Nicole Cotroneo
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SPR720, A Novel Aminobenzimidazole Gyrase Inhibitor, Demonstrates Potent Efficacy Against *Mycobacterium avium* ATCC 700898 in a Chronic C3HeBFeJ Mouse Infection Model

Deepshikha Verma[1], Chelsea Peterson[1], Suzanne Stokes[2], **Nicole Cotroneo[2]**, Diane Ordway[1]

[1]Colorado State University, Fort Collins, CO; [2]Spero Therapeutics, Cambridge, MA

Spero Therapeutics
675 Massachusetts Ave
Cambridge, MA 02139
Phone: (781) 254-5393
Ncotroneo@sperotherapeutics.com

ABSTRACT

Background: SPR719 (the active metabolite of phosphate prodrug SPR720) belongs to a novel class which targets the ATPase subunits of gyrase by a mechanism distinct from fluoroquinolones. SPR719 has potent antibacterial activity against nontuberculous mycobacteria strains (NTM), including Mycobacterium avium, and is under development for treatment of NTM pulmonary disease. Oral efficacy of SPR720 was evaluated alone and in combination treatment in the C3HeBFeJ chronic mouse infection model which produces necrotic granulomas, similar to humans.

Methods: Mice were infected with a pulmonary aerosol of 1x108.5 CFU of *M. avium* ATCC 700898, (SPR719 MIC = 2 µg/mL). Treatment started on day 28 for 8 weeks with: saline, clarithromycin 250 mg/kg (CLR) QD, SPR720 at 10, 30 and 100 mg/kg QD, or SPR720 at 50 mg/kg BID. SPR720 at 30 mg/kg QD was also combined with CLR +/- ethambutol at 100 mg/kg (EMB), or CLR + rifabutin at 100 mg/kg (RFB) +/- EMB. Mice were evaluated for bacterial burden (CFU) on days 1, 27 and 60 after infection by plating serial dilutions of organ homogenates on nutrient 7H11 and charcoal agar plates. Lung pathology was evaluated by assessing prevalence and size of pulmonary lesions.

Results: CLR treatment for 28 days showed a significant reduction in the bacterial burden in the lung, spleen, and liver compared to the untreated control. SPR720 demonstrated a dose dependent reduction in bacterial burden, including at 100 mg/kg which showed a statistically significant reduction in the bacterial burden in the lung, spleen, and liver. CLR + EMB + SPR720 at 30 mg/kg showed the greatest reduction in the bacterial burden in the lung, spleen, and liver. RFB when added to the treatment regimen did not demonstrate enhanced efficacy compared the additive effect of EMB + CLR +/- SPR720. Lung pathology showed that lesions were less numerous and smaller in infected mice treated with all regimens.

Conclusions: Oral administration of SPR720 demonstrated a statistically significant reduction in the bacterial burden in all tissues with concomitant improvement in lung pathology, both alone and in combination with standard of care agents. These results support the continued development of SPR720 for treatment of NTM pulmonary infections.

INTRODUCTION

Nontuberculous mycobacterial (NTM) pulmonary infection is a chronic, progressive disease that occurs through inhalation of mycobacteria from environmental sources. NTM are found worldwide and NTM pulmonary infections are primarily due to *Mycobacterium avium* complex (MAC), *M. abscessus*, and *M. kansasii* (FDA 2016). There are currently no systemic antimicrobial agents specifically approved for the treatment of pulmonary NTM infections, and no formal FDA guidance for the clinical development of drugs for this indication. Thus, there is an urgent need for the introduction of new agents the treatment of NTM pulmonary disease (NTM-PD). The increasing resistance and poor tolerability to current standard of care (SoC) agents, as well as the high relapse and mortality rates, highlight the unmet need and priority for development of new agents for NTM-PD.

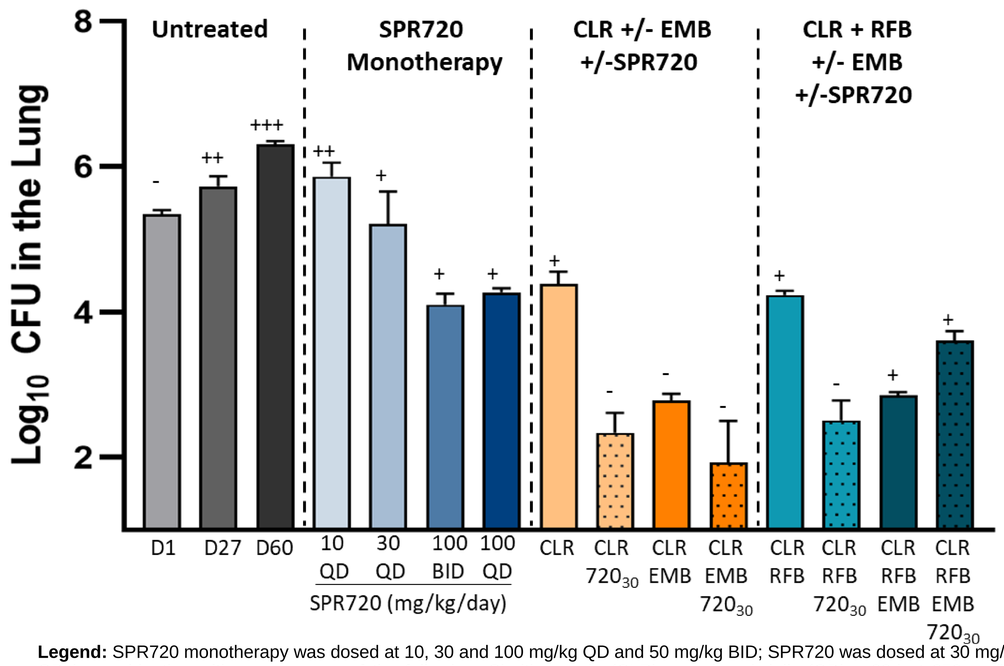
SPR720, a phosphate prodrug, converts rapidly to SPR719, the active metabolite, *in vivo*. SPR719 is an aminobenzimidazole which belongs to a novel class of antibacterial agents which targets the ATPase subunits of gyrase and topoisomerase via a mechanism distinct from that of the fluoroquinolones. These enzymes are highly conserved and essential in bacteria for DNA replication (Grillot et al 2014; O'Dowd et al 2015), and in mycobacteria, only gyrase enzymes have been identified. Oral efficacy of SPR720 was evaluated alone and in combination treatment in the C3HeBFeJ chronic mouse infection model which produces necrotic granulomas, similar to humans.

METHODS

The minimum inhibitory concentration (MIC) of SPR719 was determined using methods established by the CLSI. The efficacy of SPR720 was evaluated in a 60-day chronic C3HeBFeJ mouse infection model, a model which was developed to be representative of non-tuberculosis mycobacteria (NTM) pulmonary infection (Verma et. al 2019). C3HeBFeJ mice were infected by aerosol delivery of $1x10^{8.5}$ CFU/mL of *Mycobacterium avium* ATCC 700898, a broadly susceptible isolate with an SPR719 MIC of 2 µg/mL, Three mice were sacrificed on Day 2 and Day 27 post-infection to determine bacterial uptake. On Day 28 post-infection, SPR720 was administered by oral gavage at 10 mg/kg/day, 30 mg/kg/day, and 100 mg/kg/day delivered every 24 hours (QD), and 50 mg/kg was delivered twice a day (BID) for a total of 100 mg/kg/day. Positive control clarithromycin was administered by oral gavage at 250 mg/kg/day. Ethambutol was administered by oral gavage at 100 mg/kg/day, and rifabutin was administered by oral gavage at 100 mg/kg/day in combination treatments. Animals were dosed from Day 28 to 61 consecutively. At the end of the dosing phase (Day 61), whole lung, spleen and liver were aseptically removed, processed, and plated on 7H11 agar plates for 21-25 days to determine bacterial burdens as colony forming units (CFUs).

RESULTS

Figure 1: Pulmonary Bacterial Burden (log_{10} CFU/lung) after Treatment with SPR720 Alone and in Combination with Clarithromycin, Rifabutin, and/or Ethambutol



Legend: SPR720 monotherapy was dosed at 10, 30 and 100 mg/kg QD and 50 mg/kg BID; SPR720 was dosed at 30 mg/kg QD in combination with one or more of the following: Clarithromycin (CLR) was dosed daily (QD) x 28d at 250 mg/kg; Ethambutol (EMB) was dosed QD x 28d at 100 mg/kg; Rifabutin (RFB) was dosed QD x 28d at 100 mg/kg.
Lung appearance: - Uninfected; + slightly infected; ++ moderately infected; +++ heavily infected

- ***M. avium* ATCC 700898 SPR719 MIC was 2 µg/mL. RFB, CLR and EMB MICs are 0.25, 1 and 4 µg/mL, respectively.**
- **SPR720 monotherapy resulted in a dose-dependent reduction in pulmonary bacterial burden of *M. avium* ATCC 700898 at 10 – 100 mg/kg/day in comparison to the Day 60 untreated control group. In the lung, liver and spleen, SPR720 similarly reduced the burden ($p<0.001$) at 50 mg/kg BID and 100 mg/kg QD (Table 1).**
- **The reduction in log_{10} CFU achieved with all CLR combinations was statistically significant in comparison to the Day 60 control ($p<0.0001$). SPR720 at 30 mg/kg/day improved the efficacy of CLR (-3.96 log_{10}) and was similar to CLR + EMB (-3.5 log_{10}).**
- **The combination of CLR (250 mg/kg), EMB (100 mg/kg) and SPR720 (30 mg/kg) demonstrated the greatest reduction of 4.37 log_{10} CFU in the average bacterial burden in all tissues studied.**
- **The addition of RFB did not enhance the activity of CLR +/- EMB, regardless of whether SPR720 was included in the treatment regimen.**
- **All treatment regimens improved lung appearance vs. the untreated control. The lungs of mice treated with CLR + SPR720 at 30 mg/kg/day did not have any visual indication of infection, similar to the lungs of mice treated with CLR + EMB +/- SPR720 at 30 mg/kg/day as well as CLR + RFB +/- SPR720 at 30 mg/kg/day.**
- **Various combination treatments of SPR720, CLR, RFB and EMB reduced the bacterial burden in the lung, liver, and spleen compared to Day 60 controls.**

Table 1: Bacterial Burden (log_{10} CFU/organ) in Lung, Spleen and Liver

Group	N	Lung Log_{10} CFU ±SEM	Spleen Log_{10} CFU ±SEM	Liver Log_{10} CFU ±SEM
D1 Inoculation Control	3	5.53±0.05	0±0	0±0
D27 Treatment Initiation Control	3	5.72±0.14	3.16±0.24	3.33±0.05
D60 Untreated Control	3	6.3±0.04	4.20±0.04	5.1±0.08
SPR720 10 mg/kg QD	6	5.86±0.19	3.80±0.07	3.88±0.10
SPR720 30 mg/kg QD	6	5.21±0.44	3.31±0.17	3.40±0.18
SPR720 50 mg/kg BID	6	4.10±0.14	3.07±0.23	3.34±0.89
SPR720 100 mg/kg QD	6	4.26±0.05	3.21±0.45	3.5±0.52
CLR 250 mg/kg QD	6	4.38±0.17	2.41±0.56	2.69±0.11
CLR + SPR720 QD	6	2.34±0.27	2.24±0.52	2.68±0.73
CLR + EMB QD	6	2.78±0.09	2.2±0.54	3.6 ±0.52
CLR + EMB + SPR720 QD	6	1.93±0.57	1.7±0.40	2.13±0.67
CLR + RFB QD	6	3.64±0.06	2.80±0.15	3.76±0.06
CLR + RFB + SPR720 QD	6	2.5±0.27	2.40±0.64	2.73±0.57
CLR + EMB + RFB QD	6	2.85±0.04	2.81±0.18	3.73±0.03
CLR + EMB + RFB + SPR720 QD	6	3.61±0.12	2.85±0.16	3.6 ±0.52

Legend: SPR720 monotherapy was dosed at 10, 30 and 100 mg/kg QD and 50 mg/kg BID; SPR720 was dosed at 30 mg/kg QD in combination with one or more of the following: Clarithromycin (CLR) was dosed daily (QD) x 28d at 250 mg/kg; Ethambutol (EMB) was dosed QD x 28d at 100 mg/kg; Rifabutin (RFB) was dosed QD x 28d at 100 mg/kg.

CONCLUSIONS

- **The oral administration of SPR720 demonstrated a statistically significant reduction in the bacterial burden in all tissues with concomitant improvement in lung pathology, both alone and in combination with standard of care agents.**
- **These data support the further clinical development of SPR720 as an oral option for the treatment of pulmonary NTM infections in humans.**

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