ALDENINE® PBC
CODE: P10-PD050

Date: November 2007 Revision:0

GENERAL DESCRIPTION

Human skin is directly exposed to environmental aggression, mainly in the form of chemicals, air pollutants and UV irradiation. These factors generate reactive species (free radicals and others) responsible for extensive skin cell damage and aging. Although Reactive Oxygen Species (ROS) have been known and studied for years, Reactive Carbonyl Species (RCS), another important class of intermediates, have only recently been related to skin deterioration.

RCS are small molecular weight carbonyls (Fig.1) that are activated by $\alpha,\beta$-insaturation (such as 4-hydroxynonenal and acrolein), $\alpha$-oxo-substitution (such as glyoxal) and $\beta$-oxo-substitution (such as malondialdehyde).

![Fig.1. Several Reactive Carbonyl Species (RCS)](image-url)
RCS are especially dangerous because they are a by-product of cellular metabolism, including lipid peroxidation, glycation, autooxidation of sugars, etc.

Among the damage caused by RCS we find DNA damage, proteosome degradation as well as cellular and extracellular protein alteration. This last effect has been linked to skin deterioration, and indeed skin collagen, the primary component of the extracellular matrix, is an important target for RCS. Young collagen fibres are tough, elastic, and in bulk appear white, but with age they undergo crosslinking, lose their elasticity and become yellow. Crosslinking is responsible for the hardening of collagen as well as for the deterioration of skin with age, and the molecules responsible for crosslinking are RCS.

RCS, especially dicarbonyl compounds, react with proteins to form a variety of adducts in a reaction known as the Maillard reaction. These adducts are collectively known as Advanced Glycation End-products (AGEs) and are effective indicators of protein damage and are highly increased with age.

If we concentrate on HNE, one of the most abundant and toxic of these aldehydes, we see that HNE-protein adducts are detected in photodamaged and aged skin but not in young healthy skin. Keratinocytes detoxify endogenously-formed HNE by forming adducts with GSH (glutathione), the skin’s natural hydrophilic antioxidant. However, when submitted to UVB, keratinocytes are depleted of GSH, they can no longer detoxify from HNE and they die. So an external agent is needed to quench HNE and other RCS when keratinocytes receive UVB, that is, when the skin is exposed to the sunlight.

Ultraviolet radiation causes significant changes to the cells of the skin, including DNA damage. UVA radiation constitutes more of the 90% of the environmentally relevant solar UV radiation and plays an important role in skin aging. DNA damage contributes greatly to these age-associated skin changes and DNA lesions caused by UVA radiation trigger photoaging of human skin. Therefore, a product which can protect skin cells from UVA-induced DNA damage could prevent photoaging, improving the skin’s appearance.

Lipotec has developed a formula based on recent research from the University of Milan, which is able to detoxify the skin from these noxious RCS and protect it from DNA damage induced by UVA radiation. The formula contains a cluster formed by two peptides: a vegetal protein hydrolysate (INCI: Hydrolysed Wheat Protein, Hydrolysed Soy Protein) and a synthetic tripeptide named GHK (INCI: Tripeptide-1). The combination of the two peptides is ALDENINE®. The tripeptide becomes entrapped within the tertiary structure of the vegetal protein, therefore becomes stabilised. The formation of the cluster can be demonstrated by comparing HPLC retention times for both separate molecules with the retention time of the cluster, which is different.

GHK is able to covalently sequester HNE and other reactive aldehydes, thereby acting as a cellular detoxifier. The tripeptide is more active than carnosine, a dipeptide (β-alanyl-L-histidine) present in high concentration in skeletal muscle, which also acts as a cellular scavenger.
The hydrolysed vegetal protein (HVP) is a stimulator of Collagen III as proved by in vitro tests on fibroblasts. Collagen III is the type of collagen produced by extremely youthful skin. When you are 50 years old, approximately 90% of your collagen is Collagen I, when you were 4 years old, approximately 90% of your collagen was Collagen III. During the aging process, cells gradually lose their ability to produce Collagen III through functional impairment.

The HVP contained in ALDENINE® boosts Collagen III production by almost 300% in 7 days, while it does not affect Collagen I, already plentiful in the skin.

**COSMETIC PROPERTIES AND APPLICATIONS**

- GHK captures noxious RCS, which skin cells cannot detoxify by themselves.

- The plant protein hydrolysate selectively boosts synthesis of Collagen III.

- ALDENINE® PBC is particularly formulated for stabilisation of the GHK tripeptide.

- Since keratinocytes cannot eliminate RCS when submitted to UVB, ALDENINE® can protect cells from photodamage.

- ALDENINE® PBC protects skin cells from UVA-induced DNA damage, thus preventing photoaging.
TECHNICAL DATA

PRODUCT SPECIFICATIONS

Appearance Translucent solution
Colour Amber to Brown
Active ingredient content 0.1% Tripeptide-1
6.25% Hydrolysed Soy Protein (25% Protein Extract)
3.75% Hydrolysed Wheat Protein (15% Protein Extract)
Preservative 1.3% Phenoxyethanol
1.2% Butylene Glycol
0.5% Caprylyl Glycol

PRESENTATION AND DOSAGE

ALDENINE® PBC is presented as a liquid solution that is ready to be incorporated in the final formulation. We recommend adding it to the final product in the last step of manufacture at temperatures no higher than 45 ºC.

The recommended dosage for effectiveness is between 2 and 5%.

STORAGE

ALDENINE® PBC must be stored in a cool, clean and dark place. If kept in these conditions shelf life is at least 18 months.

SAFETY

All the raw materials involved in the preparation of the product have been tested for the evaluation of primary skin irritation potential, sensitisation, ophthalmic irritation, oral and percutaneous toxicity. No signs of irritation or allergic reactions were observed.
Efficacy

Several tests were performed to prove the efficacy of both components, in both the RCS scavenger and Collagen III synthesis claims:

Relative capture of RCS
The graph (Fig. 2.) shows how GHK is more effective than the endogenous scavenger carnosine (CAR) at capturing HNE in vitro.

![Comparative Quenching Activity](image)

**Fig. 2.** HNE-scavenging effect

Synthesis of Collagen III
Human Dermal Fibroblasts (HDF) were seeded at two different densities in 96-well culture plates and treated with Hydrolysed Vegetal Protein at different concentrations for 24 hours and 7 days. Collagen I and III were detected using an ELISA test with monoclonal antibodies. The increase in Collagen III can be seen even after 24 hours but a dose dependent result is obtained after 7 days (Fig. 3)

![Collagen III](image)

**Fig. 3.** Synthesis of Collagen III
**UVB protection**

Keratinocytes were photographed as: non-treated, treated with GHK, treated with HNE and treated with both. The pictures were repeated after irradiating the keratinocytes with a low UVB dose (50 mJ/cm²).

Note: this dose is enough to provoke erythema in people whose skin is phototype I to III (see table below)

<table>
<thead>
<tr>
<th>Phototype</th>
<th>Description</th>
<th>Tolerance (mJ/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPT I</td>
<td>Always burns, never tans</td>
<td>18</td>
</tr>
<tr>
<td>SPT II</td>
<td>Burns easily, tans minimally</td>
<td>30</td>
</tr>
<tr>
<td>SPT III</td>
<td>Burns moderately, tans gradually to light brown</td>
<td>48</td>
</tr>
<tr>
<td>SPT IV</td>
<td>Burns minimally, always tans well to moderately brown</td>
<td>72</td>
</tr>
<tr>
<td>SPT V</td>
<td>Rarely burns, tans profusely to dark</td>
<td>84</td>
</tr>
<tr>
<td>SPT VI</td>
<td>Never burns, deeply pigmented</td>
<td>108</td>
</tr>
</tbody>
</table>

The results presented in Fig. 4 show that irradiating keratinocytes with a low dose of UVB does not modify cellular morphology (top left photograph). GHK does not show a cytotoxic effect (top right photograph). In the bottom left photograph we can see that the cells cannot eliminate HNE since UVB depletes them of GSH, their natural scavenger. The picture shows clearly the deep morphological alterations due to HNE toxicity – non-confluent cells and vast areas of necrosis. The bottom right photograph proves that GHK is able to prevent damage by scavenging HNE and detoxifying the cell.

**Fig. 4. Irradiated keratinocytes**
**Absolute capture of RCS**

The following figures show the quenching activity of GHK versus HNE and ACR as a function of molar ratio, at 1 and 2 hours. It is clear that the results are dose-dependent and activity is continuing after 2 hours.

The potent RCS-scavenging activity of GHK is under patent by Lipotec SA.

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**Fig. 5. Efficacy of GHK as an RCS scavenger**
Glycation inhibitory activity of GHK

It is known that some enzymes can suffer glycation *in vivo*. Among those enzymes we can find the Cu, Zn-Superoxide Dismutase (SOD). The SOD is an enzyme that converts superoxide radicals to hydrogen peroxide and oxygen. The incubation of SOD with glucose or other monosaccharides gives rise to glycation, which inactivates the enzyme. Some compounds can inhibit SOD glycation and, therefore, maintain its activity.

In this study, the inactivation of SOD by its reaction with fructose is used as a model of glycation. The effect of GHK as an inhibitor of glycation is evaluated. A method to assess the SOD activity by the inhibition of the transformation of xanthine to uric acid with the enzyme xanthine oxidase is used. With this reaction, the WST-1 (2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) is transformed to formazan, a compound which absorbs at 470nm. If SOD is added to this reaction, radical $O_2^-$ is captured and the formation of this coloured compound is avoided.

The results show there is a increase in the SOD activity, which means that GHK inhibits its glycation.
Comet Assay

The internal photoprotection capacity of ALDENINE® against UVA radiation was assayed in primary cultures of human melanocytes using the alkaline comet assay.

Melanocytes (10^5 cells/plate) were incubated with three different concentrations of ALDENINE® (1%, 2% and 4%) for two hours at 37°C. After this contact period, cells were irradiated with UVA (0.8J/cm²) for no more than 3 min at 4°C. Finally, UV-induced DNA breaks were analyzed by the alkaline Comet assay.

The single cell gel electrophoresis assay (also known as the Comet assay) is a simple, rapid and sensitive technique for analysing and quantifying DNA damage in individual eukaryotic cells. Cells are embedded in agarose on a microscope slide, lysed with detergent and then electrophoresed at high pH. The DNA of the nuclei is drawn to the anode and the slides are stained with a fluorescent stain and analysed with a fluorescence microscope. The damage is represented by an increase of DNA fragments that have migrated out of the cell nucleus in the form of a characteristic streak similar to the tail of a comet. The DNA fragments are generated by DNA double strand breaks, single strand breaks and/or strand breaks induced by alkali-labile sites.

Negative controls included non-irradiated untreated cells and non-irradiated cells treated with 1%, 2% and 4% ALDENINE®. UVA irradiated cells without ALDENINE® were used as positive controls.

Results show that no increase in DNA damage could be detected in the melanocytes treated with three different concentrations of ALDENINE® when compared to the control cells.

Fig 6. Negative control cells
Pre-treatment with increasing concentrations of ALDENINE® decreased UVA-induced DNA lesions in a non linear manner. ALDENINE® showed to have an internal photoprotection capacity against UVA light with a dose-response relationship.

<table>
<thead>
<tr>
<th>Sample (concentration)</th>
<th>% Protection</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>UVA</td>
<td>0.0</td>
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<tr>
<td>ALDENINE® (1%) + UVA</td>
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<tr>
<td>ALDENINE® (2%) + UVA</td>
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<tr>
<td>ALDENINE® (4%) + UVA</td>
<td>98.5</td>
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GENERAL PRODUCT INFORMATION

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<tr>
<th>Trade name</th>
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<tr>
<td>Product code</td>
<td>P10-PD050</td>
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INGREDIENTS

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<th>CAS No</th>
<th>EINECS No</th>
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<td>CAPRYLYL GLYCOL</td>
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¹ Not Listed

Note: Graphs and photographs are available for customer use provided that the final product contains the same concentration of active as the formulations in our tests. Customers must request written permission for use of the graphic material and/or ingredient tradenames to Lipotec. Customers are responsible for compliance with local and international advertising regulations. Lipotec uses the ™ symbol for EU trademark applications. The symbol is changed to ® when the EU trademark is granted. The specific situation of the trademark in each country may vary and we recommend that you contact us for updated information.