Human milk oligosaccharides attenuate solar irradiation induced inflammation and oxidative stress in human skin

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Campiche, Remo[1]*, Kala, Rishabh[2], Mandary, Madiiha[3], Philibert, Evans[3], Gempeler, Mathias[1], Hueber, Aline[1]

[1]DSM Nutritional Products, Kaiseraugst, Switzerland
[2]Genemarkers LLC, Kalamazoo, United States
[3]Centre International de Développement Pharmaceutique (CIDP), Phoenix, Mauritius

Introduction:

Human milk oligosaccharides (HMOs) can make up to 15% of total weight of human breast milk and there are about 200 different HMOs identified. In comparison, bovine milk oligosaccharides make up less than 1% of bovine milk and there are only about 40 different species. The amount and complexity of HMOs are unique among mammals. HMOs have found their way into infant formula to better mimic the natural composition of human breast milk. HMOs were reported to beneficially modulate the gut microbiome [1], gut epithelial barrier [2], they are anti-inflammatory [3] and good for innate immunity development [4]. Such effects are also interesting for skin care. Particularly the rise of the skin microbiome topic is prone to be a target for HMOs with their known benefits for the infant gut microbiome. In addition, other topics are of interest, too, as HMOs are implicated in protecting infants from atopic disorders [5]. It was found for example that 2'-Fucosyllactose (2'FL) was able to attenuate particulate matter induced inflammation in keratinocytes [6].

Our study is aimed at testing various HMOs for their anti-inflammatory and anti-oxidative stress activity in human skin as a response to solar irradiation such as blue light 412 nm, blue light 450 nm, and UV-B.

Materials & Methods:

The human milk oligosaccharides use in this study were obtained from Glycom A/S (DSM Early Life Nutrition), Hørsholm, Denmark.

UVB irradiation and gene-expression analysis

Human full thickness reconstructed skin (EpidermFT-400, MatTek Life Sciences, Ashland, MA, USA) was maintained at 37°C in a 5% CO_2 atmosphere with 95% relative humidity. Irradiation was done using a Hönle SOL 500 solar simulator with an H2 filter attached. UVB (200 mJ/cm^2 total dose) was monitored using a PMA2106 UVB detector (SolarLight). Tissues were placed in Dulbecco's phosphate buffered saline (DPBS) during irradiation. Cytotoxicity was monitored by Takara MK401 lactate dehydrogenase (LDH) assay (TakaraBio, Shiga, Japan). Tissues were first pre-treated topically with 0.5% HMOs in water for 24 hours and then exposed to solar irradiation. After irradiation, test substances were re-applied and incubated for an additional 24 hours before RNA isolation. RNA quality was assessed by the A260/280 method. cDNA was synthesized and amplified by qPCR using Taqman® gene expression assays in an OpenArray format on a QuantStudio 12K Flex instrument (Life Technologies, Carlsbad, CA, USA). Data analysis and statistics were run by ThemoFisher Connect Software (Life Technologies, Carlsbad, CA, USA). Linear relative quantitation (RQ) values were converted to linear fold-change values to simplify data interpretation.

Intracellular Reactive Oxygen Species (ROS) Scavenging Activity Assay

Full skin explants (Ø 8mm from surgical waste, phototype III-IV according to Fitzpatrick scale) were treated with the test items (20 µl homogeneously spread on the epidermis side with a finger cot) overnight. Afterwards a non-fluorescent probe, 2', 7'-Dichlorofluorescein diacetate (DCFH-DA) (Sigma Aldrich, MA, USA) was added. DCFH-DA is taken up by cells by passive diffusion and DCFH-DA is deacetylated by cellular esterases to non-fluorescent DCFH, which was trapped within cells. The fluorescent 2', 7'- dichlorofluorescein (DCF) was generated upon enzymatic reduction and subsequent oxidation by ROS. After treatment with DCFH-DA, the skin explants were exposed to different environmental conditions: either blue light 412nm 20 J/cm^2, blue light 450nm 20 J/cm^2 or unexposed in the dark. After exposure, tissues were lysed with 2% Triton X-100. Fluorescence was measured at 465/88nm excitation and 528/30nm emission in a Synergy HTX multimode microplate reader (BioTek, Winooski, VT, USA). Results were correlated to the test item's ability to scavenge or exacerbate ROS.

Conclusions:

In summary, we provide evidence that HMOs are attractive novel skin care molecules. Our findings highlight anti-inflammatory and anti-oxidative stress activities with distinct efficacies. As such, specific HMOs like here LNnT or 3'SL could find their way into anti-aging, photoprotection or after sun care applications to name a few.

Acknowledgements:

We are grateful to our colleagues at DSM Early Life Nutrition (Glycom) for providing us with the HMOs.

Results & Discussion:

We first investigated the effect of our UVB source on inflammation markers in the EpidermFT-400 tissue (Fig. 1).

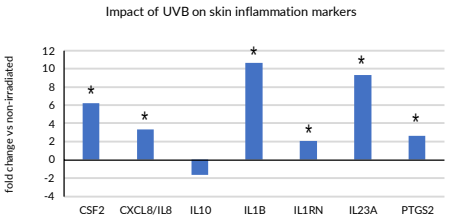

Figure 1: UV-irradiation induced inflammation markers in reconstructed human skin. Various markers such as colony-stimulating factor (CSF, interleukins (IL) and prostaglandin synthease (PTGS) were induced. *p<0.05 vs non-irradiated control.

We then looked at the effect of HMOs on the inflammation markers (Fig. 2).

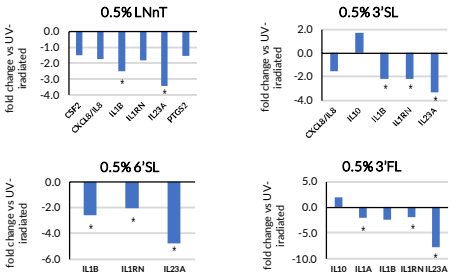

Figure 2: Effect of four HMOs on UV-induced expression of inflammation markers in reconstructed human skin. The HMOs were able to significantly reduce the expression of these markers. *p<0.05 vs UV-irradiated control.

We further investigated the anti-oxidative activity of the HMOs in response to high energy visible (blue) light irradiation (HEV) (Figs. 3 and 4).

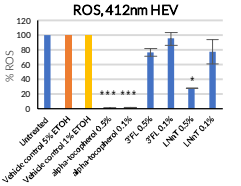

Figure 3: Effect of HMOs on HEV 412nm irradiated skin and ROS formation. Particularly LNnT was able to inhibit the formation of ROS. Alpha-tocopherol served as a reference. ***p<0.001 vs EtOH control, *p<0.05 vs untreated.

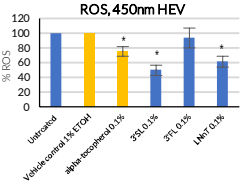

Figure 4: Effect of HMOs on HEV 450nm irradiated skin and ROS formation. 3'SL and LNnT were able to significantly inhibit ROS formation. Alpha-tocopherol served as a reference. *p<0.05 vs untreated or EtOH.

The different HMOs tested in this study showed differential anti-inflammatory activity with LNnT having the broadest activity and 6'SL having the narrowest activity against inflammatory cytokines, highlighting the various specificities of these HMOs. In addition, we found a significant down-regulation of ROS in skin after HEV irradiation at 412nm and 450nm by LNnT and 3'SL underlining the potential of HMOs to protect from major skin aging pathways.

References:

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