

# Sulforaphane Pre-treatment Improves Cytoprotection Against Opportunistic Pathogens

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## Introduction

Alcohol is associated with increased mortality and morbidity globally. Pulmonary infections with opportunistic pathogens can occur in healthy humans; however, binge alcohol intoxication ( $\geq 0.08\%$  BAC) is a major risk factor. We have previously shown that a single dose of alcohol comparable to binge alcohol intoxication increases infection by reducing alveolar macrophage function in vivo.

The aim of this study was to:

- 1) Test the therapeutic potential of the phytonutrient sulforaphane (SFN) given as a pre-treatment.
- 2) Test the alcohol-induced effects on phagocytic function in murine and human macrophages in vitro.

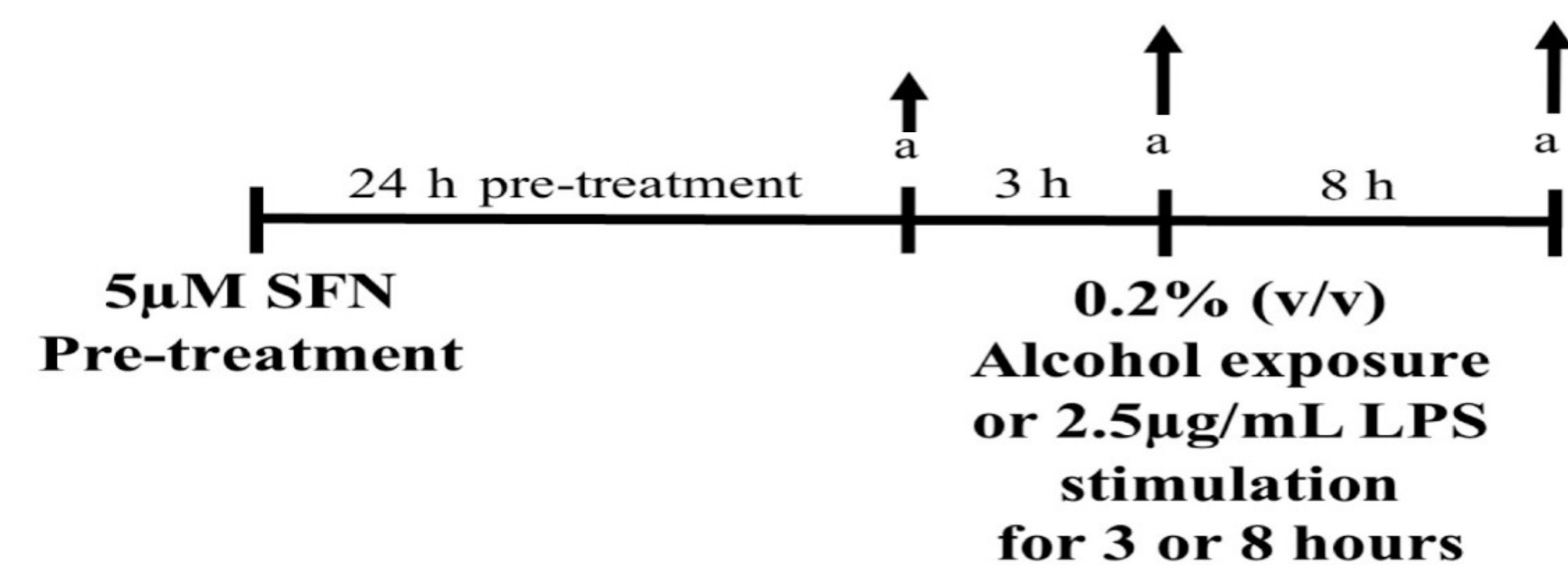
## Hypothesis

Pre-treatment of MH-S and THP-1 cells with SFN will prevent alcohol-induced dysfunction.

## Materials and Methods

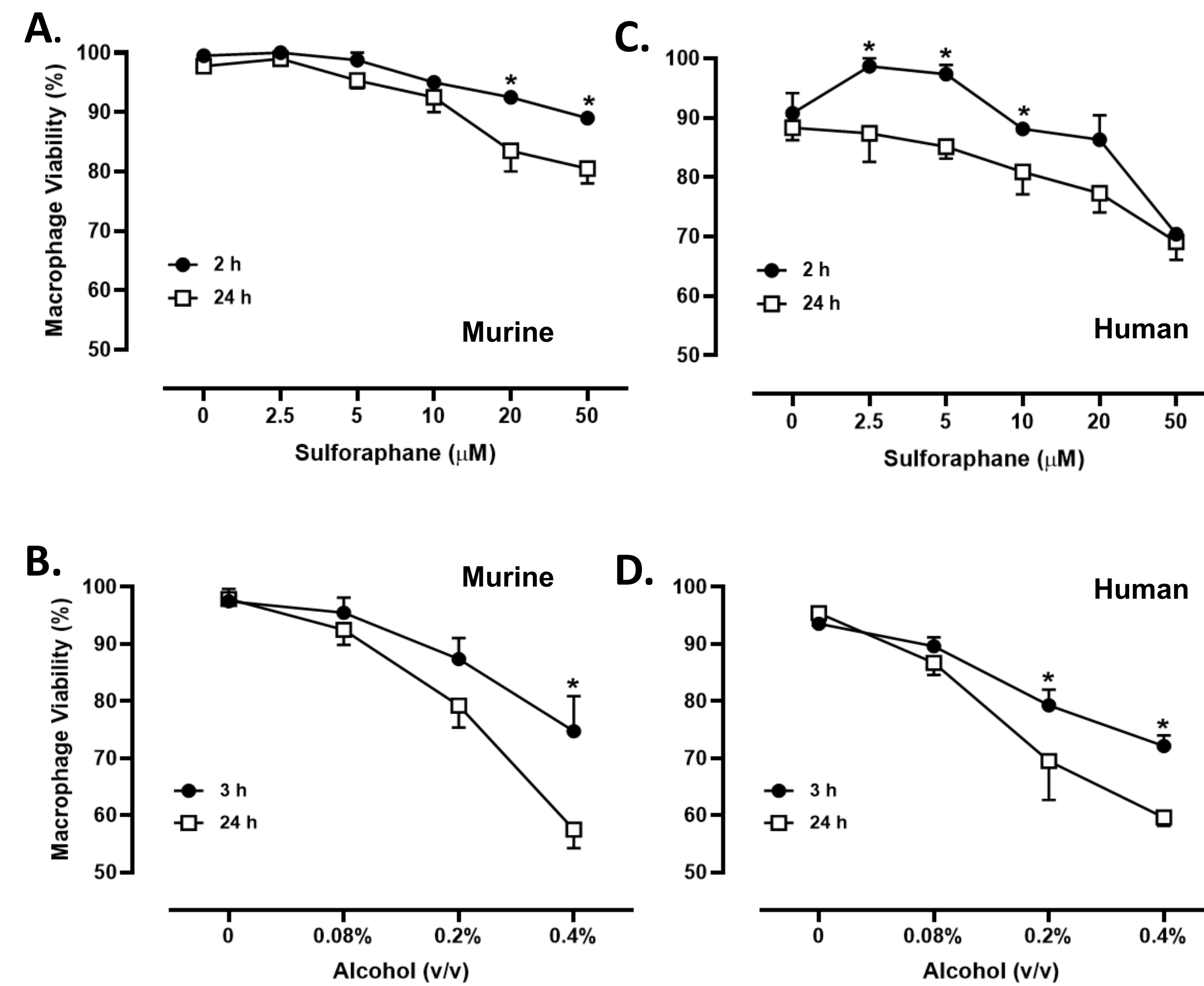
Cells were cultured with 0, 2.5, 5, 10, 20, 50  $\mu\text{M}$  SFN for 2 or 24 h. MH-S cells were then infected with live *B. thailandensis* E264, and THP-1 cells were infected with live *S. epidermidis*. After 3 and 8 hours, cells were washed, lysed, and remaining intracellular bacteria were plated for CFU. Cytokine analysis was performed by ELISA to quantify TNF- $\alpha$ , IL-10, and IFN- $\gamma$ .

SFN Pre-treatment and alcohol or LPS exposure



**Legend:** Assays run at several timepoints after various treatments  
a. Live Infection Assay

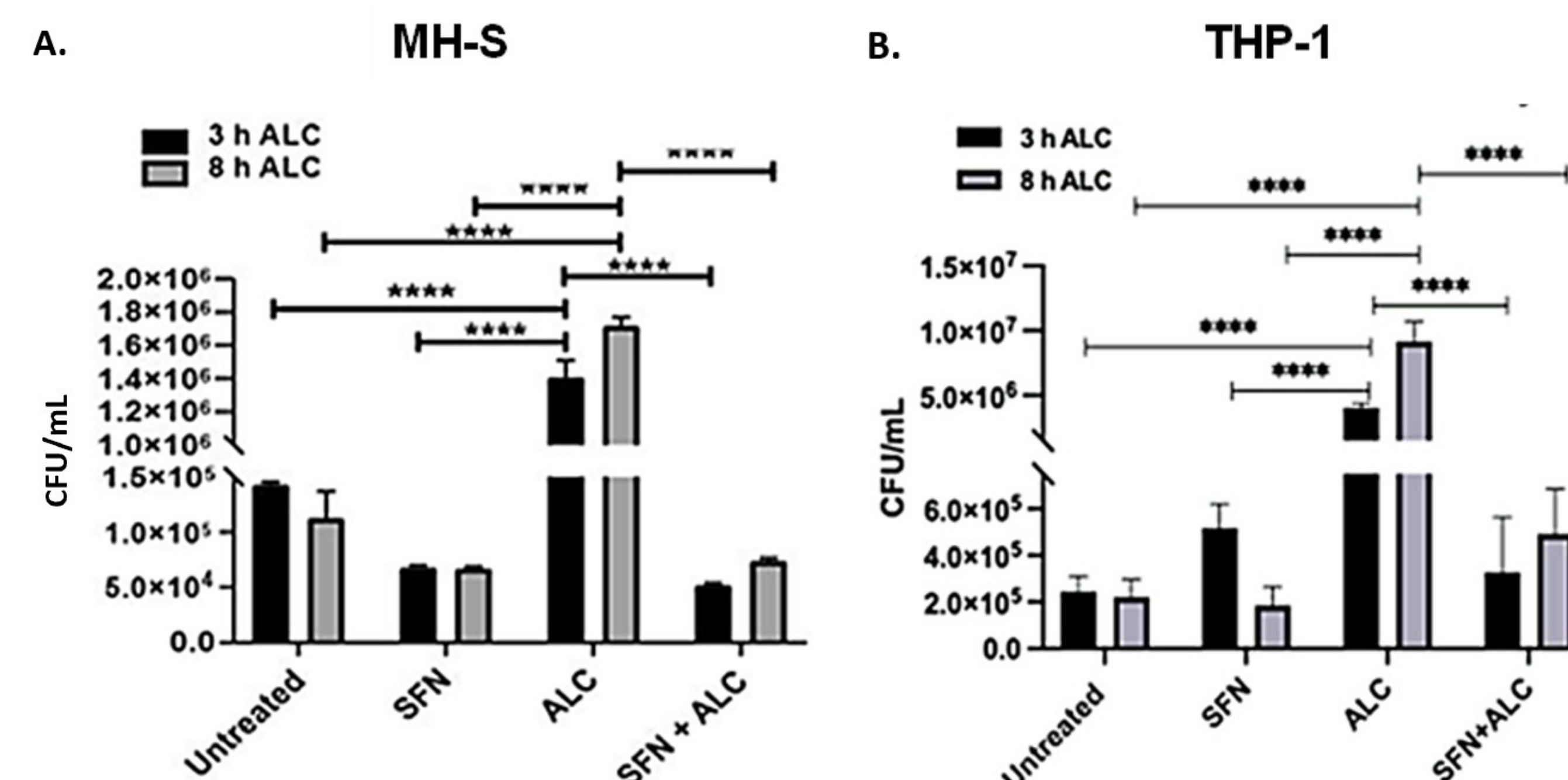
## Results



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

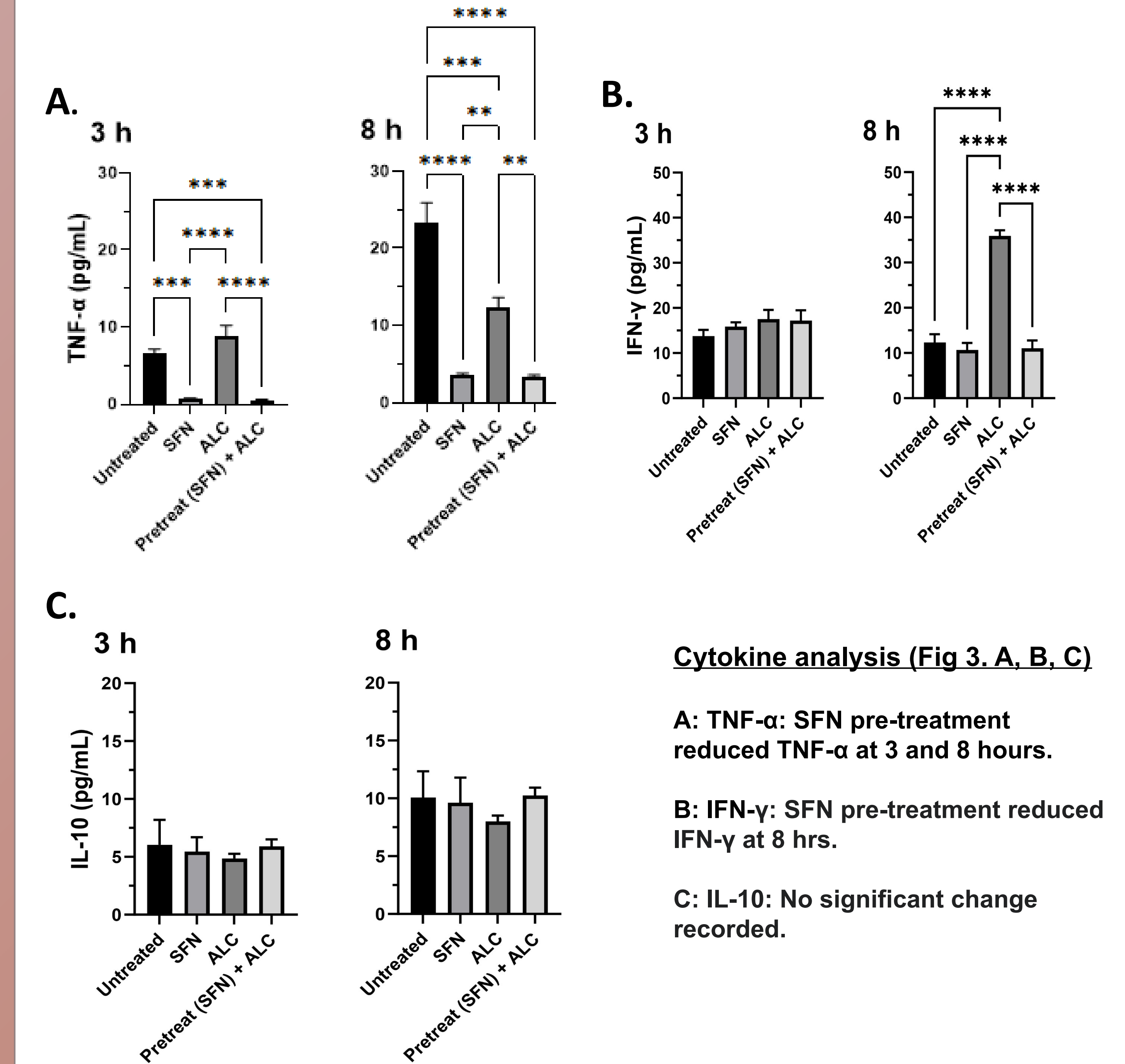
A: MH-S cell viability with increasing concentrations of SFN.  
B: MH-S cell viability measured with increasing concentrations of alcohol.

C: THP-1 cell viability with increasing concentrations of SFN.  
D: THP-1 cell viability with increasing concentrations of alcohol.



Macrophage killing (Fig 2. A,B)

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



Cytokine analysis (Fig 3. A, B, C)

A: TNF- $\alpha$ : SFN pre-treatment reduced TNF- $\alpha$  at 3 and 8 hours.

B: IFN- $\gamma$ : SFN pre-treatment reduced IFN- $\gamma$  at 8 hrs.

C: IL-10: No significant change recorded.

## Discussion/Conclusion

Pre-treatment of MH-S and THP-1 cells with SFN:

- 1) Are biologically relevant and safe at the doses tested.
- 2) Restore critical macrophage phagocytic responses,
- 3) Provide consistent results in both murine MH-S and human THP-1 cell lines.
- 4) Provide protection against both gram-positive and negative pathogens.

## Proposed Future Directions:

Administering SFN post-exposure to alcohol to determine the efficacy of treatment in other settings.

Repeat the experiment in vivo using a rat model to gauge efficacy in living organisms.