AGRICULTURAL RESEARCH FOUNDATION
INTERIM REPORT
FUNDING CYCLE 2020 – 2023

TITLE: A Novel In Vitro Model to Assess Phytochemical Intervention In Respiratory Disease

RESEARCH LEADER: Susan Tilton

EXECUTIVE SUMMARY:

Overall, the role of diet and nutrition in respiratory health is not very well understood; however, evidence suggests an important role of diet in lung disease. We have previously observed that animals on a high fat diet show enhanced inflammation and reduced antioxidant capacity in the lung contributing to an increased susceptibility for inflammation-based respiratory diseases, such as asthma and chronic obstructive pulmonary disease (COPD), after inhalation exposure to chemical pollutants. In addition, several studies indicate an important role for dietary phytochemicals in protecting against specific respiratory disease mechanisms through regulation of anti-inflammatory and anti-oxidative processes. In particular, studies indicate that sulforaphane, an isothiocyanate derived from cruciferous vegetables, may also be protective towards chemical-induced airway inflammation and oxidative stress that contributes to respiratory disease. Historically, the lack of appropriate in vitro models has limited effective evaluation of phytochemicals on respiratory disease endpoints. Traditional *in vitro* culture systems, which include a number of transformed cell lines amenable to unlimited passaging, lack the structure, function, multicellular communication and metabolic capacity of the tissues they are intended to model. To address current limitations associated with *in vitro* studies of chemical toxicity, we are utilizing a 3D reconstructed human bronchial epithelial (HBEC) culture model to evaluate the effectiveness of sulforaphane on respiratory disease endpoints *in vitro*. To our knowledge, these studies are the first to evaluate the effects of sulforaphane using advanced organotypic lung culture models to better recapitulate a response in humans.

OBJECTIVES:

We hypothesize that the phytochemical sulforaphane will have anti-oxidant effects in HBEC and will protect against barrier dysfunction cause by PAH chemical treatments through induction of NQO1 and subsequent reduction in oxidative stress and inflammation endpoints. We propose the following aims:

Aim 1. Assess the role of sulforaphane, an isothiocyanate from cruciferous vegetables, to modulate NRF2 targets NQO1 and GSTP1 in normal and diseased HBEC both in the presence and absence of chemical toxicants BAP and DBC.

Aim 2. Identify global mechanisms regulated by sulforaphane in HBEC using RNAsequencing.

PROCEDURES:

For Aim 1, HBEC cells (passage 1-2; Lonza, Walkersville, MD) from normal and asthmatic donors will be cultured and expanded in bronchial epithelial growth medium (Lonza). Transepithelial electrical

resistance (TEER) will also be quantified as a functional measure of differentiation. Differentiated HBEC cells (normal and diseased donor) will be exposed to a dose-response of sulforphane for 48 hrs (N=4 replicates) in the presence or absence of 250 ug/ml BAP and 10 ug/ml DBC, based on prior studies. Cytotoxicity, TEER, pro-inflammatory cytokine IL-8 release, and oxidative stress will be measured.

For Aim 2, total RNA will be isolated from treated normal and asthmatic HBEC (N=4) treated for 48 hrs (Qiagen RNAeasy mini kit) and quantified by NanoDrop ND-8000 (ThermoScientific, Cole-Palmer, Vernon Hills, IL) for sequencing through the OSU Center for Genome Research and Biotechnology. We will identify mechanisms of toxicity through bioinformatics approaches, including unsupervised clustering, functional pathway analysis and transcription factor enrichment of differentially expressed mRNA and lncRNA ($q<0.05$).

SIGNIFICANT ACCOMPLISHMENTS TO DATE:

We have completed important parts of Aim 1 to optimize testing of sulforaphane (SFN) and another dietary micronutrient, diindolylmethane (DIM), in combination with PAH chemicals in our 3D lung cell model and to optimize specific assays with our cells for oxidative stress and cytotoxicity in a 96-well plate format using fluorescent-based assays. Our current data shows that SFN, in particular, reduces oxidative stress induced in lung cells after treatment with different PAH chemicals, including benzo[a]pyrene (BAP) and dibenzo[def,p]chrysene (DBC) (Figure 1). These range-finding studies will provide preliminary data needed for testing chemicals in combination with SFN in the 3D lung model for sequencing in

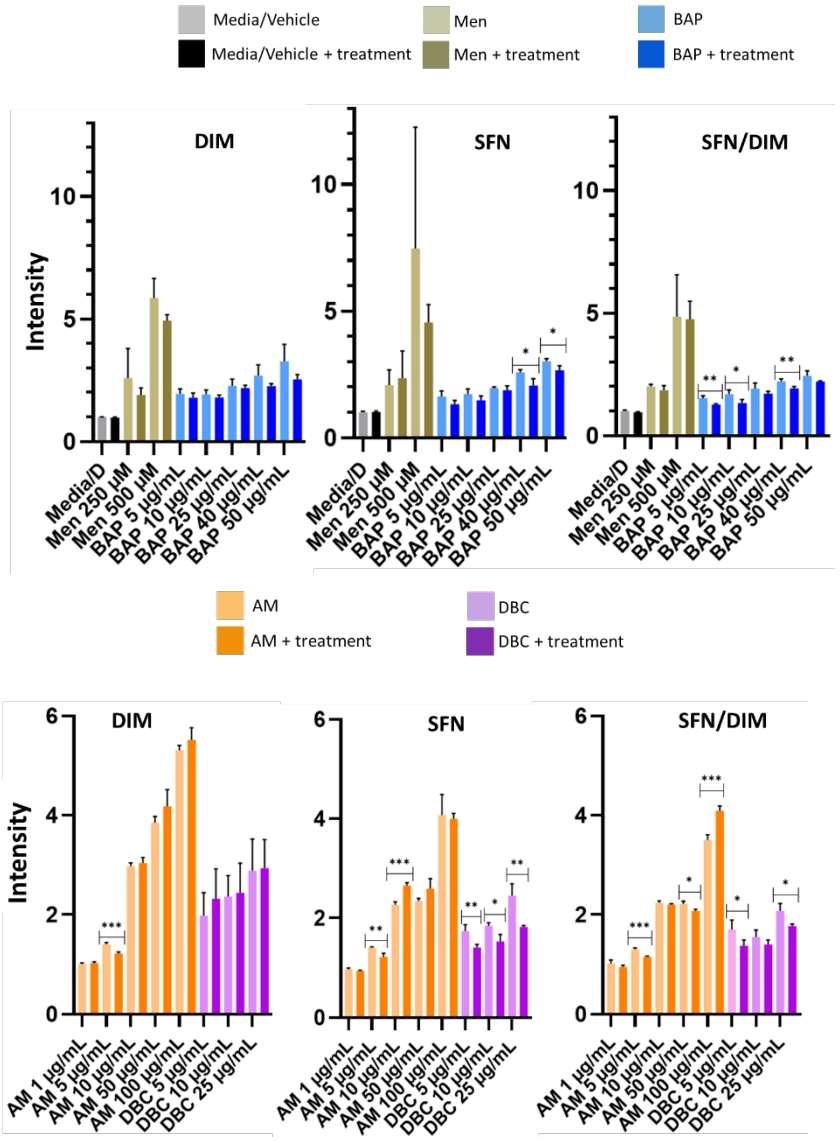

Figure 1. Oxidative stress measured in lung epithelial cells by 2',7' – dichlorofluorescin diacetate (DCFDA) fluorescent-based assay. Cells were treated with several PAH chemicals or mixtures, including BAP, DBC or a simulated air mixture from Beijing, China, in the presence or absence for sulforaphane (SFN) or diindolymethane (DIM) micronutrients.

Aim 2. We are also in the process of developing new assays related to our endpoints and collecting samples for RNAseq analysis.

ADDITIONAL FUNDING RECEIVED DURING PROJECT TERM:

A new 5-year grant was awarded from NIEHS titled, "Linking PAH Exposure to Health Outcomes Using Human Primary *In Vitro* Respiratory Model" from NIEHS as part of the OSU Superfund Research Program. Funding for this award began in April 2020.

FUTURE FUNDING POSSIBILITIES:

We will plan to submit data collected as part of this project as preliminary data for grant submissions to the NIH and explore opportunities with USDA NIFA.