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Rebalancing the Th1 / Th2 Immune Response in Atopic Skin with Magnesium Carboxymethyl Beta-Glucan

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INTRODUCTION

Atopic dermatitis is a chronic inflammatory skin disease associated with red, itchy, and dry skin eczema which can appear on any body part but is most prevalent on the extremities [1]. Constant scratching of the very itchy skin patches by sufferers of atopic dermatitis leads to skin lesions and barrier impairment while introducing bacteria into the skin. This renders eczema vulnerable to infections, most commonly by the bacterium Staphylococcus aureus [2], which further contribute to irritation in a vicious itch-scratch cycle.

The causes of atopic dermatitis are a mixture of hereditary and environmental influences. While genetic predisposition plays an important role in the probability of developing eczema, it cannot be dismissed that people living in small families in urban surroundings are far more likely to experience atopic reactions than those in rural communities [3]. Furthermore, the prevalence of skin eczema and other allergies has greatly increased in the industrialized world in the past decades. These observations led to the hygiene hypothesis that reduced contact with pathogens during childhood leads to a skewed immune system development manifested in overreaction to allergens [4]. Later molecular studies substantiated the hypothesis by uncovering how the immune response is unbalanced in atopic dermatitis. Upon encountering a pathogen antigen-presenting cells in the skin activate T helper cells, which secrete cytokines to mediate the downstream response. Two different types of T helper cells, type 1 and type 2 (Th1 and Th2, respectively) can be activated, leading to different consequences. Th1 activates macrophages, part of the innate immune system, which engulf and destroy microbial intruders. Th2 elicits an adaptive immune response, characterized by B cell activation and immunoglobulin E (IgE) antibody production [5], which evokes a release of histamine potentially leading to allergic sensitization. A balanced immune system reacts with the suitable pathway according to the threat, whereas in atopic dermatitis skin the response is skewed towards Th2 activation and it was shown that the severity of eczema correlates with IgE levels [6]. Another marker of atopic dermatitis is elevated expression of the pro-inflammatory cytokine interleukin-8 (IL-8) [7].

Rebalancing the immune response to an appropriate reaction could improve atopic skin conditions. Notably, activation of the

ABSTRACT

The rise of allergies in the industrialized world can be partly explained by an unbalanced immune system due to insufficient exposure to allergens at a young age. This evokes a defense reaction against allergens that involves overactivation of the T helper cell Th2 pathway, which causes Immunoglobulin E antibody production and ultimately leads to histamine production and an allergic reaction characterized in the skin by dryness, redness and itchiness. Suppression of the Th2 pathway by Th1 could alleviate allergic reactions. To investigate the effect of a potential Th1 activator on eczematous skin, a magnesium salt of a baker's yeast cell wall component, Magnesium Carboxymethyl Beta-Glucan, was tested in in vitro atopic dermatitis models. Treatment with Magnesium Carboxymethyl Beta-Glucan inhibits the formation of IgE antibodies as well as the expression of inflammation markers that are elevated in eczematous skin. Magnesium Carboxymethyl Beta-Glucan also impairs binding to reconstructed human epidermis of Staphylococcus aureus bacteria, which are often responsible for superinfections in eczema where the skin barrier is disrupted. In addition to suppressing a Th2-activated allergic response, Magnesium Carboxymethyl Beta-Glucan improves the skin barrier, smoothness and hydration in volunteers that are diagnosed with atopic dermatitis.

Th1 pathway leads to suppression of the Th2 path. Therefore, activating the Th1 instead of the Th2 response towards allergens would correct the immune response imbalance. Interestingly, it has been shown that Toll-like receptors (TLR) in keratinocytes play an important role in the activation of the Th1 response. These receptors belong to the class of pattern recognition receptors and their function is to recognize foreign material, for example, microorganisms, and activate the innate immune system via Th1. When the skin is exposed to an allergen together with a TLR ligand, the Th2 immune response is reduced [8], which prevents Th2-mediated oversensitization of the skin.

Interesting compounds that could act as a TLR pattern recognition molecule are beta-glucans. 1,3-β-Glucans are polysaccharides composed of glucopyranose with (1,3) glycosidic linkages and branches at carbons 1 and 6 **(Figure 1)**. These molecules are derived from microbial origins and have been shown to positively influence wound healing. Interestingly, 1,3-β-glucan was shown to bind the Toll-like receptor TLR2 in keratinocytes and thereby rebalance the Th1/Th2 response in allergic rhinitis patients [9]. Therefore, beta-glucans are promising candidates to alleviate atopic dermatitis symptoms, such as redness and itching.

This work investigates the effect of a novel magnesium salt version of beta-glucan derived from baker's yeast cell wall, which was carboxymethylated for increased water solubility (INCI: Magnesium Carboxymethyl Beta-Glucan, from here on: MgCM-Glucan) in *in vitro* atopic dermatitis models. Additionally, a clinical study was performed to assess whether MgCM-Glucan is able to ameliorate the skin condition in atopic dermatitis patients.

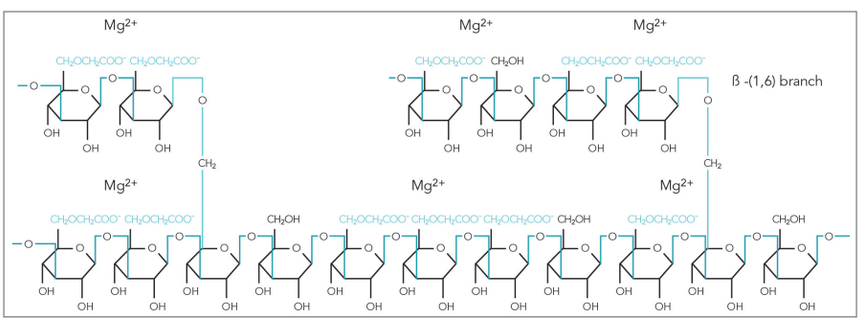

Figure 1 Chemical structure of Magnesium Carboxymethyl Beta-Glucan

EXPERIMENTAL

Helix-Coil transition analysis

The helix-coil transition analysis was based on [13]. MgCM-Glucan, laminarin (Sigma-Aldrich, St. Louis, USA) as the control triple helix molecule, or dextran (Sigma-Aldrich, St. Louis, USA) as the random-coil control was dissolved in NaOH solutions ranging from 0.01 M to 1 M. Helix formation was determined by adding Congo red (Sigma-Aldrich, St. Louis, USA) and measuring the maximum absorbance wavelength of the solutions using a UV/VIS Spectrophotometer (Shimadzu, Kyoto, Japan).

IgE release

CD19+B lymphocytes were incubated in assay medium alone or supplemented with 0.8 mg/ml MgCM-Glucan for 2 h. Cells were then treated with a mix of anti-CD40 and IL-4 to stimulate IgE production. After incubation for 14 days, culture supernatants were harvested and IgE levels were quantified using an ELISA kit (Mabtech, Stockholm, Sweden).

IL-8 release

10-day-old reconstructed human epidermis models were pre-incubated with assay medium with or without 0.5 mg/ml MgCM-Glucan for 2 h. Then the reconstructed human epidermis was stimulated or not (unstimulated control) with a cytokine mix of IL-4, IL-13, IL-22 and TNF-α (3 ng/ml) for 24 h to mimic the chronic inflammation phase of atopic dermatitis. The reconstructed human epidermis was washed in phosphate buffered saline (PBS) solution, RNA was extracted using TriPure Isolation Reagent (Roche, Basel, Switzerland), and IL-8 expression was quantified by RT-qPCR using the LightCycler system (Roche, Basel, Switzerland).

Staphylococcus aureus adhesion to reconstructed human epidermis

Staphylococcus aureus bacteria were cultured in L broth medium and radiolabeled with [3H]-adenine (10 µCi/ml) (Analytic, Braunschweig, Germany) for 24 h and washed four times with PBS. A suspension of bacteria was prepared with an optical density of 0.5 at 525 nm.

Reconstructed human epidermis was pretreated with different concentrations of MgCM-Glucan or PBS for 2 h. The reconstructed human epidermis was then cleaned and treated topically with either PBS and bacteria or MgCM-Glucan and bacteria in a 1:1 volume ratio for 1 h. After washing the reconstructed human epidermis four times with PBS, the remaining radioactivity was measured using liquid scintillation counting (Beckmann Coulter, Brea, USA).

Skin parameter measurements

A double-blind, placebo-controlled clinical trial was performed with 20 volunteers (14 female, 6 male, mean age 44.3 years) that had been previously diagnosed with atopic dermatitis but were in a symptom-free interval during the study. Placebo cream and the same cream containing 0.1% MgCM-Glucan were applied to the inside of each forearm twice daily for 28 days. After day 14 and day 28 of the treatment the skin hydration was measured using a Corneometer MPA5 CPU (Courage & Khazaka GmbH, Cologne, Germany), skin roughness was determined by means of PRIMOS 5.7 high-res (GFMesstechnik, Teltow, Berlin) and TEWL was measured with a Tewameter TM 210 (Courage & Khazaka GmbH, Cologne, Germany).

RESULTS AND DISCUSSION

Magnesium Carboxymethyl Beta-Glucan forms highly stable helices

Various methods, such as X-ray diffraction [10] and NMR spectroscopy [11], have demonstrated that beta-glucans form triple

helical structures, which are believed to be stabilized by intermolecular hydrogen bonds [12]. To further improve stability of carboxymethyl beta-glucan, a magnesium salt (Magnesium Carboxymethyl Beta-Glucan, MgCM-Glucan) was prepared. Adding the divalent counterion Mg2+ to the negatively charged carboxymethyl groups ***(Figure 1)*** allows additional intermolecular bridging to stabilize the helical structure.

To assess the helical stability of MgCM-Glucan, a helix-coil transition analysis was carried out. This assay is based on the observation that alkaline solutions interfere with hydrogen bonding, which disrupts triple helix formation and leads to a random coil structure. To measure helix formation, the dye Congo red was used, which exhibits a shift in the maximum absorption wavelength when associated with helical polysaccharides. Laminarin was used as a control triple helix molecule and dextran served as a random-coil control. While laminarin changes its conformation from a triple helix to a random coil formation, as visualized by the absorption shift between 0.05 and 0.1 M NaOH towards that of dextran and Congo red alone, MgCM-Glucan remains stable as a triple helix even at 1 M NaOH ***(Figure 2)***. This suggests that the magnesium salt of carboxymethyl beta-glucan possesses a highly stable helical structure.

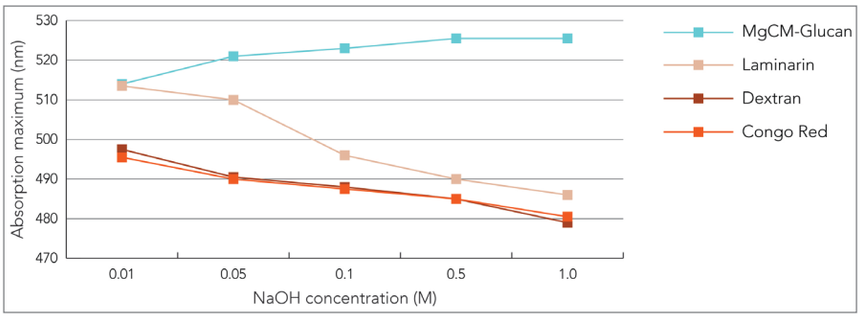

Figure 2 Helix-coil transition assay: absorption maximum of MgCM-Glucan in the presence of Congo red at different concentrations of NaOH

Reduction of an immune response in *in vitro* atopic dermatitis models

An *in vitro* atopic dermatitis model was used to investigate how treatment with MgCM-Glucan influences the immune response. Cultured B cells were stimulated by cytokines to produce IgE, an indicator for the severity of atopic dermatitis. Treatment with anti-CD40 and IL-4 drastically increased IgE expression. When cells were additionally pre-treated with MgCM-Glucan before cytokine stimulation, IgE expression was reduced by 26% compared with control pretreated, stimulated cells.

To simulate the chronic stage of atopic dermatitis, reconstructed human epidermis was stimulated with a pro-inflammatory cytokine mix. In reconstructed human epidermis pretreated with MgCM-Glucan, IL-8 expression was decreased by 53% compared with stimulated but not pretreated reconstructed human epidermis. Taken together, these results demonstrate that treatment with MgCM-Glucan reduces inflammation markers in two different atopic dermatitis models.

Impairment of *S. aureus* adhesion to reconstructed human epidermis

Another common issue with eczematous skin is infection with *S. aureus* bacteria, which exacerbates the inflammation of the skin. Thus, it was assessed whether MgCM-Glucan has an influence on the adhesion of *S. aureus* to reconstructed human epidermis. Interestingly, MgCM-Glucan strongly impaired *S. aureus* binding to reconstructed human epidermis in a concentration-dependent manner ***(Figure 3)***. This could be an additional aid in combating the chronic inflammation in eczematous skin.

Improvement of skin barrier in atopic dermatitis patients

To test whether MgCM-Glucan ameliorates atopic skin, a double-blind, placebo-controlled clinical study was carried

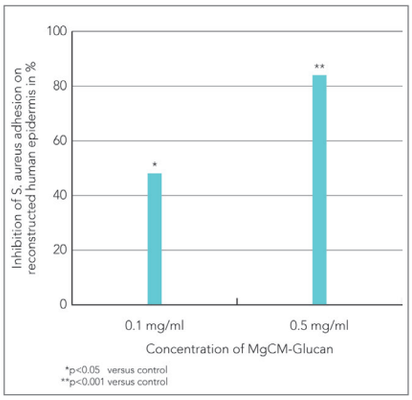

Figure 3 Inhibition of *S. aureus* adhesion to reconstructed human epidermis by different concentrations of MgCM-Glucan compared with buffer-treated control

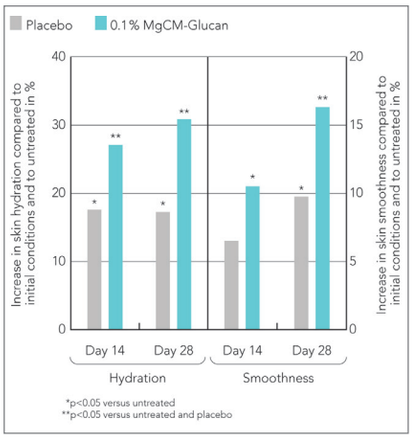

Figure 4 Measurement of skin hydration by corneometer (left) and skin smoothness by PRIMOS (right) after 14 and 28 days of topical application of 0.1% MgCM-Glucan to volunteers that were previously diagnosed with atopic dermatitis

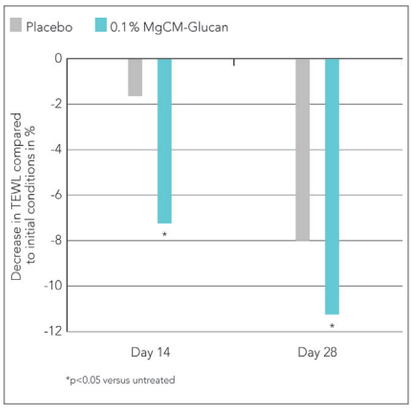

Figure 5 Measurement of transepidermal water loss (TEWL) by tewameter after 14 and 28 days of topical application of 0.1% MgCM-Glucan to volunteers that were previously diagnosed with atopic dermatitis

out. After topical application of 0.1% MgCM-Glucan in an emulsion twice daily for 28 days, a significant increase in skin hydration and smoothness was measured ***(Figure 4)***. Furthermore, TEWL decreased significantly ***(Figure 5)***, indicating an improved skin barrier function with treatment with MgCM-Glucan.

CONCLUSION

The newly developed magnesium salt of carboxymethyl beta-glucan, MgCM-Glucan, is a highly stable molecule that adopts a helical conformation. Tests in *in vitro* atopic dermatitis models demonstrated that MgCM-Glucan is able to reduce the inflammatory response, which is upregulated by an overactive Th2 pathway in atopic dermatitis. Additionally, MgCM-Glucan improved skin barrier function and hydration in atopic dermatitis patients. Therefore, MgCM-Glucan is a promising compound for ameliorating atopic skin conditions.

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