International Journal of Cosmetic Science **20**, 79–86 (1998)

Improving skin function with CM-glucan, a biological response modifier from yeast

F. ZÜLLI* and F. SUTER
Mibelle AG Cosmetics, Bolimattstr. 1, 5024 Buchs, Switzerland

H. BILTZ and H.P. NISSEN
Derma Consult GmbH, Brunnenstr. 61, 53347 Alfter, Germany

Poster Presentation at the 19th IFSCC International Congress, Sydney, Australia, 22–25 October 1996
Received 23 June 1997
Accepted 29 September 1997

Keywords: CM-glucan; skin protection; UV-A; yeast polysaccharide

Summary

Preparations from yeast have been used for a long time for cosmetic and pharmaceutical purposes. Studies have identified glucan from the cell wall of baker's yeast as an immunologically active agent. Glucan is a poly beta-(1-3)-linked glucopyranose of high molecular weight and belongs to the class of compounds known as biological response modifiers. Glucan preparations are involved in the activation of the body's natural defence mechanisms and in the acceleration of the skin's wound healing processes. In the skin, Langerhans' cells and keratinocytes are the immunologically competent cells. Recent studies indicate that UV irradiation can deplete the number and viability of these cells (immunosuppression). The use of non-specific immune-stimulators, such as glucan, is a new approach for improving the function of stressed skin. 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 formulations. The functional properties of this new compound have been investigated *in vitro* and *in vivo*. Cell culture experiments showed that CM-glucan protects skin cells against the depletion of antioxidant molecules upon UV-A irradiation 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 a detergent challenge or UV-A irradiation. In addition, CM-glucan enhanced the renewal rate of the stratum corneum.

Résumé

Les levures ont longtemps constitué une source pour les préparations cosmétiques et pharmaceutiques. Des études ont identifié le glucan extrait de la membrane cellulaire des levures comme un agent d'efficacité immunologique de ces préparations. Le glucan est un poly béta (1–3) glucopyranose de haut poids moléculaire et appartient à la classe des drogues connues comme agent modifiants de réponse biologique. Les préparations de glucan jouent leur rôle dans l'activation des mécanismes de défense naturelle du corps, et dans les processus de cicatrisation de la peau. Dans la peau, les cellules de Langerhans et les kératinocytes sont les cellules responsables de l'activité immunologique. De récentes etudes indiquent que les rayonnements UV peuvent provoquer une diminution du nombre et de la viabilité de ces cellules (immuno-suppression). L'utilisation de stimulateurs non spécifiques de cellules immuno-compétentes, tels que le glucan, constitue une nouvelle approche pour améliorer la fonction de la peau stressée. Nous avons développé un procédé permettant de modifier le glucan pur extrait de la levure de boulanger, pour le transformer en carboxymethyl glucan (CM-glucan), un produit hydro-soluble adapté aux applications topiques. Au cours de différentes expérimentations, les propriétes de la nouvelle matière première cosmétique CM-glucan ont été recherchées. Les expériences de

* To whom correspondence should be addressed.

culture cellulaire ont montré que le CM-glucan protège les cellules de la peau contre la réduction des molécules antioxydantes sous rayonnement UV-A et favorise la croissance des kératinocytes. Dans des études contre placebo, effectuées sur volontaires sains, le prétraitement de le peau avec CM-glucan a généré une protection substantielle contre les détériorations de la peau causées par les détergents ou les rayonnements UV-A. En outre, le CM-glucan a aussi augmenté le taux de régénération du stratum corneum.

Introduction

Crude extracts from yeast have been used for a long time for cosmetic and pharmaceutical purposes. These products have been found useful in treating various diseases and skin conditions [1].

In 1941, biochemical investigations of yeast components led to the discovery of the first defined pharmaceutical yeast product, zymosan [2]. Further studies have shown that water insoluble zymosan has immune-stimulating activity. This product is a cell wall preparation composed of glucan, other polysaccharides, proteins and lipids. Over the last two decades, glucan from yeast cell walls has been identified as a single immunologically active component.

Glucan is a beta-(1-3)-linked polyglucose of high molecular weight and belongs to the class of compounds known today as biological response modifiers. Glucan from baker's yeast is a very potent stimulator of the immune system by activating macrophages and other cells. Therefore, glucan preparations have been extensively studied in wound healing [3], infectiology [4] and oncology [5, 6]. In all these applications, different beta-(1-3)-glucan preparations from yeast have been shown to be very active. Recently, the tolerability and efficacy of a soluble yeast glucan has been proven in a phase II study [7].

Development of a glucan preparation for cosmetic use

Recent studies indicate that UV-B irradiation suppresses the activity of immunocompetent cells (keratinocytes and Langerhans' cells) within the epidermis. The immunosuppression is caused by a reduction of the number and viability of these cells [8, 9]. The use of non-specific stimulators of the immune system in cosmetic formulations, and especially in sun care products, is thus a new approach which gives beneficial functions to these products.

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. We have developed a process to modify it to carboxymethyl glucan (CM-glucan), a water soluble product (Fig. 1). The carboxymethylation takes place under specific conditions in a reaction that yields a product with a substitution degree of 0.75. This still allows the molecule to adopt a helical conformation which is important for the activity of glucan preparations. The chemical identity of the structure could be confirmed by ^{13}C-NMR spectroscopy [10].

The dermatological tolerance of CM-glucan has been carefully monitored in dermatologically healthy volunteers using a 2% aqueous solution. The results prove that this material is neither an irritant/photo-irritant nor a sensitizer/photo-sensitizer.

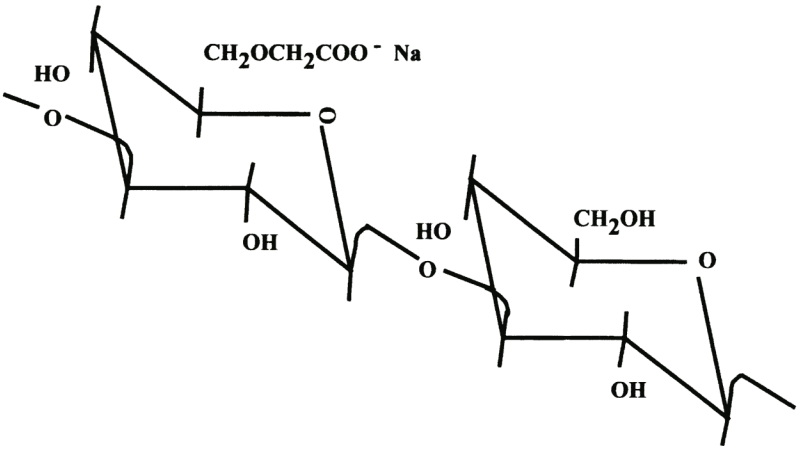

Figure 1. Chemical structure of carboxymethylated beta-(1-3)-glucan (CM-glucan) from baker's yeast. On average, three out of four glucose units of the beta-(1-3)-linked polymer are modified at position 6.

Evaluation of the CM-glucan activity by using cell culture techniques

Because glucan preparations have shown activity at very low concentrations, cell culture experiments should reveal any activity. We have investigated the activity of our CM-glucan on porcine keratinocytes. The addition of the polysaccharide to the culture medium containing 10% calf serum showed a significant stimulation of keratinocyte proliferation (Fig. 2) At a concentration of 0.01%, the relative cell count was increased by more than 40% after 120 h.

In other experiments, CM-glucan has been tested for its ability to protect human skin cells against oxidative stress. In addition to immunosuppression, oxidative stress induced by UV-A irradiation has been shown to be involved in carcinogenesis [11]. Keratinocyte cultures of human skin were developed from normal adult skin, samples of which were extracted by biopsy. The cell cultures from various donors were pretreated with CM-glucan for 18 h at a concentration of 0.01% before being exposed to UV-A irradiation (320–450 nm) at a dose rate of 300 W m^{-2} for two different time periods.

To demonstrate protection against oxidative stress induced by UV-A irradiation from CM-glucan pretreatment, researchers measured changes in the intracellular concentrations of glutathione and ferritin [12]. These cell culture experiments showed that CM-glucan pretreatment protects keratinocytes from the depletion of antioxidant molecules. The effects were similar to those of DL-α-tocopherol, an antioxidant used as a control because of its known ability to protect cells from UV-A induced oxidative stress.

CM-glucan improves human skin barrier function

To study the efficacy of the yeast polysaccharide *in vivo*, CM-glucan was formulated at different concentrations in a hydrogel and in an oil-in-water emulsion. The products were applied twice daily to the forearm skin of five volunteers over a period of 14 days. During this time, all formulations enhanced the skin humidity (corneometer units) compared to the

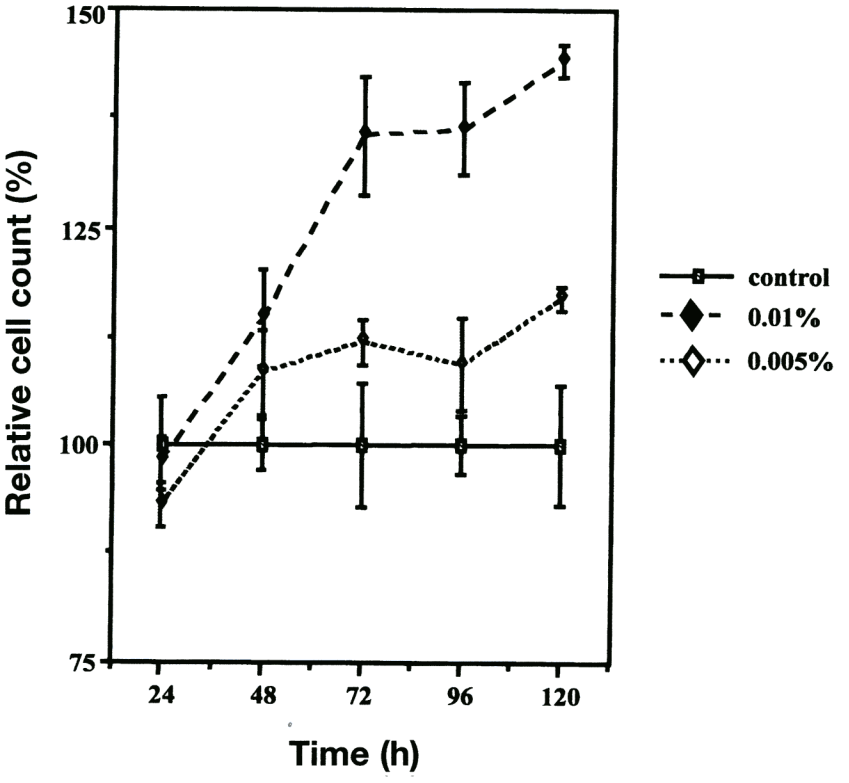

Figure 2. Influence of two different concentrations of carboxymethylated beta-(1-3)-glucan (CM-glucan) on the growth of porcine keratinocytes in M199 culture medium containing 10% calf serum. ($n = 4$, values shown are the means ± SD)

untreated skin. After the pretreatment, the skin was damaged with sodium dodecyl sulfate (10%) for 2 h. This subsequent challenge led to a drastic reduction of skin humidity. However, in skin pretreated with the products containing CM-glucan, the reduction of skin humidity was much less pronounced than in untreated skin. The strength of the protective effect seems to depend on the CM-glucan concentration in the formulations (Fig. 3). The application of the products over a period of 14 days did not influence the transepidermal water loss of healthy skin. The damage of the skin barrier function by the subsequent detergent challenge led to a drastic increase of the values for transepidermal water loss, but again, a concentration dependent protection by the formulations containing CM-glucan could be observed compared to untreated skin (data not shown).

CM-glucan accelerates the renewal of the stratum corneum

An enhancement of the cell renewal rate could be observed in skin treated with formulations containing CM-glucan. The renewal of the stratum corneum was measured by the gradual diminution of a fluorescence set at the onset of the experiment by dansyl chloride. The increase of the cell renewal rate depended on the CM-glucan concentration

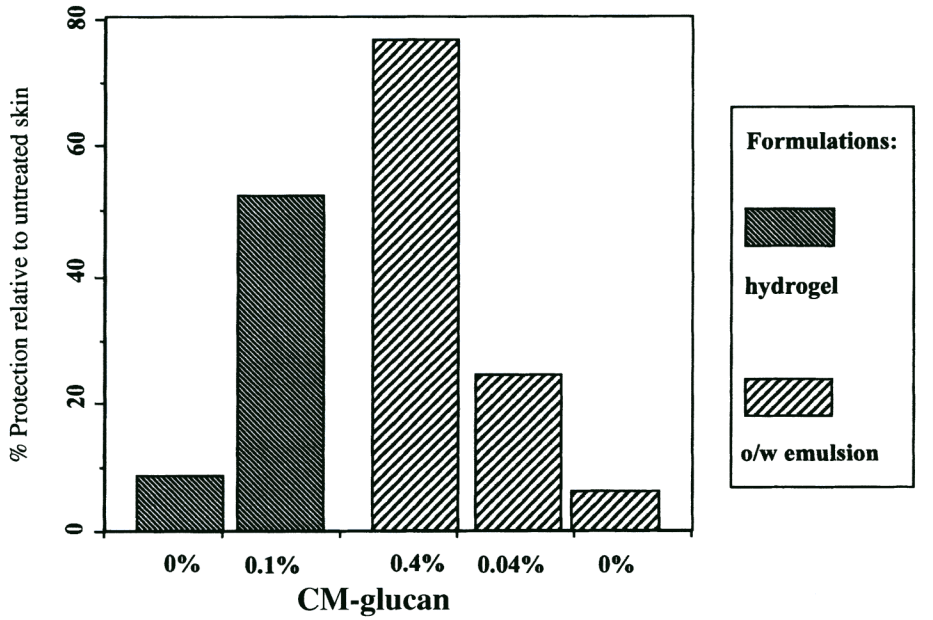

Figure 3. Protective effects of carboxymethylated beta-(1-3)-glucan (CM-glucan) in cosmetic formulations against the reduction of skin humidity caused by a challenge with 10% sodium dodecyl sulfate. The skin of five volunteers was pretreated with the cosmetic products for 2 weeks. The skin humidity was measured on day 14 before and after the challenge. The protective effects are expressed as percentages relative to untreated skin ($n = 5$, mean values are given).

in the cosmetic formulations. At a concentration of 0.4% CM-glucan in an oil-in-water emulsion, the renewal rate of the stratum corneum was enhanced by more than 30% compared to untreated skin. This *in vivo* result corresponds favourably with our *in vitro* observation that CM-glucan enhances the proliferation of porcine keratinocytes (Fig. 2).

GM-glucan inhibits squalene peroxidation

Our *in vitro* studies have shown substantial protective effects against cell damage induced by UV-A irradiation by a pretreatment of the cultures with CM-glucan. To evaluate a corresponding *in vivo* efficacy of the polysaccharide in protecting skin against oxidative stress induced by UV-A irradiation, we used the non-invasive technique for identifying squalene hydroperoxides formation [13].

Squalene is a major component of the sebum and is particularly susceptible to photo-oxidation. Colin *et al.* [13] showed that even low dose UV-A irradiation of the skin leads to the formation of squalene hydroperoxides. However, topical application of strong free radical scavengers protected squalene against peroxidation.

In our study, three oil-in-water emulsions containing 0.2%, 0.04% and 0% CM-glucan were applied twice daily on the forearm of 10 volunteers. On the fifth day, the pretreated skin and a non-treated area were exposed to UV-A irradiation (10 J cm^{-2}). Subsequently, skin lipids were extracted with 1 ml of ethanol from all irradiated areas and, as a control,

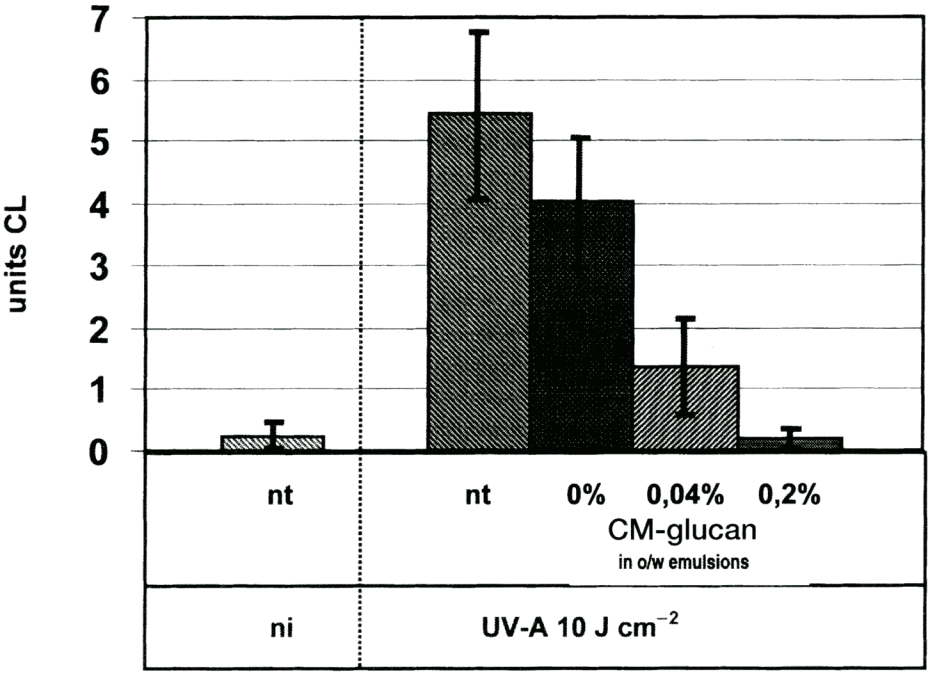

Figure 4. *In vivo* formation of squalène hydroperoxides caused by UV-A irradiation (10 J cm^{-2}). Skin sites of 10 volunteers were pretreated for 5 days with oil-in-water (o/w) emulsions containing different concentrations of carboxymethylated beta-(1-3)-glucan (CM-glucan). Then the pretreated skin sites and a non-treated (nt) site were exposed to UV-A. Squalene hydroperoxides concentrations were measured in chemiluminescence units after lipid extractions from irradiated skin sites and as a control from a non-irradiated (ni) area. (n = 10, values shown are the means ± SD)

also from non–irradiated skin. Squalene and squalene hydroperoxides were then determined in these extracts by using HPLC techniques [13–16].

In all subjects, the UV-A irradiation led to a substantial increase of the squalene hydroperoxide concentrations in untreated skin. The pretreatment of the skin with the oil-in-water emulsion without CM-glucan resulted in an average squalene hydroperoxide concentration (chemiluminescence units) of 74.2% of that of untreated skin. The incorporation of 0.04% CM-glucan into the same emulsion reduced this value to 25.3%. The application of 0.2% CM-glucan resulted in a lipid peroxides value similar to that of non–irradiated skin. Therefore in this test, an almost complete protection against UV-A induced squalene peroxidation could be observed with this product (Fig. 4).

Conclusion

Our work shows that carboxymethylated beta-(1-3)-glucan (CM-glucan) from baker's yeast with an optimized degree of substitution is a promising new active ingredient in cosmetic and dermatological preparations. CM-glucan is water soluble and can therefore easily be incorporated into appropriate formulations.

In cell culture experiments, we observed a stimulation of keratinocyte proliferation at very low concentrations (0.01%). The pretreatment of human keratinocytes with CM-glucan rendered them less sensitive to oxidative stress induced by UV-A irradiation. Corresponding activity could be verified *in vivo*. The pretreatment of skin with cosmetic formulations containing CM-glucan showed substantial protection against skin damage caused by a detergent challenge. In these studies, CM-glucan protected the skin against a decrease of skin humidity and an increase of transepidermal water loss. To some extent, a second skin effect from the film-forming properties of the polysaccharide may be present; but the concentrations used in our experiments were too low for CM-glucan to have produced such profound effects solely through its film-forming behaviour. However, the exact molecular protecting mechanism warrants further investigation.

CM-glucan also provided a concentration-dependent protection against UV-A irradiation. Skin pretreatment with a formulation containing 0.2% CM-glucan showed an almost complete inhibition of lipid peroxidation. Protection against lipid peroxidation induced by UV-A irradiation is usually only observed by topical application of antioxidants. Colin *et al.* [13] reported a significant inhibition of squalene peroxidation upon the application of 0.2% D-α-tocopherol, but with the cosmetically stable vitamin E acetate only a very small protection against UV-A induced lipid peroxidation could be observed at the same concentration.

Since CM-glucan is neither an antioxidant nor an iron chelator, its mechanism of action must involve the stimulation of an endogenous cellular defence system to produce factors which protect the skin against oxidative stress and other environmental insults.

References

1. Hänsel, R. Polysaccharide die immun-stimulierend wirken: Eine Uebersicht über entsprechende Fertigarzneimittel. *Farmaceutisch Tijdschrift voor Belgie* **64**, 313–26 (1987).
2. Pillemer, L. and Ecker, E.E. Anticomplementary factor in fresh yeast. *J. Biol. Chem.* **137**, 139–42 (1941).
3. Wolk, M. and Danon, D. Promotion of wound healing by yeast glucan evaluated on single animals. *Med. Biol.* **63**, 73–80 (1985).
4. Kokashis, P.L., Williams, D.L., Cook, J.A. and Di Luzio, N.R. Increased resistance to *Staphylococcus aureus* infection and enhancement in serum lysozyme activity by glucan. *Science* **199**, 1340–2 (1978).
5. Di Luzio, N.R., Williams, D.L., McNamee, R.B., Edwards, B.F. and Kitahama, A. Comparative tumor-inhibitory and anti-bacterial activity of soluble and particulate glucan. *Int. J. Cancer* **24**, 773–9 (1979).
6. Hofer, M., Pospisil, M., Bohacek, J., Pipalova, I. and Sandula, J. Enhancement by carboxymethylglucan of early cellular damage in 1 Gy-irradiated mice. *Folia Biola. Praha* **41**, 112–7 (1995).
7. Babineau, T.J., Hackford, A., Kenler, A., Bistrian, B., Forse, R.A., Fairchild, P.G., Heard, S., Keroack, M., Caushaj, P. and Benotti, P. A phase II multicenter, double-blind, randomized, placebo-controlled study of three dosages of an immunomodulator (PGG-Glucan) in high-risk surgical patients. *Arch. Surg.* **129**, 1204–10 (1994).
8. Tang, A. and Udey, M.C. Doses of ultraviolet radiation that modulate accessory cell activity and ICAM-1 expression are ultimately cytotoxic for murine epidermal Langerhans cells. *J. Invest. Dermatol.* **99**, 571–3 (1992).

9. Mommaas, A.M., Mulder, A.A. and Vermeer, B.J. Short-term and long-term UVB-induced immunesuppression in human skin exhibit different ultrastructural features. *Eur. J. Morphol.* **31**, 30–4 (1993).
10. Zülli, F. and Saecker, C. CM-Glucan a new yeast polysaccharide for cosmetic use. *Cosmet. Toil. Manufacture Worldwide* 131–6 (1994).
11. Kripke, M.L. and Applegate, L.A. Alterations in the immune response by ultraviolet radiation. In *Biochemistry and physiology of the skin.* (Goldsmith, L., ed.) pp. 1304–28. New York, Oxford University Press (1991).
12. Zülli, F., Applegate, L.A., Frenk, E. and Suter, F. Photoprotective effects of CM-Glucan on cultured human skin cells. *Eurocosemtics* 11, 46–50 (1995).
13. Colin, C., Boussouira, B., Bernard, D., Moyal, D. and Nguyen, Q.L. Non invasive methods of evaluation of oxidative stress induced by low doses of ultra violet in humans. IFSCC Congress, Venezia **A105**, 50–72 (1994).
14. Nissen, H.P. and Kreysel, H.W. The use of HPLC for the determination of lipids in biological materials. Chromatographia **30**, 686–90 (1990).
15. Zhang, J.R., Cazers, A.R., Lutzke, B.S. and Hall, E.D. HPLC–chemiluminescence and thermospray LC–MS study of hydroperoxides generated from phosphatidylcholine. *Free Radical. Biol. Med.* **18**, 1–10 (1995).
16. Holley, A.E. and Slater, T.F. Measurement of lipid hydroperioxides in normal human blood-plasma using HPLC–chemiluminescence linked to a diodearray detector for measuring conjugated dienes. *Free Radical Res. Comm.* **15**, 51–63 (1991).