Study data confirm global antipollution activity

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Abstract

Latest scientific evidence confirms, that air pollution contributes to premature skin ageing and may even be the primary cause of ageing in some cases. Especially for particulate matter and polycyclic aromatic hydrocarbons (PAHs) negative influence on skin integrity has been shown. The natural stress-protection molecule Ectoin® is well known to strengthen the skin barrier function, to work against variable stressors such as UVA/UVB radiation, IR-A, visible light or allergens, and for its anti-inflammatory activity. This study demonstrates the global protection and prevention efficacy of Ectoin from air pollution induced skin damage. Ectoin® is well suited for topical antipollution products targeting skin pigmentation and accelerated ageing as well as particle stressed and irritated skin.

Skin ageing is a complex process and underlies the involvement of multiple factors. Even though some of the fundamental mechanisms of aged skin are still not yet fully understood one indisputable fact stands out: cutaneous ageing is mainly driven by external influences and only a small percentage is attributed to intrinsic factors like genetic predisposition. Protecting the skin from external stress-factors and the prevention of cell damage thus are logical and effective strategies to retard premature skin ageing.

In recent years, stress-protection molecules, so-called extremolytes became more and more popular as cosmetic active ingredients. Stress-protection molecules are found inside of extremophilic microorganisms and plants, which prosper in the hostile conditions of deserts, hot springs, arctic ice, the deep sea or salt lakes. They protect the extremophiles from damage caused by total desiccation, extreme UV-radiation, osmotic stress or damage caused by total desiccation, extreme UV-radiation, osmotic stress or thermal fluctuation. One of the best-known stress-protection molecules is Ectoin® (INCI: Ectoin). The amino acid derivate was discovered in 1985 as the self-defence substance of extremophiles found in a salt puddle in Wadi El Natrun (Egyptian desert). Today, natural Ectoin (now referred to by its chemical term ‘ectoine’) as well as the synthetic molecule are available as cosmetic active ingredients. The following findings refer to natural ectoine.

Ectoine, is a safe and well-studied multifunctional active ingredient with global skin protection and repairing properties. It is formulated in numerous skin care as well as sun protection products. Furthermore, ectoine is the main ingredient in OTC medical devices for the treatment of allergies, atopic dermatitis, dry eyes, rhinitis or the treatment for pollution induced COPD and asthma. It is approved for very sensitive skin including children and infants.

Ectoine protects and stabilises proteins, enzymes, nucleic acids as well as cell membranes, when applied to human tissues. It restores and stabilises the skin barrier and consequently increases the degree of skin hydration, which can be preserved for 7 days without further treatment. Various in vitro, in vivo studies and clinical trials confirm ectoine activity, with regard to protection of Langerhans cells, protection from heat/IR-A, UVA/UVB4, and visible light,7 inflammation reduction1 and treatment of atopic dermatitis.9 Furthermore, several clinical studies were conducted with the natural stress-protection molecule for the development of an inhalation solution and other medical devices. The inhalation of an ectoine solution by patients suffering from pollution induced COPD resulted in the reduction of lung inflammation.10 Moreover ectoine has been described to act preventive against neutrophilic inflammation induced by environmental nanoparticles and showed a reduction on PM induced exacerbation of allergen sensation.11 Based on these study results, the use of natural ectoine as an anti-pollution active ingredient in cosmetic formulations appears to be a meaningful approach.

Besides UV-exposure, ambient pollution can be seen as one of the main contributors to premature skin ageing. In the past years, several epidemiological and mechanistic studies concluded a connection between pollutants and accelerating wrinkles as well as pigmentation. Air pollutants may not only induce skin ageing but are also linked to causing or worsening skin conditions such as acne, atopic dermatitis, eczema and allergic reactions, telangiectasia or even skin cancer.15 The first study, showing that particulate matter (PM) may have negative impact on human skin was conducted by Vierkötter et al. in 2010. In this epidemiological, cross-sectional study with 400 women participating in the evaluation of skin ageing, the association between airborne particle exposure and extrinsic skin ageing, particularly pigment spot formation was shown for the first time.13 The results revealed the strongest association between exposure to soot and the formation of pigment spots in the cheek area of the test persons.

Depending on the size and depth of penetration of pollution particles, the effects on the skin are different. Skin exposure to PAHs can cause oxidative stress and thus induce melanocyte proliferation and inflammatory diseases. PAH and PM are known to bind with specificity to the aryl hydrocarbon receptor (AhR), modifying the expression of Cyp1A1 (cytochrome P450, family 1, subfamily A, polypeptide-1) and the release of POMC (pro-opiomelanocortin), MMP1 (matrilysin/ matrixmetallopeptidase-1) and IL-6 (interleukin-6) resulting in inflammation, formation of pigment spots, collagen breakdown and wrinkles.17,18 The clinical relevance of these scientific findings, showing the damaging impact of air pollution on human skin, touches upon...
Aspects of both, prevention and therapy. Anti-ageing concepts, which do not include pollution protection may need to be reconsidered.

The following study will present data, which show that ectoine protects cells against the damaging stress of air pollution and thus prevents pollutant-induced skin ageing like wrinkle and age spot formation.

Methods and results: in vitro study

For this in vitro study fresh human epidermal keratinocytes from female Asian and female Caucasian donors were used. Cells were untreated and pre-treated (24h) with 2 mM ectoine solution. Afterwards, cells were stressed with fine and ultrafine carbon black particles and different surrogates for authentic street particulate matter such as SRM 1650 and SRM 2975 (Table 1).

After the particle stress, the expression of POMC, MMP1 and Cyp1A1 mRNA was measured in keratinocytes by using real time PCR. POMC, MMP1 and Cyp1A1 are marker genes which can be activated by pollution particles. The results in Figure 1 show that fine and ultrafine carbon black particles and diesel particulate matter induced POMC, MMP1 and Cyp1A1 mRNA expression. POMC is known for melanogenesis stimulation in human melanocytes and to cause dark spot formations. It can therefore be used as a marker gene for pigmentation. MMPs play a role in collagens and elastase breakdown in the extracellular matrix of the dermis and can be used as marker for wrinkle formation. Cyp1A1 mRNA expression induces oxidative stress in human skin which results in inflammation or cancer.

Ectoine-protected keratinocytes significantly down regulated PM induced overexpression of marker genes. POMC mRNA expression is down regulated in all tested cases by 100% or close to 100%. In addition, ectoine also protected from up-regulation of MMP1 and Cyp1A1.

In vivo study

To test in vivo the anti-pollution activity and efficacy of a cream containing ectoine, a specialised dermatological center in Germany was chosen. The study design (placebo controlled, randomised, double blind) is the most innovative, standardised in vivo pollution test method currently available. Six volunteers applied the cream with placebo or 1% ectoine on volar forearm for 5 days twice daily. Furthermore, areas on the volar forearm were tested untreated and unstressed (negative control) as well as untreated and stressed with cigarette smoke (positive control). On day 5, skin was stressed with cigarette smoke as pollutant for 15 minutes to induce oxidative stress to the skin in a standardised pollution chamber system (Fig 2).

The protective activity of the test products was evaluated by analysis of barrier lipid oxidation levels (measured by malondialdehyde, MDA) of ex vivo samples from the skin surface. MDA results from lipid peroxidation of polyunsaturated fatty acids and is one of the reactive electrophile species that cause toxic stress in skin cells and can therefore be used as a marker for air pollution induced damage.

Five days of cream application with 1% ectoine showed a positive effect. The pollution-induced MDA overexpression was 48% lower compared to placebo and 47% lower compared to untreated but stressed control. A clear trend towards efficacy in

Table 1: Different types and sizes of particulate matter were used.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Size</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Printex 90</td>
<td>Ultrafine particle</td>
<td>0.014 µm diameter</td>
<td>Both particles are much smaller than PM$<em>{2.5}$ and PM,$</em>{10}$</td>
</tr>
<tr>
<td>Huber 990</td>
<td>Fine particle</td>
<td>0.26 µm diameter</td>
<td></td>
</tr>
<tr>
<td>SRM 1650</td>
<td>Diesel engine soot</td>
<td></td>
<td>Both materials contain PAHs, heavy metals as well as PM$<em>{2.5}$, PM$</em>{10}$, PM,</td>
</tr>
<tr>
<td>SRM 2975</td>
<td>Diesel soot from a forklift engine</td>
<td></td>
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protection against pollution induced skin damage was observable.

Summary and discussion
Ectoin strengthens the skin barrier function and is capable to shield the skin from the whole spectrum of air pollution and allergens. This includes clouding metals and PAHs as well as all particle sizes (PM₁₀, PM₂.₅, PM₁, and smaller) for a global, complete and instant protection and prevention of pollution induced skin damage and ageing.

Ectoin forms a so-called ‘Ectoin Hydro Complex’ around skin cells, thus protects the skin at cellular level. Particle induced damage will be prevented and repaired. Air pollution induced expression of POMC, MMP1 and Cyp1A1 in skin cells (Asian and Caucasian) are significantly reduced by Ectoin treatment. The protective activity of Ectoin-containing cream was evaluated in vivo by analysis of barrier lipid oxidation levels from the skin surface. In conclusion, Ectoin is well-suited for topical cosmetic products for protection against pollution induced skin pigmentation, irritation and ageing.

References