PHOTOPROTECTIVE EFFECTS OF CM-GLUCAN ON CULTURED HUMAN SKIN CELLS

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Summary

It has become increasingly apparent that exposure to solar ultraviolet radiation mediates a number of harmful effects in humans. Recent studies indicate that UV radiation can be a potent stimulator of the oxidative stress in the epidermis and can have a profound influence on the immune system. It is also known that sunscreens do not offer a complete protection against all biological effects of sunlight. The use of non-specific stimulators of the immune system in sun care formulations is a new approach to lend beneficial functions to these products. Glucan extracted from the cell wall of baker's yeast is a very potent stimulator of the immune system. In this report, we present the development of CM-Glucan, a new water-soluble glucan derivative. The protective effects of this modified yeast polysaccharide regarding oxidative stress in human skin keratinocytes and fibroblasts induced by UV-A radiation could be demonstrated by measuring intracellular glutathion and ferritin concentrations as endpoints.

Lichtschutz-Wirkung von CM-Glucan auf kultivierte Zellen der humanen Haut

Die Gefährlichkeit des ultravioletten Sonnenlichtes für den menschlichen Körper ist heute allgemein bekannt. Neuste Studien weisen darauf hin, daß UV-Strahlung nicht nur einen entscheidenden Einfluß auf das Immunsystem hat, sondern auch den oxidativen Streß in der Epidermis fördert. Weiterhin ist heute bekannt, daß Sonnenfilter keinen vollständigen Schutz gegen alle biologischen Effekte des Sonnenlichtes bieten. Die Verwendung von nicht spezifischen Immunstimulatoren in Sonnenschutzprodukten ist daher eine neue Möglichkeit, diesen Produkten verbesserte Eigenschaften zuzuführen. Das Glucan aus der Zellwand der Bäckerhefe ist ein sehr wirksamer Stimulator des Immunsystems. In dieser Arbeit präsentieren wir die Entwicklung von CM-Glucan, einem neuen wasserlöslichen Glucan-Derivat. Es konnte im Zellkultur-Versuch gezeigt werden, daß dieses modifizierte Polysaccharid aus der Hefe vor oxidativem Streß, induziert durch UV-A Strahlung, schützt. CM-Glucan verhinderte dabei eine Erniedrigung der intrazellulären Konzentrationen von Glutathion und Ferritin in bestrahlten Keratinocyten und Fibroblasten der humanen Haut.

Effet photoprotecteur du CM-Glucan sur des cellules de la peau humaine cultivées

Il est bien connu que les rayons ultraviolets de la lumière solaire sont dangereux pour le corps humain. De récentes études indiquent que la radiation UV peut-être un facteur stimulant important du système immunitaire, mais aussi du stress oxydant dans l'épiderme.
Il est également connu que les filtres solaires n' offrent pas une protection complète contre tous les effets biologiques de la lumière solaire. L' utilisation de stimulateurs non spécifiques du système immunitaire dans les produits solaires est une nouvelle possibilité d' améliorer les propriétés de ces produits.

Le glucan de la membrane cellulaire de la levure de boulanger est un stimulateur très efficace du système immunitaire. Dans cette étude nous vous présentons le développement du CM-Glucan, un nouveau dérivé du glucan, soluble dans l' eau.

Il a pu être montré dans un essai de cultures cellulaires, que ce polysaccharide de levure protège du stress oxydant, induit par les rayons UVA. Ceci est dû à l'effet que le CM-Glucan empêche une dégradation des concentrations intercellulaires de glutathion et de ferritin dans les keratinocytes et les fibroblastes irradiés.

Introduction

In recent years, sun care products have gained considerable attention. This can be attributed to a perceived depletion of ozone in the stratosphere which has resulted in an increased concern for today's consumers about premature skin ageing and skin cancer. Commercially available sunscreens inhibit the development of many UV-induced alterations like erythema in mammalian skin [1]. However, it appears that sunscreens do not offer complete protection against the biological effects of sunlight [2, 3]. This is important considering the number of humans who expose themselves to sunlight for extended periods of time while using sunscreen products with high SPF values.

Recent studies indicate that UV-B radiation induces a suppression of immunological activity by a depletion of the number and viability of the immunocompetent cells (keratinocytes and Langerhans cells) within the epidermis [4, 5]. Immunosupression induced by UV-radiation plays a crucial role in the development of skin cancer in mice [6]. There is increasing evidence now that UV radiation may also contribute to the development of skin cancers in humans by way of debilitating the immune system [7, 8].

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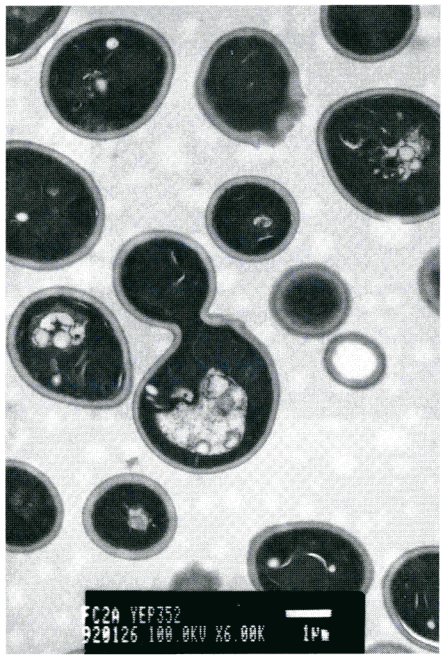

Figure 1. The cell wall of budding yeast (Saccharomyces cerevisiae) appears as small white border.

In addition to immunosuppression, oxidative stress has also been shown to be involved in carcinogenesis [9, 10]. UV-A radiation is known to be a very potent stimulator of the oxidative stress in the epidermis resulting in phototoxic and photoallergic reactions in the skin [11]. The induction of heme oxygenase appears to be a general mammalian response to oxidant stress [12]. However, a number of effects on other molecules such as epidermal growth factor have been reported [13].

The use of non-specific stimulators of the immune system in sun care formulations is a new approach to lend beneficial functions to these products. Polysaccharides from various sources have been known for some time to be such stimulators. Glucan extracted from the cell wall of baker's yeast is a very potent stimulator of the immune system and has gained a lot of interest over the last decade in various pharmaceutical indications.

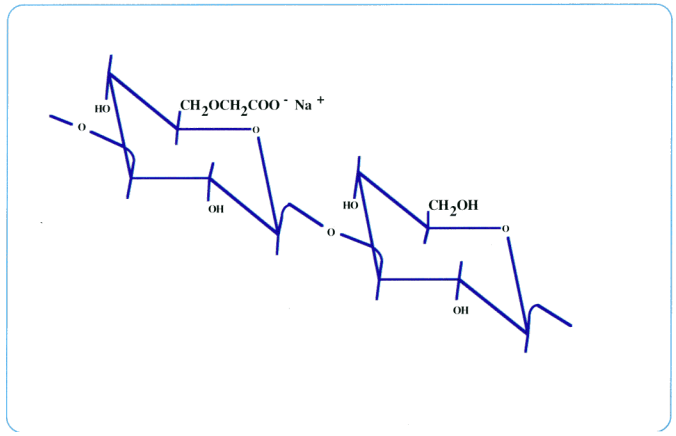

Figure 2. Structure of CM-Glucan (CTFA declaration: Sodium Carboxymethyl Betaglucan); three out of four glucose units are modified by carboxymethyl groups.

Glucan, an immune stimulating polysaccharide from yeast

For centuries, crude preparations from yeast and living yeast cells have been used for pharmaceutical and cosmetic purposes [14]. In 1941, the first defined pharmaceutical yeast product, Zymosan, was described [15]. Over the last decade, glucan from yeast cell walls has been identified to be the immunologically effective agent of Zymosan. It stimulates macrophage-mediated phagocytotic defense mechanisms [16].

Glucan is a (1—>3)-Beta-linked polyglucose of high molecular weight and belongs to the class of drugs known as biological response modifiers (BRMs). Yeast-derived glucan preparations have been studied in interesting fields such as wound healing [17, 18, 19], infectiology [20] or oncology [21].

Development of a glucan preparation for cosmetic use

Despite its polar nature, glucan isolated from the cell walls of baker's yeast is a water-insoluble particulate polymer which is not suitable for personal care products. To render it cosmetically applicable, it necessitates a conversion into a water-soluble and biologically effective form.

In our laboratory, we have developed a process for the isolation of pure glucan from the cell wall of baker's yeast (Fig. 1). Through derivation to carboxymethyl glucan (CM-Glucan), we obtained a water-soluble product. The degree of carboxymethyl substitution was 0.75 measured using a titration/dialysis method. This means that on an average, three out of four glucose units are modified.

The chemical identity of the structure of CM-Glucan (Fig. 2) was confirmed by 13C-nuclear magnetic resonance (NMR) spectroscopy [22].

Previous reports indicate that the immunological activity of certain Beta (1—>3)-D-Glucan BRM's is related to the helical conformation of the polymer [23]. Therefore, the active helical structure of the new compound CM-Glucan was confirmed by a helix/coil transition experiment [22].

Photoprotection by CM-Glucan against effects of UV-A radiation

CM-Glucan prepared by our standard high quality

process was tested for its ability to protect cultured human skin cells against oxidative stress, including UV-A radiation (MUTZHAS 3000 sunlamp).
Cell cultures of human skin fibroblasts and keratinocytes were developed from normal adult skin [24]. These cells from different donors were then used for bioassays using alpha-tocopherol as a control.
The cells were pre-treated for 18 hours in the tissue culture medium at a final concentration of 100 µg CM-Glucan per ml before they were exposed to UV radiation (320-450 nm) at a dose rate of 300 W/m2 for two different time periods. Immediately after the UV-A radiation, the intracellular concentration of reduced glutathion (GSH) was determined by an enzymatic assay. Intracellular concentrations of ferritin (ELISA) and total protein were determined 24 hours later.

The normal response seen in skin keratinocytes is portrayed by a dose dependent decrease in GSH immediately (one to two hours) after UV-A radiation. The decrease can vary to some degree between different cell lines. Normal concentrations of GSH are found only after a recovery of more than 24 hours (data not shown). Human skin fibroblasts are much more sensitive and will show a decrease of GSH with lower doses of UV-A irradiation (1x105 J/m2).

Figure 3 shows one example of a keratinocytes cell culture which was irradiated with two different doses of UV-A. The culture which was pre-treated with CM-Glucan was clearly less sensitive to UV-A radiation compared to the untreated control regarding the depletion of GSH. Pre-treatment with alpha-tocopherol (final concentration 10 µM) also showed substantial protection against depletion of the antioxidant GSH but with a different kinetic.
Figure 4 shows that CM-Glucan markedly protects keratinocytes against depletion of the iron binding protein ferritin when the cells are under oxidative stress caused by UV-A radiation. In all experiments, we observed first an increase of the intracellular ferritin concentrations at low doses of UV-A radiation (4x105 J/m2). However, at higher doses the cells could no longer counter the oxidative stress by overexpression of ferritin and a pronounced drop of the ferritin concentrations could be observed in all non treated cells and in some experiments also in the alpha-tocopherol treated cultures.

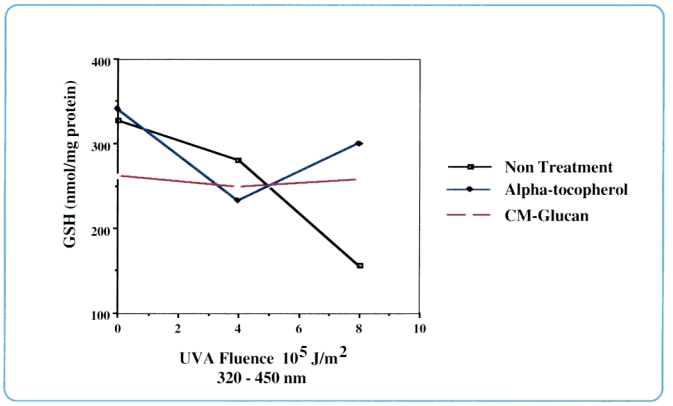

Figure 3. Influence of CM-Glucan (100 µg/ml) regarding glutathion (GSH) depletion in human keratinocytes induced by UV-A radiation.

Conclusion

Our studies have shown conclusively that CM-Glucan does not have any phototoxic effects on cultured human skin fibroblasts and keratinocytes. In addition, CM-Glucan clearly has protective effects regarding oxidative stress induced by UV-A radiation using cellular glutathion and ferritin levels as endpoints. Oxidative stress is known to be involved in carcinogenesis and the prevention of such stress has been shown to be highly beneficial [9, 25, 10].
The cell culture experiments have proven that CM-Glucan is a very potent new agent to protect fibroblasts and keratinocytes from the depletion of antioxidant molecules. In our investigations, CM-Glucan was active at concentrations as low as 100 µg/ml.
At the same concentration, CM-Glucan also stimulated the growth of porcine keratinocytes. The supplementation of the culture medium with this polysaccharide resulted in a 30% enhancement of the proliferation of these cells (Schneider and Graeve, Frauenhofer Institut Stuttgart, Germany, unpublished results).
The data presented in this report, together with the results of other investigations of topically applied glucan preparations regarding wound-healing [17, 18, 19] show that CM-Glucan is a new potent biological agent for cosmetic and dermatological formulations to be used in various indications [22]. By inducing the body's own defense mechanism, CM-Glucan acts in a "holistic" way and meets the demands of today's consumers for more natural but active compounds.

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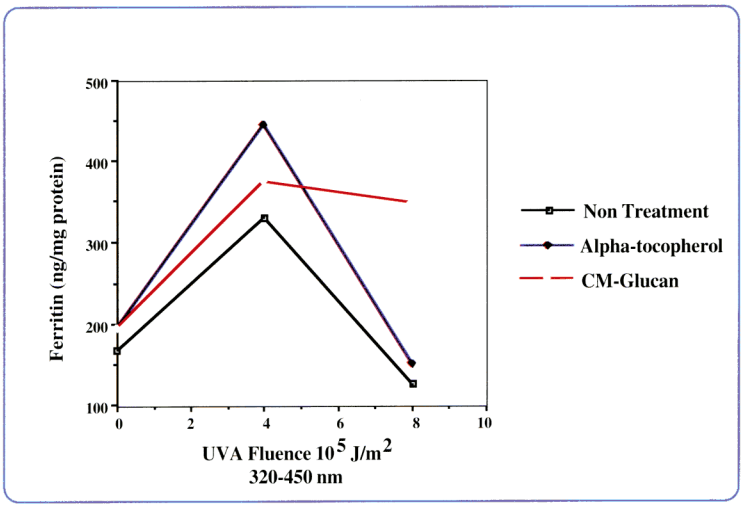

Figure 4. Influence of CM-Glucan (100 µg/ml) regarding the concentration of the antioxidant molecule ferritin in human fibroblasts upon UV-A radiation.

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