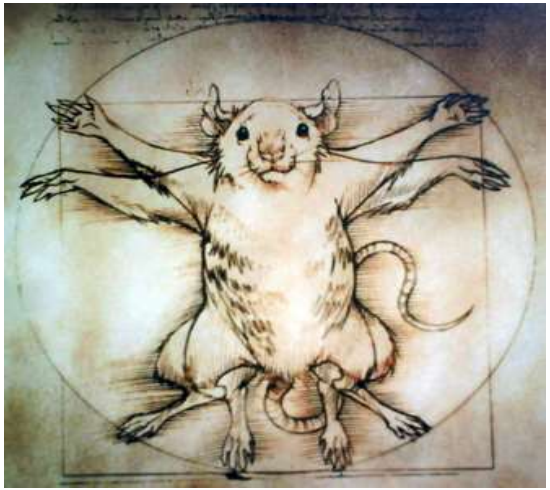
Translatable Therapeutic Effects of Extracellular Superoxide Dismutase (ecSOD) in the Treatment of *Staphylococcus aureus* Infections



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ecSOD Background

Extracellular Superoxide Dismutase (ecSOD)

- Is a major regulator of extracellular superoxide, hydrogen peroxide (H_2O_2) and nitric oxide concentrations.
- Is anchored to the extracellular matrix (EM) and protects it from oxidative damage.
  - Circulating form is a result of proteolytic cleavage of the heparin-binding domain.
- Is primarily localized to vascular endothelium.
- Human polymorphisms are associated with several inflammatory diseases.

Proposed roles of ecSOD during immune responses:

- Contributes to increased bacterial clearance due to H_2O_2 production.
- H_2O_2 induces neutrophil recruitment and pro- inflammatory cytokines/chemokines.
- Conversely, modulation of superoxide levels by ecSOD can reduce neutrophil recruitment and dampen inflammation during noninfectious insults.

ecSOD activity during an infection could impact the clearance of pathogens and subsequent disease severity.

Role of ecSOD during bacterial infections

Previous work[1] with a mouse model of Listeriosis demonstrated that:

- Neutrophils mobilized to infected mouse livers in proportion to increasing ecSOD activity, but…
- Neutrophils had a diminished ability to generate an oxidative burst.
- Higher incidence of neutrophil apoptosis with increased ecSOD activity.
- ecSOD KO mice were resistant to infection while wild-type mice with variable ecSOD activities were not as resistant.

These results suggest a novel role for ecSOD in the pathogenesis of bacterial infections.

Are these results pathogen specific or can they be reproduced with other pathogens/infections?

[1]Break et. al., *J. Immunol.* 188:3342-3350, 2012.

Staphylococcus aureus

Characteristics/Phenotype

- Capable of producing multiple virulence factors.
- Multi-drug resistant strains present (MRSA, VISA, VRSA).
  - Concerning to serious hazard level threats (CDC, 2013 AR Threats Report).

Carriage, Infection and Associated Diseases

- Primarily colonizes the anterior nares and transmitted by contact.
- Infections primarily occur due to compromised epithelial barrier (skin).
- Diseases range in severity:
  - Skin-associated, toxinoses, and systemic/life-threatening conditions
- Similar to *Listeria monocytogenes*, neutrophils play an critical role in immunity to *S. aureus* infections.

Therefore, a set of animal experiments were conducted to evaluate the impact of ecSOD activity on disease severity in three *S. aureus* infection models.

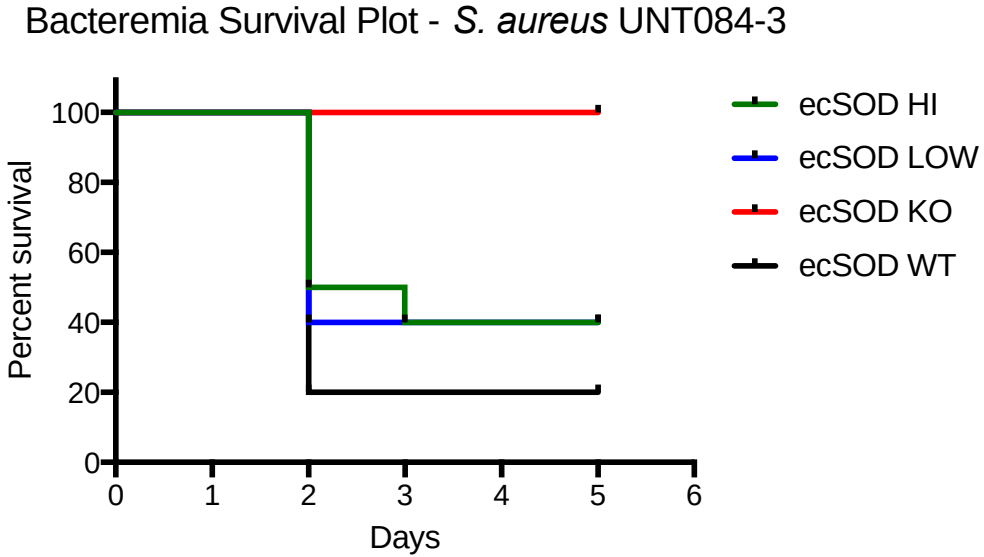
Experimental Designs

Used congenic mice (C57BL/6 background) with varying ecSOD activities that included:

- High levels of ecSOD (ecSOD HI), low levels of ecSOD (ecSOD LOW), null ecSOD (ecSOD KO), and wild-type levels of ecSOD (ecSOD WT)

1. Bacteremia model
   - ✓ Pathogen: *S. aureus* UNT084-3 (USA100)
   - ✓ Procedure: Mice were IP infected with 7.1 $\log_{10}$ CFU (5% mucin), and survival was monitored for 5 days.
2. Pneumonia model
   - ✓ Pathogen: *S. aureus* UNT003-3 (USA300)
   - ✓ Procedure: Anesthetized mice were infected by intranasally instilling 7.8 $\log_{10}$ CFU. Lung CFU determined at 24 hrs, or survival was monitored for 7 days.
3. Subcutaneous abscess model
   - ✓ Pathogen: *S. aureus* UNT013-4 (RN6390, elevated toxin producer)
   - ✓ Procedure: Anesthetized mice were SC injected with 7.8 $\log_{10}$ CFU diluted 1:4 in sterile dextran beads (20 mg/mL). Abscesses were imaged and harvested 4 days after infection for CFU enumeration.

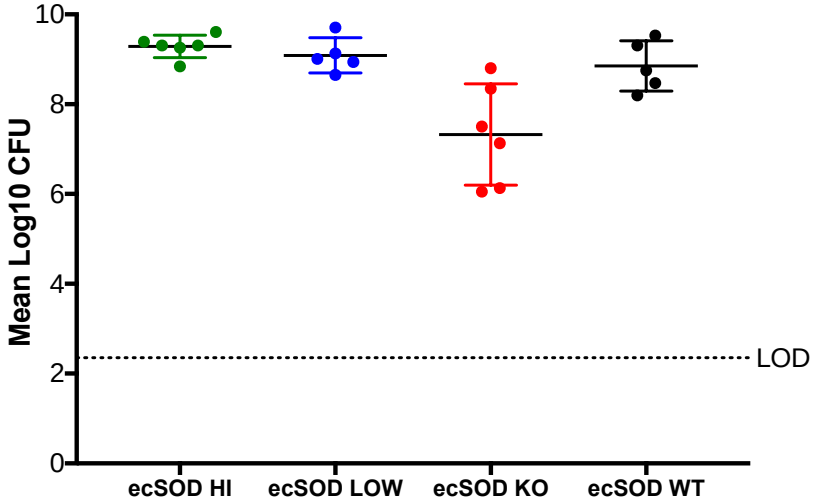
Results – Bacteremia



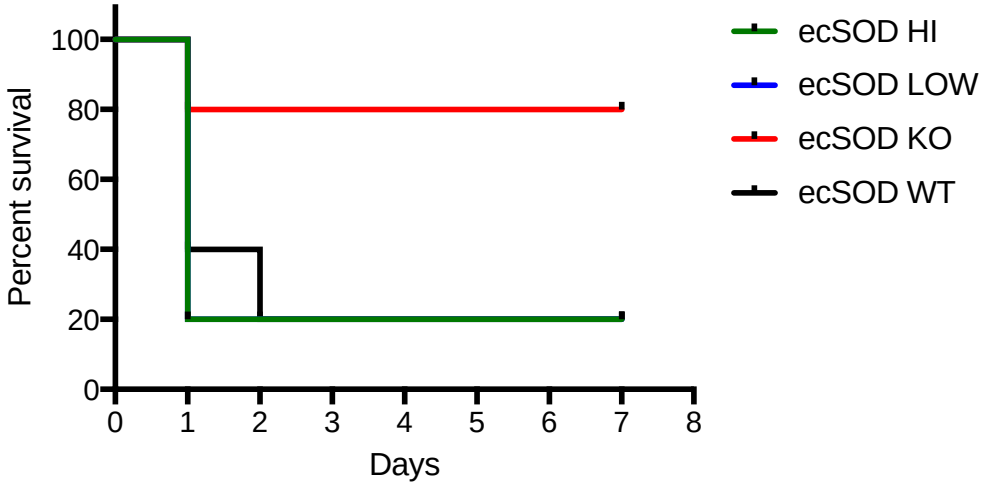
- For ecSOD HI, LOW and WT mice, 20 - 40% of the infected mice survived by the end of the 5 day census period. (n=10)
- All (100%) of the infected ecSOD KO mice survived by the end of 5 day census period. (n=10)
- Survival was significantly different ($p<0.05$, Log-rank) for infected ecSOD KO mice as compared to the survival results of the other strains.

Results – Pneumonia

24 hr Mean Lung CFU Counts - *S. aureus* UNT003-3



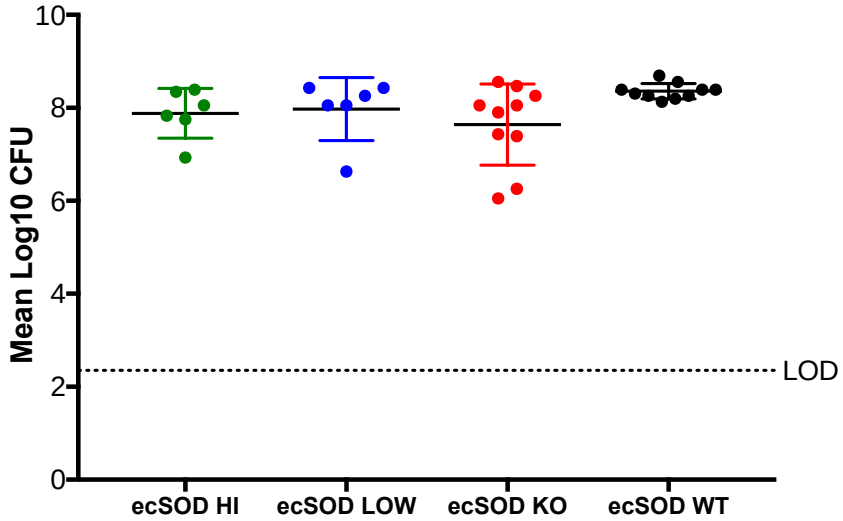
Pneumonia Survival Plot - *S. aureus* UNT003-3

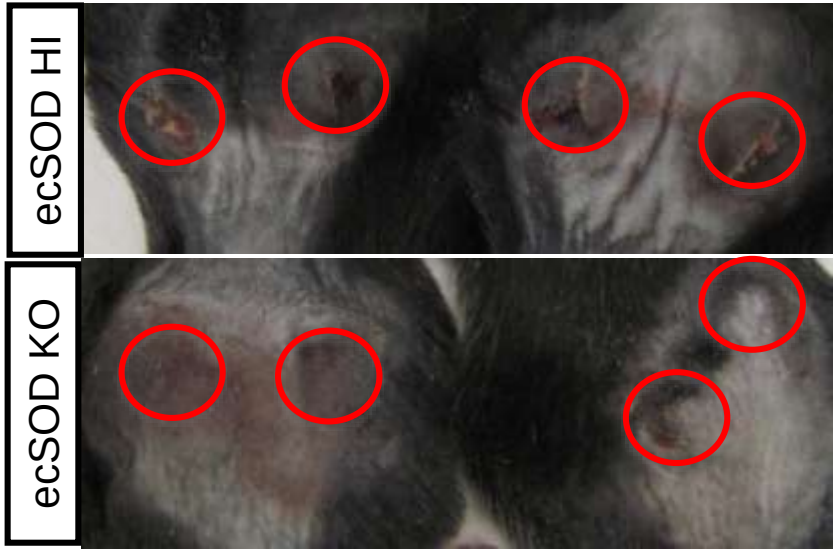


- Mean lung CFU for ecSOD KO mice was ~7.5 $\log_{10}$ 24hrs after infection, while the 24hr lung counts for the other strains was ~9 $\log_{10}$ CFU. (n=5,6)
- For the ecSOD KO mice, 80% of the infected mice survived by the end of the 7 day census period, while the 7-day survival rate was 20% for the other strains. (n=10)
- Survival and CFU results were significantly different (p<0.05, Log-rank OR Tukey's post-hoc analysis) for infected ecSOD KO mice when compared to the other strains.

Results – Subcutaneous Abscess

Day 4 Mean Abcess CFU Counts - *S. aureus* UNT006-4



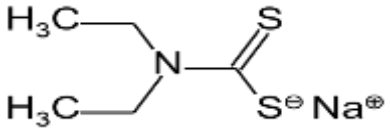


- Abscess CFU for all 4 mouse strains were not significantly different 4 days after infection. (n=6,10)
- 60-80% of the abscesses for ecSOD HI, ecSOD LOW, and ecSOD WT had severe cellulitis with associated damage to subdermal tissues 4 days post-infection.
- Only 22% of the abscesses for ecSOD KO mice had severe cellulitis and associated subdermal damage 4 days post-infection.

Experimental Design – Therapeutic treatment of SC abscess

- Subcutaneous abscess model
  - ✓ Animal: C57BL/6 (female) mice
  - ✓ Pathogen: *S. aureus* UNT013-4 (RN6390, elevated toxin producer)
  - ✓ Infection: Anesthetized mice were SC injected with 7.6 $\log_{10}$ CFU diluted 1:4 in sterile dextran beads (20 mg/mL).
  - ✓ Treatment: Infected animals were IP administered 700 mg/kg diethyldithiocarbamate (DDC) ± 5 mg/kg vancomycin (Vanco), 5 mg/kg Vanco, or vehicle starting 4 hours after infection and continuing for 3 days.
  - ✓ Endpoint: Abscesses were imaged and harvested 4 days after infection for CFU enumeration.

Diethyldithiocarbamate (DDC)

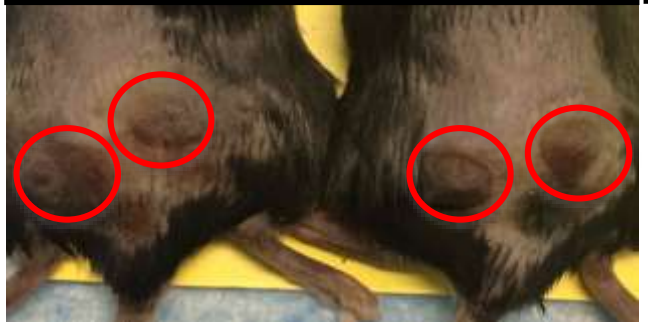


  - ✓ Copper chelator (ecSOD is a Cu/Zn enzyme)
  - ✓ Metabolite of disulfiram – used for 60 years in the treatment of alcoholism.

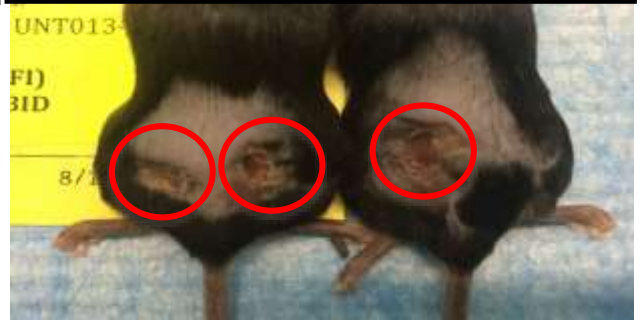
Results – Therapeutic treatment of SC abscess

- Gross observations of abscesses after 4 days of sub-optimal vanco or vehicle treatment revealed abscess cavitation and severe subdermal tissue damage.
- Comparatively, animals treated with 4 days of DDC (± sub-optimal vanco) had noticeably diminished abscess-associated tissue damage.

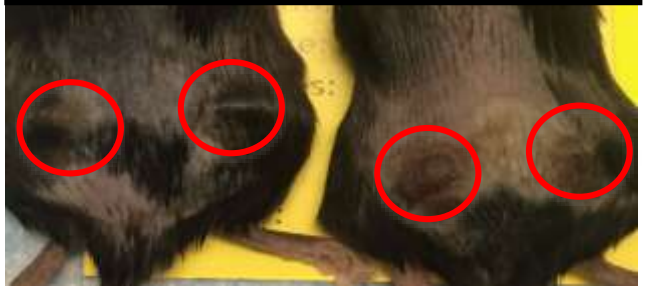
DDC 700 mg/kg, 4 days BID



Vanco 5 mg/kg, 4 days QD



DDC 700 mg/kg, 4 days BID & Vanco 5 mg/kg, 4 days QD



Vehicle, 4 days BID

Conclusions

- ecSOD KO mice lethally infected (bacteremia, pneumonia) with *S. aureus* had significantly higher survival rates than the ecSOD expressing mouse strains.
- Likewise, 24 hour lung-associated CFU counts were significantly lower in respiratory infected ecSOD KO mice.
- Even though abscess-associated *S. aureus* CFU counts were similar for all ecSOD mouse strains, the gross pathology was noticeably lessened in ecSOD KO mice.
- Treatment with DDC, a non-specific inhibitor of ecSOD activity, did not decrease abscess-associated CFU.
    - However, DDC treatment did diminish the pathological severity of *S. aureus* infected abscesses.
- DDC treatment in the SC abscess model generated results that were similar the ecSOD KO mice, which suggests a potentially novel therapeutic target.
- Future work will be focused of optimizing DDC dosing in other models and exploring new methods to inhibit ecSOD activity.

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