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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

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## ABSTRACT

Understanding of 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[1]
- ***In vitro* activity**: Minimal inhibitory concentrations (MICs) were determined by microbroth dilution using cation-adjusted Mueller-Hinton broth (Oxoid, CM0405) in 96-well polypropylene microtitre plates according to CLSI guidelines[2]
- ***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 4h 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. Levels of urinary biomarkers KIM-1, cystatin-C and albumin in the urine were measured by ELISA. Compounds were scored as Low, Medium, or High toxicity based on a combination of biomarker levels and histopathology, compared to Polymyxin B which was scored as Medium

## RESULTS

**Figure 1** Structures of Polymyxin nonapeptides and Polymyxin B

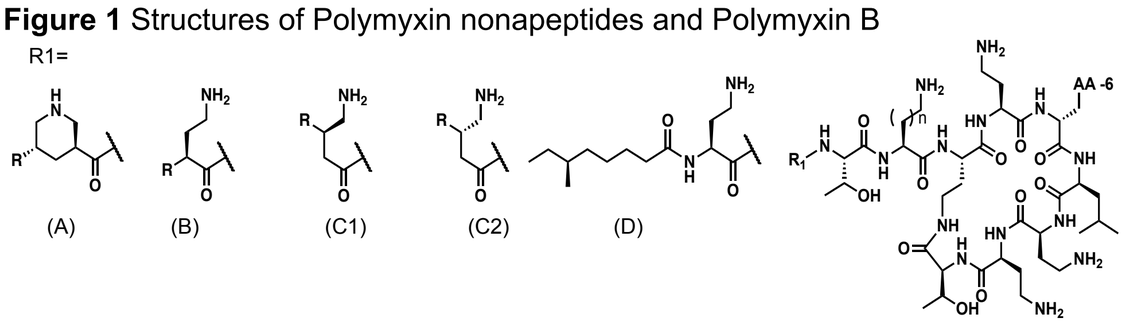
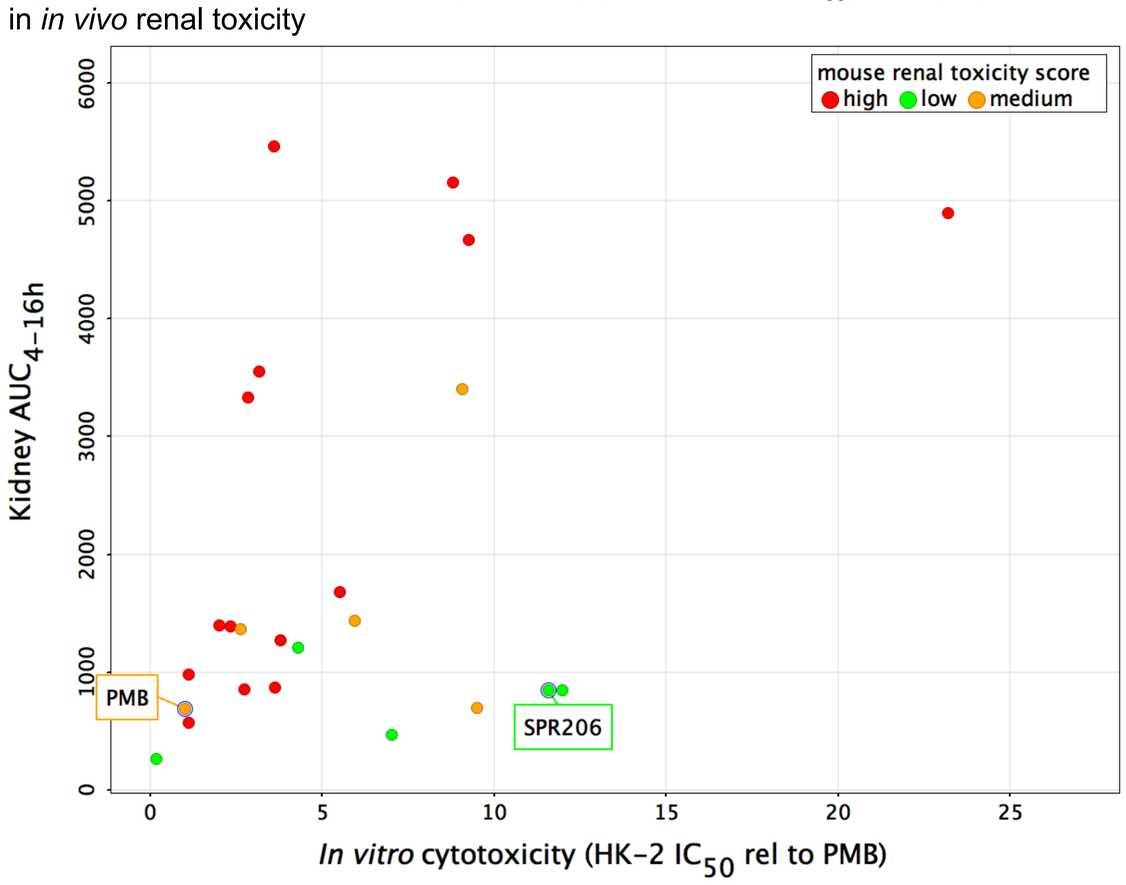



**Figure 2.** Kidney exposure and cytotoxicity (relative HK-2 $IC_{50}$) both play a role in *in vivo* renal toxicity




Relationship between cytotoxicity and kidney exposure of 32 polymyxin nonapeptides of general structures shown in Figure 1, and coloured by renal toxicity score

**Table 1.** Nonapeptides with differing N-terminal moieties have very different kidney exposure

| | AA-6 | Structure class | n | R | *E.coli* ATCC 25922 | *K.pneumoniae* ATCC 13882 | *P.aeruginosa* ATCC 27853 | *A.baumannii* NCTC 13424 | $IC_{50}$ rel to PMB | Kidney level 4h (µg/g) | Kidney $AUC_{4-16h}$ (µg.h/g) | *In vivo* tox |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SPR1355 | D-Phe | A | 0 | isobutyl | 0.03 | 0.03 | 0.25 | 0.125 | 8.8 | 469 | 5161 | H |
| SPR1356 | D-Phe | A | 0 | cyclohexyl | 0.125 | 0.125 | 0.5 | 0.125 | 5 | 603 | 4430 | H |
| SPR1357 | D-Leu | A | 0 | isobutyl | 0.06 | 0.06 | 0.5 | 0.06 | 23.2 | 516 | 4902 | H |
| SPR1358 | D-Cha | B | 0 | phenyl | 0.06 | 0.125 | 0.25 | 0.03 | 9.2 | 567 | 4674 | H |
| SPR1359 | D-Phe | B | 0 | 3-chlorophenyl | 0.125 | 0.125 | 0.03 | 0.125 | 5 | 589 | ND | ND |
| SPR1360 | D-Phe | B | 0 | 4-chlorophenyl | 0.06 | 0.125 | 0.125 | 0.06 | 4.8 | 533 | ND | ND |
| SPR206 | D-Phe | C1 | 0 | 3-chlorophenyl | 0.125 | 0.125 | 0.25 | 0.06 | 11.6 | 170 | 850 | L |
| SPR1361 | D-Phe | C1 | 0 | 4-chlorophenyl | 0.125 | 0.125 | 0.06 | 0.06 | 8.8 | 267 | 1632 | ND |
| SPR1362 | D-Phe | C1 | 0 | benzyl | 0.25 | 0.25 | 0.125 | 0.125 | 12.0 | 159 | 847 | L |
| PMB | D-Phe | D | 1 | | 0.25 | 0.25 | 0.5 | 0.25 | 1 | 128 | 688 | M |

MICs in µg/ml. ND=not determined. In vivo tox score: H=High, M=Medium, L=Low. Cha: cyclohexylalanine

- 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

**Table 2.** Differences in kidney exposure for β-branched aminopropyl nonapeptides diastereomers

| | AA-6 | Structure class | n | R | *E.coli* ATCC 25922 | *K.pneumoniae* ATCC 13882 | *P.aeruginosa* ATCC 27853 | *A.baumannii* NCTC 13424 | $IC_{50}$ rel to PMB | Kidney level 4h (µg/g) | *In vivo* tox |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SPR206 | D-Phe | C1 | 0 | 3-chlorophenyl | 0.125 | 0.125 | 0.25 | 0.06 | 11.6 | 170 | L |
| SPR1363 | D-Phe | C2 | 0 | | 0.125 | 0.125 | 0.125 | 0.5 | 7.8 | 381 | ND |
| SPR1361 | D-Phe | C1 | 0 | 4-chlorophenyl | 0.125 | 0.125 | 0.06 | 0.06 | 8.8 | 267 | ND |
| SPR1364 | D-Phe | C2 | 0 | | 0.25 | 0.25 | 0.25 | 0.25 | 7.2 | 538 | ND |
| SPR1205 | D-Phe | C1 | 0 | n-pentyl | 0.125 | 0.25 | 0.125 | 0.125 | 5.9 | 224 | M |
| SPR1365 | D-Phe | C2 | 0 | | 0.06 | 0.25 | 0.06 | 0.06 | 6.6 | 470 | ND |
| SPR1362 | D-Phe | C1 | 0 | benzyl | 0.25 | 0.25 | 0.125 | 0.125 | 12.0 | 159 | L |
| SPR1366 | D-Phe | C2 | 0 | | 0.125 | 0.5 | 0.125 | 0.25 | 8 | 346 | ND |
| SPR1367 | D-Phe | C1 | 0 | 3-methylphenyl | 0.125 | 0.25 | 0.25 | 0.125 | 26.7 | 209 | ND |
| SPR1368 | D-Phe | C1 | 0 | 3-chlorobenzyl | 0.5 | 0.5 | 0.25 | 0.25 | 5.2 | 163 | ND |

MICs in µg/ml. ND=not determined. In vivo tox score: H=High, M=Medium, L=Low

- Although both diastereomers in the beta-substituted series show similar antibacterial activity diastereomer C1 consistently shows lower kidney exposure
- Beta-aryl/benzyl aminobutyrates, such as SPR206 and SPR1362, with kidney exposure similar to PMB, but 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

1. Brown, P. *et al.* Synthesis and structure-activity relationships of polymyxin nonapeptide derivatives with N-terminal aminoacyl moieties. *Poster F-739, 55th Intersci. Conf. Antimicrob. Agents Chemother., San Diego, USA* (2015)
2. CLSI M07-A10: Methods for Dilution Antimicrobial Susceptibility Testing for Aerobic Bacteria

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