Investigation of Sulfur Scavenging In a Mouse Model of Systemic *Staphylococcus aureus* Infection Using Imaging Mass Spectrometry

Justin M. Ellenburg[1], Joshua Lensmire[2], Paige J. Kies[2], Nick R. Ellin[1], Neal D. Hammer[2], Boone M. Prentice[1]

1 Department of Chemistry, University of Florida, Gainesville FL, USA
2 Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing MI, USA

Overview

- **Purpose:** The goal is to provide *in situ* evidence of scavenging of sulfur-containing metabolites by *S. aureus* from host tissues and to better understand the role of sulfur in pathogenicity.
- **Approach:** Imaging mass spectrometry was used to map the distribution of various sulfur-containing compounds in infected and control tissues.
- **Results:** Glutathione (GSH) and oxidized glutathione (GSSG) are detected at higher abundances with altered spatial distributions in *S. aureus* infected tissues compared to controls. L-cysteine-glutathione disulfide (GSH-Cys) was detected at lower abundance in infected tissue. Cysteinylglycine has also been detected.
- **Significance:** Imaging mass spectrometry was used to observe evidence of sulfur scavenging from host tissue by *S. aureus* during infection, demonstrating the disturbed distribution of sulfur-containing metabolites during *S. aureus* infection.

Introduction

During systemic infection, bacterial pathogens can infiltrate organs and proliferate within the soft tissue forming deep-seated abscesses.[1] The host responds by encapsulating the damaged and infected tissue in layers of neutrophils and other immune cells (Figure 1).[2] Immune cells work to clear the damaged tissue and pathogens, while the pathogen continues to replicate inside the abscess.[1] In order to replicate, various pathogens have been shown to scavenge transition metals and carbon sources from host tissue during infection.[3] Macronutrients, like sulfur, are readily available in host tissue and are required for bacterial proliferation, but sulfur acquisition is less well understood.[2,3] *In vitro* studies have shown that *Staphylococcus aureus* scavenges organosulfur metabolites, such as glutathione, from its environment.[3] Evidence of sulfur scavenging from host tissues is lacking, however. Herein, we utilize matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI IMS) to investigate the distribution and role of sulfur-containing metabolites in a mouse model of systemic *S. aureus* infection.

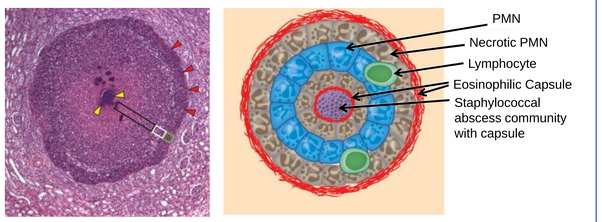

Figure 1. *S. aureus* lesion in murine model. A) H&E stain of abscess in renal tissue showing necrotic neutrophils (black box), healthy neutrophils (white box), and necrotic immune cells (green box). B) Schematic illustrating histopathology. Obtained from Lithgow et. al.

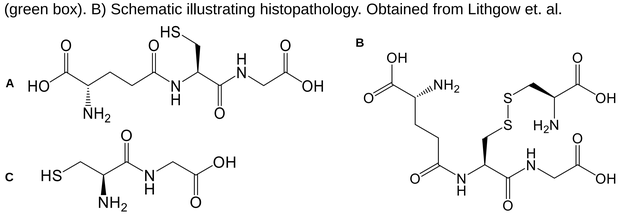

Figure 2. A) Glutathione (GSH) B) L-Cysteine-glutathione disulfide (GSH-Cys), C) Cysteinylglycine

Methods

Infection and organ harvesting: 8-week-old female C57BL/6 mice were retro-orbitally infected with 10^7 CFU of *S. aureus* resuspended in PBS. Mice were euthanized 96 hours post-infection and the kidneys, liver, and heart were harvested and stored on dry ice for shipment.

Tissue sample preparation: Tissues were sectioned at 10µm on a Leica CM3050S Cryostat. One section of *S. aureus* infected mouse kidney and one section of phosphate-buffered saline (PBS) inoculated mouse kidney (control) were thaw mounted onto an ITO coated slide.

Matrix application: A 5 mg/mL solution of 9-aminoacridine was prepared using 90% MeOH. 9AA was applied by robotic sprayer at 85ºC with a 0.11 mL/min flow rate. Nozzle velocity was set to 700 mm/min with a 2 mm track spacing and crisscross pattern. Nitrogen pressure was set at 10 psi with a 2 L/min flow rate.

IMS: IMS was performed on a 7T solariX FTICR mass spectrometer (Bruker Daltonics) in negative ion mode. Continuous Accumulation of Selected Ions (CASI) was used to improve the ion signal over the mass window of *m/z* 280-620. All IMS experiments were preformed at 150µm spatial resolution. Mass resolution at *m/z* 400 is 67,000 measured by FWHM. Image process performed with flexImaging and SCiLS software (Buker Daltonics)

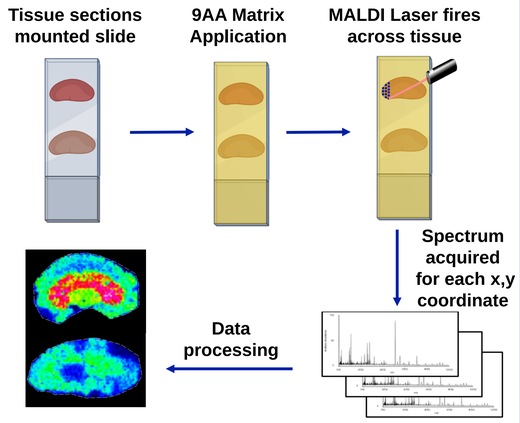

Figure 3. Imaging mass spectrometry workflow. Created with BioRender.com

Results- Detection of Sulfur-Containing Metabolites

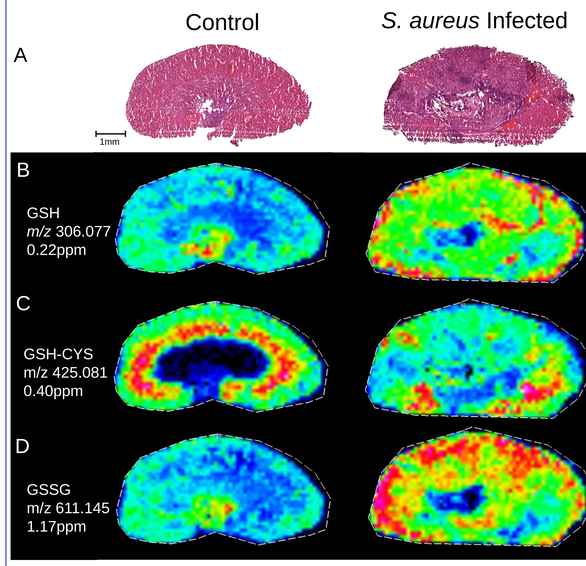

Figure 4. A) H&E stain of PBS inoculated mouse kidney (left) and *S aureus* infected mouse kidney (right). Spatial distributions of B) GSH, C) GSH-Cys, and D) Glutathione disulfide (GSSG) were obtained at a150µm spatial resolution.

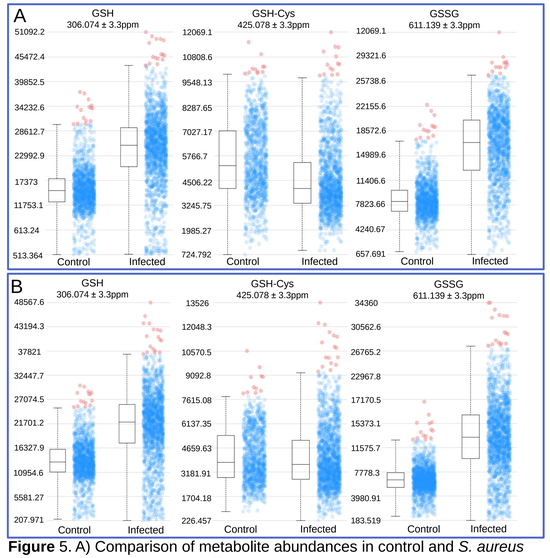

Figure 5. A) Comparison of metabolite abundances in control and *S. aureus* infected tissues from figure 4. Each data point represents the abundance of the given metabolites in one spectrum/pixel from the MSI image after root mean square normalization . B) Comparison of metabolite abundances from serial sections of A (Ion images now shown).

Results- Detection of Cysteinylglycine Using Alternative DHB Matrix

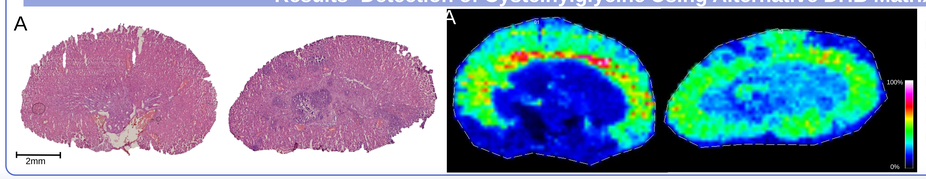

Figure 6. A) H&E stain of PBS inoculated mouse kidney (left) and *S aureus* infected mouse kidney (right). B) Spatial distribution of Cysteinylglycine detected using Dihydroxybenzoic acid (DHB) used as matrix. IMS experiment performed at 150µm spatial resolution.

Results- In vitro Scavenging

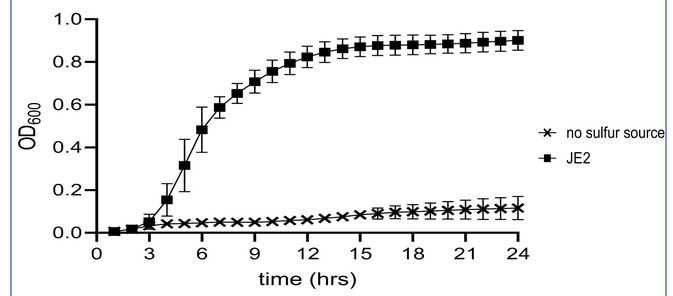

Figure 7. The optical density of *S. aureus* colonies grown *in vitro* measured at a wavelength of 600nm . Colonies were grown in the presence or absence of 25µM cysteine-glutathione disulfide as a sulfur source.

Conclusions

- GSH, GSSG, GSH-Cys have all been detected in mouse kidney. Through improved sample preparation and MALDI imaging techniques, L-Cysteinylglycine has been detected as well. Localization of these metabolites to abscesses is not seen, however.
- SCiLS analysis of MALDI images reveals that GSH and GSSG have a higher abundance in infected tissue compared to controls. Little difference is seen in the abundance of GSH-Cys abundance between the two tissues.
- Differing spatial distribution of these compounds provides support for sulfur scavenging by *S. aureus*, but the distribution of sulfur containing compounds during infection appears complex and likely depends on the progression of the abscess as well as the depth within the abscess.

Future Work

- Image the distribution of sulfur-containing metabolites in liver and heart tissue. Comparison between organs may be limited by natural differences in metabolite abundance in different tissues.
- Image the distribution of sulfur-containing metabolites through the entire 3-dimensional structure of a *S. aureus* abscess.

References

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2. *Lensmirer, Joshua M.; Hammer, Neal D. Nutrient sulfur acquisition strategies employed by bacterial pathogens. Current Opinion in Microbiology,* ***2019****, 47, 52-58.*
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