The Effects of Antioxidant Therapy on Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2) Expression in Phagocytic Cells

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Oxidative stress is an imbalance of reactive oxygen species (ROS) and antioxidant defenses resulting in cell damage and chronic inflammation. ROS are unstable oxygen molecules produced during regular metabolic processes in the cell or accumulated from exogenous sources, including radiation, infection, and a high-fat diet. Chronic oxidative stress contributes to many disease state pathologies, including neurodegenerative disorders, cardiovascular disease, diabetes, and cancer. All cells express nuclear factor-erythroid 2-related factor 2 (Nrf2) to mitigate excess ROS production. Nrf2 is a transcription factor that promotes the expression of antioxidant enzymes, such as heme oxygenase-1 (HO-1). Our study targets the expression and activation of Nrf2 in cells treated with L2, a compound created by Dr. Kayla Green (TCU Chemistry) and her colleagues. Our lab previously demonstrated the antioxidant capability of L2 and its ability to protect microglial and neuronal cells from oxidative stress. Our current research aims to examine the therapeutic potential of this compound by monitoring Nrf2 and antioxidant levels in macrophages and microglia. This research could provide preliminary evidence for the efficacy of this compound as a treatment option for oxidative stress diseases.

Introduction

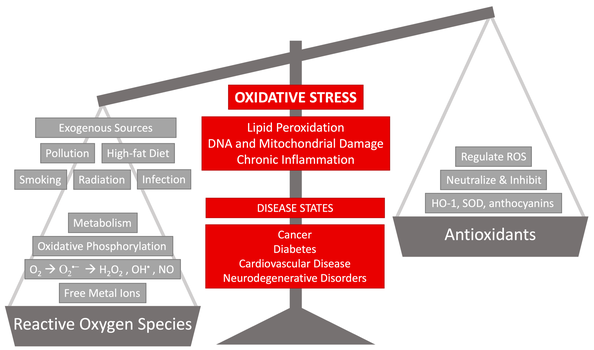


Figure 1. An imbalance between ROS and antioxidant defenses in oxidative stress.

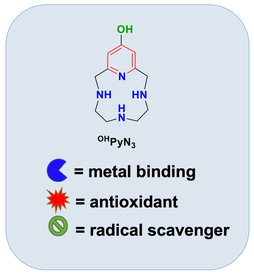


Figure 2. L2 antioxidant defense properties.

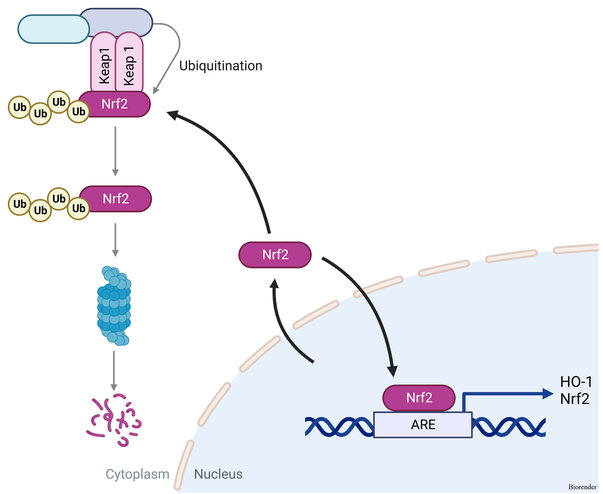
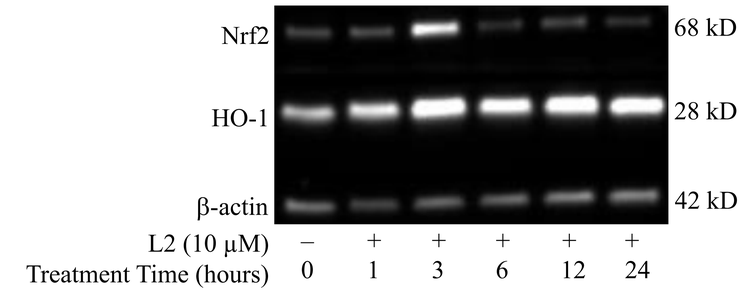


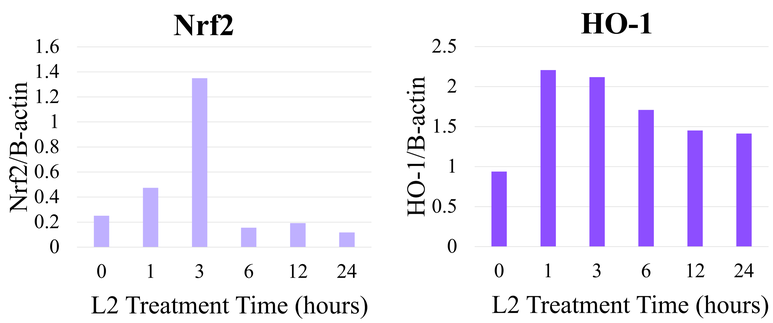
Figure 3. The Nrf2 pathway and regulation mechanisms.

Methods

- Immortalized BV2 microglial cells and RAW 264.7 macrophage cells were cultured in complete DMEM media and maintained at 37 ºC and 5% CO_2. Cells were passed around 80% confluency .
- Cells were treated with 10 µM L2 for varying timepoints (0, 1, 2, 3, 6, 12, or 24 hours). Following treatment, cells were lysed, and protein levels were analyzed using Western blots.

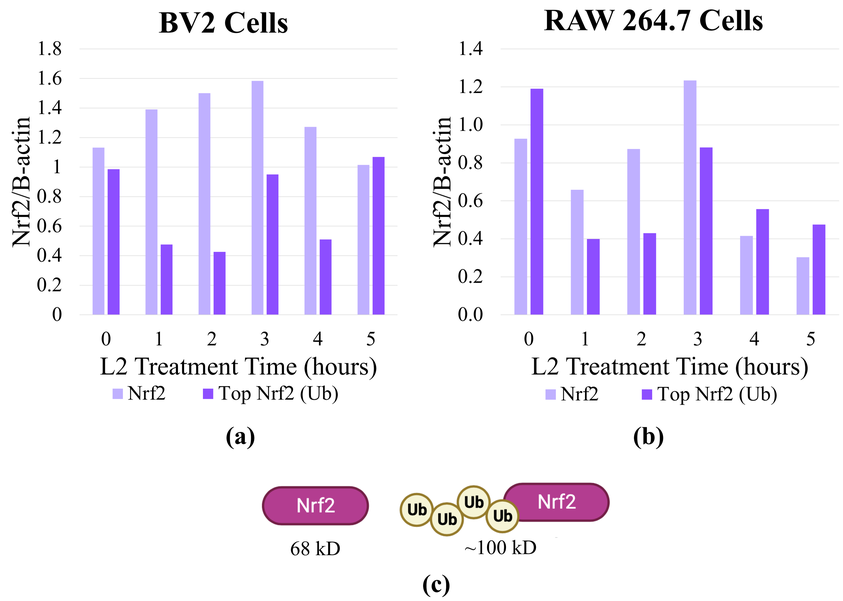
Results





(a) **(b)**

Figure 3. Evaluation of Nrf2 and HO-1 levels. RAW 264.7 cells were treated with 10 µM L2 for 0, 1, 3, 6, 12, or 24 hours. Western blots were used to analyze the concentration of (**a**) Nrf2 and (**b**) HO-1. Densitometric estimation was conducted using β-actin as a loading control.



(a) **(b)** **(c)**

Figure 3. Evaluation of Nrf2 and ubiquitinated Nrf2 levels in (**a**) BV2 and (**b**) RAW 264.7 cells. Cells were treated with 10 µM L2 for 0, 1, 2, 3, 4, or 5 hours. Western blots were used to analyze Nrf2 concentration and densitometric estimation was conducted using β-actin as a loading control. (**c**) Bands appearing at 68 kD is native Nrf2 and bands appearing at 100 kD is likely ubiquitinated Nrf2.

Conclusions

- Repeat experiments for statistical analysis and replicate experiments using other novel compounds created by Dr. Green and colleagues.
- Analyze nuclear Nrf2 levels and mRNA levels to investigate potential L2 mechanisms.

Future Directions

- Nrf2 increases following L2 treatment.
- Ubiquitinated Nrf2 decreases following L2 treatment.
- HO-1 levels increase following L2 treatment.

Funding

This research was funded by an undergraduate Science and Engineering Research Center award, a grant provided by the John V. Roach Honors College, and a grant provided by the Pre-Health Professions Institute. The project is also funded by the National Institute of Health (NIH).

Resources

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