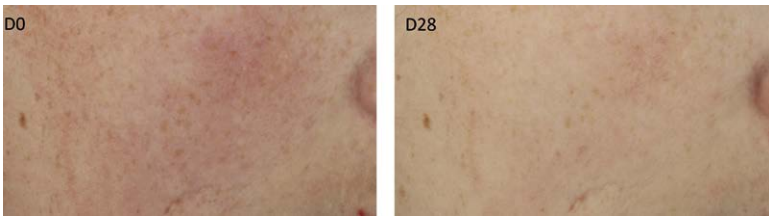

Fig. 4 Reduction of redness induced by 28 days of *Hd extract* application.

In vivo Study

Significant decreases of skin sensitivity, irritability, heat sensations, pain, flushes, and redness were reported using the SS-10 scale [9] after 28 days of repeated applications of the *HD extract* (**Tab. 1**). A significant decrease of the surface of erythrosis was also observed with our extract (**Fig.3** and **4**). Finally, 90% of the women declared that the product had a soothing effect when applied after the stinging test (**Fig. 5**).

Discussion

There is a growing body of litterature attempting to gain an understanding of the exposome of human skin and its contribution to the skin aging process and sensitive skin [10], sensitive skin being defined as a sensory reaction triggered by frictions and/or environmental factors, usually without a visible clinical manifestation. Subjects with reactive skin induced by the exposome present discomfort mainly based on the presence of subjective symptoms such as itching, stinging, burning or pain, which has a high impact on the quality of life. The treatment of reactive skin is challenging and generally based on continuous and topical application of antisensitive moisturizing extreme tolerance products that improve skin features associated with itching, stinging, dryness, tightness, burning, or pain [10]. To maintain a healthy skin, it is recommended to hydrate and protect it. In this study, we investigated whether *Halymenia durvillei* extract, rich in polysaccharides and particularely in Galactose, could calm feelings of discomfort, redness.

Neurosensory dysfunction in the skin might represent one of the pathomechanisms of sensitive skin. Another mechanism through which the cutaneous nervous system could contribute to sensitive skin might be by functional hyperreactivity of cutaneous nerves. Cutaneous nerve fibres, such as unmyelinated C fibres mediating pain, itch and warmth, are equipped with sensory neuroreceptors such as endothelin and transient receptor potential (TRP) channels. Interestingly, the activation of TRP family members results in a combination of afferent functions with effector roles. Among them, TRPV-1, initially named capsaicin, or vanilloid 1 receptor, was identified on nociceptive sensory nerve endings and is known to mediate sensations of pain, itch, warmth and afferent functions to chemical stimuli. Additionally, TRPV-1 controls the release of neuropeptides in local neurogenic inflammation. [11]. TRPV-1 results in the local cutaneous release of neuropeptides such as substance P (SP), which subsequently activates different types of cells in the skin, e.g. keratinocytes, mast cells, antigen-presenting cells and T cells located in close vicinity to the sensory nerve endings [12]. It is considered as a major mediator of neurogenic inflammation and itch [13], through activation of tissular Neurokinin 1 receptors (NK1-R), inducing the release of pro-inflammatory cytokines and chemokines, resulting in

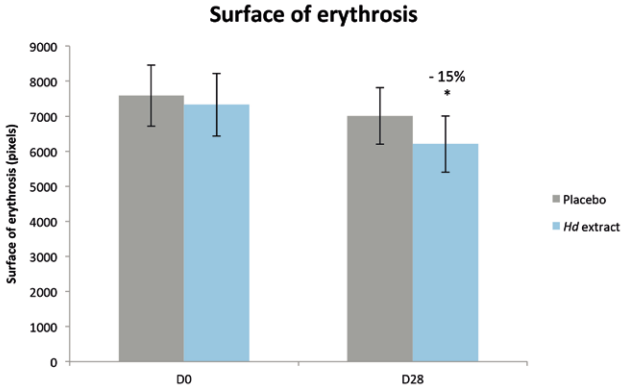

Fig. 3 Effect of *Hd extract* on erythrosis.

Fig. 5 Words cloud chart reported by women on Day 28 regarding their comments concerning the algae extract.

the recruitment of further immune cell subsets to the skin. Our results are in line with the pathophysiology of sensitive skin, *HD extract* inducing a decrease of TRPV-1 and NK1-R expression, as well as reduction VEGF and TNF-α.

The Sensitive Scale (SS-10) [10], which enables to measure the severity of skin sensitivity, was also used in a clinical study including twenty-five females, who declared having a sensitive, irritable and reactive skin on the face area. Significant decreases of skin sensitivity, irritability, heat sensations, pain, flushes, and redness were reported after 28 days of repeated applications of *Hd extract* (**Tab. 1**). Erythrosis takes the form of diffuse, persistent red patches on the face. It appears as the result of exposome, such as shaving or temperature fluctuations. It occurs mainly around the nose, chin, forehead and cheeks, and is associated with a sensitive skin. We also note that *Hd extract* decreases erythrosis after 28 days of application (**Fig. 3**).

In a previous article [14], we showed that a Schisandra chinensis extract developped by Greentech R&D protected cells from oxidative stress induced by environmental pollutants. In fact, this extract was able to stimulate the expression of Nrf2 and DJ-1, as well as the decrease of NF-kB and AhR, suggesting that this extract may provide wide protection for skin against daily environmental factors. As the exposome is much wider than urban pollution and considering that there is currently no model that mimics all the factors of the exposome, our new *Hd extract* has been tested on reactive skin (induced by the exposome) and we showed a good efficiency of this extract, as presented in the results part.

Conclusion

In summary, using an *ex vivo* model, we showed that *Hd extract* was able to reduce the expression of TRPV-1 and NK1-R as well as to decrease the TNF-α, and VEGF suggesting that this active extract may provide protection for sensitive and reactive skin by daily environmental insults, named exposome. The *in vivo* study shows that this algae extract calms feelings of discomfort and redness, and controls the microvascularization. Volunteers' questionnaire revealed large self-perceived benefits consistent with expert visual grading. It will now be interesting to evaluate whether this *Hd extract* can modulate the skin microbiota, the relationship between the skin microbiota, reactive skin and the skin barrier function, having been recently reported [15].

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