

Synergic benefits of probiotics and selective antioxidants from TCM on cytokine storm-induced protein damages on skin

Andrea Cavagnino¹, Michel Frey², Martin Baraibar¹ & Lionel Breton^{2,3,*}.

¹OxiProteomics SAS, Créteil, France; ²IDEC Therapeutic/Telostim, Paris, France; ³Cilia Consulting SAS, Versailles, France.
*Corresponding author Email: btwocg@gmail.com

Introduction:

Interleukin 6 (IL-6), a pro-inflammatory cytokine, mediates a wide range of inflammatory and immune responses and its expression is elevated in inflammatory or immunodeficient diseases, being also involved in the COVID-19 pandemic characterized by elevated serum levels in the cytokine inflammatory storm [1-4].
Probiotics are known to be beneficial for immune functions and for some of them, control of infectious diseases and inflammation. A symbiotic mixture composed of these specific probiotics' strains associated to antioxidants known to be efficient against both, oxidative and inflammatory events could be relevant to provide a global beneficial effect on early events of cytokines' induced stress. Protein damage leading to the formation of carbonyl groups derives from direct oxidation of several amino acid side chains but can also derive through protein adducts formation with lipid peroxidation products and dicarbonyl glycation compounds. Probiotics activities on skin disorders by topical route, can be explained by i) a direct effect at the site of application by enhancing the skins natural defense barriers and ii) the production of antimicrobial peptides that benefit cutaneous immune responses and eliminate pathogens as commensal strains.
Regarding viral or bacterial infections, the benefit of probiotics is increasingly mentioned and their contribution to the overall immune balance should be permanently confirmed over the next decade, for future adaptation to new infections (5,6). Therefore, certain probiotic strains have recently been described as having the capacity to reduce the severity of certain viral infections and already influencing strains of coronavirus (7,8). The anti-inflammatory potential of *Bifidobacterium adolescentis* against noroviruses has been for example, recently described (9).
In this study we assessed the synergic effectiveness of probiotics combined with well known antioxidants to counteract the negative effects of IL-6 mediated oxidative stress on skin fibroblasts.

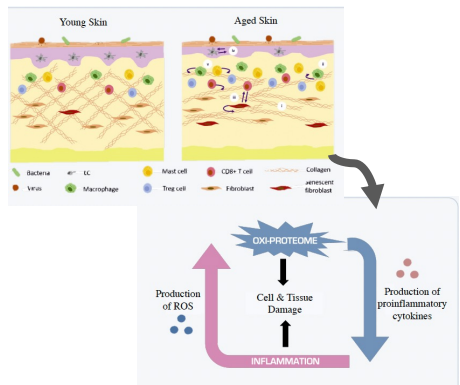
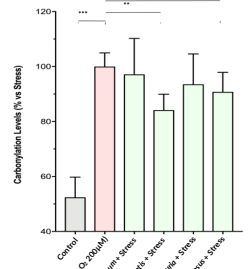


Figure 1. ECM disorganization occurs with aging ("I" figure above) influencing migration and activity of skin-resident/infiltrating immune cells ("II" figure above). Increased number of senescent fibroblasts is also observed. Taking together, those phenomenon lead to increased levels of protease and pro-inflammatory molecules in skin, such as IL-6/IL-8, TNF α , resulting in a self-sustaining negative loop of inflammation, oxidative stress, and cell and tissues damages. [Adapted from 11]

Results & Discussion:

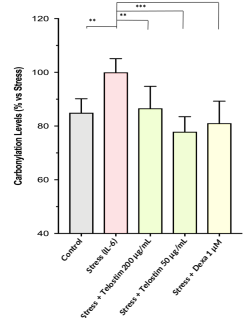
Postbiotics and oxidative stress.

Lactobacillus rhamnosus has been linked to a reduction in adult acne [12]. Here, we present the efficacy of 4 probiotics strands in reducing protein carbonylation induced by H2O2 exposure of skin cells (keratinocytes). The postbiotics of *Lactobacillus rhamnosus* and *Bifidobacterium adolescentis* presented a significant efficacy in the protection of cells from oxidative stress-induced deleterious effects (protein oxidation) (Figure 2).



Probiotics and inflammatory (IL-6) stress.

A patented symbiotic mixture composed of specific probiotics' strains associated to an anti-oxidants was evaluated against IL-6-induced stress. IL-6 alone resulted in a significant increase of carbonylated proteins, which was efficiently inhibited by the treatment with a reference compound (dexamethasone). The presence of the patented symbiotic mixture protected skin cells from IL-6-induced increase of protein carbonylation (Figure 3).



Conclusions:

Interleukin 6 (IL-6), a pro-inflammatory cytokine showed a significant increase in oxidized proteins was observed after IL-6 treatment of human dermal fibroblasts. A patented symbiotic mixture composed of specific probiotics' strains associated to an anti-oxidants showed a significant protective effect against IL-6-induced proteins' oxidation versus dexamethasone. The symbiotic mix showed to be efficient against both, oxidative and inflammatory events suggesting to provide a global beneficial effect on early events of cytokines' storm and associated consequences on skin.
In this study, we have evidenced the ability of selected probiotics strains known for their antiviral and anti-inflammatory profiles in association with antioxidants from traditional Chinese medicine, to prevent oxidative damage on human fibroblasts upon cytokines' stress. In addition, our model provides relevant early targets in early event of infectious disease and associated inflammation specifically in the skin.

Materials & Methods:

Human skin cells in vitro culture.

Human keratinocytes (HaCat) were cultured in calcium-free DMEM, with 2% SVF, at 37 °C and humid atmosphere, supplemented with 5% CO₂. Cells were plated (30,000 cells/well) at Day 0 (D0) in 96 well plates containing culture medium and distributed in several experimental groups, containing 9 replicates per group (n=9), as depicted in the following Table. The experimental groups were incubated for 16 hours with the postbiotics (10% v/v), then exposed to stress (H₂O₂ 200 µM, 30 minutes). Human dermal fibroblasts were cultured in calcium-free DMEM, with 2% SVF, at 37 °C and humid atmosphere, supplemented with 5% CO₂. Cells were plated (20,000 cells/well) at Day 0 (D0) in 96 well plates containing culture medium and distributed in several experimental groups, containing 6 replicates per group (n=6). The experimental groups were incubated for 24 hours with the 3 symbiotic mixture, in presence of IL-6 (8 ng/mL). The Stress groups received only the treatment with H₂O₂ (200µM) or IL-6 (8 ng/mL), while the Control group was untreated. Dexamethasone (1µM) has been used as reference against IL-6 triggering.

Carbonylated proteins assessment.

Upon cell fixation, carbonylated proteins were labeled in situ with a specific fluorophore as described previously (10). Their fluorescence emission was detected and recorded using a flow cytometry plate reader. Data management and statistical analyses were accomplished using GraphPad Software.

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