Sulforaphane Pre-treatment Improves Cytoprotection Against Opportunistic Pathogens

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Introduction

Binge alcohol intoxication (≥0.08% BAC) has been recognized as a serious risk factor for opportunistic pulmonary bacterial infections. These infections include but are not limited to melioidosis and pneumonia caused *by B. pseudomallei* and *S. epidermidis*, respectively. The exact mechanisms by which alcohol causes multi-organ injury remains unclear, but a major focal point appears to be alveolar macrophage dysfunction. Currently, immuno-treatments to mitigate the toxic effects of binge alcohol intoxication have not been established. One promising phytonutrient that can be used as an immuno-treatment is sulforaphane (SFN). Well known for its antioxidant effects, we set out to determine whether SFN could help prevent infectious onset in alcoholic states.

Objectives of this experiment were to 1) measure the effects of SFN + alcohol on macrophage viability, 2) measure the macrophage killing effect when challenged with SFN + alcohol 3) compare the consistency of phagocytic ability of SFN treated macrophages in both murine and human cell lines, 4) compare phagocytic ability of SFN treated macrophages against both gram-positive and gram-negative pathogens, and 5) measure changes in expression of cellular oxidant regulator Nrf2.

Hypothesis

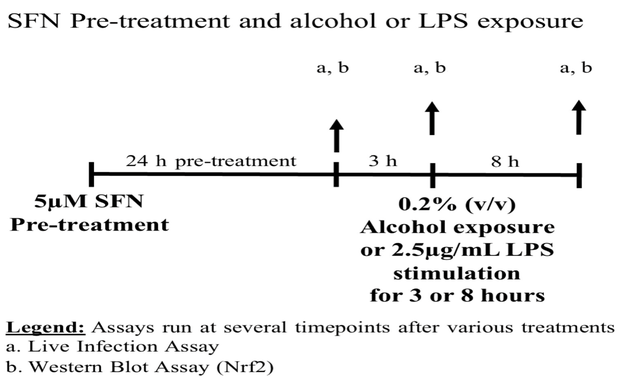
Pre-treatment of MH-S and THP-1 cells with SFN would 1) be biologically relevant and safe, 2) restore critical macrophage phagocytic responses, 3) provide consistent results in both MH-S and THP-1 cell lines, 4) provide protection against both gram negative, and positive pathogens and 5) increase Nrf2 expression in response to infection. .

Materials and Methods

Cells were cultured with 0, 2.5, 5, 10, 20, 50 uM SFN for 2 or 24 h (Fig. 1). Similar procedure done with alcohol (Fig.1).

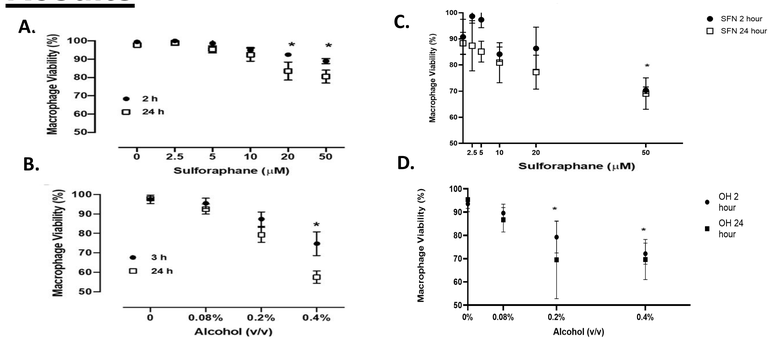
MH-S cells were then infected with live *B. thailandensis* E264, and at 3 and 8 hours washed to remove extracellular bacteria, and then lysed. Intracellular bacteria were plated for CFU count (Fig. 2 A). THP-1 cells were infected with live *S. epidermidis,* at 3 and 8 hours washed, and then lysed. Remaining intracellular bacteria were plated for CFU count of surviving bacteria (Fig. 2 B).

To determine Nrf2 change, Western blot was performed using rabbit monoclonal antibodies. Quantitative analysis was performed using fluorescent densitometry (Fig 3.).



Legend: Assays run at several timepoints after various treatments
a. Live Infection Assay
b. Western Blot Assay (Nrf2)

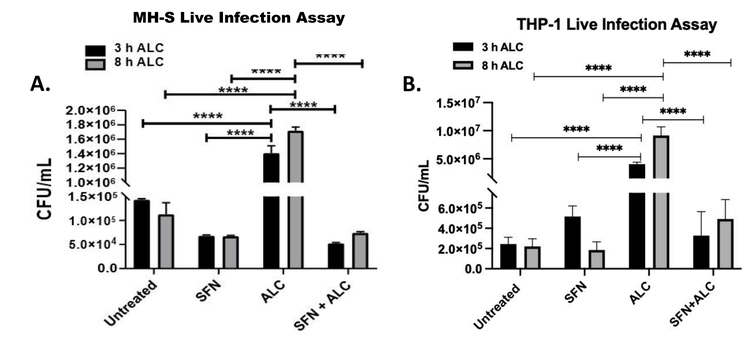
Results



Cell Viability (Fig 1. A,B,C,D)

SFN:
A: MH-S cells treated with increasing concentrations of SFN.
C: THP-1 Cells treated with increasing concentrations of SFN. TD50 was calculated to be 90 uM.

Alcohol:
B: MH-S Cell viability measured at increasing concentrations of alcohol.
D: THP-1 Cell viability measured at increasing concentrations of alcohol.



Macrophage killing (Fig 2. A,B)

Fig A:
MH-S cells pre-treated with SFN resulted in decreased intracellular survival of B. thailandensis ~15-fold compared to MH-S cells only exposed to alcohol (** = p-value of <0.0001).**
Fig. B:
THP-1 cells pre-treated with SFN resulted in decreased intracellular survival of S. epidermidis ~10 fold compared to THP-1 cells only exposed to alcohol.

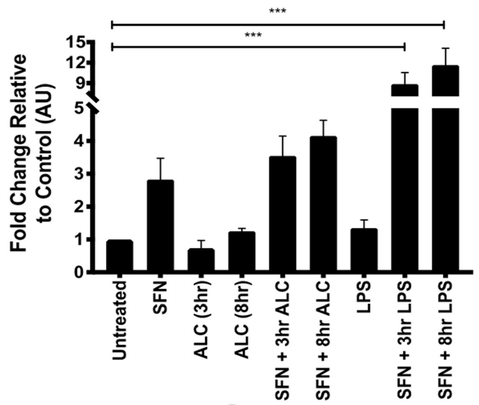


Fig 3. Nrf2 expression:

SFN:
5 µM SFN increased Nrf2 protein content approximately 2-fold compared to untreated control.

Alcohol:
Did not change Nrf2 expression compared to untreated control.

SFN + Alcohol:
Increased Nrf2 expression ~ 3 or 3.5-fold compared to MH-S cells treated with alcohol alone.

SFN + Alcohol + LPS:
LPS stimulus increased Nrf2 expression ~10 or 12-fold compared to LPS stimulus for 8 h alone.

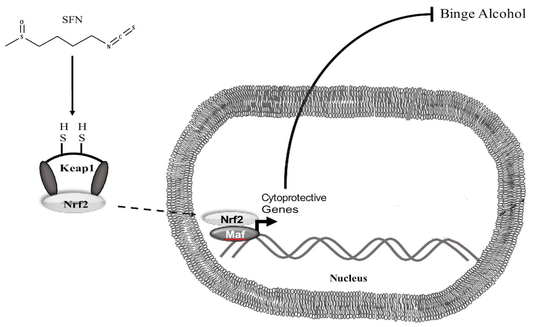
Discussion

At concentrations ≤10 uM SFN was found to be nontoxic, and biologically relevant to the alveolar macrophages in both mouse (MH-S) and Human cell lines (THP-1). TD-50 of THP-1 cells was 90 uM indicating therapeutic levels are potentially available.

SFN pre-treatment rescued alveolar macrophage killing effectiveness consistently across both MH-S and THP-1 cells, suggesting that SFN can be effective in multiple cell lines, and against both gram negative and positive bacteria.

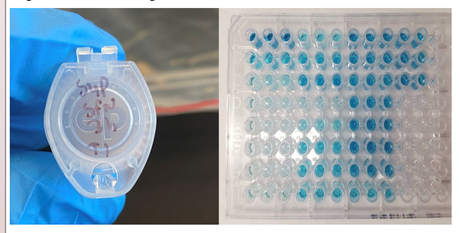
SFN pre-treatment induced a transcriptional change to increase Nrf2 expression in the presence of infection.

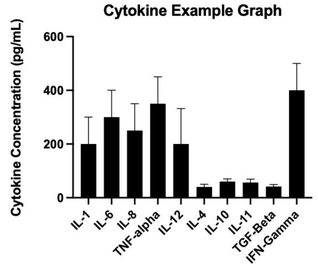
SFN may be an effective pre-treatment option to prevent alcohol mediated immune dysfunction by restoring macrophage phagocytic killing and providing protections against oxidants via Nrf2 expression.



Future Directions

Cytokine Analysis:





Proposed Mouse Model: