In vitro kidney tox systems with increased sensitivity and throughput evolving

human kidney cells > opossum kidney cells > NRK-52 cells

Do they reflect the in vivo situation?

How can we better capture kidney injury onset?
Reference molecules to build *in vitro* to *in vivo* correlation

- **Colistin sulfate**
  - used as a drug directly in Asia
  - available as USP material

- **Polymyxin B nonapeptide**
  - reported to be much safer in *in vitro* assays and in a dog *in vivo* study (AAC1989, p1428)
  - Synergistic (lowering MIC) with some other antibiotics (MurF, LpxC inhibitors, AAC (2009) 53, 3240-3247), PF1090; ICAAC 2011, A2-1170)
  - production/purification relatively straight forward

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<tr>
<td>Colistin (Polymyxin E)</td>
<td>D-Leu⁶</td>
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<td>Polymyxin B (PMB)</td>
<td>D-Phe⁶</td>
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<td>Polymyxin B Nonapeptide (PMBN)</td>
<td>D-Phe⁶</td>
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**In Vitro Safety Tools**

In vitro HK2 kidney toxicity assay and microbiological activity

*In vitro* HK2 data indicate possible opportunities for improved renal tox profile while preserving MIC

In *vivo* translation?

**Nonapeptide**

**PMB, Colistin**

AstraZeneca
Choice of animal model:

Mouse: difficult to produce kidney lesions

Rat: kidney lesions reported for cumulative doses >37 mg/kg

Dog/monkey: animal welfare, translatability?
Translational Kidney Biomarkers
(beyond creatinine and BUN)

AZ is collaborating in a Predictive Safety Testing Consortium on nephrotoxicity. The working Group interacts with industry and regulatory bodies to facilitate biomarker qualification.

Goal: Gain experience assessing and interpreting novel kidney premonitory and diagnostic biomarker data in all preclinical repeat-dose in vivo studies
- KIM-1, Clusterin, NGAL (Lipocalin), Osteopontin*
- DMPK bioanalysis of tissue levels

*These biomarkers are qualified in rat but are being used routinely for other species by the industry, including humans. All 4 are premonitory

A, nephron segment-specific biomarkers; B, drugs that elicit site-specific tox
Nat Biotechnol 2010 28(5): 436
In Vitro to In Vivo Correlation

Biomarkers indicate kidney injury in colistin- but not PMBN-dosed animals in 7-day iv rat studies

- Unexpected \(^1\) lack of kidney histopathology for both colistin and PMBN
- 4 biomarker signals elevated @ day8 only for colistin
- no change in the Biomarkers Kim-1 and Lipocalin.
- No significant serum chemistry change

\(^1\) J. A. C. 2012, p. 452; AAC 2011, p. 4044
SC Dosing: Tolerability, Exposures & PK

Dose_group | Cmax (ug/ml) | Half-life (hrs) | AUC (hr*μg/mL) |
--- | --- | --- | --- |
0.5 mg/kg- IV | 0.57 | 0.8 | 0.98 |
5 mg/kg- SC | 1.96 | 1.34 | 10.32 |
10 mg/kg-SC | 3.64 | 1.88 | 26.86 |
20 mg/kg-SC | 7.33 | 2.75 | 79.13 |
40 mg/kg-SC | 13.16 | 3.31 | 170.85 |

IV dosing (60 min): 1 mg/Kg was not tolerated
SC dosing: Not tested beyond 40 mg/Kg

C<sub>max</sub> and AUC differences do not explain the tolerability issues. SC dosing is better tolerated for colistin sulfate exposure exceeds iv > 10 fold.
Histamine response does not track with tolerability issues

**Single IV Dose of Colistin**
- Strong histamine response @ 1mg/kg
- Tolerability issues within a few minutes
- Rats do not tolerate 1mg/kg (<60 min)

**Ascending IV Dose of Colistin Day 2**
- Histamine response varies
- Rats tolerate 1 mg/kg

**Single Subcutaneous Dose of Colistin**
- High histamine levels sustained over 8h
- Rats tolerate high doses
2 Day SC Dosing in Rats with Colistin Sulfate & PMBN Indicates Differentiation in Renal Toxicity

Sc dosing combined with QID dosing adequate to induce lesions quickly

kidney lesions observed in Colistin group only

Differentiation between colistin & PMBN supports exploration of novel analogs

At 10x mag.:
- tubules in colistin-treated rats appeared plump with prominent pale, basophilic cytoplasm

At 20x mag.:
- colistin-treated rats:
  • mitotic figures (←)
  • Increased number of necrotic cells in tubular lumina (*)
  • affected tubules have sloughed epithelium and nuclei are irregular and hyperchromatic.

- In both colistin- and PMBN- treated rats, cytoplasmic vacuolization of tubules is more prominent than in controls.
Summary

- Next generation kidney injury biomarkers detect early onset of kidney injury and differentiate colistin from PMBN.
- Consistent with these findings, in vitro studies of human renal epithelial cells show higher sensitivity to colistin and polymyxin B than PMBN.
- Novel analogs show reduced toxicity in HK2 assay while maintaining microbiological activity.
- Tolerability issues cannot be explained by a simple histamine response.
- Subcutaneous dosing in rats increases exposures above those obtained for intravenous dosing.
- In a 2-day SC study, differentiation in the renal histopathology between colistin sulfate and PMBN is observable.
- These studies support that in vitro studies with human renal kidney cells can be used to assess the nephrotoxicity for novel analogs.
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