Panigrahi et al. (2017) Can J Biotech, Volume 1, Special Issue, Page 145
https://doi.org/10.24870/cjb.2017-a131

Canadian Journal of Biotechnology

ISSN 2560-8304

Poster Presentation **OPEN ACCESS**

Category: Miscellaneous

Modulation of inflammation and autophagy pathways by trehalose containing eye drop formulation in corneal epithelial cells: implications for dry eye disease

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Abstract

Ocular surface inflammation is an immunological perturbation activated in response to various adverse conditions and is a key biomarker to understand the disease pathology and its underlying immunological landscape [1]. The molecular link between Inflammation and autophagy, often implicated in disease conditions, is poorly understood. The aim of this study is to understand the regulation of inflammation signaling pathways by using a well-established modulator of autophagy, trehalose (TRE), on desiccation stress-induced inflammation in SV40 immortalized human corneal epithelial cells. To mimic the dry eye condition, HCE cells were exposed to desiccation stress at 80% confluency in a six well tissue culture plate. The medium was completely aspirated and cells were kept for drying at room temperature for 10 min. Fresh medium with TRE was added and incubated for 6 hrs. The regulation of induced inflammatory and autophagic gene expression and protein activation by TRE formulation (1.2%) was studied. Optimal drug treatment concentrations were determined by dose escalation cytotoxicity studies. Gene expression was evaluated by quantitative PCR, while protein expression and functions were tested by immunoblotting and fluorescence imaging (Cyto-ID, Lysotracker Red). TRE formulation was able to rescue the morphological changes due to desiccation stress. Live to dead cell ratio increased upon TRE treatment. TRE treatment reduced inflammation induced gene expression of IL-6 (2%), MCP-1 (33.31%), IL-8 (9.56%), MMP-9 (18.96%), and TNFα (58.16%) in HCE. Active form of p38, p44/42, and p65 protein levels were altered significantly by TRE treatment. LAMP1 and LC3 autophagy protein markers were also altered with desiccation stress and TRE treatment. The data demonstrate that TRE formulation is effective in reducing desiccation stress induced inflammation in HCE. Further increased phosphorylation of p38, p44/42 and elevated levels of LC3 and LAMP1 suggest that induction of autophagy. This could be a protective mechanism of autophagy in the desiccation stress model. All together our data suggest that TRE may have a novel role on reducing inflammation through autophagy in HCE. Therefore, TRE might be a potential therapeutic for ocular surface treatment.

References

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Citation: Panigrahi, T., Shetty, R., Shivapriya, S., Nelson, E.J.R., Jeyabalan, N. and Ghosh, A. Modulation of inflammation and autophagy pathways by trehalose containing eye drop formulation in corneal epithelial cells: implications for dry eye disease [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 145. https://doi.org/10.24870/cjb.2017-a131