Evaluating the Sterilizing Activity of SPR720 in Combination Therapy Against *Mycobacterium tuberculosis* Infection in Mice.

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Background: The emergence of multidrug resistant (MDR) and extensively drug resistant (XDR) strains of *M. tuberculosis* (Mtb) has made TB control difficult worldwide. New drugs are urgently needed. SPR720 (SPR) has been shown to be as active as moxifloxacin (MOX). The goal of this study was to compare the sterilizing activity of SPR in a regimen with rifampin (RIF) and pyrazinamide (PZA) to MOX with RIF and PZA. SPR720 is an orally bioavailable prodrug of SPR719, an aminobenzimidazole inhibitor of gyrase B/ParE with broad spectrum antibacterial activity. Spero Therapeutics is developing SPR720 for the treatment of infections caused by non-tuberculous mycobacteria.
Methods: Six-week old female BALB/c mice were infected with 3.24 Log_{10} CFU of Mtb Erdman (ATCC 35801) via aerosol delivery. Treatment started 3 weeks post-infection and was delivered by oral gavage 5 days per week. At treatment initiation, 6 mice were euthanized to determine the infection load. A drug control regimen consisting of INH (25 mg/kg), RIF (10 mg/kg), and PZA (150 mg/kg) (INH/RIF/PZA) was administered for 16 weeks. There were two regimens that contained SPR at 100 mg/kg, RIF at either 10 or 30 mg/kg, and PZA at 150mg/kg (SPR/RIF10/PZA and SPR/RIF30/PZA). These two regimens were compared to MOX (100 mg/kg), RIF (10mg/kg), and PZA (150 mg/kg) (MOX/RIF/PZA). The SPR and MOX regimens were administered for either 8 or 12 weeks with 14 mice in each arm of the experiment. Six mice were euthanized at the completion of therapy and 8 mice in each group were left untreated for an additional 12 weeks to measure the level of reactivation of Mtb following treatment completion. Six mice left untreated were euthanized at the 8 week timepoint to determine the infection load.
Results: The mice were infected with 7.68 ± 0.38 Log_{10} CFU Mtb at the time treatment began. No detectable colonies were recovered after 16 weeks of INH/RIF/PZA or 8 and 12 weeks of SPR/RIF10/PZA, SPR/RIF30/PZA, and MOX/RIF/PZA treatments. After 12 weeks post-treatment, there was Mtb regrowth observed in all groups with the exception of SPR/RIF30/PZA given for 12 weeks. There was more regrowth observed in the 16-week INH/RIF/PZA regimen than the 12-week SPR and MOX regimens. No differences in regrowth were observed between the SPR/RIF10/PZA and MOX/RIF/PZA groups at 8 and 12 weeks.
Conclusion: SPR was as effective as MOX in combination regimens with RIF and PZA. SPR combined with increased doses of RIF might be a better regimen to treat drug-susceptible TB and for XDR TB infections, SPR would be a viable option in second-line drug regimens.

Title: Potent Activity of a Novel Gyrase Inhibitor (SPR719/SPR720) *In Vitro* and in a Prolonged Acute *Mycobacterium abscessus* Mouse Model of Infection

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Background: *Mycobacterium abscessus,* a fast-growing mycobacterial species, has emerged in recent years as an important opportunistic pathogen increasingly responsible for mortality with extremely limited therapeutic options available. Here we evaluate the *in vitro* and *in vivo* activity of SPR719 and it's phosphate prodrug, SPR720, against *M. abscessus* 103 in a prolonged acute SCID mouse model of *M. abscessus* infection.

Methods: MIC testing was performed by microbroth dilution method using cation adjusted Mueller Hinton broth, consistent with M7-A7 CLSI methodology against clinical strains of *M. abscessus*. To assess efficacy, SCID female mice received an intravenous infection with $1x10^6$ CFU/mouse of *M. abcessus subsp* boletti strain 103. Three mice were sacrificed after day one of infection (PI) to determine bacterial uptake (lungs, spleens and livers). SPR720 was administered orally (PO) (400, 300, 200, 100, 50 and 25 mg/kg q24h) starting 2 days PI and continued for 16 days. Clarithromycin (CLR) was administered PO at 250 mg/kg q24h. Mice were sacrificed 24 h after the last dose with whole organ bacterial loads evaluated.

Results: SPR719 had potent activity against *M. abscessus* 103, *M. abscessus* 21 and *M. abscessus* 1513 strains (MIC in mg/L of 1, 1 and 2, respectively) compared to CLR (MIC >4 mg/L) and amikacin (>8 mg/L). In the efficacy study, no significant weight loss or clinical observations in the lungs, spleens or livers were noted for any of the SPR720 treated groups. Resulting burdens are shown in the table below.

Treatment Group	N	Burden (Log_{10} CFU)		
		Lung	Spleen	Liver
Day 2	3	4.1	5.1	6.1
Day 17	5	5.7	5.6	6.2
CLR	5	3.9	3.8	5.6
SPR720 – 400	5	3.1	3.7	5.3
SPR720 – 300	5	3.4	3.7	5.6
SPR720 – 200	5	3.4	3.1	4.9
SPR720 – 100	5	2.6	2.9	4.0
SPR720 – 50	5	3.6	3.6	5.6
SPR720 – 25	5	3.9	3.6	5.3

CLR served as a positive control and behaved as expected. SPR720 at 100 mg/kg q24h demonstrated the greatest reduction in bacterial burden in the lung ($p<0.0001$), spleen ($p<0.0001$), and liver ($p<0.0001$) compared to the *M. abscessus* day 17 infected control.

Conclusions: SPR719 displayed potent activity in all of the clinical strains tested *in vitro*. SPR720 siginificantly reduced the bacterial burden in lungs, spleens and livers in a prolonged SCID treatment mouse model. These findings support the further advancement of SPR720 for the treatment of nontuberculosis mycobacterial disease.