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Photoprotective effects of glucomannan isolated from *Candida utilis*

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Abstract—Glucomannans belong to yeast and fungal cell wall polysaccharides with known immunostimulatory and radioprotective effects. However, glucomannan protective effects against pathological consequences of skin exposure to short wavelength solar light, ultraviolet (UV) radiation, are unclear. Herein, a highly branched glucomannan (GM) isolated from the cell wall of *Candida utilis*, a member of the α-(1→6)-D-mannan group, was tested for its photoprotective effects in an in vitro model of UVB-irradiated human keratinocytes and an in vivo model of UV-induced erythema formation in human volunteers. GM suppressed the UVB-induced decrease of keratinocyte viability, which was connected with the suppression of UVB-induced keratinocyte apoptosis. GM reduced UVB-mediated caspase activation together with suppression of DNA fragment release into the cytoplasm. Furthermore, GM suppressed UVB-induced gene expression of pro-inflammatory markers including nuclear factor kappa B, inducible nitric oxide synthase, interleukins 8 and 1, together with suppression of prostaglandin E2 and interleukin 1α protein release. In vivo, GM decreased UV-induced skin erythema formation, which was correlated with a decrease of phosholipase A_2 activity within the stratum corneum. It could be concluded that GM isolated from *C. utilis* possesses significant photoprotective effects on human keratinocytes in vitro as well as in vivo.

Keywords: Glucomannan; *Candida utilis*; HaCaT keratinocytes; UV-protection; Polysaccharide; Apoptosis

1. Introduction

It is well recognized that skin exposure to solar radiation has detrimental consequences, both acute and chronic. In particular, the short wavelength part of solar light, ultraviolet (UV) radiation, contributes significantly to undesirable effects, which could lead to the development of cutaneous malignancies.[1–3] The impact of cutaneous malignancies is significant since melanoma and non-melanoma skin cancer are the most abundant carcinomas in western countries.[1,4] Therefore, development and improvement of skin protection strategies against solar radiation is a critical issue.

Different mechanisms are suggested to contribute to the adverse effects of UV radiation on the skin. One of the most significant mechanisms is UV-induced suppression of immune functions connected with massive cutaneous cell death. Decrease of cutaneous cell viability is related to UV-induced cell damage due to the formation of free radicals that destruct cellular structures including lipids, nucleic acids, and proteins.[1,2] Physiological functions associated with UV-induced impairment of skin are directly associated with the inflammatory response provoked by the damaged keratinocytes, which leads to the release of a wide range of inflammatory mediators. Cytokines released during this early phase of UV-induced skin reaction are considered to be impor-

Abbreviations: αMSH, alpha melanocyte stimulating hormone; CR3, complement receptor 3; GM, glucomannan; iNOS, inducible nitric oxide synthase; IL-1α, interleukin 1 alpha; MR, mannose receptor; NO, nitric oxide; NFκB, nuclear factor κB; PAMPs, pathogen-associated molecular patterns; PLA_2, phospholipase A_2; PGE_2, prostaglandin E_2; TLR, toll-like receptor; UVB, ultraviolet light B

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tant mediators of the consequent immune suppression in skin.[5] Therefore, the prevention of UV-induced keratinocyte injury could significantly reduce the detrimental effect of UV radiation on skin.[1,2]

Increased resistance of cells to different types of noxious stimuli is connected with an increase in the activity of various intracellular protective and repairing mechanisms, which resemble adaptation to cellular stress.[6,7] Activation of stress-induced signaling pathways by non-specific stressors, such as heat or radiation leads to the activation of cytoprotective responses.[8] Similar cell responses could be induced through the activation of specific cell surface receptors including receptors for microbial molecular patterns (or pathogen-associated molecular patterns, PAMPs) comprising lipids, carbohydrates, proteins, and nucleic acids because their molecular structure is distinct from those expressed on the surface of mammalian cells.[9–12] Polysaccharides that are part of the cell wall of yeasts and fungi are among the molecular structures recognized by these receptors.[9–12] Therefore, biologically active polysaccharides with the ability to active cellular responses in skin could be skin-protective agents.

Yeast and fungal polysaccharides consist of glucose and mannose units joined together by glycosidic linkages via different positions and in different ratios. The biological activity of purified glucans and glucomannans, including decrease of infectious complications and inhibition of tumor growth, is known to depend on their structure. Parameters such as primary structure, degree of branching, molecular weight, solubility, solution conformation, and ionic charge were suggested to play a role in determining the biological activity of these molecules.[13,14] In a previous study, we observed strong immunostimulatory effects of two structurally different polysaccharides, schizophyllan, and carboxymethylglucan, which were isolated from *Schizophyllum communae* and *Saccharomyces cerevisiae* cell walls, respectively.[15] However, the relationships between the structure of glucans and glucomannans and their stimulatory activities still remain unclear. Glucomannan (GM) isolated from *Candida utilis* consists of the α-(1→6)-D-mannopyranosyl backbone carrying mannooligosaccharidic side chains (1–5 units) containing α-(1→2) linkages, where some of the side chains are terminated with non-reducing D-glucopyranosyl residues.[16] Generally, the mass of this polysaccharide varies between 30 and 70 kDa and the mannose/glucose ratio is 2–3:1 (Fig. 1).

Glucans and glucomannans exhibit various biological activities, which are mediated by interaction with cell surface receptors. GM is recognized by several types of receptors including complement receptor 3 (CR3), the mannose receptor (MR), toll-like receptors (TLRs), and other lectin receptors, which are widely expressed on leukocytes and mediate cellular response to different types of PAMPs.[10] However, mannan/glucan binding

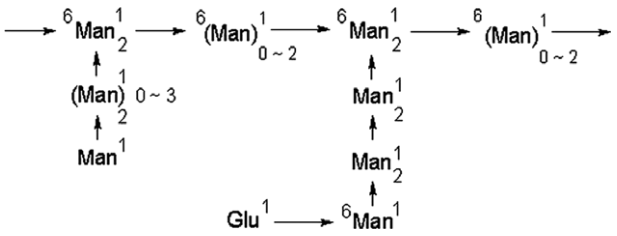

Figure 1. Structure of GM isolated from *Candida utilis*, which consists of an α-(1→6)-D-mannopyranosyl backbone carrying mannooligosaccharidic side chains (1–5 units) composed of α-(1→2) linkages, where some of the side chains are terminated with non-reducing D-glucopyranosyl residues.

sites have also been described on other cell types including fibroblasts.[10] and different epithelial cells.[17,18] These glucan/mannan receptors could also be assumed to be present on keratinocytes. Indeed, keratinocytes were already shown to express lectin receptors and toll-like receptor 4, which were suggested to be crucial for the recognition of O-linked mannosyl polymers such as glucomannans.[7,19,20]

Given the above premises, it could be hypothesized that GM acts as a photoprotective agent and prevents UVB-induced damage of cutaneous cells. The photoprotective properties of the GM isolated from *C. utilis* were evaluated in vitro on UVB-irradiated primary human keratinocytes and the immortalized keratinocyte cell line HaCaT. Furthermore, the photoprotective effects of the GM were confirmed in vivo by the measurement of UV-induced erythema formation in human volunteers. Parameters of the skin inflammatory response (phospholipase A_2 activity) were determined in stripped layers of stratum corneum. The data obtained show significant GM protective effects against UVB-induced death of human keratinocytes and suggest GM as a potent photoprotective agent.

2. Results

2.1. UVB exposure induced inflammatory response and apoptosis in keratinocytes

To explore the photoprotective properties of GM, model of primary human keratinocytes and human keratinocyte cell line HaCaT irradiated by UVB light was employed. Doses of UVB radiation (10 and 20 mJ/cm^2) and concentrations of GM (50 and 500, 1000 μg/mL) were selected based on the preliminary results (data not shown). Upon irradiation with 10 mJ/cm^2 UVB, the viability of both keratinocyte cell cultures decreased significantly after 24 h. Thus, the dose 10 mJ/cm^2 of UVB was selected for cell viability evaluation and for the induction of the cell inflammatory response. A higher dose of UVB (20 mJ/cm^2) was selected for the