ANTIOXIDANT EFFECTS OF ANTHOCYANINS IN THE BV2 MICROGLIAL CELL LINE

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Introduction

Microglial cells are the macrophages of the Central Nervous System (CNS) and are involved in the development and protection of the brain, promoting its normal function. Microglial activation refers to the conversion of the M0 phenotype into two functional phenotypes, M1 and M2. Continuous stimulation of the M1 phenotype results in chronic neuroinflammation and oxidative stress, which are hallmarks of neurodegeneration (Figure 1).

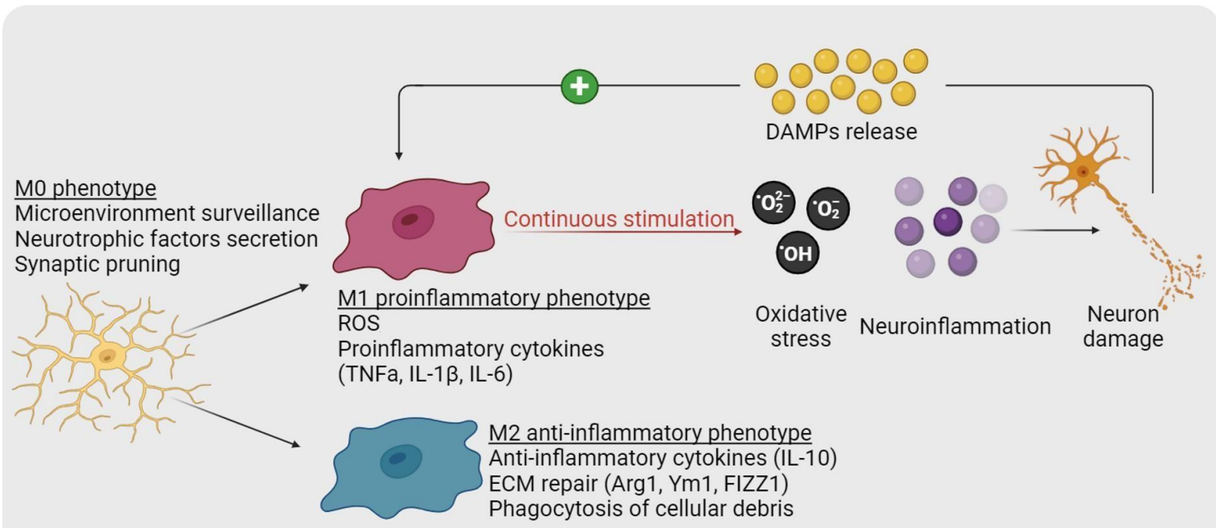

Figure 1. During the M0 phenotype, microglial cells secrete neurotrophic factors, participate in synaptic pruning, and detect changes in their microenvironment. Depending on the stimulus, microglial cells acquire M1 pro-inflammatory or M2 anti-inflammatory phenotype. In the M1 phenotype, microglial cells produce ROS as well as pro-inflammatory cytokines. On the other hand, the M2 phenotype is characterized by the production of anti-inflammatory cytokines, extracellular matrix (ECM) repair, and phagocytosis of cellular debris. Continuous stimulation of the M1 phenotype results in oxidative stress and neuroinflammation, which damage the neurons. Damaged neurons release damage-associated molecular patterns (DAMPs), which are endogenous danger signals. DAMPs can further induce inflammatory responses, thus generating a positive feedback loop that maintains inflammation and oxidative stress in the CNS. This process results in neurodegeneration and progressive neuron death.

Anthocyanins (ACNs) are water-soluble natural pigments, and they belong to the chemical class of polyphenols, which are phytochemical compounds with well-known antioxidant activities. Their antioxidant activities include the neutralization of Reactive Oxygen Species (ROS), as well as the induction of the *Keap1-Nrf2* pathway. Nrf2 is a transcription factor, which activates the expression of antioxidant genes, such as SOD, CAT, and GPx.

Materials and Methods

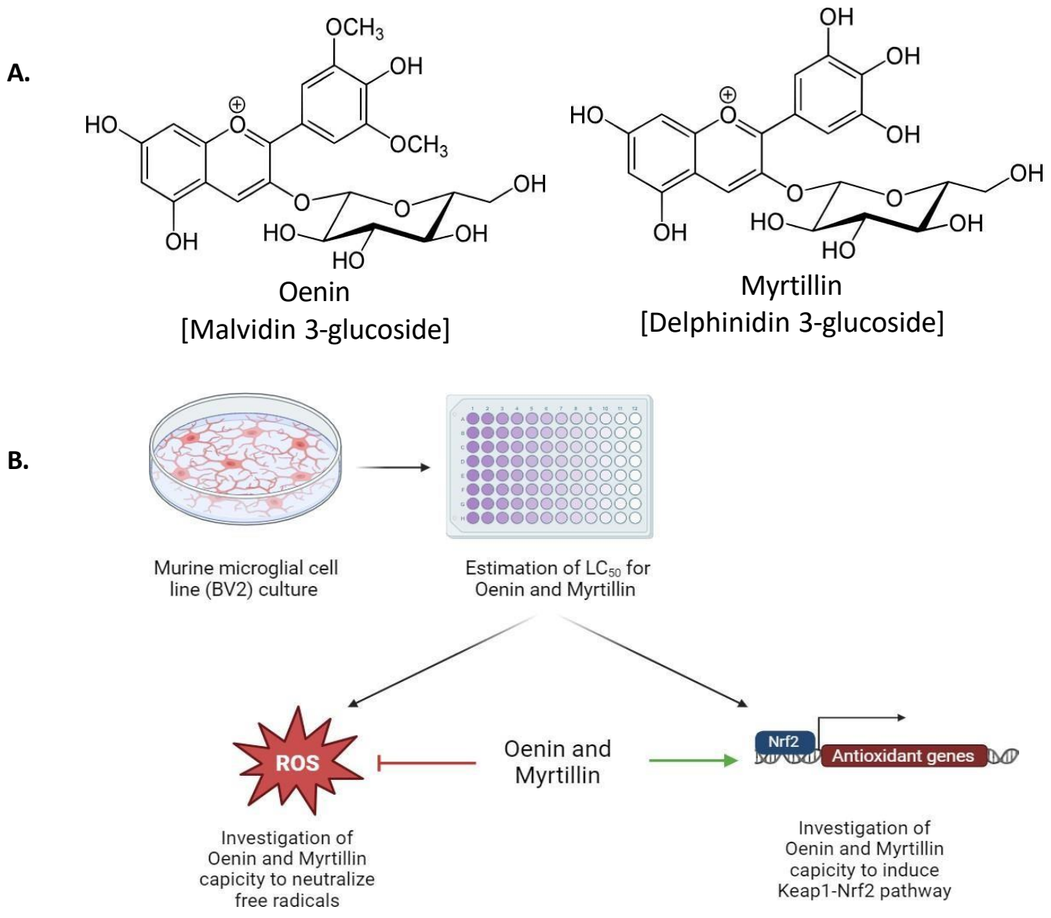

Figure 2. A) Molecular structure of two of most common ACNs in grapes, Oenin and Myrtillin. Oenin and Myrtillin are used throughout our study. **B)** The murine microglial cell line BV2 was used to assess antioxidant activity of Oenin and Myrtillin. Firstly, we estimated the Lethal Concentration 50% (LC_{50}) of both ACNs, which is the concentration of a given agent that is lethal to 50% of the cells, to calculate the bioactive concentration of ACNs (0.5 × LC_{50}). Then, their antioxidant activity was estimated by both their ability to neutralize ROS and to induce cellular antioxidant defense through the transcription factor Nrf2.

Results

Toxicity levels of Oenin and Myrtillin are comparable for BV2 Cell line

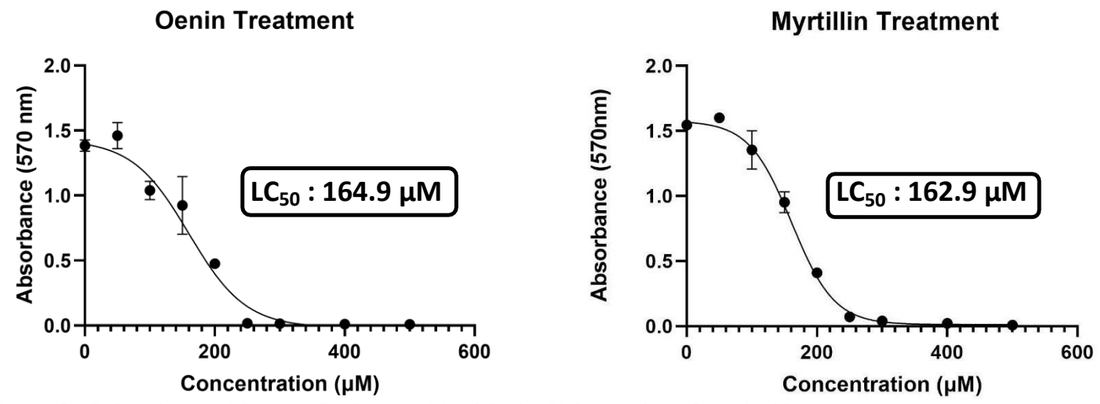

Figure 3. Estimation of LC_{50} for Oenin and Myrtillin in BV2 cell line. Cell viability was assessed by means of the 3-(4,5-dimethylthiazol-2- yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay for both ACNs following 48 hours of incubation in BV2 cells. In viable cells, the yellow MTT is converted into purple formazan crystals. Graphs depict the absorbance at 570 nm determined for BV2 cells following treatment with the indicated compounds at different concentrations. The background absorbance of the plates at 630 nm was also measured and subtracted from 570 nm measurement. LC_{50} was estimated for each compound based on non-linear regression analysis for curve fitting using the GraphPad software (v 8.0.2). The black dots in graphs for ACNs represent the fitted curve in each case; determined LC_{50} values are depicted in each graph.

Oenin and Myrtillin reduce Reactive Oxygen Species (ROS) Levels

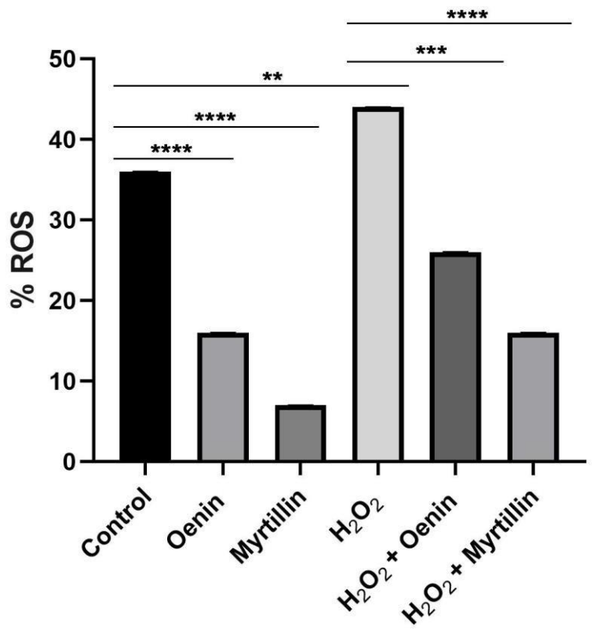

Figure 4. Reactive Oxygen Species (ROS) were measured using H_2DCFDA in BV2 cells after treatment with 0.5 × LC_{50} of each ACN, without or following pre-treatment with 600 µM H_2O_2. The % ROS was calculated based on the maximum ROS production value (3 mM). Data represent means ± SD; stars denote statistical significance (unpaired, one-tailed t-test); *: p-value < 0.05, **: p-value < 0.01, ***: p-value < 0.001.

Oenin and Myrtillin upregulate *Keap1-Nrf2* pathway

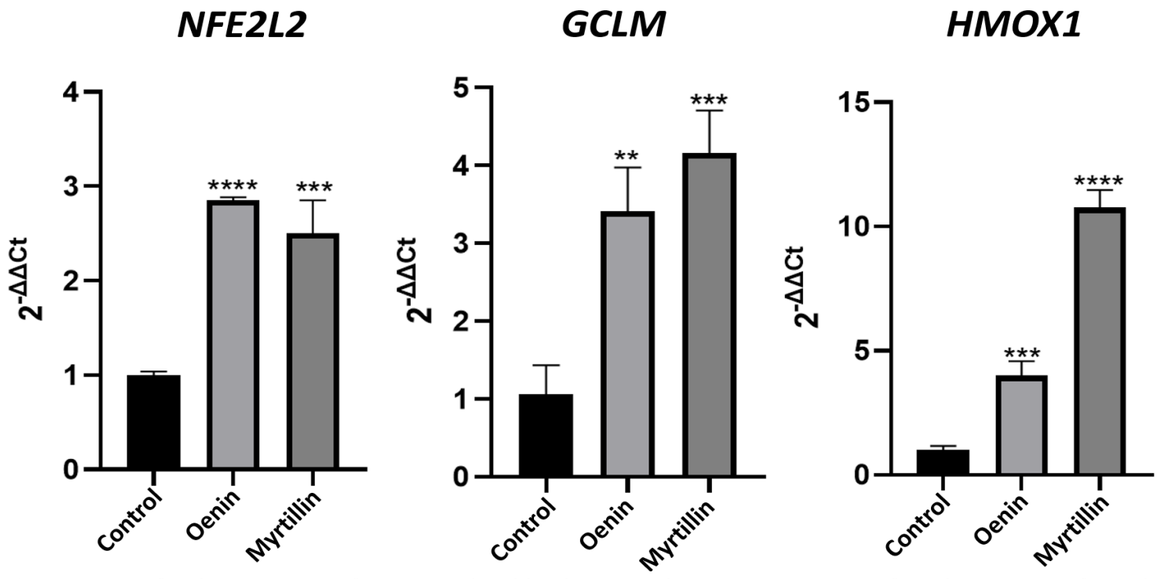

Figure 5. qPCR for the expression of genes regulated by the antioxidant response *Keap1-Nrf2* pathway was performed using mRNA extracted from BV2 cells treated with 0.5 × LC_{50} of each anthocyanin for 48 hours and compared to non-treated control cells. Data represent means ± SD of three independent experiments. Stars denote statistical significance (unpaired, one-tailed t-test); *: p-value < 0.05, **: p-value < 0.01, ***: p-value < 0.001, ****: p-value < 0.0001.

Conclusions

Our results suggest that ACNs have antioxidant activities in the BV2 cell line, as they can eliminate Reactive Oxygen Species. Furthermore, they can induce the signaling pathway of the transcription factor Nrf2, which activates the expression of cellular antioxidant defenses. Thus, ANCs could be preventative and/or therapeutic agents against neurodegenerative diseases.

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