In Vitro Pharmacodynamics of Tigecycline and Tetracycline Combinations against *Pseudomonas aeruginosa*

Amelia N. Deitchman, PharmD; Ravi Shankar P. Singh, PhD; Anna Zoehner; Hartmut Derendorf, PhD
Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL

ABSTRACT

OBJECTIVES: Tigecycline (TIG) is a glycylcycline antibiotic with a broad spectrum of activity. Recent research has shown that TIG may be combined with tetracycline (TET) to have synergistic activity against *P. aeruginosa*. The objective of this research was to perform *in vitro* static time-kill curves to characterize the bacterial killing effect of TIG alone and in the presence of varying TET concentrations for *P. aeruginosa*.
METHODS: *In vitro* static time-kill curves were performed for *P. aeruginosa* (ATCC 27853) for varying TIG concentrations (0.25x, 0.5x, 1x, 2x, 4x, and 8x MIC) alone and in combination with TET 4, 8, and 12 mg/L. Experiments were performed over 24 hours with time points at 0, 2, 4, 6, 8, 10, 12, 16, and 24 h.
RESULTS: When comparing TIG alone and in combination with TET, differences of greater than 1-log colony-forming units/mL (CFU/mL) at 24 h were not observed at TIG concentrations ≤2 mg/L. The time-kill curve separation was much more evident for TIG 4 mg/L with a CFU/mL decrease of approximately 1.5-, 2-, 3.5-log in the presence of TET 4, 8, and 12 mg/L, respectively. The kill effect of tigecycline at higher concentrations (≥8 mg/L) was indifferent to tetracycline addition.
CONCLUSION: These results further demonstrate the novel synergistic activity of TIG-TET combinations against *P. aeruginosa*. The effect of TET is most pronounced at TIG 4 mg/L, which is reasonable, given a TIG MIC of 8 mg/L. With a further mechanistic understanding, pharmacokinetic-pharmacodynamic modeling of this data could be used to determine an optimal dosing regimen for future trials.

BACKGROUND

- Tigecycline, a novel glycylcycline antibiotic and minocycline analog
- Approved by the FDA in 2005 for complicated intra-abdominal infection (cIAI), complicated skin and skin structure infection (cSSSI) and community acquired pneumonia
- FDA added a black box warning to label for increased mortality risk in 2013[1]
  - Not associated with adverse effects
  - Likely to be related with the failure of treatment[2]
- Exhibits atypical nonlinear plasma protein binding (Figure 1A)
- Previous research shows that this nonlinear behavior is normalized with the addition of EDTA and induced with the addition of calcium chloride, leading to an increased unbound (active) fraction of tigecycline (Figure 1B,C)

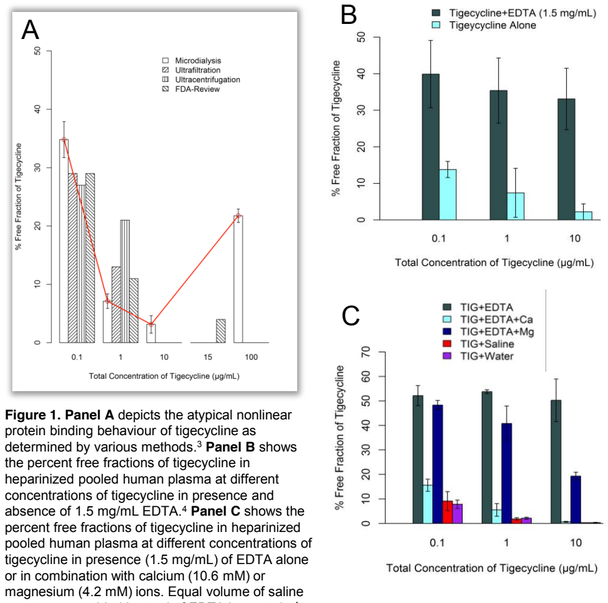


Figure 1. Panel A depicts the atypical nonlinear protein binding behaviour of tigecycline as determined by various methods.[3] **Panel B** shows the percent free fractions of tigecycline in heparinized pooled human plasma at different concentrations of tigecycline in presence and absence of 1.5 mg/mL EDTA.[4] **Panel C** shows the percent free fractions of tigecycline in heparinized pooled human plasma at different concentrations of tigecycline in presence (1.5 mg/mL) of EDTA alone or in combination with calcium (10.6 mM) or magnesium (4.2 mM) ions. Equal volume of saline or water was added instead of EDTA in controls.[4]

- Our group hypothesizes that a the complex protein-metal tigecycline interaction is responsible for this atypical behavior.
- Previous MIC experiments with metal ions and EDTA suggest that calcium chelation is likely responsible for decreased tigecycline activity and that another drug that chelates calcium (such as tetracycline) may be added to enhance the effect of tigecycline against *P. aeruginosa* (Table 1)[5]

Table 1. Effect of tetracycline addition on MICs of tigecycline against *P. aeruginosa*. MIC of Tetracycline alone is 16 mg/L.

Tetracycline Concentration (mg/L)	P. aeruginosa MIC
	Tigecycline (mg/L)
0	8
4	4
8	2
12	1

METHODS

***In Vitro* Static Time-Kill Curves (Figure 2)**

- 50mL-culture flasks inoculated with 5×10^5 CFU/mL and incubated for 2 h
- Varying concentrations of tigecycline (0, 0.25, 0.5, 1, 2, 4, 8×MIC) alone and in combination with tetracycline 4, 8, 12 mg/L added to flasks
- Samples from each flask taken, serial dilutions performed, and plating at 0, 2, 4, 6, 8, 10, 12, 16, and 24 h
- Incubated for 16-24 hours
- # Colonies counted 16-24 h post-plating
- $\log_{10}$(CFU/mL) calculated

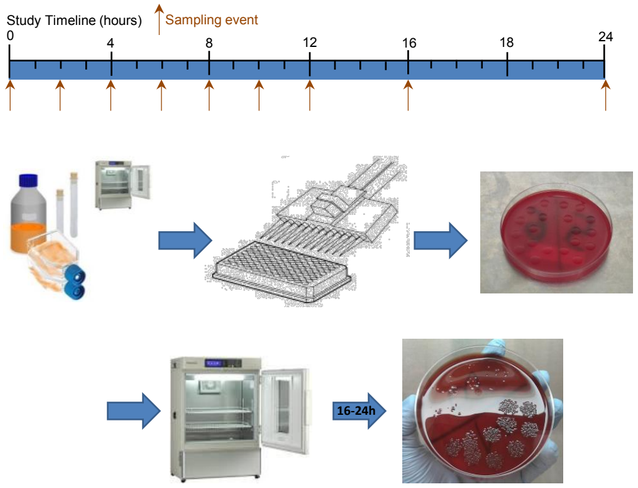
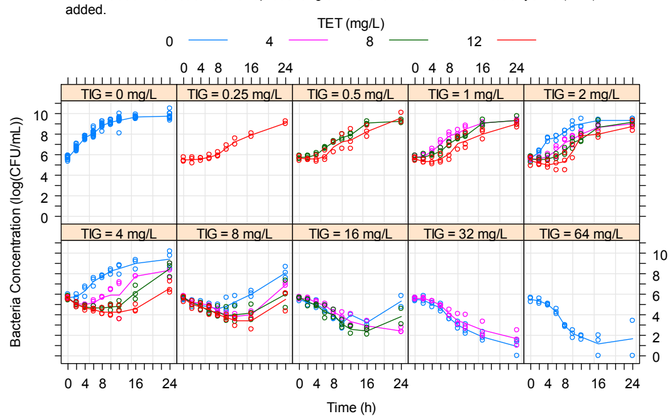


Figure 2 shows the plating process for time-kill curve experiments. A sample is taken from incubated flask at each time point a sampling event occurs and serial dilutions with normal saline are performed. Select dilutions are plated on blood agar to be incubated and subsequently counted to determine the concentration of bacteria present.

RESULTS

Figure 3. Results from time-kill curve experiments. Each panel shows a tigecycline (TIG) concentration with each curve representing a different concentration of tetracycline (TET) added.



- Differences of greater than 1- $\log_{10}$(CFU/mL) at 24 h were not observed between TET concentrations for TIG concentrations ≤2 mg/L
- The time-kill curve separation was most evident for TIG 4 mg/L with an average CFU/mL decrease of 1.38-, 3.42-, 4.21- $\log_{10}$ at 12 h and approximately 0.31-, 0.66-, 2.40- $\log_{10}$ at 24 h, in the presence of TET 4, 8, and 12 mg/L, respectively.
- The antimicrobial effect of tigecycline at concentrations >8 mg/L was indifferent to the addition of tetracycline.

CONCLUSIONS

- Enhanced antimicrobial effect of tigecycline-tetracycline combinations against *P. aeruginosa* observed in MIC studies is confirmed by time-kill curve studies, where enhanced activity was most pronounced when TIG 4 mg/L
- Decline in effect after 12 h could be due to drug degradation and must be accounted for to determine the full antimicrobial effect
- With better mechanistic understanding, pharmacokinetic-pharmacodynamic modeling of this data could be used to determine an optimal dosing regimen for future trials
- If future studies are successful, this novel combination has the potential to broadening the spectrum of tigecycline activity when co-administered with tetracycline, also allowing for lower doses of tigecycline to be administered.

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