ABSTRACT

Understanding the parameters that lead to antimicrobial activity, kidney cell toxicity and kidney accumulation of polymyxin analogues, has facilitated the design of novel polymyxin analogues that exhibit reduced in vivo toxicity and retain excellent antibacterial properties. We demonstrate that compounds with an aminobutyrate N-terminus substituted at the β-position with an aryl-containing moiety, as exemplified by SPR206, offer an excellent combination of low cytotoxicity and kidney exposure. These promising attributes support further evaluation of SPR206 towards clinical development.

INTRODUCTION

We have previously shown that polymyxin nonapeptides with an N-terminal group containing an appropriately positioned amine branched from an alkyl or aralkyl chain, show promising antibacterial activity and lower cytotoxicity compared to polymyxin B (PMB). Here we show that in vivo toxicity is related to both kidney exposure and in vitro cytotoxicity. We have determined the relationship of these parameters to key structural features and subsequently exploited this understanding to design less toxic analogues of PMB that retain potent antimicrobial activity.

METHODS

- **Chemical synthesis:** Polymyxin nonapeptide derivatives were synthesised as previously described.
- **In vitro activity:** Minimal inhibitory concentrations (MICs) were determined by microbroth dilution using cation-adjusted Mueller-Hinton broth (Oxoid, CM0405) in 96-well polystyrene microtitre plates according to CLSI guidelines.
- **In vitro cytotoxicity:** Mammalian cell toxicity was measured using confluent monolayers of the human HK-2 proximal tubule epithelial cell line. Cell viability was measured using resazurin blue. IC_{50} is expressed relative to that of PMB in the same experiment.

**Kidney PK:** Levels of drug in kidneys were determined by LC-MS/MS following subcutaneous (SC) administration in mice (17.2 mg/kg free base) with kidneys collected 4, 8, and 16 hours after dosing (n=3 mice/timepoint).

**In vivo renal toxicity:** Renal toxicity in the mouse was determined after dosing SC three times a day (17.2 mg/kg/dose free base) at 4 hours intervals for 4 consecutive days, or after dosing SC at 25mg/kg free base at 8h intervals for 24 hours. Starting immediately after the last dose, mice were transferred to individual metabolic cages and urine was collected over the following 24 hours for determination of biomarker levels. At termination kidneys were excised and stored in neutral buffered formalin for histopathology.

**Results**

- Piperidines (A), although showing reduced cytotoxicity compared to PMB, show extremely high kidney exposure resulting in high in vivo renal toxicity.
- Alpha-substituted aminobutyrates (B) show a similarly high kidney exposure, leading to high in vivo toxicity (CA1104).
- Beta-substituted aminobutyrates (C), show reduced kidney exposure compared to alpha-substituted, as shown by comparing SPR1359/SPR206, SPR1360/SPR1361.

- Although both diastereomers in the beta-substituted series show similar antibacterial activity diastereomer C1 consistently shows lower kidney exposure.
- Beta-aryl/benzyl aminobutyric acidides, such as SPR206 and SPR1362, with kidney exposure similar to PMB, with reduced cytotoxicity, show very low in vivo renal toxicity, while retaining excellent in vitro potency.

CONCLUSIONS

- **In vitro cytotoxicity** alone is not a predictor of **in vivo toxicity.** Kidney accumulation also plays a significant role in toxicity.
- **Compounds** with an aminobutyrate N-terminus substituted at the β-position with an aryl-containing moiety, e.g. SPR206, offer an excellent combination of low cytotoxicity and kidney exposure, leading to low in vivo renal toxicity, while retaining excellent antibacterial activity in vitro.
- These promising attributes support further evaluation of SPR206 towards clinical development.

REFERENCES

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Understanding the SAR Interplay for Kidney Exposure and Cytotoxicity Facilitates the Design of Improved Polymyxin Derivatives – Identification of SPR206 as a Development Candidate

P. Brown1, S. Boakes2, E. Duperchey2, D. Rivers2, J. Singh2 and M. J. Dawson1

1Spero Therapeutics, Cambridge, MA, USA; 2Cantab Anti-infectives, Welwyn Garden City, UK.