Up-regulation of inflammatory biomarkers in BEAS-2B lung cells exposed to biodiesel PM

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Introduction

Biodiesel is a more sustainable fuel, but research is contradictory on the effects of particulate matter (PM) from combustion of biodiesel on bronchial inflammation in vitro. The literature suggests that diesel exhaust particles induce CYP1A1 at low concentrations in BEAS-2B cells (a human lung epithelial line), whereas COX-2 expression occurs at higher concentrations (Totslandal et al. 2010; Fukigawa et al. 2013). CYP1A1 has been suggested as a sensitive biomarker for diesel exposure, but few studies exist for biodiesel exposure (Caughey et al. 2001). No known studies exist that examine biodiesel particulate matter from real world operation of nonroad diesel engines. Questions remain though, regarding the specific pathway of inflammation. Our goals are to compare the expression of CYP1A1 and COX-2 mRNA in BEAS-2B cells after exposure to PM from biodiesel and diesel fuel. Quantitative real time PCR will be used to monitor these sensitive biomarkers. Previous work in the Traviss lab suggests the following model of potential pathways for Reactive Oxygen Species production (Figure 1).

Our main hypothesis is as follows:

(1)There is a significant difference in levels of CYP1A1 and COX2 expression in BEAS-2B cells treated with PM from diesel vs. biodiesel fuel combustion.

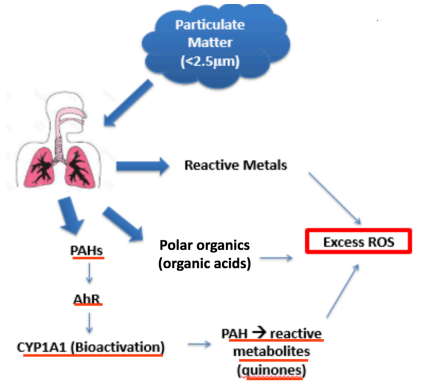

Figure 1: Potential pathways linking $PM_{2.5}$ exposure to increased ROS production. High ROS levels have been associated with diabetes, neurodegenerative disorders, and age-related diseases such as Alzheimer's disease, Parkinson's disease and cardiovascular diseases (Qin et al. 2006; Zhang et al. 2000; Brieger et al. 2012). Respiratory silhouette image courtesy of http://www.123rf.com/clipart-vector/human_lung.html

Methods

Field Site & Sample Collection:

- During the summer of 2018, real world biodiesel particles were collected from the tailpipe of a non-road front loader (John Deere 624K Tier 3 engine) that is used daily at the Keene Recycling/Transfer Station.
- The John Deere 624K is fueled by a commercial B20 blend (20% biodiesel, 80% petroleum diesel).

Cell Culture:

- BEAS2-B cells will be cultured in Lonza BEGM in 75 mL flasks with vented caps.
- When the cells can be seeded at around 120,000 cells per well, they will be transferred to different 6 well plates.
- According to current literature, BEAS2-B lung cells will express CYP1a1 in a higher quantity than PTGS2 when exposed to diesel particles as opposed to biodiesel.

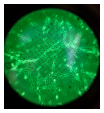

Figure 2. BEAS-2B epithelial lung cells at approximately 40% confluency under 400x magnification

Experimental Design:

- In the case of diesel particles, NIST particles will be used in place of real world diesel particles.
- NIST particles are well analyzed, and are quality controlled.
- Real world biodiesel particles have been collected from the tailpipe of a non-road vehicle.
- This John Deere 624K Tier 3 engine is used daily at the Keene Transfer station.
- It runs on B20 biodiesel.
- BEAS2-B cells will be cultured in Bronchial life basal medium, and passaged nine times in 75 mL flasks with vented caps
- When the cells can be seeded at around 120,000 cells per well, they will be transferred to different 6 well plates.
- Each 6 well plate will contain a row of positive controls, and a row of a treatment.
- The three treatment levels of particles will be as follows: 0 µg/mL, 25 µg/mL, and 100 µg/mL
- After 4 hours, which is around when most of these genes are most highly expressed, the cells will be taken from the wells, and total RNA will be extracted.

qrt-PCR:

- Once the RNA has been isolated from the sample of cells from the given well plate, the Applied Biosystems High Capacity cDNA archive kit will be used to make cDNA.
- 200ng RNA in total volume of 10 µL of water is used for each reaction.
- The gene that codes for 18S rRNA will be used as a housekeeping gene, to standardize the expression of the other genes, PTGS2 and COX-2

Anticipated Results

- When exposed to DEP, the genes CYP1A1 and PTGS2 are more highly expressed in BEAS-2B cells compared to when they are exposed to biodiesel particles, and the level of mRNA found in the cells for these genes compared to a housekeeping gene (18S rRNA) will increase with higher levels of exposure to DEP (Totslandal et al. 2010).
- The Traviss Lab along with Nathan Martin and Rachel Klaski showed in their cytokine profile of the pro-inflammatory response to diesel when compared to real world biodiesel particles (Figure 3), and found that IL-8 was more abundant in cells exposed to diesel particles than biodiesel. This would indicate that our results should show more of an inflammatory response from diesel than biodiesel, meaning that PTGS2 and CYP1A1 will not be as highly expressed in the biodiesel group (Martin et al. 2017).

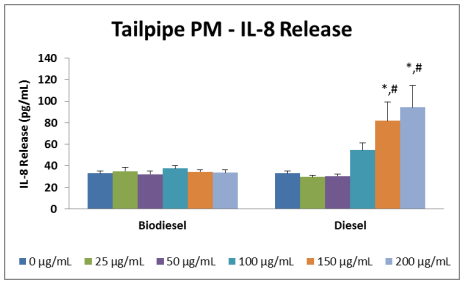

Figure 3: Pro-inflammatory response measured by IL-8 release from BEAS-2B epithelial lung cells in response to increasing concentrations of petroleum diesel and B20 PM. Asterisk (*) indicates significant difference from control ($p < 0.05$). Pound (#) indicates a significant difference between fuel types at the 150 and 200 µg/ml treatment concentrations ($p < 0.05$). Error bars represent standard error from the mean.

- The abundance of IL-8 is a good indicator of the same nature of pro-inflammatory response by BEAS-2B cells, particularly as it relates to COX-2, and some studies have found that the pathway that triggers NF-kB and IL-8 actually leads to the transcription of COX-2, thus creating protein that oxidizes diesel exhaust particles, and can create more ROS (Totslandal et al. 2010; Hawley et al. 2014).
- After 4 hours, we will see upregulation of CYP1A1 and COX-2, proportional with the concentration of DEP (Fukagawa et al. 2013).

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