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A GLP 14 Day Repeat Dose Toxicology Study of SPR206 in Monkeys

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ABSTRACT

Background: SPR206 is a polymyxin analog with potent, broad-spectrum direct antibacterial activity being investigated for the treatment of multi-drug resistant Gram-negative infections. The GLP 14-day toxicology assessment of SPR206 is reported here, including a CNS assessment

Methods: SPR206 was assessed for toxicity in male and female cynomolgus monkeys at 15, 30, and 45 mg/kg/day delivered via three one-hour infusions (8 hours apart; TID) for 14 consecutive days. A 28-day recovery period also was included in the study design. Parameters assessed during the study included body weights, clinical observations, food consumption, neurological examinations, electrocardiography, ophthalmology, hematology, coagulation, serum chemistry, urinalysis, and urine and plasma toxicokinetics. Macroscopic findings, organ weights, and histopathology were performed on a full panel of tissues.

Results: The NOAEL of SPR206 following 14 days of TID dosing to cynomolgus monkeys was 30 mg/kg/day. There were no SPR206 related effects on food consumption, electrocardiography, or ophthalmology at any SPR206 dose. The target organ of toxicity for SPR206 in monkeys was the kidney. SPR206 at 45 mg/kg/day resulted in significant clinical observations, including one moribund female, and mild to moderate increases in blood urea nitrogen (BUN) and serum creatinine (SrCr). Changes in BUN and SrCr correlated with pale kidneys (one female), higher kidney weights, and histopathological changes in the kidney of tubular regeneration, degeneration/necrosis, casts, and dilation. The observed nephrotoxicity at 45 mg/kg/day was partially reversible following a 28-day recovery period. SPR206 was associated with sporadic hypoactivity, ataxia, and balance at ≥30 mg/kg/day, however, no neurological effects were evident at any dose level including the highest dose tested of 45 mg/kg/day. The toxicokinetics of SPR206 were dose proportional and dose linear; the AUC0-24h exposure of SPR206 at the NOAEL dose was 342 µg*hr/mL.

Conclusion: As anticipated, the target organ of toxicity for SPR206 in this study was the kidney. This toxicity is monitorable, reversible, and the NOAEL AUC0-24h exposure compares favorably to that of clinically validated progenitor SPR741 therefore supporting advancement of SPR206 into Phase 1 studies. In other GLP safety testing, SPR206 was not genotoxic, and there were no significant alterations in cardiovascular or respiratory safety pharmacology parameters.

INTRODUCTION

SPR206 is a polymyxin analog with potent, broad-spectrum, direct antibacterial activity the four major Gram-negative ESKAPE pathogens (*K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* species), as well as *E. coli*, including those possessing prominent resistant mechanisms. SPR206 exhibits non-clinical efficacy similar or superior to PMB and colistin and is being investigated for the treatment of serious Gram-negative hospital infections. SPR206 was assessed for potential target organs of toxicity, recovery of any toxicity, and biomarkers to monitor any potential toxicity in a GLP 14-day toxicology study in cynomolgus monkeys per ICH guidance to enable Phase 1 studies in normal healthy volunteers.

METHODS

SPR206 or control article/vehicle (0.9% Sodium Chloride Injection, USP) was administered by IV, 1-hour infusion through a chronic femoral IV catheter. Doses were administered three times per day (8 hours ± 30 minutes apart), for fourteen consecutive days. The dosing volume was constant among groups, at 10 mL/kg. The SPR206 dose levels in the current study were 15, 30, or 45 mg/kg/day. Parameters assessed during the in-life phase of the study included weekly body weights, clinical observations (daily during the dosing period), food consumption, neurological examinations, electrocardiography, ophthalmology, clinical pathology (hematology, coagulation, serum chemistry including blood urea nitrogen and creatinine, urinalysis), and plasma toxicokinetics (TK). At necropsy, gross observations were recorded, organ weights were measured, and specific tissues were collected. Histopathologic assessment was conducted on tissue sections stained with hematoxylin and eosin (H&E).

For toxicokinetic assessment, blood samples were collected from the SPR206 dosed monkeys prior to dose administration, immediately following the end of dose administration (within 5 minutes), and at approximately 30 minutes and 1, 2, 4, and 8 hours (immediately prior to the second TID dose) post end of the first TID dose on study days 1 and 14. The plasma from these samples was analyzed for SPR206 using a validated bioanalytical method.

RESULTS

The NOAEL of SPR206 following 14 days of repeated three times per day one hour infusions in male and female cynomolgus monkeys was 30 mg/kg/day based upon histopathological observations and accompanying BUN and serum creatinine biomarker evaluations (**Table 1**). SPR206 at doses of ≤ 30 mg/kg/day was associated with non-adverse changes in tubular degeneration/regeneration and tubular necrosis with slight increases in renal biomarkers. At 45 mg/kg/day, SPR206 was associated with a higher incidence and greater severity of tubular degeneration/regeneration and tubular necrosis with associated increases in renal biomarkers. Histopathological changes were partially revesible at 30 and 45 mg/kg/day doses.

The functional observational battery assessment for potential CNS-related impact of SPR206 noted an isolated incident in one animal at 30 mg/kg/day on day 3, that resolved. Additionally, there were no SPR206-realted injection site reactions either macroscopically or microscopically.

In other GLP and non-GLP assessments, SPR206 was determined to have no impact on CV/pulmonary pharmacology and to be negative for chromosomal aberration, Ames, hERG, micronucleus, flocculation, hemolysis, and local irritation. Additionally, SPR206 is not metabolized by human and non-human intestinal or liver S9 fractions, and is not a direct, or time-dependent inhibitor of human CYP isoforms (>100 µM).

Table 1: SPR206 Demonstrates Non-adverse Findings Following 14-days of Repeated Dosing at 30 mg/kg/day

Dose (mg/kg/day)	% Increase Control BUN (mg/dL)	% Increase Control CRN (mg/dL)	Renal Histopathology
Saline	N/A	N/A	1/6 minimal tubular degeneration/ necrosis
15	104	97	No findings
30	125	103	2/6 minimal tubular degeneration/ necrosis 1/6 mild tubular degeneration/ necrosis
45	117	127	2/6 minimal tubular degeneration/ necrosis 1/6 mild tubular degeneration/ necrosis 1/6 moderate tubular degeneration/ necrosis

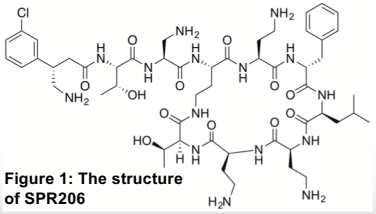

Figure 1: The structure of SPR206

Toxicokinetic analysis revealed dose linear and dose proportional plasma exposure of SPR206 after IV dosing and no apparent accumulation over 14 days of dosing. The daily AUC of SPR206 at the NOAEL dose of 30 mg/kg/day was determined to be 345 mg*hr/mL, with a Cmax of 42 mg/mL (Table 2).

Table 2: Dose Proportional Exposure of SPR206 Following 14 Days of Repeated Dosing

Dose (mg/kg/day)	Study Day	Cmax (µg/mL)	$AUC_{(0\text{-}24)}$ (µg*hr/mL)
15	1	18.7	135.2
	14	19.9	131.9
30	1	41.2	321.6
	14	43.3	343.2
45	1	70.9	636.0
	14	69.6	559.5

Importantly, the plasma exposure of SPR206 at the NOAEL dose of 30 mg/kg/day compares favorably to the plasma exposure recorded for SPR741 at its NOAEL dose of 45 mg/kg/day when administered in an identical manner during an identical, but separate GLP study (**Table 3**). This result is crucial to the progression of SPR206, since SPR741 has successfully completed Phase 1 clinical evaluations in which safe and tolerated human doses corresponded to plasma exposure similar to those recorded at the NOAEL in monkey.

Table 3: Comparison of SPR206 vs. SPR741 toxicokinetics at respective NOAEL dose

Compound	Dose (mg/kg/day)	Cmax (µg/mL)	$AUC_{(0\text{-}24)}$ (µg*hr/mL)	AUC/dose
SPR206	30	42	345	11.5
SPR741	40	47	363	9.1

CONCLUSIONS

As anticipated, the target organ of toxicity for SPR206 in this study is the kidney. The toxicity is monitorable and reversible. TK analysis at the NOAEL dose indicates high plasma exposure, consistent with a previously evaluated molecule, SPR741, that successfully completed Phase 1 evaluations. Additional toxicology and safety pharmacology studies of SPR206 reveal the risk for CNS, CV/pulmonary, genotoxic and metabolic issues is low. The totality of data supports advancement of SPR206 into clinical evaluation and we anticipate initiating Phase 1 studies around year end.

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