Abstract N°: 3497

NAcM-OPT Protects Keratinocytes from H2O2-Induced Cell Damage via Promotion of Autophagy

Renxue Xiong1, Qingmei Shen2, Yan Zhao2, Xiuzu Song1, Cuiping Guan1

1Hangzhou Third People’s Hospital, Department of Dermatology, 2Zhejiang Chinese Medical University, School of Basic Medical Sciences

Introduction & Objectives: To investigate the protective effect of NAcM-OPT, a small molecule inhibitor of ubiquitine proteasome, on H2O2-induced oxidative damage in keratinocytes.

Materials & Methods: HaCaT cells were treated with NAcM-OPT under the stimulation of oxidative stress, and cell activity was detected by CCK-8 method; cell proliferation was detected by EdU method; the changes of autophagic flux were detected by mGFP-RFP-LC3 dual fluorescent autophagy indicator system; intracellular ROS were detected by DCF method; apoptosis was detected by DAPI staining; mitochondrial activity was detected by mitochondrial membrane potential. The expression of autophagy-related proteins Beclin1 and LC3 was detected by western blotting; mitochondrial morphology was observed by transmission electron microscopy; the expression of antioxidant genes was examined by qRT-PCR. Keratinocytes were supplemented with the autophagy activator rapamycin, melanocytes were added to the keratinocyte cell supernatant, and qRT-PCR was used to identify tyrosinase expression.

Results: In HaCaT cells, H2O2 stimulation led to an increase in intracellular ROS, a significant decrease in apoptosis, a slowdown in cell division, an impairment of mitochondrial activity, a decrease in the expression of the autophagy-related proteins Beclin1 and LC3, and a reduction in the antioxidant genes Nrf2, HO-1, NQO-1, and GCLM. The H2O2-induced increase in ROS and apoptosis in HaCaT cells could be attenuated by NAcM-OPT pretreatment, which also reduced cell proliferation and mitochondrial activity. In vivo experiments confirming that NAcM-OPT reduces the lack of melanin content in H2O2-stimulated mice and in vitro studies that NAcM-OPT enhances melanocyte tyrosinase expression by adding the autophagy activator rapamycin.

Conclusion: NAcM-OPT was shown to increase cell viability and cell proliferation. Moreover, NAcM-OPT showed the ability to alleviate ROS accumulation and cell apoptosis in HaCaT cells under oxidative stress. Importantly, we found that autophagic flux was improved due to increased autophagy protein expression with NAcM-OPT treated under H2O2-induced oxidative stress, which reduced susceptibility to excessive ROS. Furthermore, Rap enhanced the mRNA levels of TYR in melanocytes. In addition, NAcM-OPT depicted the ability to alleviate mitochondrial damage and restore mitochondrial function and significantly upregulated the expression of Nrf2, HO-1, NQO-1, and GCLM. Most importantly, NAcM-OPT increased epidermal thickness, follicle length, and melanin synthesis under oxidative stress in vivo. Based on these findings, NAcM-OPT activated the expression of autophagy to alleviate mitochondrial damage in HaCaT cells and enhance TYR expression in melanocytes. Therefore, NAcM-OPT has clear potential as a promising small molecule antioxidant drug for the treatment of vitiligo.