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Hydrolysis of sphingomyelin to ceramide induced by heat-killed *Lactoplantibacillus plantarum* APsulloc 331261 via neutral sphingomyelinase 2 activation protects against Staphylococcal α-toxin–induced cytotoxicity in keratinocytes

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Introduction:

Atopic dermatitis (AD) is a chronic inflammatory skin disease associated with skin barrier dysfunction as the initial step of the development of AD. The function of skin barrier is based on lipid matrix, which are composed of ceramides, cholesterol and free fatty acids in the outermost layer of the skin, the stratum corneum (SC). In particularly, reduction of ceramides in the SC is involved in barrier impairment in AD [1]. In addition, the colonization of *Staphylococcus aureus* (*S. aureus*) in the skin have an important role in the pathogenesis of AD [2]. Recently, oral or topical application of probiotics has shown to provide incredible health benefits to AD treatment [3]. Although the effect of a selected probiotic extract in increasing ceramide levels on the SC in AD patients is reported [3], there was no direct evidence how probiotic extract can modulate ceramide production and *S. aureus* α-toxin sensitivity in human keratinocytes.

Materials & Methods:

APsulloc 331261 adjusted to 10^9 cfu/ml was heat-killed at 80°C for 10 min in a water bath. Normal human epidermal keratinocytes (NHEKs) were incubated with heat-killed APsulloc 331261 at various concentrations (0, 10^7, and 10^8 cfu/ml) under either low calcium (50 μM) or high calcium (1.2 mM). mRNA expression responsible for ceramide synthesis was determined using quantitative real time PCR (qRT-PCR) and SMPD3 activity was measured by neutral sphingomyelinase activity assay kit. Lipids of NHEKs were extracted by Bligh Dyer method and analyzed ceramide species (NDS, NS, NP, AP, and AS) by liquid chromatography-mass spectrometry. Alpha-toxin-induced cytotoxicity was measured by lactate dehydrogenase release. Statistical comparisons were performed using Student's *t*-test between two groups or one-way ANOVA test within multiple groups, followed by Turkey's post hoc test.

Results & Discussion:

Fig 1. Heat-killed APsullloc 331261 stimulates SMPD3 expression in NHEKs under both low and high calcium cond...

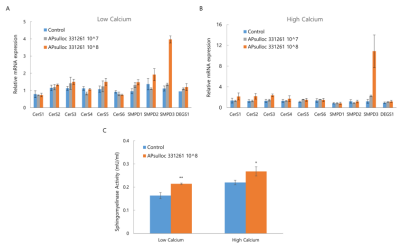


Fig 2. SMPD3 is upregulated during keratinocyte differentiation and the induction of SMPD3 expression by heat-killed APsulloc 331261 is mediated via PPAR delta

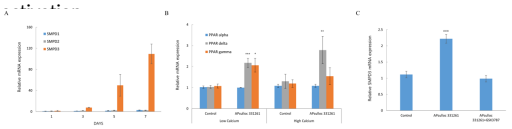


Fig 3. Upregulation of Ceramide NS synthesis is induced by APsulloc 331261 via SMPD3 activation

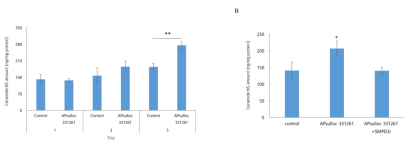
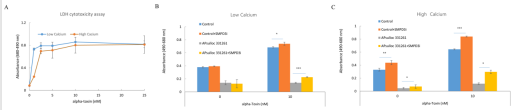


Fig 4. Heat-killed APsulloc 331261 protects against staphylococcal alpha-toxin-mediated cell death via SMPD3



The present study demonstrated that heat-killed APsulloc 331261 stimulated SMPD3 expression, resulting in the increase of ceramide presumably at the expense of sphingomyelin. Heat-killed APsulloc 331261 could confer protection against α-toxin–induced cell death. The action mechanism may be attributed to possibly the reduction of the number of α-toxin binding sites on the cell surface due to enzymatic activity of SMPD3-mediated cleavage of sphingomyelin which is one of the known α-toxin receptors (4).

Conclusions:

In current study, heat-killed APsulloc 331261 was shown to significantly upregulate SMPD3 expression, which hydrolyze sphingomyelin to ceramide and subsequently increase the production of ceramides from keratinocyres. Moreover, heat-killed APsulloc 331261 protected against staphylococcal alpha-toxin-induced keratinocytes death. These results suggest that heat-killed APsulloc 331261 are potential supplements for AD treatment.

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