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# Treatment of *Mycobacterium avium* subspecies *hominissuis* (MAH) Infection with a Novel Gyrase Inhibitor (SPR719/SPR720) was Associated with a Significant Decrease in Bacterial Load as Assessed in Macrophages, Biofilm and in Mice

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## REVISED ABSTRACT

**Background:** *Mycobacterium* avium subsp. *hominissuis* (MAH) is a nontuberculosis mycobacterial (NTM) pathogen increasingly associated with pulmonary infection in individuals with underlying lung conditions, such as cystic fibrosis, emphysema, and bronchiectasis. Current treatment is lengthy and commonly results in emergence of resistant organisms. Therefore, new therapies are needed. A novel DNA gyrase inhibitor (SPR719, active moiety and SPR720, orally available pro-drug of SPR719) was evaluated for the ability to inhibit or kill MAH using macrophage and mouse model systems.

**Methods:** MIC testing of MAH strains MAC104 and A5 were performed by microbroth dilution, consistent with M7-A7 CLSI methodology. THP-1 macrophages were infected and treated for 6d. To evaluate efficacy, C57BL/6 mice were infected intranasally with 3 x $10^7$ MAC104 and allowed to establish for 3 weeks. Then, SPR720 at various concentrations and dosing regimens (q24h, q48h and q72h) were administered PO for 4 weeks. Clarithromycin (CLR at 100mg/kg/d) was used as a positive control.

**Results:** SPR719 had potency against MAH strains MAC104 and A5 of 1 and 0.5 mg/L, respectively. MAC104 infected macrophages treated with SPR719 at concentrations of 2 - 16 mg/L had a decrease of 83 – 89% in bacteria in 6d, compared with 95% reduction obtained with 8 mg/L of CLR. In the murine model of infection, the most efficacious regimen (lowest average lung CFU and most number of animals with cleared lungs) was obtained when SPR720 was dosed at 50 mg/kg q24h. The resulting lung CFU from all treatment groups can be found in the table.

**Conclusions:** Taken altogether, SPR719 showed significant activity *in vitro* and SPR720 showed significant activity *in vivo* models of MAH infection of the lung. These findings support the further advancement of SPR720 for the treatment of NTM lung disease.

## INTRODUCTION

- Infections caused by nontuberculosis mycobacteria (NTM) are increasing in prevalence due to improved recognition and diagnosis.
- NTM infections are generally difficult to treat since these organisms are resistant to most of the anti-mycobacterial drugs and therefore new agents are needed
- *M. avium* complex (MAC) and *M. abscessus* are the most common pathogens found
- *Mycobacterium* avium subsp. *hominissuis* (MAH) is a pathogen increasingly associated with pulmonary infection in individuals with underlying lung conditions, such as cystic fibrosis, emphysema, and bronchiectasis
- The *in vitro* and *in vivo* activity of SPR719 and it's phosphate prodrug, SPR720, was evaluated for the ability to inhibit or kill MAH using macrophage and mouse model systems

## RESULTS

**Table 1.** MIC (mg/L) values against MAH strains

| Compound | *M. avium* subspecies *hominssuis* strain number MIC in mg/L | |
|---|---|---|
| | 104 | A5 |
| SPR719 | 1 | 0.5 |
| Clarithromycin (CLR) | 2 | 1 |

- SPR719 was more potent than CLR against *M. avium* subspecies *hominissuis* strains 104 and A5

**Table 2.** Percent decrease of MAH104 after treatment of infected THP-1 macrophages

| Compound | Concentration | Percent decrease over t = 0 |
|---|---|---|
| SPR719 | 2 | 83 |
| | 4 | 83 |
| | 8 | 83 |
| | 16 | 89 |
| SPR720 | 2 | 75 |
| | 4 | 77 |
| | 8 | 84 |
| | 16 | 84 |
| CLR | 2 | 92 |
| | 4 | 93 |
| | 8 | 95 |
| | 16 | 97 |

- MAC104 infected macrophages treated with SPR719 at concentrations of 2 - 16 mg/L had a decrease of 83 – 89% in bacteria in 6d, compared with 95% reduction obtained with 8 mg/L of CLR

**Figure 1.** Structures of SPR719 (active) and SPR720 (prodrug)

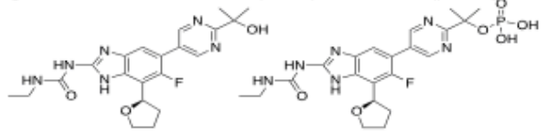

**Table 3.** Burden and number of animals with no CFU in lungs after 4 weeks of treatment

| Treatment Group | Average Lung CFU | N with no CFU in the lung |
|---|---|---|
| Pre-treatment | 6.8 x $10^5$ | 1 |
| Vehicle | 2.4 x $10^6$ | 1 |
| CLR 100 mg/kg/q24h | 9.4 x $10^4$ | 0 |
| SPR720 50 mg/kg/q24h | 2.6 x $10^5$ | 5 |
| SPR720 50 mg/kg/q48h | 4.4 x $10^5$ | 4 |
| SPR720 100 mg/kg/q48h | 4 x $10^5$ | 1 |
| SPR720 200 mg/kg/q48h | 3.2 x $10^5$ | 1 |
| SPR720 100 mg/kg/q72h | 4 x $10^5$ | 1 |
| SPR720 200 mg/kg/q72h | 5 x $10^5$ | 0 |

- In the murine model of infection, the most efficacious regimen (lowest average lung CFU and most number of animals with cleared lungs) was obtained when SPR720 was dosed at 50 mg/kg q24h.

## METHODS

- MIC testing of SPR719 against MAH strains MAC104 and A5 were performed by microbroth dilution, consistent with M24-A2 CLSI methodology[1]
- THP-1 macrophages were infected with MAH strains and treated for 6d with SPR719, SPR720 or comparators as described in Rose *et al*[2]
- To assess efficacy, C57BL/6 mice were infected intranasally with 3 x $10^7$ MAC104 and allowed to establish for 3 weeks as described previously[2]
- SPR720 (at 50, 100 or 200 mg/kg dosed either q24h, q48h or q72h) or clarithromycin (at 100 mg/kg q24h) was administered PO starting after the 3 week period and continued for 4 weeks

## CONCLUSIONS

- The novel gyrase inhibitor displayed potent activity *in vitro* against strains of *M. avium*
- SPR719 decreased bacterial load in MAC104 infected macrophages, similar to CLR
- SPR720 treatment significantly reduced the bacterial burden in the lungs of mice infected with strains of *M. avium*
- These findings support the further development of SPR720 for the treatment of NTM infections

## REFERENCES

1. CLSI (2011) M24-A2
2. Rose, SJ *et al.* 2014. Delivery of aerosolized liposomal amikacin as a novel approach for the treatment of nontuberculosis mycobacteria in an experimental model of pulmonary infection. PLOS One 9(9):e108703