Streamlining Drug Screening and Early Diagnostics for Bloodstream Infections

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Abstract

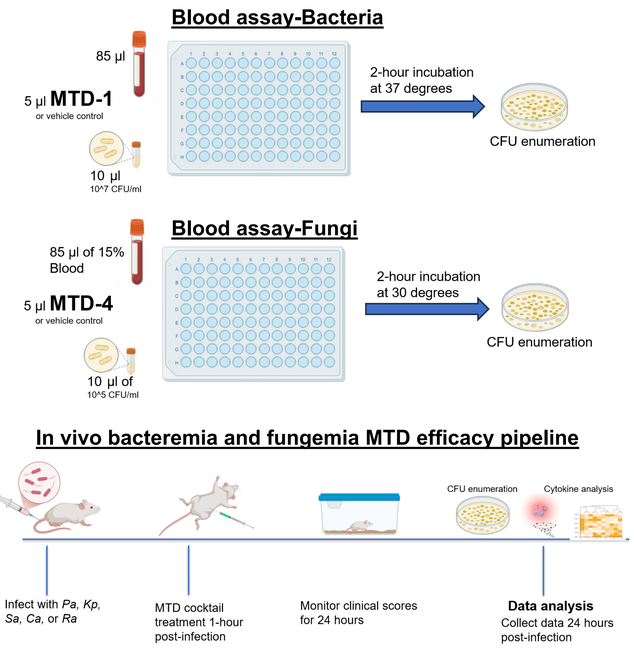
Introduction: Bacterial and fungal bloodborne infections remain a major complication to battlefield injuries for Service Members. Many of these infections are recalcitrant to standard-of-care treatments, highlighting the need for alternative approaches. We have developed blood assays and bacterial and fungal sepsis models to screen novel drugs. Moreover, we conducted immunoprofiling of blood from infected animals to develop an early, pathogen-specific diagnostic test and to assess therapeutic efficacy. We identified minimized theta-defensins (MTDs), peptide analogs of naturally occurring theta-defensins, that are active against bacterial and fungal pathogens in our blood assays. We further investigated effects of MTDs on immune response to different pathogens in vivo.

Materials and Methods: A 96-well assay using murine blood infected with six selected pathogens was developed. Infected blood was treated with MTDs, and enumerated for CFU after 2 h. For immunological profiling, we developed a 24 h survivable blood infection model via optimization of pathogen concentrations using intravenous injection. The highest survivable inoculum was used for in vivo testing of MTDs, administered 1 h after infection via intraperitoneal injection. 24 h post infection, blood was collected for cytokine analysis via the 19-plex Mesoscale diagnostic platform, a sensitive and rapid assay for analyzing immunological factors.

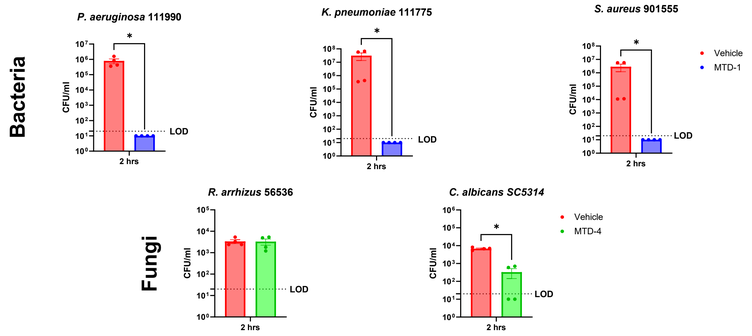
Results: MTD treatment eradicated bacterial pathogens and reduced fungal burden of *Candida albicans* in the blood assays. We determined inoculum concentrations for blood infections of 3 MDR bacterial and 2 fungal pathogens for the analysis of immunological factors. Immunoprofiling revealed pathogen specific profiles in vehicle-treated animals. MTD treatment resulted in significant differences in IL5 and IL1β for mice infected with *K. pneumoniae* and IL33 and MIP2 for mice infected with *P. aeruginosa.* For animals infected with fungal pathogens, MTD treatment yielded significant differences in IL33 and IL2.

Conclusions: We developed in vitro blood assays for testing therapeutics and a 24 h in vivo blood infection model which identified immunological factors specific to infection and treatment. Immunoprofiling revealed the potential for pathogen identification and elucidated the immunomodulatory role of MTDs. Future studies will include in vivo MTD efficacy testing in battlefield relevant trauma infection models.

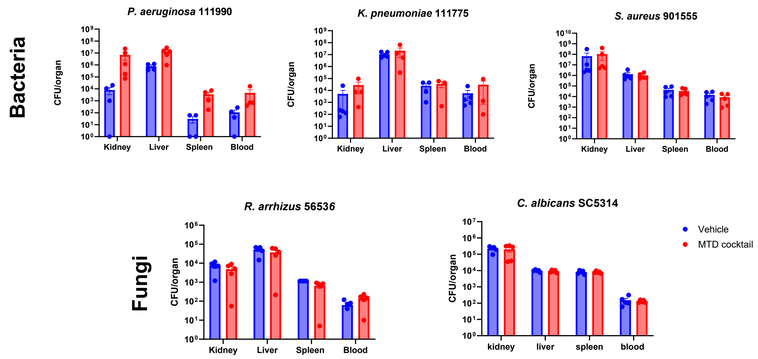
Experimental Design for MTD Efficacy



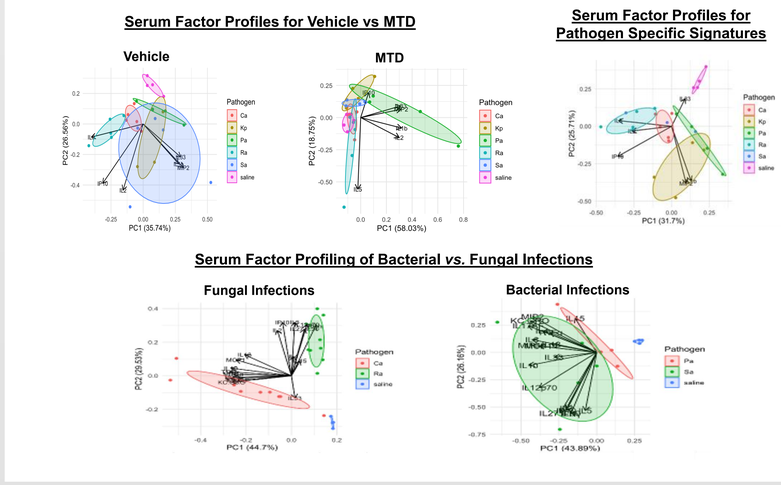
MTD Treatment in Blood Assays Resulted in Decreased Bacterial Viability



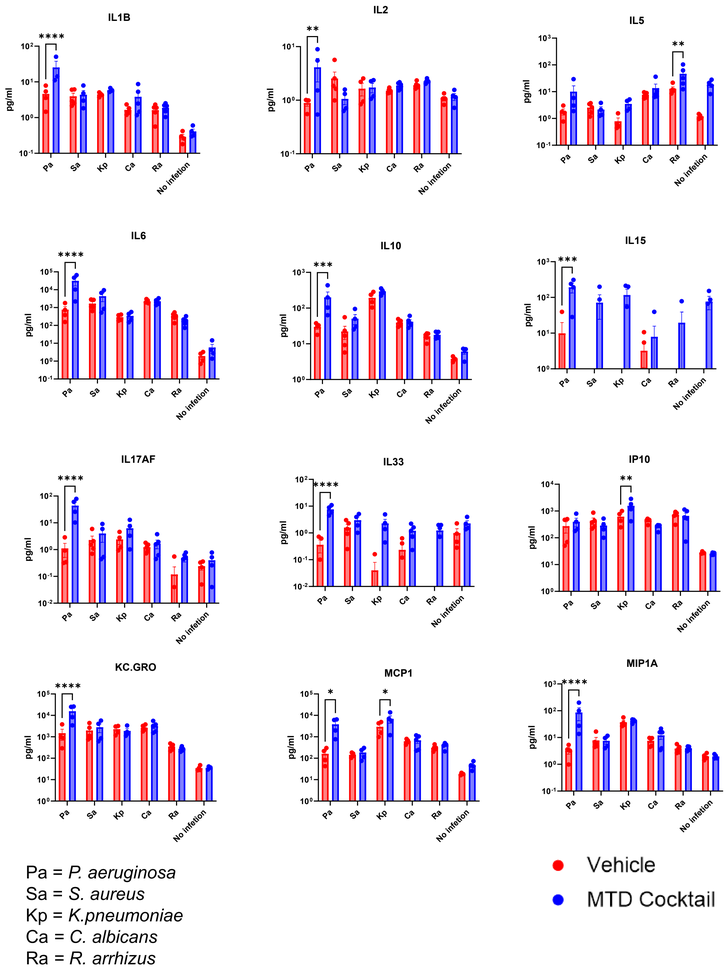
24-hour Bacteremia and Fungemia CFU Analysis



PCA Displays Pathogen Specific Cytokine Signatures



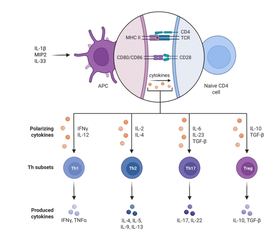
MTD Cocktail Resulted in Alterations of Immunological Factors



Pa = *P. aeruginosa*
Sa = *S. aureus*
Kp = *K.pneumoniae*
Ca = *C. albicans*
Ra = *R. arrhizus*

Summary

- This project has led to the development of an ex vivo blood assay and in vivo bacteremia and fungemia models to test the MTD immunomodulators
- MTDs were efficacious against *Pa*, *Sa*, *Kp*, and *Ca* in ex vivo blood assays
- MTD treatment in a 24-hour bacteremia and fungemia model did not result in decreased bioburden indicating that alterations in immunological factors were due to immunomodulation and not due to decreased CFU.



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