Testing the Effect of Novel Antioxidant Compounds on the Activation of the Antioxidant Gene Activator Nrf2

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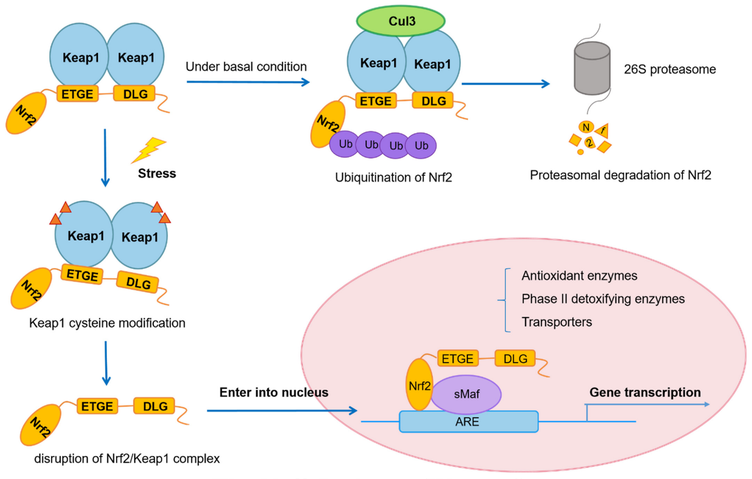
The presence of Reactive Oxidative Species (ROS) in the brain have been linked to the etiology of Alzheimer's disease and neurodegeneration. In this project, novel antioxidant Indole derivative drugs were tested on BV-2 microglial cells using RT-qPCR to assess their ability to activate antioxidant gene expression. Nuclear factor erythroid 2–related factor 2 (Nrf2) is a gene transcription factor that is activated by oxidative stress and binds to a sequence called the Antioxidant Response Element (ARE), a region upstream of the DNA promoter sequence. Nrf2 activates transcription of antioxidative genes. Based on theoretical docking studies, we hypothesize that the novel compounds will disrupt the interaction between Nrf2 and its inhibitor KEAP, releasing Nrf-2 and enabling it to translocate to the nucleus. The novel antioxidant drugs should either increase the transcription of Nrf2-activated genes or reduce overall levels of antioxidative stress within cells. We tested for antioxidant properties by measuring Hemeoxygenase-1 (HO-1) and Nrf2 mRNA levels in BV-2 cells in the presence of these compounds

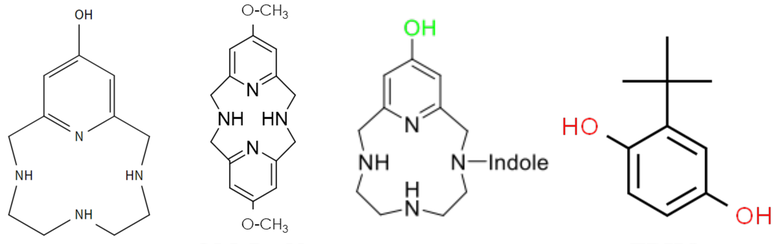
Introduction

- Alzheimer's disease (AD) is the leading form of dementia
- Pathology stems from an accumulation of beta-amyloid plaques and neurofibrillary tangles (NFT) made up of hyperphosphorylated tau fibers in the brain
- More than 6 million Americans suffer from AD
- Accumulation of plaques and NFTs leads to inflammation which increases oxidative stress
- Oxidative stress activates the innate antioxidative cellular response via activation of the Nrf2 pathway
- Hemeoxygenase-1 (HO-1) is an Nrf2-activated antioxidative gene present within microglial and neuronal cells

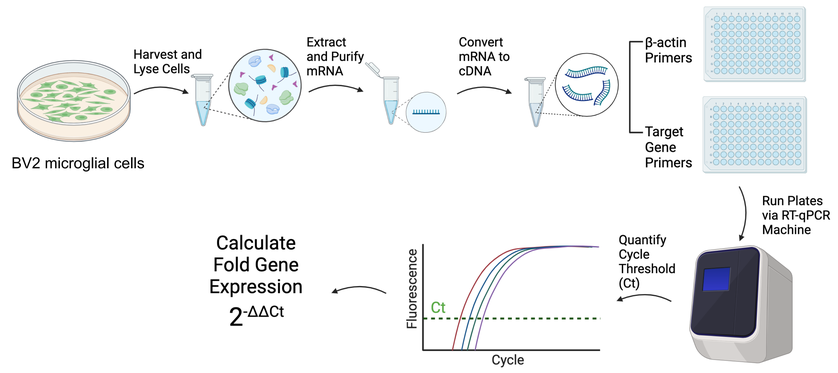


The Nrf2 Pathway (Wu et. al)

Compounds used in this study



Methods: quantitative RT-PCR (qPCR)



Conclusions

Controls

- TBHQ, a known inhibitor of Nrf2/KEAP1 binding, was shown to consistently activate Nrf2-induced expression of HO-1
- BSO, a known inducer of direct oxidative stress, was shown to consistently activate Nrf2-induced expression of HO-1 in a dose-dependent manner

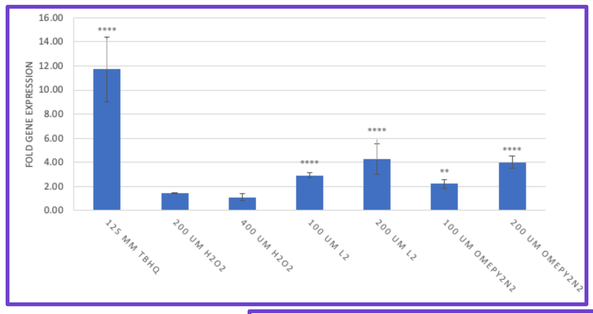
Experimental

- PK60, at 12 μM, was shown to induce a 5-fold increase in HO-1 gene expression, signaling a disruption in the inhibition of KEAP1 on Nrf2
- L2 and $OMePy_2N_2$ were both shown to increase HO-1 gene expression in a dose-dependent manner, confirming computer modeling of antagonism against Nrf2/KEAP1 binding

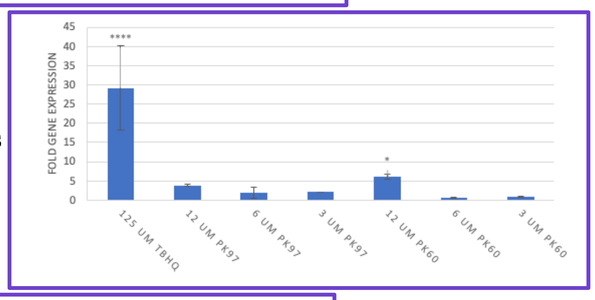
Funding and Acknowledgements

- Thank you to Dr. Akkaraju and Dr. Green for your support!
- And to Dr. Luque and Dr. Gil for serving on my honors committee!

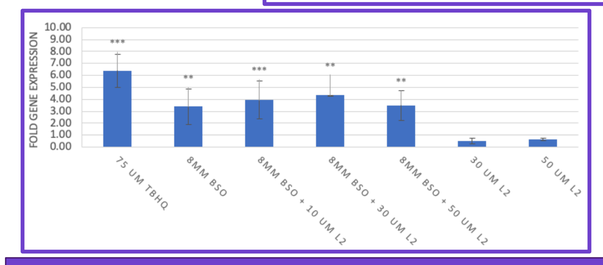
Results



Fold Gene expression of HO-1 in BV2 cells treated with TBHQ, H_2O_2, L2, and $OMePy_2N_2$



Fold Gene expression of HO-1 in BV2 cells treated with TBHQ, PK97, and PK60



Fold Gene expression of HO-1 in BV2 cells with TBHQ, BSO, and L2

Future Directions

- Find consistent Negative Controls for future experiments
- Further explore the mechanism of action of the multimodal drugs to better understand how Nrf2 activation

Resources

Johnston, Hannah M., et al. "Enhancement of the antioxidant activity and neurotherapeutic features through Pyridol addition to tetraazamacrocyclic molecules." *Inorganic Chemistry,* vol. 58, no. 24, 27 Nov. 2019, pp. 16771–16784, https://doi.org/10.1021/acs.inorgchem.9b02932.

Wu, Shijia, et al. "Nrf2 in Cancers: A Double-edged Sword." *Cancer Medicine*, vol. 8, no. 5, 2019, pp. 2252-2267, https://doi.org/10.1002/cam4.2101. Accessed 28 Mar. 2024.

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