Poster # AAR-791

SPR741 in combination with minocycline increases antibacterial efficacy *in vitro* and *in vivo* against XDR-*Acinetobacter baumannii* in a pulmonary model of infection

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Abstract

Background: *Acinetobacter baumannii* is a significant health problem for civilians and Wounded Warriors. *A. baumannii* makes up about 10% of all nosocomial infections, and has high mortality rates (>50%) in the ICU. Recent increases in antibiotic resistance have been published to include the emergence of extensively drug-resistant (XDR) and pandrug-resistant (PDR) strains that cannot be treated by the standard of care. Therefore, there is an urgent need to develop novel therapeutics for this bacterial species. One potential approach is the use of antibiotic adjuvants, small molecules that can potentiate the activity of other drugs. Based on previous data, we hypothesized that a combination of SPR741 (potentiator) and minocycline would increase efficacy against extensively drug-resistant (XDR) strains of *A. baumannii*.

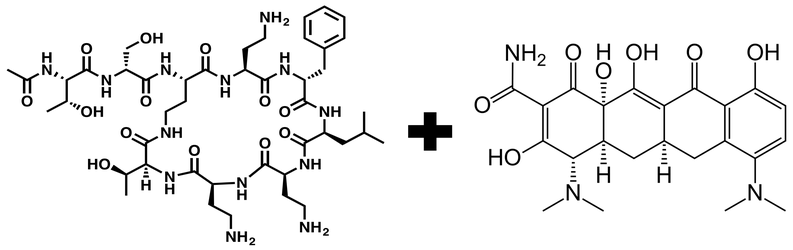
Methods: MICs of SPR741 and minocycline alone and in combination were determined against a diversity set of *A. baumannii* strains. A checkerboard assay was used to determine the FIC. Time-kill assays were done according to standard methods. A murine pulmonary model of infection was used to evaluate the activity of SPR741 and minocycline in combination against AB5075, an XDR *A. baumannii* strain. Mice were treated with either PBS, minocycline alone, SPR741 alone, or the combination at 4 hours post-infection, and subsequently, BID for 3 days. Survival was ascertained after one week, and bacterial load was measured via CFU/g on Day 2 when control animals were still alive. A similar approach will be used for other bacterial species and will also incorporate a wound model of infection.

Results: We found that the combination of SPR741 and minocycline was efficacious against XDR-*A. baumannii* strains *in vitro* via time-kill and MIC assays and reduced the MIC by 10-fold. Mice treated with the SPR741/minocycline combination had a >80% survival rate (16/20 mice) where the results were statistically significant when compared to all the other groups via the log-rank test ($P < 0.0001$). In contrast, mice treated with minocycline alone (1.0 mg/kg BID), only had a 30% survival rate, and when SPR741 was used alone, no mice survived. When CFU/g of lung tissue was examined, a statistically significant 4-5 $\log_{10}$ reduction of bacterial burden was observed with the drug combination.

Conclusions: SPR741 can be used to enhance the treatment efficacy with current FDA-approved antibiotics and minocycline is one of the best partners with regard to *in vitro* and *in vivo* results. Future *in vivo* work will assess other antibiotic combinations with SPR741 against other XDR gram-negative bacterial species.

Background

- **SPR741 is a potentiator molecule that disrupts the outer membrane of Gram negative bacteria. This facilitates the entry of antibiotics, resulting in better efficacy and bacterial kill.**
- **Our previous work has shown that SPR741 can potentiate rifampin, and we eradicated XDR-*A. baumannii*, both *in vitro* and in relevant *in vivo* model systems[1].**
- **The goal of this study is to evaluate SPR741 with clinically relevant partner, minocycline, and validate the activity against *A. baumannii*, but also include other Gram-negative species.**



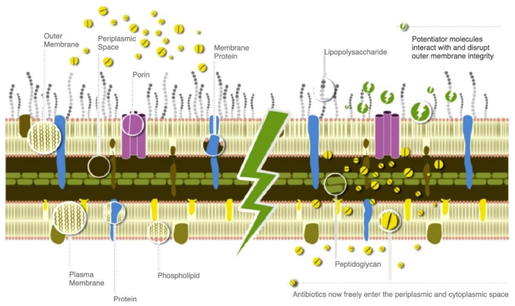


Figure 1 – SPR741 has three positive charges that disrupt the Gram-negative outer membrane, which facilitates the entry of another antibiotic, in this case, minocycline. The drug combination was hoped to provide a synergistic efficacy to defeat *A. baumannnii* and other Gram-negative bacterial infections. Image from Zabawa et. al. 2016[2].

Results

Susceptibility to minocycline and SPR741

Strain of bacteria	Minocycline (µg/mL)	SPR741 (µg/mL)	Combination	Combination (Fold change MIC)
AB5075	0.5	>64	0.0125	40-fold
KP4640	32	>64	2	16-fold

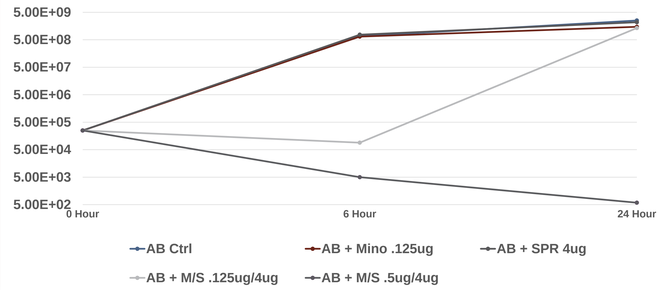


Figure 2 - **MIC and Time-Kill**
Minimal Inhibitory Concentration is determined doing a dilution series of minocycline and SPR741 in cation-adjusted Mueller-Hinton broth (CAMHB) in 96-well plates according to CLSI[3] at range from 0.0625 – 64 ug/mL.

Time-kill evaluated SPR741 and minocycline alone and in combination at 1/4X and 1X the MIC. The combination at 1X yielded synergistic, bactericidal killing.

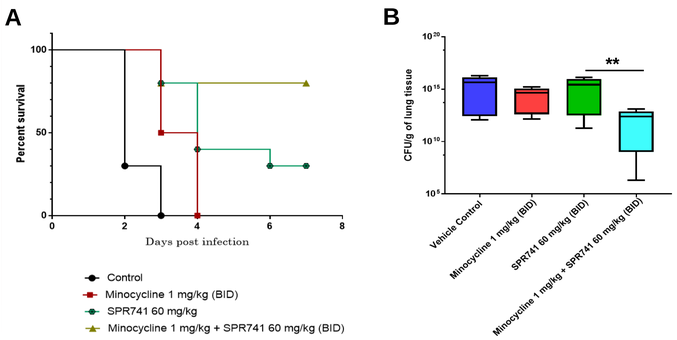


Figure 3 - ***A. baumannii* Pulmonary Model of Infection**
(A) BALB/c mice (n=20) are pretreated with cyclophosphamide and infected intranasally with 5.0 x 10^6 CFU AB5075. Mice are treated with minocycline, SPR741, or the combination twice a day for three days. Survival is monitored until Day 7. Results are significant using the Mantel-Cox test. (B) BALB/c mice (n=16) are infected as above, but on Day 2, they are sacrificed, and the bacterial burden is calculated for lung tissue. Boxes show median and interquartile ranges, while whiskers represent 95% CI. Groups were compared via Mann-Whitney U-test. ** represent $P <$ values of 0.01 ($P = 0.0029$)

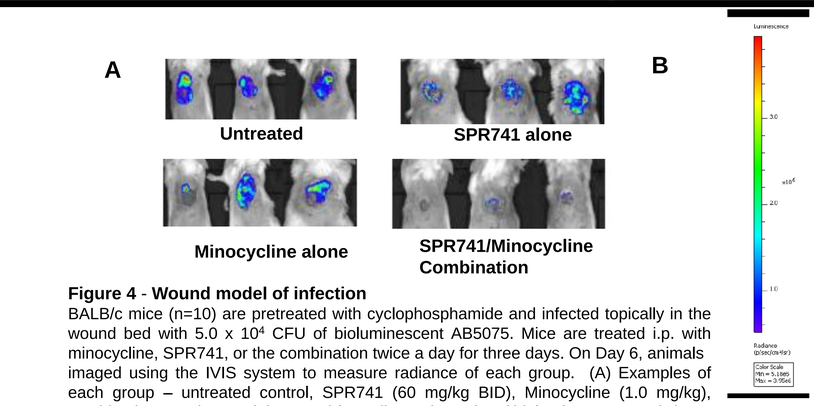


Figure 4 - **Wound model of infection**
BALB/c mice (n=10) are pretreated with cyclophosphamide and infected topically in the wound bed with 5.0 x 10^4 CFU of bioluminescent AB5075. Mice are treated i.p. with minocycline, SPR741, or the combination twice a day for three days. On Day 6, animals imaged using the IVIS system to measure radiance of each group. (A) Examples of each group – untreated control, SPR741 (60 mg/kg BID), Minocycline (1.0 mg/kg), combination are shown. (B) Legend for radiance (quantity of bioluminescence units).

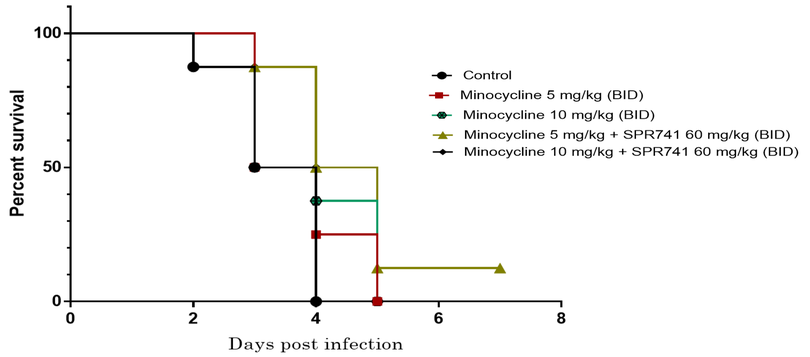


Figure 5 - ***K. pneumoniae* Pulmonary Model of Infection**
BALB/c mice (n=20) are pretreated with cyclophosphamide and infected intranasally with 2.0 x 10^7 CFU KP4640. Mice are treated with minocycline, SPR741, or the combination twice a day for three days. Survival is monitored until Day 7. Results are significant using the Mantel-Cox test. Unfortunately, because of minocycline resistance of KP4640, SPR741 potentiation is not enough to promote survival in this animal model.

Conclusions

- **SPR741 potentiator molecule lowers the MIC of minocycline against *A. baumannii* and *K. pneumoniae*. *K. pneumoniae* that was once resistant, now becomes susceptible.**
- **The combination results in bactericidal activity as visualized via time-kill assay.**
- ***In vivo*, in the lung model of infection the combination of SPR741 and minocycline results in a significant 2-3_{log} reduction in *A. baumannii* burden promoting survival.**
- ***In vivo*, in the wound model of infection the combination also shows a reduction in *A. baumannii* burden, which also improved the wound inflammation and tissue damage.**

Ongoing Investigations - Future Directions

1) **Evaluate bacterial burden and wound closure in the wound model of infection.**
2) **Identify *E. coli* and *K. pneumoniae* minocycline susceptible strains for animal testing.**
3) **Evaluate SPR206, another polymyxin-like compound with other antibiotic combinations.**
4) **Evaluate topical application along with systemic delivery.**

References

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Research was conducted under an approved animal protocol in an AAALACi accredited facility in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, NRC Publication, 2011 edition.

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