Introduction and Purpose

TP-6076 is a novel, fully synthetic tetracycline antibiotic developed for the treatment of life-threatening bacterial infections. It has demonstrated in vitro potency against carbapenem-resistant Acinetobacter baumannii (CRAB), with MIC50 value of 0.983 μg/mL. The current study examined the in vivo efficacy of TP-6076 in murine thigh and lung infection models challenged with A. baumannii.

Methods

Minimum Inhibitory Concentration (MIC) assay

The MIC of TP-6076 against A. baumannii clinical isolates was tested in MIC assays conducted according to CLSI guidelines. 5 μL of the 500 μg/mL stock solution of TP-6076 in DMSO was added to respective wells of microtiter plates followed by 5 μL of overnight cultures (10^5 CFU/mL). Plates were incubated at 37°C for 24 hours. The MIC was defined as the lowest concentration of the drug that prevented visible growth of the bacteria.

Mouse infection models

Female CD-1 mice (22-25 g) were quarantined at least 3 days prior to use and housed 5 per cage in accordance with approved IACUC protocols. Animals were rendered neutropenic by intraperitoneal injection of cyclophosphamide at 150 mg/kg (Day -4) and 100 mg/kg (Day -1) pre-infection. In the thigh infection model, mice were inoculated intramuscularly with 0.1 mL of the designated strain (6 log10 colony forming units (CFUs)). While, for the lung infection model, mice were inoculated intranasally (7 log10 CFUs).

Pharmacokinetics

Pharmacokinetics of TP-6076 were determined in the murine thigh infection model. The Pharmacokinetics of TP-6076 in female CD-1 mice were determined using non-invasive PK/PD methods. The AUCs for TP-6076 concentration-time profiles were calculated using non-compartmental analysis.

In vivo efficacy

For in vivo efficacy studies, TP-6076 was administered intravenously 2 hours post-infection at various dose levels. Mice were euthanized via CO2 inhalation at 24 hours (baseline) or 26 hours post-infection, and CFUs in tissues were determined. A sigmoid IC50 model was used to describe the relationship between MIC and the average change of bacterial burden (equation is shown below). Both plasma and ELF AUCs were calculated as the area under the plasma/ELF concentration-time curve. The ELF AUC was evaluated in the lung infection model, while only plasma AUCs were evaluated in the thigh infection model.

Results

In vivo efficacy of TP-6076 in murine thigh and lung infection models challenged with Acinetobacter baumannii

Table 1. TP-6076 MICs for the tested A. baumannii strains

<table>
<thead>
<tr>
<th>Strain</th>
<th>TP-6076 MIC (μg/mL)</th>
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<tbody>
<tr>
<td>AB3116</td>
<td>0.0312</td>
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<tr>
<td>AB3117</td>
<td>0.0325</td>
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<tr>
<td>AB3118</td>
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</tr>
<tr>
<td>AB3123</td>
<td>0.0312</td>
</tr>
</tbody>
</table>

Figure 1. Nonlinear Pharmacokinetics for TP-6076 in female CD-1 mice

E = Emax X (X / (XIC50 + XIC50))

• E is the top of the curve
• Emax is the maximum effect
• X is plasma/ELF AUC
• IC50 is the required value of PK/PD index to achieve 50% of the Emax
• HI is the Hill slope of the curve

• TP-6076 demonstrated potent in vivo efficacy against A. baumannii in murine neutropenic thigh/lung infection models. Full exposure-response relationships were obtained in both models, and the profiles were comparable.
• Since the determination of TP-6076 free fraction has proven challenging, only total plasma and ELF exposures were used in the current study. A better understanding of TP-6076’s protein binding relationships in mouse and human plasma is needed before the results can be used to predict outcomes in patients.

Conclusion

Nonlinear TP-6076 pharmacokinetics was observed in female CD-1 mice with single IV bolus injection. The increase in total plasma/ELF AUCs was more than dose-proportional over the 0.25 to 40 mg/kg dose range. The relationship between Log-transformed plasma/ELF exposures and dose levels can be described by a log-log equation, which was used to predict AUCs for other dosing levels within the range.

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References