In Vivo Efficacy of Novel, Fully Synthetic Tetracyclines in a Murine Lung Infection Model Challenged with KPC-producing Klebsiella pneumoniae

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Objectives: To examine the in vivo efficacy of novel, fully synthetic tetracyclines with potent in vitro activity against multidrug-resistant (MDR) Gram-negative pathogens (including carbapenem-resistant Enterobacteriaceae) in a murine lung infection model challenged with Klebsiella pneumoniae carbapenemase (KPC)-producing K. pneumoniae.

Methods: Minimal inhibitory concentration (MIC) assays were conducted according to CLSI guidelines. Groups of six female CD-1 mice were made neutropenic by administration of 150 mg/kg cyclophosphamide intraperitoneally on Day 0. On Day 0, mice were inoculated intranasally with KPC-producing K. pneumoniae KP1906 (6.7 log CFU/mouse). Controls and CFUs were administered intravenously (IV) at 5, 10, and 40 mg/kg twice at 12 hr post-infection. Tetracycline was administered IV at 80 mg/kg twice at 12 hr post-infection and colistin was administered subcutaneously at 10 mg/kg once at 2 hr post-infection. All mice were euthanized via CO2 inhalation and were homogenized, diluted, and plated for CFU determination.

Results: The MIC values of TP-076, TP-138, TP-600, tigecycline, and colistin against KP1906 were 0.0625, 0.0625, 1.0, and 0.5 μg/mL, respectively. At the 24 hr untreated controls, all strains demonstrated a log reduction of at least 2 log CFU/mL. TP-076 showed colony reductions of 0.36, 1.39, and 2.81 log10, respectively, and TP-600 showed colony reductions of 1.88, 2.58, and 3.21 log10. Tigecycline had a modest effect at 80 mg/kg, producing only a 1.23 log10 CFU reduction. Subcutaneous administration of a single 10 mg/kg dose of colistin resulted in counts comparable to the untreated control group.

Conclusions: Novel tetracyclines, TP-076, TP-138, and TP-600, demonstrated potent in vivo efficacy in a murine lung infection model challenged with KPC-producing K. pneumoniae. Additional studies are in progress to further develop these and related new tetracycline analogs to combat serious and life-threatening Gram-negative infections.

Materials and Methods

Compounds: Novel, fully synthetic tetracycline and polycyclic tetracycline analogs such as 6 and 7 were prepared by coupling a D-ring precursor 1 or a DE-ring precursor 2 to the AB-ring enone 3 via a Michael/Diels-Alder reaction to form the polycyclic intermediates 4 and 5. These were further extended to the general synthetic scheme. Lead compounds TP-076, TP-138, and TP-600 are included in this study belonging to general subclasses 6 or 7.

Bacterial strains: Klebsiella pneumoniae KP1906 (KPC-producing strain; UNI 205-1) was used by the lab of Dr. William J. Weiss at UNI Health Science Center, Fort Worth, TX. It is resistant to gentamicin, tobramycin, ceftazidime/ceftaxime, and imipenem by Clinical and Laboratory Standards Institute (CLSI) breakpoints, but susceptible to tigecycline and colistin.

In vitro susceptibility assays were performed according to CLSI guidelines. Minimal inhibitory concentrations (MICs) of each strain were determined against each compound by using a microbroth dilution method (CLSI document M31-A2, CLSI, Wayne, PA).

Lung infection model: Female CD-1 mice (22 ± 2 g, Harlan laboratory) were inoculated intranasally with 150 mg/kg (IP) on day 4 for neutropenic bacteria (based on previous data). Mice were anesthetized by IP injection of 5-15 ml of a mixture of ketamine-HCl (40 mg/kg) and xylazine (14 mg/kg) and intranasally (IN) inoculated with 0.15 ml of the designated inoculum (final infective dose of 108 CFU/mouse). For IN inoculation, intranasal drops were placed onto the internal nares for inhalation. After inoculation, mice were placed back into cages and monitored for recovery. During infection, mice were weighed and monitored for general health. After mice were challenged with 1 μg/mL (TP-076), 10 μg/mL (TP-138), and 100 μg/mL (TP-600) of each compound, the mice were euthanized via CO2 inhalation and lungs homogenized, diluted, and plated for colony-forming units (CFU) determination.

Table 1: MIC Values against Carbapenem-resistant Klebsiella pneumoniae KP1906

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC [μg/mL]</th>
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<tbody>
<tr>
<td>TP-076</td>
<td>0.0625</td>
</tr>
<tr>
<td>TP-138</td>
<td>0.0625</td>
</tr>
<tr>
<td>TP-600</td>
<td>0.125</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>1</td>
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<tr>
<td>Imipenem</td>
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</tbody>
</table>

Conclusions

The potent in vitro antibacterial activity of novel, fully synthetic tetracyclines (TP-076, TP-138, and TP-600) against serious Gram-negative pathogens translated to superior in vivo efficacy in a murine lung infection model challenged with KPC-producing K. pneumoniae, as compared to tigecycline.

In vivo efficacy observed was dose proportional.

Further studies are in progress to develop those new potent tetracyclines active against lethal infections, especially those caused by MDR Gram-negative bacteria including carbapenem-resistant Enterobacteriaceae (CRE).

References & Acknowledgements

2) We thank Dr. William Weiss and colleagues for conducting the in vivo experiments.