Understanding the Interaction Between a Potential Oxidative Stress Therapeutic and the Nrf2 Antioxidant Pathway

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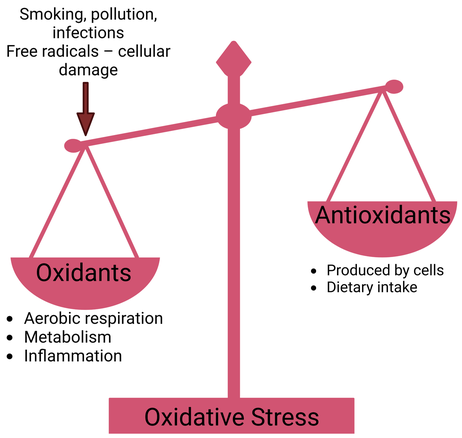
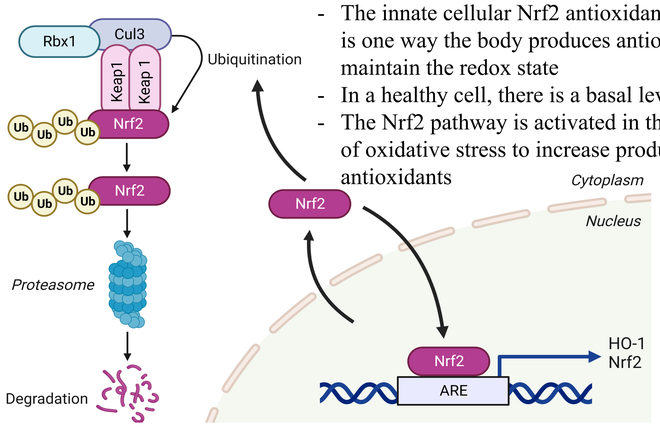
Oxidative stress caused by the imbalance between antioxidants and oxidative species is a major component of several chronic diseases such as cardiovascular disease, cancer, and some neurodegenerative diseases. Potential therapeutics have previously been explored to address the role of oxidative stress in disease, but many have been unsuccessful or only target one aspect of this multifaceted disease pathway. To address this, Dr. Green's lab at TCU created the L2 compound to act as a multimodal antioxidant therapy. Specifically, preliminary studies analyzing whole cell lysates have shown the L2 compound activating the natural antioxidant pathway of the cell – the Nrf2 pathway. Once this pathway is activated due to oxidative stress, Nrf2 is released from its inhibitor in the cytoplasm and migrates to the nucleus where it binds to various parts of the genome and begins transcription of antioxidant genes and detoxifying enzymes. This data was unexpected as the addition of antioxidant compound L2 should mitigate the need to activate the Nrf2 antioxidant pathway. Therefore, it is the purpose of this study to understand the interaction between L2 and the apparent increase of Nrf2 in cells by measuring its activation through increased Nrf2 levels in the nucleus.

Background

- The redox balance between oxidants and antioxidants plays a major role in health
- Oxidants produced in cellular processes are in balance with naturally produced or consumed antioxidants
- Excess exogenous oxidants shift the redox balance into a state of oxidative stress
- Chronic oxidative stressed is associated with several diseases

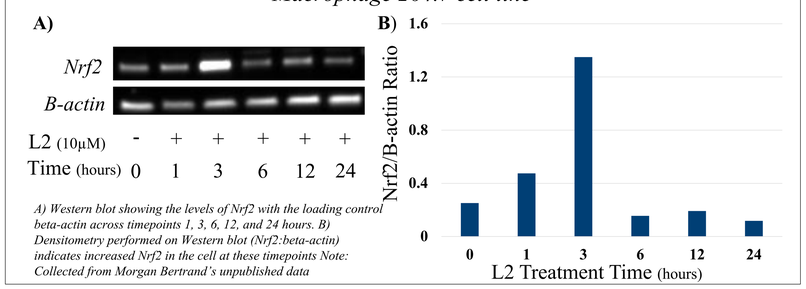


- The innate cellular Nrf2 antioxidant pathway is one way the body produces antioxidants to maintain the redox state
- In a healthy cell, there is a basal level of Nrf2
- The Nrf2 pathway is activated in the presence of oxidative stress to increase production of antioxidants



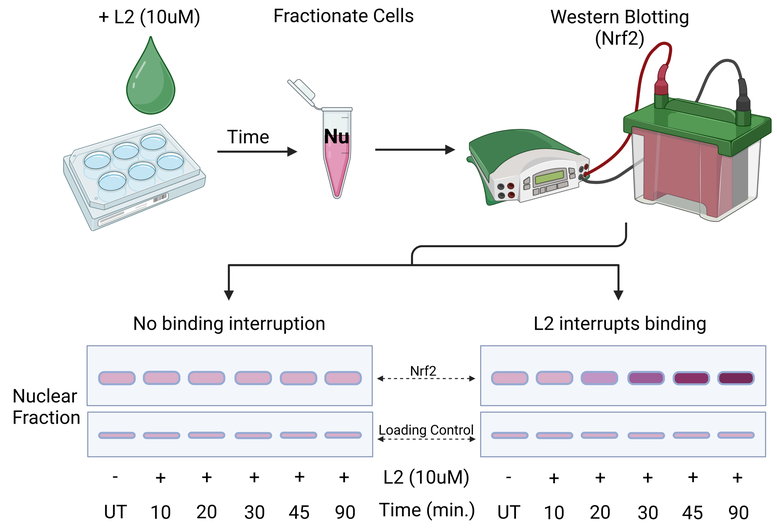
- Analysis of nuclear Nrf2 protein levels will indicate possible interference by potential antioxidant therapeutic L2 between Nrf2 and inhibitor Keap1

Effect of 10μM L2 at different time points on levels of Nrf2 in Raw Macrophage 264.7 cell line



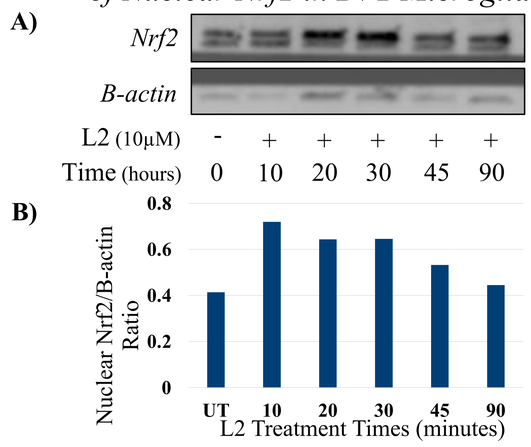
A) Western blot showing the levels of Nrf2 with the loading control beta-actin across timepoints 1, 3, 6, 12, and 24 hours. B) Densitometry performed on Western blot (Nrf2:beta-actin) indicates increased Nrf2 in the cell at these timepoints Note: Collected from Morgan Bertrand's unpublished data

Methods



Preliminary Results

Effect of 10μM L2 at different time points on levels of Nuclear Nrf2 in BV2 Microglial Cell Line



A) Western blot showing the levels of nuclear Nrf2 with loading control beta-actin across 10, 20, 30, 45, and 90 minutes. B) Densitometry performed on Western blot (nuclear Nrf2:beta-actin) indicates increased Nrf2 in nuclear fraction at 10, 20, and 30 minute timepoints.

Discussion

- Previous data shows L2 interacts with Nrf2 pathway at the 3-hour timepoint, indicating its mechanism of action could be by decreasing Nrf2 degradation or increasing transcription and/or translation
- L2 treatment at shorter timepoints should eliminate transcription or translation as the cause of the increase
- Therefore, nuclear Nrf2 protein level increase at 10, 20, and 30 minutes could be due to L2 interfering with Nrf2's ability to bind to its inhibitor Keap1, causing a reduction in proteasomal degradation of Nrf2

Future Directions

- Understand the mechanisms by which L2 induces an apparent increase in Nrf2 by examining if its effect is due to:
  a. an increase in Nrf2 gene expression
  b. interference between Nrf2 and its inhibitor Keap1
- Analyze Nrf2 gene expression by analyzing the alteration of Nrf2 gene transcription through qRT-PCR and the alteration of Nrf2 protein synthesis through treatment with puromycin
- Analyze Nrf2:Keap1 interaction through repeating nuclear Nrf2 level isolation and transfecting cells with tagged Keap1 to analyze inhibited binding

References

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