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Combination Therapy for the Investigational Polymyxin SPR206 and Meropenem (MEM) Increases the Rapidity and Extent of Killing of *Pseudomonas aeruginosa* (PA) and Prevents the Bacterium from Emerging Resistant to Both Antibiotics

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Abstract

Background: The CDC lists multidrug-resistant PA as a Serious Threat pathogen causing 32,200 hospital infections and 2,700 deaths in the US in 2017. PA resistance is frequent with antibiotic monotherapies. SPR206 is a next generation polymyxin that, in a first in human study, was generally safe and well tolerated at the potential therapeutic dose of 100 mg IV Q8h. The aim of this study was to quantify the dose-range PA killing activity of SPR206 monotherapy in a hollow fiber infection model (HFIM) and to assess if the combination of SPR206 and MEM more effectively reduced bacterial burden and prevented development of resistance than either drug alone.

Methods: Agar dilution MICs and mutation frequencies (MFs) for SPR206, polymyxin B (PMB), & MEM were performed for PA ATCC 27853. A 10-day HFIM dose range study simulating the free PK profiles for SPR206 (50 – 600 mg IV Q8h) quantified PA killing and possible regrowth. A HFIM study compared single vs combination SPR206 100 mg IV Q8h and MEM 2g IV Q8h regimens for enhanced PA killing and resistance suppression.

Results: The SPR206, PMB, & MEM MICs were 0.5, 0.5, and 0.25 mg/L, respectively. The MFs for SPR206 and PMB to 3x, 5x and 8x MIC were -6.2, -6.9, & -7.7 log CFU and -5.3, -6.4, & -7.7 log CFU, respectively. The MF for 3x MIC of MEM was -6.8 log CFU. In a dose-range HFIM, simulated regimens for 50 – 600 mg IV Q8h showed a dose-response effect, with 2.9 – 7.3 log CFU/mL reductions in PA seen at 5h. All regimens had regrowth by isolates with SPR206 MICs of 1 – 8 mg/L. In a HFIM study simulating the SPR206 and MEM clinical regimens, alone and in combination, SPR206 alone killed 5 log CFU/mL of PA at 5h, followed by regrowth. MEM alone killed 3.5 log CFU/mL of PA at 5h, with maximum kill seen on Day 4, followed by regrowth. SPR206 plus MEM killed 0.8 log CFU/mL more PA at 5h vs SPR206 alone and had undetectable PA counts by day 4. Combination therapy prevented regrowth.

Conclusions: SPR206 (100 mg IV Q8h) killed 5 log CFU/mL of PA at 5h, but regrowth ensued. SPR206 plus MEM produced 0.8 log CFU/mL more killing of PA at 5h. The killing activity of the 2-drug regimen combined with its resistance prevention effect resulted in undetectable PA counts by Day 4 of treatment.

Background

- The CDC lists multidrug-resistant PA as a Serious Threat pathogen causing 32,200 hospital infections and 2,700 deaths in the US in 2017.[1] PA resistance is frequent with antibiotic monotherapies. Polymyxins, including polymyxin B and polymyxin E (colistin) are considered the drugs of last resort for the treatment of infections due to multidrug-resistant PA. However, the nephrotoxicity of these antibiotics and the emergence of resistance to the polymyxins have limited their use in the clinic.[2]
- SPR206 is a next generation polymyxin compound that has MIC_{50} values for PA and *Acinetobacter baumannii* that are 4x lower than PMB and MIC_{90} values that are 2x-4x lower than PMB.[3] It is also less nephrotoxic than PMB in nonclinical studies.[4,5] In a first in human study SPR206 was generally safe and well tolerated at the potential therapeutic dose of 100 mg IV Q8h.[6]
- The two aims for this project were: (1) to quantify the dose-range killing of PA by SPR206 in a hollow fiber infection model (HFIM) and (2) to assess if the combination of SPR206 and MEM was more effective in reducing bacterial burden and in preventing the development of resistance than either drug alone.

References:
1. CDC. Antibiotic resistance threats report, 2019. Altanta, GA: U.S. Dept. of Health and Human Services, CDC; 2019
2. Tsuji BT, et al. Pharmacotherapy. 2019, 39:10-39. PMID: 30719469
3. Zhang Y, et al. J. Antimicrob. Chemother. 2020, 75:2609-2615. PMID: 32591806
4. Brown P, et al.. ACS Infect Dis. 2019, 5:1645–1656. PMID: 31525992
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Methods

- **Bacterium.** *P. aeruginosa* (PA) ATCC 27853 was purchased from the American Type Culture Collection (Manassas, VA). For each study, an aliquot of a stock culture was grown in cation-adjusted Mueller-Hinton broth (CA-MHB) to log-phase growth. The suspension was diluted to the desired conc. and used immediately.
- **Drugs**. The structures of SPR206 and PMB are shown in **Figure 1.** SPR206 was provided by Spero Therapeutics (Cambridge, MA). PMB and MEM were purchased from Curascript (Lake Mary, FL).

Fig 1. Structure of SPR206 and PMB

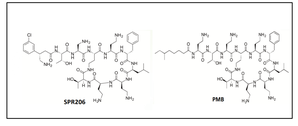
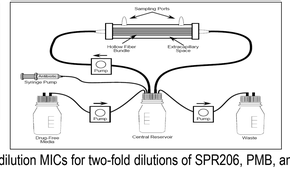


Figure. 2. Schematic of a hollow fiber arm.



- **Susceptibility studies**. Microdilution broth and agar dilution MICs for two-fold dilutions of SPR206, PMB, and MEM were determined in CA-MHB and on Mueller-Hinton agar. Final bacterial densities in the broth and on agar were 5 x 10^5 CFU/mL and 10^4 CFU/spot, respectively. Cultures were incubated at 35°C, ambient air, for 16-20 h before the MICs were read.
- **Mutation frequencies studies.** The mutation frequencies for 3x, 5x and 8x MICs of SPR206 and PMB and 3x MIC for MEM were quantified using standard methods.
- **Hollow fiber infection model (HFIM).** HFIMs **(Figure 2)** examine the effect of simulated drug PK profiles on the killing of bacteria and on the amplification of drug resistance. For the **SPR206 dose-range HFIM experiment**, 7.3 log CFU/mL (12 mL) were inoculated into cellulosic HFIM cartridges (FiberCell Systems, New Market, MD) and were exposed to simulated regimens of SPR206 50 – 600 mg given IV Q8h. PMB 1.25 mg/kg given IV Q12h (70 kg person) was the comparator. The drugs were given as 1h infusions. Treatment lasted for 10 days.

The **second HFIM study** evaluated the killing of PA (10^8 CFU/mL) by regimens of SPR206 100 mg IV Q8h and MEM 2g IV Q8h alone and in combination for the killing of PA and for resistance prevention.

Table 1. PK-PD parameter values for SPR206 and MEM administered alone and in combination in the second HFIM study.

Clinical regimen	serum protein binding (%)	serum half-life (h)	*f*Cmax (mg/L)	*f*AUC_{24h} (mg*h/L)	*f*T>MIC (%)
SPR206 100 mg IV Q8h	8.6%[5]	2.6	4.5	49.7	100%
MEM 2g IV Q8h	2%	0.64	99.8	455.4	88%

- PK-PD parameter values for SPR206 100 mg IV Q8h and MEM 2g IV Q8h simulated in the second HFIM study are shown in **Table 1**.
- Throughout both HFIM studies, bacterial specimens taken from the study arms were cultured quantitatively on drug-free agar and agar supplemented with 3x and 5x MIC of SPR206 and 3x MIC of MEM to evaluate the effect of each regimen on the total and less-susceptible bacterial populations.

Results

- **MIC (Table 2) and mutation frequency values (Table 3) for SPR206, PMB, and MEM:**

Table 2. Broth microdilution and agar dilution MICs (mg/L) for SPR206, PMB and MEM for PA ATCC 27853

Test method	SPR206	PMB	MEM
Broth microdilution	0.5	0.5	0.5
Agar dilution	0.5	0.5	0.25

Table 3. Mutation frequency values (log CFU) for SPR206, PMB, and MEM

Multiple of MIC	SPR206	PMB	MEM
3x agar MIC	-6.2	-5.3	-6.8
5x agar MIC	-6.9	-6.4	
8x agar MIC	-7.7	-7.7	

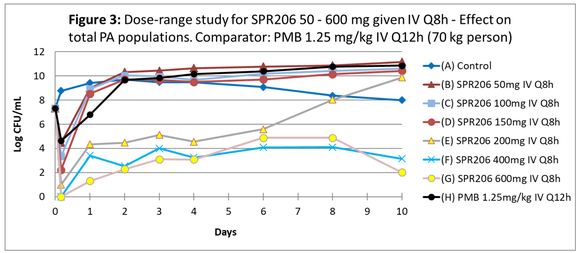
Results (Continued)

- **SPR206 dose-range HIFM study for SPR206 (Figure 3).** SPR206 produced a dose-response effect characterized by rapid and substantial dose-dependent killing of PA at the 5h time point. At this time point, SPR206 at the 100 mg dose produced 1.3 log CFU/mL more killing than PMB. However, the rapidly PA killing by all the SPR206 regimens and PMB was followed by regrowth. The regrowth was due to amplification of subpopulations with reduced susceptibilities to SPR206 and PMB.

The MICs of the less-susceptible colonies which grew on SPR206 or PMB-supplemented agars had MICs of 1 – 8 mg/L, compared to the SPR206 and PMB MICs of 0.5 mg/L for the parent isolate.

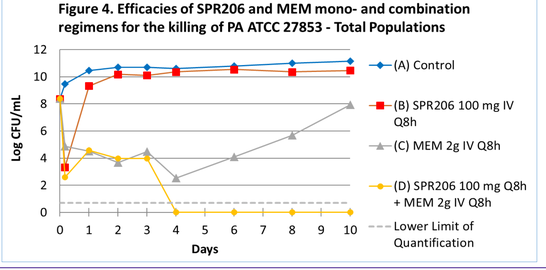
Maximum killing of PA was observed for the potential clinical regimen of SPR206 100 mg IV Q8h killed 4.00 log CFU/mL of bacteria. Emax killing of 7.33 log CFU/mL was observed with the SPR206 400 mg dose. The maximum killing by the PMB clinical regimen was 2.70 log CFU/mL **(Figure 3)**.

Figure 3: Dose-range study for SPR206 50 - 600 mg given IV Q8h - Effect on total PA populations. Comparator: PMB 1.25 mg/kg IV Q12h (70 kg person)



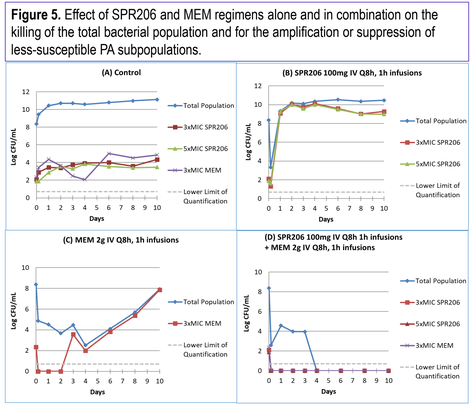
- **Improved efficacy of the combination of SPR206 and MEM is due to enhanced killing of PA and each drug counterselecting for resistance to its partner antibiotic. Figure 4** shows the effect of simulated regimens for SPR206 100 mg IV Q8h and MEM 2g IV Q8h, administered alone and in combination, on the killing of the total PA population. At 5h, SPR206 and MEM monotherapies killed 5 and 3.5 log CFU/mL, respectively. Regrowth was observed with both monotherapies, leading to treatment failure due to amplification of less-susceptible PA subpopulations **(Figure 5).** The combination regimen provided 0.8 log CFU/mL more killing than SPR206 monotherapy. Each antibiotic in the combination regimen counterselected for resistance to its partner drug, resulting in sterilization of its HFIM arm **(Figure 5).**

Figure 4. Efficacies of SPR206 and MEM mono- and combination regimens for the killing of PA ATCC 27853 - Total Populations



Results (Continued)

Figure 5. Effect of SPR206 and MEM regimens alone and in combination on the killing of the total bacterial population and for the amplification or suppression of less-susceptible PA subpopulations.



- Colonies which grew on 3x and 5x SPR206 MIC **in the control arm (Arm A)** had SPR206 MICs of 0.5 – 8 mg/L, compared to a SPR206 MIC of 0.5 mg/L for the parent isolate. The colonies which grew on agar containing 3x MIC for MEM had MEM MICs of 0.5 – 2 mg/L. The parent isolate had a MEM MIC of 0.5 mg/L.
- In Arm B (SPR206 monotherapy), regrowth was due to isolates with SPR206 MICs of 2 – 8 mg/L, compared to an SPR206 MIC of 0.5 mg/L for the parent isolate.
- In Arm C (MEM monotherapy), regrowth was also due to isolates with MEM MICs of 2 – 8 mg/L compared to an MEM MIC of 0.5 mg/L for the parent isolate.

Conclusions

- In the SPR206 dose range study, SPR206 50 – 600 mg IV Q8h rapidly killed PA in a dose-dependent manner. For all dose-regimens, the initial killing was followed by regrowth by isolates with reduced susceptibilities to this antibiotic.
- In the SPR206 and MEM mono- and combination therapy study, SPR206 100 mg IV Q8h, alone, killed 5 log CFU/mL of PA at 5h. MEM 2g IV Q8h, alone, produced less killing than SPR206 at 5h. For both monotherapies, the initial PA killing was followed by regrowth.
- At 5 h, SPR206 plus MEM produced 0.8 log CFU/mL and 2.3 log CFU/mL more killing of PA than the SPR206 and MEM monotherapies, respectively. The killing activity of the 2-drug regimen, combined with its resistance prevention effect, resulted in undetectable PA counts by Day 4 of treatment.