CM-Glucan: A Biological Response Modifier from Baker’s Yeast for Skin Care

Keywords: CM-Glucan, Beta-Glucan, Yeast Polysaccharide, UV-A, Immune Stimulation, Skin Protection, Wound Healing

Summary
Preparations from yeast have been used for a long time for cosmetic and pharmaceutical purposes. Studies have identified glucan from the yeast’s cell wall as an immunologically effective agent of these preparations. Glucan is a poly β-(1→3)-linked glucopyranose of high molecular weight and belongs to the class of drugs known as biological response modifiers (BRMs). Glucan preparations are involved in the activation of the body’s natural defense mechanisms and in wound healing processes of the skin. In the skin, Langerhans cells and keratinocytes are the immunologically active cells. Recent studies indicate that UV radiation can induce a depletion of the number and viability of these cells (immunosuppression). The use of non-specific stimulators of immunocompetent cells, such as glucan, is a new approach to improve skin function under stress. We have developed a process to modify pure glucan from baker’s yeast to carboxymethyl glucan (CM-Glucan), a water-soluble product suitable for topical applications. In different experiments, the properties of the new, common raw material CM-Glucan has been investigated. Cell culture experiments showed that CM-Glucan protects skin cells against the depletion of antioxidant molecules upon UV-A radiation and promotes the growth of keratinocytes. In placebo controlled studies with healthy volunteers, the pretreatment of skin with CM-Glucan offered substantial protection against skin damage caused by detergents or UV-A radiation. In addition, CM-Glucan also enhanced the renewal rate of the stratum corneum.

Properties of β-(1→3) Glucans
Glucans are natural polysaccharides comprised of D-glucopyranosyl units and are found in oat, barley, wheat, microorganisms and fungi. The D-glucopyranosyl units of glucans from cereal grains are linked by β-(1→4) and β-(1→3) bonds, whereas the glucans from yeast and higher fungi consist of a linear β-(1→3) backbone on which occasional (1→6) branching occurs. Glucans from cereal grains are valuable nutritional components of our diet. These preparations from oat and barley have also skin-conditioning properties similar to emollients in cosmetic formulations. On the other hand, glucans containing β-(1→3)-linked glucopyranosyl units are known to have immune stimulating activities (1, 2, 3). β-(1→3) Glucan from yeast is a very potent stimulator of the immune system with the ability to activate macrophages, neutrophils and other cells that carry specific β-glucan receptors on their surface (4). Activation of these cells with glucan stimulates the nonspecific defense mechanisms of the host. Other polysaccharides, such as mannan, galactans, α-(1→4) or β-(1→4)-linked glucose polymers, have no such activity. Glucan preparations from yeast have been extensively studied in wound healing (5), infectiology (6) and oncology (7, 8). In all these applications, different (1→3)-β-glucan preparations from yeast have been shown to be very active at low concentrations. Wolk and Danon (5) found a significant acceleration of wound healing in the animal leg model by topical application of a yeast glucan preparation at 20 - 100 μg/ml. Because insoluble glucan preparations can produce undesirable toxicological properties, such as granuloma formation, clinical interest has focused on soluble glucan preparations. Recently, a phase II clinical study showed the tolerability and efficacy of a soluble yeast glucan (9). Application of a soluble glucan preparation (0.1 mg/kg - 2.0 mg/kg) before and after thoracic or abdominal surgery lowered postoperative infection rates. In other investigations, researchers observed improved wound healing following topical administration of a soluble phosphorylated glucan preparation (10).

The Skin’s Immune System

The skin is the body’s most important primary defense system. It consists of a physical barrier function as well as a metabolic and immunologic biochemical response system. At the epidermal level, the skin’s immune system involves cytokines and the immunocompetent Langerhans cells and keratinocytes.

Langerhans Cells
Langerhans cells play a dominant role in the immune reaction of the skin, although they present only a minor cell population (2 - 4%) in the epidermis. They are theorized to be the skin’s specialized version of macrophages and have dendrites which spread out over the whole epidermis. Presumably, one function of this dendrite network is to trap antigens and initiate a general immune response. The allergen is captured and modified by the Langerhans cell which then migrates to the lymphatic system. There, the allergen associated with human leukocyte antigen (HLA) class II molecules is presented to specific T-cells (CD4). The subsequent binding that occurs stimulates the proliferation of the T-cells in the lymph nodes as well as the production of the cytokines, the most important of which is interleukin-2 (IL-2) and interferon-gamma (IFN-γ).
Polysaccharide

Keratinocytes

Keratinocytes also produce a number of cytokines involved in immune response regulation and skin inflammation (11). Keratinocytes can produce significant levels of tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1) upon stimulation through lipopolysaccharides, UV-radiation and chemicals (Fig. 1). One unique cytokine produced by keratinocytes is transforming growth factor-β (TGF-β). This cytokine suppresses most of the immune reactions and may therefore be an important factor involved in the down regulation of immune responses in the skin. In addition to cytokine production, keratinocytes can also express the major histocompatibility (MHC) genes for HLA-DR. These molecules engulf foreign proteins and present them as antigens to T-cells. Another important cell surface marker expressed by keratinocytes is the intercellular adhesion molecule-1 (ICAM-1). This molecule is crucial to the interaction of keratinocytes with T-cells and monocytes because ICAM-1 is the ligand for the cell surface markers of such cells. ICAM-1 is of special interest in allergic contact dermatitis (ACD) (Fig. 1) because the allergens themselves cause its expression (12).

UV Induced Immunosuppression

UV Induced Immunosuppression

The modulation of ICAM-1 expression is not the only immunologic response following UV radiation. It has been shown that UV radiation alters cytokine release and impairs the number and viability of the immunocompetent cells in the epidermis (13, 14). All such responses constitute examples of immunosuppression. UV induced immunosuppression has been found to play a crucial role in the development of skin cancer in mice (15) and it is likely to be among the risk factors in the development of the disease in humans (16, 17). Although there is little dispute that commercially available sunscreen filters inhibit the development of many UV-induced alterations, like erythema (18), they do not necessarily offer complete protection against other biological effects of the sunlight, such as immunosuppression (19, 20). This may have serious consequences: consider the number of people who expose their skin to sunlight for extended periods of time in the belief that the high-SPF sunscreen products they apply will fully protect them. To compound this problem, the skin becomes even more susceptible to environmental hazards with advancing age, because of a general decline in the body’s immune defense mechanisms. With our increasing awareness of how these additional UV-related interactions and mechanisms can affect the body, concern over UV exposure and the sun care products we use should also increase. A substance which is able to stimulate the viability of the immunocompetent cells in the skin could slow down the decline of these defense mechanisms and generally improve the skin condition by enhancing the turnover of crosslinked collagen, denatured enzymes and oxidized extracellular matrix components. With its well documented immune-stimulating properties, β-(1→3)-glucan from yeast looks like a very promising candidate.
Development of a Glucan Preparation for Cosmetic Use

Glucan isolated from the cell wall of baker's yeast (Saccharomyces cerevisiae) is a water-insoluble particulate polymer which is not suitable for topical applications. In our laboratory, we have developed a process to modify pure β-(1→3)-glucan to carboxymethyl glucan (CM-Glucan), a water-soluble product (Fig. 2). The carboxymethylation is performed under special conditions to reach a degree of substitution of 0.75 (21). This level of substitution is high enough to render the material water-soluble without disturbing its helical structure, the configuration responsible for its activity. The chemical identity of the structure was confirmed by 1H-NMR spectroscopy (22). CM-Glucan is water-soluble up to 4 % and is compatible with most cosmetic ingredients as well as the majority of manufacturing conditions. The dermatological tolerance of CM-Glucan has been carefully monitored in healthy volunteers using a 2 % aqueous solution. The results proved that this material is neither an irritant/photo-irritant nor a sensitizer/photo-sensitizer. In these examinations, CM-Glucan did not induce allergic reactions nor caused inflammation.

Evaluation of the Activity of CM-Glucan by Cell Culture Techniques

Glucan preparations are active at very low concentrations and are therefore suitable for cell culture experiments. At the Frauenhofer Institute Stuttgart in Germany the activity of CM-Glucan was investigated on porcine keratinocyte cultures. The addition of the polysaccharide to the culture medium (M199) containing 10 % calf serum showed a significant concentration-dependent stimulation of the keratinocytes proliferation. With 0.01 % CM-Glucan, the relative cell count increased by more than 35 % after 120 hours (23). However, the researchers observed no protective effects against 1 J/cm² UV-B radiation with 0.01 % CM-Glucan, regardless if the porcine cell cultures were treated before or after irradiation. CM-Glucan was tested for its ability to protect human skin cells against UV-A radiation which is a very potent stimulator of oxidative stress in the epidermis. This stress leads to phototoxic and photoallergic reactions in the skin (24). Like immune-suppression, the effects of oxidative stress induced by UV-A radiation have also been shown to be involved in carcinogenesis (25). The prevention of such stress is, therefore, highly desirable. Cell cultures of human skin keratinocytes were developed through biopsy of normal human skin. The cells from different donors were pretreated with 0.01 % CM-Glucan for 18 hours before they were exposed to varying doses of UV-A radiation (320–450 nm). The protective effect of this pretreatment, regarding oxidative stress in human skin cells, could be demonstrated by measuring intracellular glutathione and ferritin concentrations as endpoints (26). Keratinocytes pretreated with the polysaccharide experienced significantly less depletion of the glutathione antioxidant than what normally occurs immediately after UV-A radiation. During these experiments, CM-Glucan produced effects similar to those of DL-α-tocopherol, an antioxidant used as a control because of its known ability to protect cells form UV-A induced oxidative stress. The observed effects of CM-Glucan in cell culture experiments appear to depend on the cell's origin. Keratinocytes cultures developed from pig embryo did not respond to CM-Glucan in the same effective way as the keratinocytes obtained from adult human skin. The skin's immune defense is a very complex system of cells including predominantly keratinocytes and Langerhans cells in the epidermis and T-cells, tissue macrophages and mast cells in the dermis. In addition, different cytokines function as mediators for cell communication and regulation. Given the complexity of this immune defense system, it is likely that the keratinocytes culture derived from human skin biopsy is more immunocompetent than the porcine culture. This may result from the increased presence of Langerhans cells in the human cell samples or from the expression of different relevant markers by the human keratinocytes.

Evaluation of Beneficial Effects of CM-Glucan on Human Skin

To study the efficacy of the yeast polysaccharide in vivo, we formulated CM-Glucan into two different types of cosmetic formulations, an oil-in-water (o/w) emulsion and a hydrogel. We evaluated CM-Glucan in these systems at concentrations ranging from 0.04 % - 0.4 %. The test products and the controls without CM-Glucan were applied twice daily to the forearm skin of five to ten volunteers.

Skin Protection of CM-Glucan Against Detergent Challenge

In the course of the 14 days product application, each of the five products enhanced skin humidity, measured in corneometer units, as compared to untreated skin. The increase of skin humidity by the oil-in-water emulsion was significantly higher than that caused by hydrogels. The subsequent challenge with sodium dodecyl sulfate (SDS) led to a drastic reduction in skin humidity. However, skin pretreated with the products containing CM-Glu-
can showed a much less pronounced decrease in skin humidity. The strength of this protective effect appears to relate directly to the concentration of CM-Glucan in the formulations (Fig. 3). Although application of the five products had little influence on the rate of transdermal water loss (TEWEL) during the initial 14 days of treatment, we observed a definite increase in TEWEL once skin was challenged with SDS and its barrier function damaged. However, we observed much less of an increase in TEWEL in skin pretreated with products containing CM-Glucan compared to placebo (data not shown). Clearly, CM-Glucan provided a concentration-dependent protective effect against detergent challenge.

Enhancement of the Renewal Rate of Stratum Corneum

In an additional experiment, an enhancement of the cell renewal rate could be demonstrated in skin treated with formulations containing CM-Glucan. We measured the renewal of the stratum corneum by the diminution of the fluorescence of initially applied dansyl chloride. The rate of this increase clearly depended on the concentration of CM-Glucan in the formulations. With 0.4% CM-Glucan in an o/w emulsion, the stratum corneum experienced more than a 30% increase in cell renewal compared to untreated skin (data not shown). The increase in cell renewal obtained in vivo correlates well with the increase in cell proliferation found in cultured porcine keratinocytes in vitro.

Inhibition of Skin Squalene perOxidation Caused by UV-A Radiation

The in-vitro studies have shown substantial protective effects against cell damage induced by UV-A radiation by pretreating keratinocytes with CM-Glucan. To evaluate the in-vivo efficacy of the polysaccharide in protecting skin against oxidative stress induced by UV-A radiation, we used a non-invasive technique for identifying squalene hydroperoxides (27). Squalene is one of the main lipids of the sebum and is particularly susceptible to photo-oxidation. Even with low doses of UV-A radiation on the skin, squalene can be converted to squalene hydroperoxides (27). In our study, three o/w emulsions containing 0.2%, 0.04% and 0% CM-Glucan were applied to separate sites on the forearm of ten volunteers for five days. On the fifth day, each of the pretreated sites and a nontreated site were exposed to 10 J/cm² UV-A radiation, after which the skin lipids from these irradiated areas were extracted with 1 ml of ethanol. Lipids extracted from an unirradiated area served as a control. We then determined the concentrations of squalene and squalene hydroperoxides in these extracts by HPLC techniques (27, 28, 29, 30). A very wide range of squalene concentrations could be detected in skin of the different volunteers. But all samples taken from skin sites exposed to UV-A radiation showed high squalene hydroperoxides concentrations. However, in samples taken from skin sites that had been pretreated with one of the CM-Glucan preparations, the incidence of hydroperoxidation was noticeably reduced (Fig. 4). The protecting effects of CM-Glucan in this experiment can be calculated as percent inhibition of peroxidation relative to placebo. The

![Fig. 3 Protecting effects of CM-Glucan in cosmetic formulations against the reduction of skin humidity caused by a detergent. The skin of five volunteers was treated with the products for two weeks. The skin humidity was measured on day 14 before and after the challenge with sodium dodecyl sulfate (SDS). The protecting effects are expressed as percentages relative to an untreated control.](image1)

![Fig. 4 In vivo formation of squalene hydroperoxides caused by UV-A radiation (10 J/cm²). Skin sites of 10 volunteers were pretreated for five days with o/w emulsions containing different concentrations of CM-Glucan. Subsequently the pretreated skin sites and a nontreated site were UV-A exposed. Squalene hydroperoxides concentrations were measured in chemiluminescence (CL) units after lipid extractions from irradiated skin sites and as a control from an unirradiated area.](image2)
peroxidation itself is expressed as the ratio of squalene hydroperoxides to squalene to normalize the difference in initial squalene concentrations. As shown in Fig. 5, the use of only 0.04% CM-Glucan in the o/w emulsion resulted in a 59% inhibition of the squalene peroxidation. At 0.2%, CM-Glucan provided almost complete protection (94.9%). Such effective protection against UV-A induced lipid peroxidation usually occurs only in the presence of very efficient antioxidants. Co/in et al (27) reported an inhibition of about 90% upon the application of 0.2% D-alpha tocopherol. An inhibition of less than 25% was observed with the application of the cosmetically stable vitamin E aceta at the same concentration. From these data, we conclude that CM-Glucan protects skin lipids against oxidation very efficiently and may, in fact, offer additional benefits to UV-A stressed skin that antioxidants do not.

**Conclusion**

Our data show that carboxymethylated β-(1→3)-glucan from baker’s yeast with an appropriate degree of substitution is a promising active component for different cosmetic and dermatological applications (Table 1). CM-Glucan is active at very low concentrations (0.01%) in cell culture experiments. However, the observed effects depend on the type and history of the cells. Keratinocytes cultures developed from pig embryo did not respond as well to CM-Glucan as the keratinocytes obtained from adult human skin. In our experiments, the pretreatment of human keratinocytes with CM-Glucan rendered them less sensitive to UV-A radiation. Results of corresponding in vitro and in vivo experiments correlated well. Pretreating human keratinocytes in vitro with CM-Glucan rendered them less sensitive to UV-A radiation. The Pretreatment of human skin in vivo with cosmetic formulations containing CM-Glucan provided substantial protection against the peroxidation of squalene after UV-A radiation. In addition, CM-Glucan is able to protect the skin against a decrease of skin humidity and an increase of transepidermal water loss from detergent challenge. To some degree, a second skin effect from the film-forming properties of the polysaccharide may be present. However, the concentrations used in the experiments were too low (0.04%) for CM-Glucan to have produced such profound effects solely through its film-forming behavior. Therefore, we speculate that biochemical activation of cells provides much of these protecting effects. Since CM-Glucan is neither an antioxidant nor an iron chelator, it must use mechanisms other than extracellular radical scavenging or activities related to it. CM-Glucan appears to stimulate cells, most likely through a receptor-mediated mode. The cells produce then endogeneous products against oxidative stress and other environmental hazards. The triggering of these defense mechanisms requires CM-Glucan to penetrate into the epidermis. Despite the fact that most polar agents of high molecular weight remain on the skin’s surface, CM-Glucan does induce biochemical effects within the skin. Other negatively charged polysaccharides, like the anticoagulant heparin, can also penetrate skin much better than theorized. The exact molecular mechanism of the CM-Glucan activity has not been fully elucidated and warrants further investigations.

**References**

2. Pillerer, L. and Eckler, E.E.: Anticomplementary factor in fresh yeast, J. Biol. Chem. 137, 139-142 (1941)

**Fig. 5** Inhibition of squalene peroxidation by two o/w emulsions containing 0.2% and 0.04% CM-Glucan relative to placebo (formulation without CM-Glucan). The inhibition of the peroxidation is shown for all ten subjects and as average value. Skin sites of the volunteers were pretreated with the products for five days. Subsequently, the peroxidation of skin lipids was induced by UV-A radiation (10 J/cm²). The rate of peroxidation is expressed as ratio of squalene hydroperoxides to squalene.

**Table 1** Benefits of CM-Glucan in Consumer Products

<table>
<thead>
<tr>
<th>Sun Care Product</th>
<th>Protection from the depletion of antioxidant molecules within the epidermis</th>
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</thead>
<tbody>
<tr>
<td>After Sun Lotion</td>
<td>Regeneration of the UV stressed skin; stimulation of the viability of cells</td>
</tr>
<tr>
<td>Day Cream</td>
<td>Enhancement of the skin's self-protecting capacity</td>
</tr>
<tr>
<td>Body Lotion</td>
<td>Enhancement of the proliferation of keratinocytes</td>
</tr>
<tr>
<td>Impure Skin Treatment</td>
<td>Regeneration of damaged skin; restoring the balance of skin function</td>
</tr>
<tr>
<td>Aged Skin Products</td>
<td>Retardation of the general decline of the immune defense mechanisms with advancing age</td>
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Polysaccharide


(21) Patent pending, Zulli, F. & Suter, F., Mibelle AG


(23) Graeve, T. and Schneider, A.: Unpublished results


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