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

Andrea Cavagnino[1], Michel Frey[2], Martin Baraibar[1] & Lionel Breton[2,3,*]

[1]OxiProteomics SAS, Créteil, France; [2]IDEC Therapeutic, Paris, France;
[3]Cilia Consulting SAS, Versailles, France.

* Corresponding author
Lionel Breton, PhD.
Cilia Consulting SAS
Versailles, France
Email: btwocg@gmail.com

ABSTRACT

Interleukin 6 (IL-6) is a cytokine that mediates a wide range of inflammatory and immune responses. Its expression is elevated in many inflammatory or immunodeficient diseases, including the COVID-19 pandemic.

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 specific probiotics' strains associated to an anti-oxidants known to be efficient against both, oxidative and inflammatory events could be relevant to provide a global beneficial effect on early events of cytokines' storm and drastic associated consequences.

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 glycating compounds.

Human dermal fibroblasts were cultured in vitro and incubated for 24 hours with the synbiotic mixture, in presence of IL-6 (8 ng/mL). Carbonylated proteins were assessed. Data management and statistical analyses were accomplished using GraphPad Software.

A significant increase in oxidized proteins was observed after IL-6 treatment of human dermal fibroblasts. The symbiotic mix shows a significant protective effect against IL-6-induced proteins' oxidation *versus* dexamethasone. 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 TCM, to prevent oxidative proteome damage on human fibroblasts upon cytokins' stress. In addition, our model provides relevant early targets in early event of infectious disease and associated inflammation in the skin.

INTRODUCTION

Interleukin 6 (IL-6) is a cytokine that mediates a wide range of inflammatory and immune responses. Its expression is elevated in inflammatory or immunodeficient diseases, including psoriasis, rheumatoid arthritis, AIDS and also obesity-classic cardiovascular risk factors and coronary artery disease. IL-6 signaling is also involved in the COVID-19 pandemic and this proatherogenic cytokine reaches elevated serum levels in the cytokine inflammatory storm generated by infections, such as SARS-CoV-2 (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 cytokins' induced stress. 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 other strains of coronavirus (7,8). The anti-inflammatory potential of Bifidobacterium adolescentis against noroviruses has been for example, recently described (9).

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 glycating 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. In this study we assessed the synergic effectiveness of probiotics combined with well known anti-oxidants to counteract the negative effects of IL-6 mediated oxidative stress on skin fibroblasts.

METHODS

Human Skin cells in vitro culture.

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 3ymbiotic mixture, in presence of IL-6 (8 ng/mL). The Stress group received only the treatment with IL-6 (8 ng/mL), while the Control group was untreated. Dexamethasone (1µM) has been used as reference.

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 fluorescence plate reader. Data management and statistical analyses were accomplished using GraphPad Software.

RESULTS & DISCUSSION

Interleukin (IL)-6 induces oxidative stress in human dermal fibroblasts.

Previous studies have shown that (IL)-6 plays a central role in acute inflammation and is necessary for the timely resolution of wound healing in the skin (11). Moreover, IL-6 synthesis is regulated in part by oxidative stress. In a vicious cycle, this oxidative stress can increase ROS production, and can initiate both inflammation and pro-inflammatory cytokine activation, such as interleukin-2 (IL-2), interleukin-6 (IL-6), and tumor necrosis factor α (TNF-α), involving multiple pathways including nuclear factor kappa light chain enhancer of activated B (NF-kB), hypoxia-inducible factor 1-alpha (HIF-1a), nuclear factor erythroid 2-related factor 2 (Nrf-2), and activator protein 1 (AP-1).

In this study, a significant increase in oxidized proteins was observed after IL-6 treatment of human dermal fibroblasts (Table 1).

Table 1.

Experimental Group	Carbonylation Level (Oxidation % vs Ctrl)	Std. Dev.	PROTECTION
Control	100	6	100%
Stress (IL-6)	118	6	0%
Stress + Telostim 200 µg/mL	102	10	89%
Stress + Telostim 50 µg/mL	92	7	100%
Stress + Dexamethasone 1µM	95	10	100%

Probiotics protect human dermal fibroblasts from (IL)-6 induced oxidative stress

Probiotics are classically defined as alive bacterial strains, which are benefit for health. In viral or bacterial infections, the benefit of probiotics is increasingly mentioned and their contributions to the global immune balance should be definitively confirmed over the next decade, for future adaptation to new infections.

Thus, some probiotic strains have recently been described as being able to reduce the severity of certain viral infections and having an effect on other coronavirus strains (7,9). This is probably the reason why the International Association for Probiotics and Prebiotics (IAPP) emphasizes the need of further scientific research on probiotics in the prevention or treatment of coronaviruses. The therapeutic arsenal to fight COVID-19 could thus be expanded with other more preventive options, possibly through the known effects of certain probiotics on CD4+, CD8+ or NK-Tcells.

In this study we assessed a new formulation containing probiotic strains documented for antiviral and respiratory activities in association with antioxidants known to have an additional affinity to ACE2 like quercetin. This supplement could be part of the prophylactic therapeutic arsenal to reduce the severity of this pandemic as part of the overall strategy to flatten the curve. A protection value (%) was obtained considering control group at maximum efficiency (100%) and the stress group at minimum efficiency (at 0%). The symbiotic mix shows a significant protective effect against IL-6-induced protein oxidation: 89% at 200 µg/mL and 100% of protection when applied on cells at 50 µg/mL. In these experimental conditions, dexamethasone (1uM) showed 80% of efficacy (Figure 1).

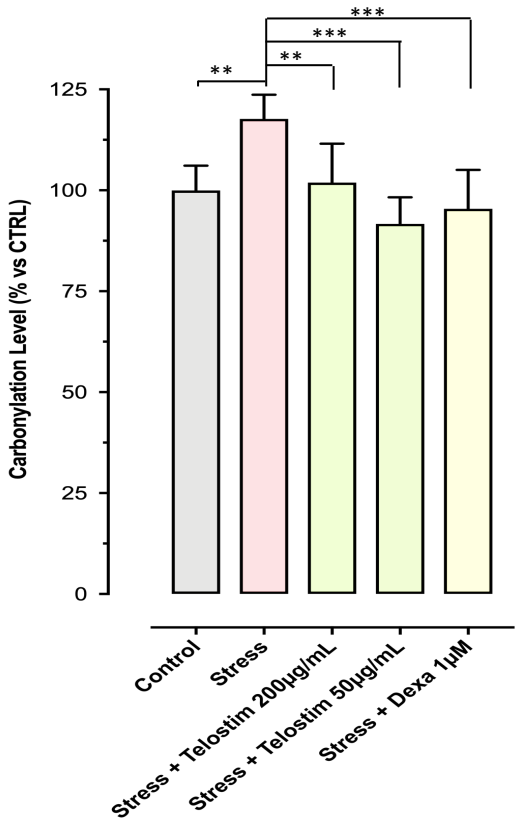

Figure 1. Protein carbonylation on human dermal fibroblasts.
Statistical analysis were performed using ANOVA
and Dunnett's post-hoc multi comparisons test ** $p<0.01$; *** $p<0.001$.

CONCLUSION

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 cytokins' stress.

In addition, our model provides relevant early targets in early event of infectious disease and associated inflammation in the skin.

Conflict of Interest Statement. NONE.

References

1. Abhay Bajaj et al, 2020. Understanding SARS-CoV-2: Genetic Diversity, Transmission and Cure in Human Indian J Microbiol. 60(3):1-4.

2. Ling Ni et al. 2020, Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals Immunity. 16;52(6):971-977

3. Qin, C. et al. (2020) Dysregulation of Immune Response in Patients with COVID-19 in Wuhan.
   China. Clin. Infect. Dis, 71(15):762-768.

4. Zuo, T. et al. (2021), Depicting SARS-CoV-2 Faecal Viral Activity in Association with Gut Microbiota Composition in Patients with COVID-19. Gut 70, 276–284.

5. Ahmad Ud Din et al. (2021) SARS-CoV-2 microbiome dysbiosis linked disorders and possible probiotics role. Biomed Pharmacother.

6. Shilia JK et al , 2021, Probiotics in Prevention and Treatment of COVID-19: Current Perspective and Future Prospects , Archives Medical research, 52,6, 582-596.

7. Baud D et al, 2020, Using Probiotics to Flatten the Curve of Coronavirus Disease COVID-2019 Pandemic. Front Public Health, 8:186.

8. Chen, J., & Vitetta, L. (2021). Modulation of gut microbiota for the prevention and treatment of COVID-19. Journal of Clinical Medicine, 10(13), 2903.

9. Li D, et al. (2016), Anti-viral Effect of Bifidobacterium adolescentis against Noroviruses. Front Microbiol. Jun 8; 7:864.

10. Baraibar MA, Ladouce R, Friguet B (2013). Proteomic quantification and identification of carbonylated proteins upon oxidative stress and during cellular aging. J Proteomics 30;92:63-70

11. Johnson BZ, Stevenson AW, Prêle CM, et al (2020). The Role of IL-6 in Skin Fibrosis and Cutaneous Wound Healing. Biomedicines. 30;8(5):101.