ASM Microbe
San Francisco
Friday, June 21st, 2019
FRIDAY-AAR-750

In Vitro Activity of SPR719 against Non-Tuberculosis Mycobacterium Strains of *Mycobacterium ulcerans*, *Mycobacterium marinum*, and *Mycobacterium chimaera*

Troy Lister
Spero Therapeutics
troy@sperotx.com

S. Pidot,[1] T. Stinear,[1] T. Lister,[2*] and A. Rubio[2]

[1]Peter Doherty Institute for Infection and Immunity, University of Melbourne, VIC, Australia, [2]Spero Therapeutics, Cambridge, MA, USA

ABSTRACT

Background: Non-tuberculosis mycobacteria (NTM) are increasingly associated with a variety of pulmonary and skin infections. Current treatment can be lengthy and variably effective, often leading to the emergence of resistance. Novel therapies are needed. This study evaluated activity of SPR719 against clinical isolates of 3 different species of NTM. SPR719 is an aminobenzimidazole inhibitor of GyrB/ParE with broad-spectrum antibacterial activity. Spero Therapeutics is developing SPR720, an orally bioavailable pro-drug of SPR719, for the treatment of NTM infections.

Methods: A panel of 20 multi-drug resistant bacterial isolates of diverse geographic origin were used: 10 strains of *M. ulcerans*, 5 strains of *M. marinum* and 5 strains of *M. chimaera*. Bacteria were grown on Brown and Buckle slopes and colonies were suspended in distilled water at ~ $4x10^5$ CFU/mL. Suspensions were diluted and approximately $4x10^2$ or $4x10^3$ bacteria were plated onto duplicate 7H10 agar plates + 10% OADC supplement containing SPR719, rifampin (RIF), or clarithromycin (CLR). Plates were incubated for 2 weeks at 30° C for *M. marinum*, 3 weeks at 37° C for *M. chimaera*, and 2.5-3 months at 30° C for *M. ulcerans*. The MIC was defined as the lowest concentration of antibiotic that inhibited visible bacterial growth compared to the negative control. In addition, *M. ulcerans* grown in mycobacterial growth indicator tubes (MGIT), each tube was inoculated with $4x10^4$ bacteria. Tubes were incubated for 3 weeks at 30° C before reading. The MIC was defined as the lowest concentration of antibiotic that did not give detectable fluorescence.

Results: Concentration ranges at which SPR719 and comparator agents inhibited each species are summarized below. SPR719 inhibited all isolates tested with MICs of 0.5-1 mg/L, ≤0.03-2 mg/L, and 0.12-2 mg/L against *M. marinum*, *M. chimaera*, and M. *ulcerans*, respectively, comparing favorably to RIF and CLR. Agar and MGIT MICs agreed within 2-fold.

Conclusion: SPR719 exhibited potent activity against all strains of Mycobateria assessed. Importantly, SPR719 demonstrated similar or superior potency against these strains compared to CLR or RIF, indicating the potential for SPR719 to be utilized as a novel treatment option for infections resulting from such Mycobacteria.

INTRODUCTION

Nontuberculous mycobacterium (NTM) infections are increasing in prevalence globally due to improved recognition and diagnosis. NTM, once thought to be merely environmental species, are responsible for a wide range of infections that are generally difficult to treat due in part to high rates of resistance and poor tolerability to current therapeutic options. Therefore, new agents are needed. SPR720 is an orally bioavailable phosphate prodrug of SPR719, a novel aminobenzimidazole which exhibits potent, broad-spectrum antibacterial activity, including against NTM species. Here, SPR719 was assessed for activity against rare NTM species *Mycobacterium ulcerans*, *Mycobacterium marinum*, and *Mycobacterium chimaera* utilizing MGIT and agar methods.

METHODS

A panel of 20 multi-drug resistant bacterial isolates of diverse geographic origin were used: 10 strains of *M. ulcerans*, 5 strains of *M. marinum* and 5 strains of *M. chimaera*. Bacteria were grown on Brown and Buckle slopes and colonies were suspended in distilled water at ~ $4x10^5$ CFU/mL. Suspensions were diluted and approximately $4x10^2$ or $4x10^3$ bacteria were plated onto duplicate 7H10 agar plates + 10% OADC supplement containing SPR719, rifampin, or clarithromycin. Control plates containing no antibiotic were also prepared. All plates were prepared fresh before use. Plates were incubated for 2 weeks at 30°C for *M. marinum*, 3 weeks at 37°C for *M. chimaera*, and 2.5-3 months at 30°C for *M. ulcerans*. The MIC was defined as the lowest concentration of antibiotic that inhibited visible bacterial growth compared to the negative control. In addition, *M. ulcerans* grown in mycobacterial growth indicator tubes (MGIT), each tube was inoculated with $4x10^4$ bacteria. Tubes were incubated for 3 weeks at 30°C before reading fluorescence by manual read. The MIC was defined as the lowest concentration of antibiotic that did not give detectable fluorescence.

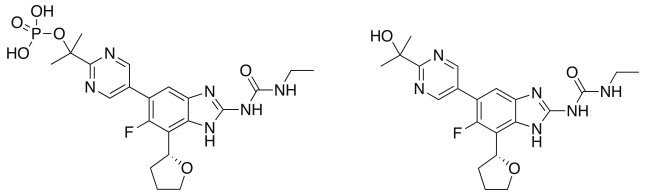

Figure 1. *Structure of SPR720 (left) and SPR719 (right)*

RESULTS

Agent	MIC (mg/L)	*M. marinum* (N=5)	*M. chimaera* (N=5)	*M. ulcerans* (N=10)
SPR719	MIC range	0.5-1	<0.03-2	0.25-0.125
	MIC_{50}	1	0.25	0.125
Rifampin	MIC range	0.5-1	ND	0.125-2
	MIC_{50}	1	ND	1
Clarithromycin	MIC range	0.5-2	0.5-4	ND
	MIC_{50}	1	0.5	ND

Table 1. *Activity of SPR719 against the tested Mycobacterium species compared to rifampin and clarithromycin. ND= not determined*

RESULTS

Table 1 shows the MIC range and MIC_{50} of SPR719 and comparator agents, rifampin and clarithromycin, against a collection of isolates from the NTM species *M. ulcerans*, *M. marinum*, and *M. chimaera*. SPR719 exhibits potent, broad-spectrum MICs against all species and isolates tested. Additionally, SPR719 comparing favorably to rifampin and clarithromycin across the isolates examined.

M. ulcerans strain #	Agar MIC (mg/L)	MGIT MIC (mg/L)	Source Country	Source Type
8063	0.125	0.125	Malaysia	Clinical
8049	0.125	0.25	Australia	Clinical
8069	0.125	0.125	Ghana	Clinical
8349	0.125	0.125	Ghana	Clinical
8384	0.125	0.25	Ivory Coast	Clinical
8386	0.25	0.25	Ivory Coast	Clinical
8387	0.125	0.125	Benin	Clinical
8489	0.25	0.125	Benin	Clinical
8537	0.125	0.125	Cameroon	Clinical
8596	0.125	0.125	Cameroon	Clinical

Table 2. *Activity of SPR719 against M. ulcerans* by agar *vs.* MGIT methods.

Mycobacterium ulcerans is a slow growing bacteria. Unlike typical Gram-positive and Gram-negative bacteria for which an MIC can be obtained after 24 hour incubation, NTM, including *M. ulcerans*, require several months to obtain MIC using traditional plate based methods. MIGT tubes allow MICs to be obtained in far shorter timeframe, but the correlation with CLSI methods has not been established. Table 2 shows that MICs obtained for SPR719 using either agar plate method or the MGIT method compare favorably, with MICs within 2-fold across the 10 strains of *M. ulcerans* tested.

CONCLUSIONS

SPR719 exhibited potent activity against all species and strains of NTM assessed. Importantly, SPR719 demonstrated similar potency compared to clarithromycin and rifampin, indicating the potential for SPR719 to be utilized as a novel, potentially better tolerated oral treatment option for infections resulting from such Mycobacteria.