Cannabigerol Inhibits Cellular Senescence in Methionine-Choline Deficient Diet Induced Non-Alcoholic Steatohepatitis Mice Model

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Background:

- Non-alcoholic steatohepatitis (NASH) is characterized by extreme fibrosis and inflammation and is the advanced form of non-alcoholic fatty liver disease (NAFLD).
- Methionine-choline deficient (MCD) diet is known to propagate NASH over short periods of time.
- Cannabigerol (CBG) is a non-psychoactive, uncontrolled cannabinoid that is available as a Hemp extract.
- CBG has shown anti-inflammatory and neuroprotective effects, but effects on NASH are unknown.
- Senescence is the process of cell aging and degradation and can be viewed as a negative in tissue.

Aims:

- Long Term Goal
  - Evaluate the therapeutic potential of CBG on the progression of NASH by using the MCD diet to induce NASH in a mouse model.
- Specific Aim
  - Evaluate the effects of CBG on cellular senescence.

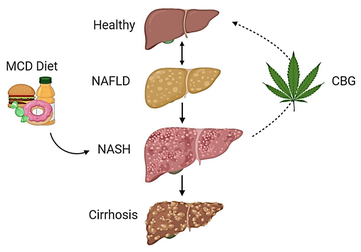

Figure 1. Schematic image of proposed hypothesis.

Methods

- **Animal**
  - Eight-week-old mice were housed for five weeks.
- **MCD diet**
  - Mice were fed three times per week and separated into either control or MCD diets; fed for five weeks.
- **CBG treatment and dosages**
  - CBG treatment was IP injected starting in the 4th week.
  - Dosages: high (24.6 mg/kg/day), low (2.46 mg/kg/day).
- **Senescence Staining**
  - Beta-galactosidase and Nuclear Fast Red Staining were utilized to visualize senescence and morphology.
- **qRT-PCR**
  - Gene expression was assessed through qRT-PCR of associated genes (p15, p16, p21, p53).

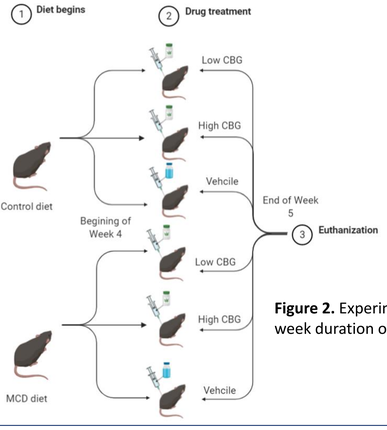

Figure 2. Experimental design during five-week duration of mouse treatments.

Increased Senescence in MCD Groups and Decreased Senescence with CBG

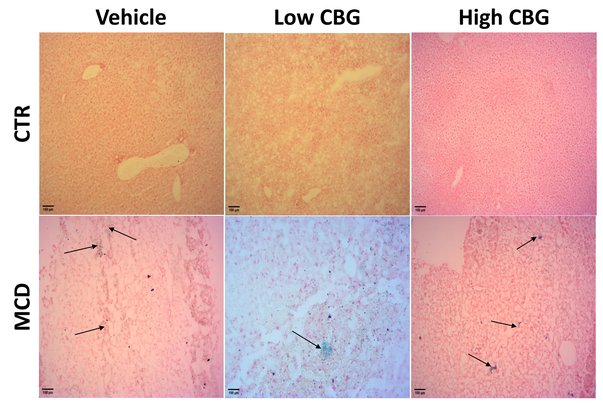

Figure 3. Beta-galactosidase (SA X-Gal) and Nuclear Fast Red staining's were obtained via frozen tissues and O.C.T Reagents. Control group tissues showed negligible levels of senescence or altered morphology. The MCD Vehicle and High CBG groups showed heightened levels of senescence compared to the control. The MCD Low CBG group showed decreased senescence compared to the MCD High or Vehicle.

Gene Expression of p15 and p16 Senescent Genes. Expression Increases in MCD Groups Compared to the Control.

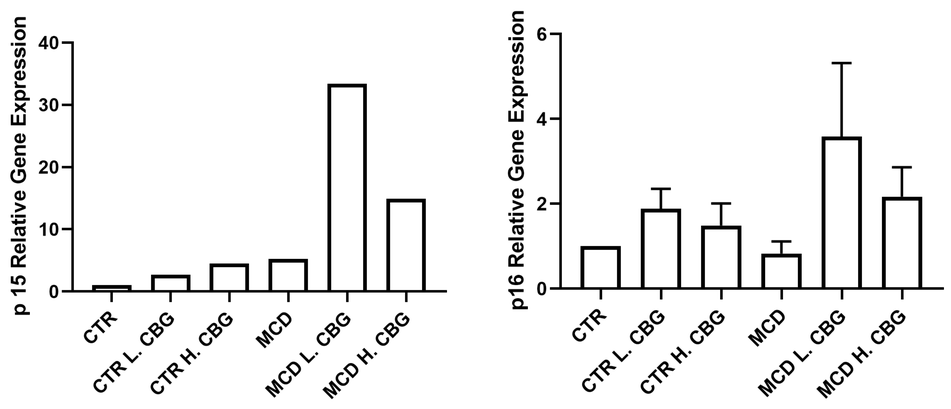

Figure 4. PCR gene expression of p15 and p16. Relative levels of expression show decreased levels in control groups compared to MCD groups. MCD Low CBG groups displayed increased levels compared to MCD Vehicle. Levels decreased with MCD High CBG compared to Low CBG.

Gene Expression of p21 and p53 Senescent Genes

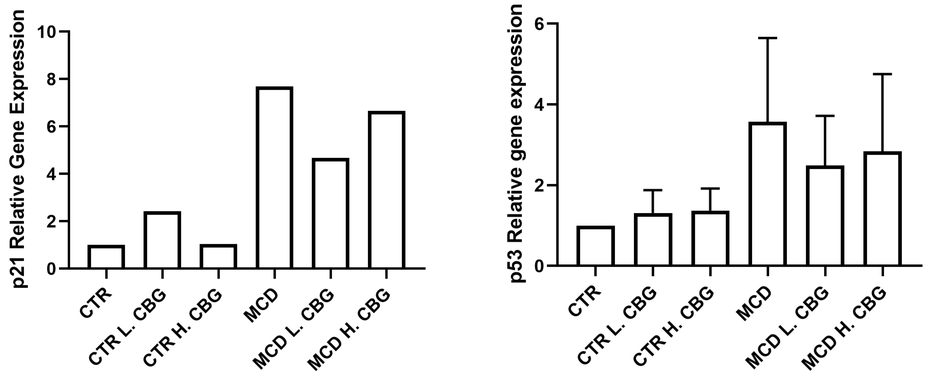

Figure 5. PCR gene expression of p21 and p53. Levels of gene expression show decreased levels in MCD Low CBG compared to MCD Vehicle. Expression increased with MCD High CBG relative to MCD Low CBG but remained below MCD Vehicle. Relative control levels remained low compared to MCD groups.

Selected References:

1. Navarro, G., Varani, K., Reyes-Resina, I., Sánchez de Medina, V., Rivas-Santisteban, R., Sánchez-Carnerero Callado, C., … Franco, R. (2018). Cannabigerol Action at Cannabinoid CB1 and CB2 Receptors and at CB1–CB2 Heteroreceptor Complexes. Frontiers in Pharmacology, 9. https://doi.org/10.3389/fphar.2018.00632
2. Pickens, M. K., Yan, J. S., Ng, R. K., Ogata, H., Grenert, J. P., Beysen, C., … Maher, J. J. (2009). Dietary sucrose is essential to the development of liver injury in the methionine-choline-deficient model of steatohepatitis. Journal of Lipid Research, 50(10), 2072–2082. https://doi.org/10.1194/jlr.m900022-jlr200

Summary:

- Senescence appears to decrease in MCD Low diets from staining.
- MCD diet induces greater gene expression of senescent genes relative to the control.
- Low dose of CBG supplementation decreased relative cellular senescence in p21 and p53 genes.
- Senescent gene expression increases in p15 and p16 genes for MCD Low compared to Vehicle.

Conclusion:

- Low CBG dose appears to show inhibition of cellular senescence compared to a vehicle in some cases. The overall association between senescence and MCD associated NASH is still to be determined.
- CBG inhibition of senescence could be better defined in future studies.

Future Direction:

- Expansion of groups for more extensive senescence profiles.
- Investigate other possible gene associations of NASH and MCD diet.
- Investigate the long-term effect of CBG on the liver
- Effects of CBG on secondary organs (pancreas, spleen).
- The relationship between Cannabinoid receptors and NASH.

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