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Characterization of the Mechanism of Action of Tebipenem (SPR859) in the *Escherichia coli* Macromolecular Synthesis Assay

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ABSTRACT

Background: Resistance to antibacterials among Enterobacteriaceae (EB) has increased in recent years. Tebipenem (SPR859) is the microbiologically active form of tebipenem-pivoxil (SPR994), an oral carbapenem, and is under development for treatment of complicated urinary tract infections (cUTI). To better understand SPR859's mechanism of action and compare it to that of other clinically relevant carbapenems, inhibition of macromolecular synthesis (MMS) pathways was studied in *Escherichia coli* ATCC 25922 with SPR859, meropenem (MEM), and imipenem (IPM).

Methods: The 25922 MICs for SPR859, MEM, and IPM were determined by standard broth microdilution methods (CLSI M7). Inhibition of DNA, RNA, protein, cell wall, and lipid MMS was evaluated against 25922 using radiolabeled precursors in the presence of SPR859 and MEM at multiples of the broth MIC, along with the appropriate positive control agents.

Results: The 25922 MICs of SPR859, MEM, and IPM were 0.015, 0.03, and 0.12 μg/mL, respectively. Exposure of exponential phase *E. coli* ATCC 25922 cells to 0.25, 0.5, 1, 2, 4, or 8-fold the MIC of either SPR859 or MEM failed to demonstrate significant inhibition of DNA, RNA, protein, or lipid synthesis. SPR859 and MEM inhibited cell wall synthesis in a dose-dependent manner, reaching a maximum inhibition of 61 and 58%, respectively, at 8-fold the MIC. Likewise, IPM inhibited cell wall synthesis reaching 84% inhibition at 8-fold the MIC.

Conclusions: SPR859 had an MIC of 0.015 μg/mL against the model organism *E. coli* ATCC 25922. There was no significant inhibition of DNA, RNA, protein, or lipid synthesis by SPR859 or MEM at 0.25x – 8x MIC. SPR859 inhibited cell wall synthesis in a dose-dependent fashion, while similar inhibition was observed with MEM and IPM. Thus, SPR859, like MEM and IMI, displayed beta-lactam-like characteristics in the macromolecular synthesis assay. All the results, including potency and degree of inhibition in these assays, suggest SPR859 has a mechanism of action consistent with the other tested carbapenems and support continued development of SPR859 as a potential cUTI treatment.

INTRODUCTION

- Resistance among Enterobacteriaceae, including resistance mediated by ESBLs, has increased.
- New agents are needed to treat antibiotic resistant Gram-negative pathogens.
- Tebipenem (SPR859; **Figure 1**) is the microbiologically active moiety of tebipenem-pivoxil (SPR994), an orally available carbapenem approved in Japan for respiratory tract infections in children; SPR994 is currently under development in the US for complicated UTI.
- Purpose: To investigate the mechanism of action of tebipenem by examining inhibition of macromolecular synthesis (MMS) in an *Escherichia coli* ATCC 25922 model.

METHODS

- The MIC of tebipenem, MEM, and IPM was determined for *E. coli* ATCC 25922 by Clinical and Laboratory Standards Institute (CLSI) guidelines M7-A101.
- *Macromolecular synthesis:* For each of the MMS reactions below, 100 μl of log phase cells grown in MHBII (DNA, RNA, lipid) or resuspended in M9 minimal medium (protein, cell wall) were added to triplicate wells containing various concentrations of test compound or control antibiotics (2.5 μl) at 40X the required final concentration. Following a 30 min pre-incubation at room temperature to allow for drug inhibition of a pathway, the assay was conducted as follows:
- *DNA, RNA, protein synthesis*: Either [^{3}H] thymidine (DNA), [^{3}H] uridine (RNA), or [^{3}H] leucine (protein) was added at 0.5 - 1 μCi per well. Reactions were allowed to proceed at room temperature for 15 min, stopped with 12 μl of cold 5% trichloroacetic acid (TCA), and incubated on ice for 30 min. The TCA precipitated material was collected on a 25 mm GF/1.2 μm PES 96 well filter plate (Corning), washed 5 times with 200 μl per well of cold 5% TCA, dried at room temperature for 1 hr, and counted using a Packard Top Count microplate scintillation counter.
- *Cell wall synthesis:* The cells were added to 1.5 ml microfuge tubes (100 μl/tube in triplicate) and [^{14}C]N-acetylglucosamine (0.4 μCi/reaction) was added to each tube and incubated for 45 min in a 37°C heating block. Reactions were stopped through the addition of 100 μl of 8% SDS, heated at 95°C for 30 min, cooled, briefly centrifuged, and spotted onto pre-wet nitrocellulose membrane filters (0.8 μm). After washing three times with 5 ml of 0.1% SDS, the filters were rinsed two times with 5 ml of deionized water, allowed to dry, and then counted using a Perkin Elmer Tri-Carb 4810TR Liquid Scintillation Analyzer.
- *Lipid synthesis:* The cells were added to 1.5 ml microfuge tubes (100 μl/tube in triplicate) and [^{3}H] glycerol was added at 0.5 μCi per reaction. Reactions were allowed to proceed at room temperature for 40 min, stopped through the addition of 375 μl chloroform/methanol (1:2), followed by vortexing for 20 sec. Chloroform (125 μl) was then added to each reaction and vortexed, followed by the addition of 125 μl dH_2O and vortexing. Reactions were centrifuged at 13,000 rpm in a microfuge for 10 min, and then 150 μl of the organic phase was transferred to a scintillation vial and allowed to dry in a fume hood for at least 1 hr. Samples were then counted using a Perkin Elmer Tri-Carb 2910TR Liquid Scintillation Analyzer.

RESULTS

- Tebipenem had an MIC of 0.015 μg/mL, compared to 0.03 and 0.12 μg/mL for MEM and IPM, respectively.
- Tebipenem, MEM, and IPM (**Figure 2**) inhibited cell wall synthesis in a dose-dependent manner, reaching a maximum inhibition of 61%, 58%, and 84%, respectively, at 8-fold the MIC.
- Exposure of exponential phase *E. coli* ATCC 25922 cells to up to 8-fold the MIC of either tebipenem (**Figure 3**) or MEM (data not shown) failed to demonstrate significant inhibition of DNA, RNA, protein, or lipid synthesis.

RESULTS

Figure 1. Structure of Tebipenem

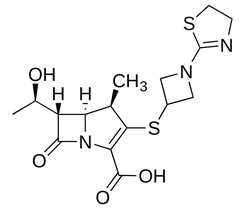


Figure 2. Cell Wall Synthesis Inhibition by Tebipenem, Meropenem, and Imipenem

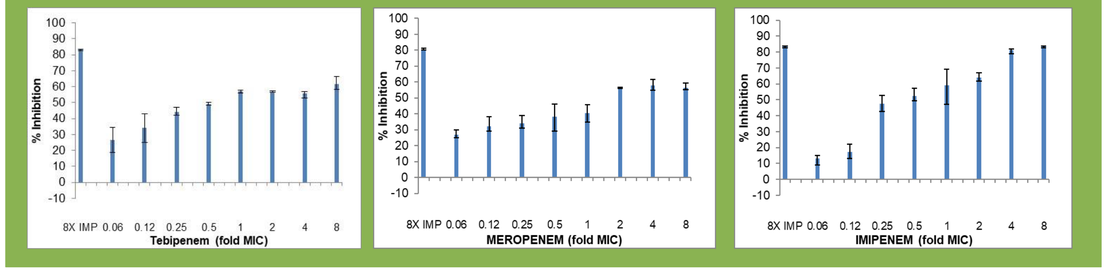
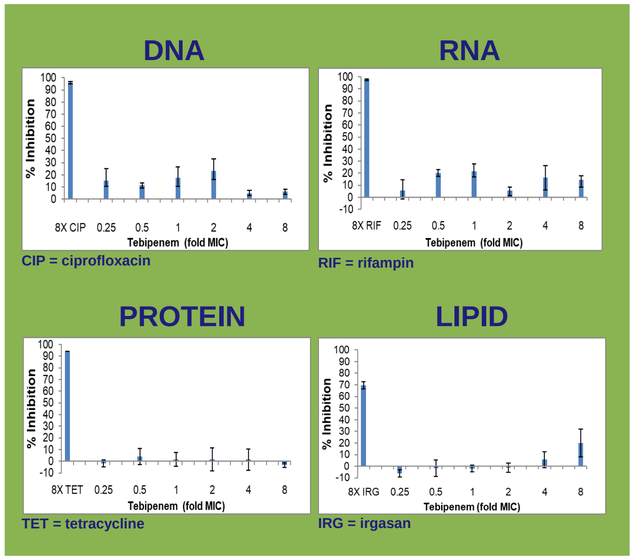


Figure 3. DNA, RNA, Protein and Lipid Synthesis Inhibition by Tebipenem



CIP = ciprofloxacin
RIF = rifampin
TET = tetracycline
IRG = irgasan

CONCLUSIONS

- Tebipenem had an MIC of 0.015 μg/mL for the macromolecular synthesis model organism *E. coli* ATCC 25922.
- Tebipenem and meropenem did not significantly inhibit DNA, RNA, protein, or lipid synthesis.
- Tebipenem exhibited clear dose-dependent inhibition of cell wall synthesis in *E. coli* in a similar fashion to that of meropenem and imipenem.