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In vitro activities of tigecycline combined with other antimicrobials against multiresistant Gram-positive and Gram-negative pathogens

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***Objectives*: To test the activity of tigecycline combined with 16 antimicrobials *in vitro* against 22 Gram-positive and 55 Gram-negative clinical isolates.**

***Methods*: Antibiotic interactions were determined by chequerboard and time–kill methods.**

***Results*: By chequerboard, of 891 organism–drug interactions tested, 97 (11%) were synergistic, 793 (89%) were indifferent and 1 (0.1%) was antagonistic. Among Gram-positive pathogens, most synergisms occurred against *Enterococcus* spp. (7/11 isolates) with the tigecycline/rifampicin combination. No antagonism was detected. Among Gram-negative organisms, synergism was observed mainly with trimethoprim/sulfamethoxazole against *Serratia marcescens* (5/5 isolates), *Proteus* spp. (2/5) and *Stenotrophomonas maltophilia* (2/5), with aztreonam against *S. maltophilia* (3/5), with cefepime and imipenem against *Enterobacter cloacae* (3/5), with ceftazidime against *Morganella morganii* (3/5), and with ceftriaxone against *Klebsiella pneumoniae* (3/5). The only case of antagonism occurred against one *S. marcescens* with the tigecycline/imipenem combination. Selected time–kill assays confirmed the bacteriostatic interactions observed by the chequerboard method. Moreover, they revealed a bactericidal synergism of tigecycline with piperacillin/tazobactam against one penicillin-resistant *Streptococcus pneumoniae* and with amikacin against *Proteus vulgaris*.**

***Conclusions*: Combinations of tigecycline with other antimicrobials produce primarily an indifferent response. Specific synergisms, especially against enterococci and problematic Gram-negative isolates, might be worth investigating in *in vitro* models and/or in animal models simulating the human environment.**

Keywords: glycylcycline, chequerboard, killing, indifference, synergism

Introduction

Tigecycline is the first glycylcycline antibiotic available for clinical use.[1] Tigecycline is highly active *in vitro* against most common Gram-positive and Gram-negative pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), glycopeptide-intermediate *S. aureus* (GISA), penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant enterococci and extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*.[1–3] The activity of tigecycline is not affected by common tetracycline resistance mechanisms, including tetracycline-specific efflux pumps and ribosomal protection. Nevertheless, some isolates tend to have decreased susceptibility to tigecycline (*Proteus mirabilis*, indole-positive *Proteus* spp., *Morganella morganii*, *Providencia* spp. and a few strains of *Serratia marcescens* and *K. pneumoniae*) or demonstrate resistance (*Pseudomonas aeruginosa*).[2,3] These strains have constitutively overexpressed multidrug efflux pump systems for which tigecycline is a substrate, such as AcrAB in *P. mirabilis*, *M. morganii* and *K. pneumoniae*[4–6] and MexXY in *P. aeruginosa*.[7]

The present study investigated the effect of combining tigecycline with other antibacterials against representative clinical isolates of Gram-positive and Gram-negative organisms.

Materials and methods

Microorganisms

A total of 77 bacterial isolates (22 Gram-positive and 55 Gram-negative) were tested (Table 1). These isolates were recovered

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Table 1. Results of chequerboard testing of tigecycline and a second antibacterial agent against Gram-positive and Gram-negative bacteria

	No. of strains showing synergy/total no. of strains															
	AMC	TZP	CRO	CAZ	FEP	IPM	MEM	ATM	AMK	SXT	CIP	MXF	RIF	VAN	TEC	LZD
Gram-positive																
E. faecalis	1/6	0/6	1/6	ND	1/6	ND	1/6	ND	1/6	0/6	0/6	0/6	4/6	0/6	1/6	0/6
E. faecium	1/5	0/5	0/5	ND	0/5	ND	0/5	ND	0/5	0/5	0/5	0/5	3/5	0/5	0/5	1/5
S. aureus	2/6	0/6	1/6	ND	0/6	ND	0/6	ND	0/6	0/6	0/6	0/6	0/6	0/6	0/6	1/6
S. pneumoniae	1/5	1/5	0/5	ND	0/5	ND	0/5	ND	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
total	5/22	1/22	2/22		1/22		1/22		1/22	0/22	0/22	0/22	7/22	0/22	1/22	2/22
Gram-negative																
Acinetobacter spp.	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	ND	ND	ND	ND	ND
C. freundii	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	ND	ND	ND	ND	ND
E. aerogenes	0/5	0/5	0/5	0/5	0/5	1/5	0/5	1/5	2/5	0/5	0/5	ND	ND	ND	ND	ND
E. cloacae	0/5	1/5	2/5	2/5	3/5	3/5	1/5	2/5	2/5	1/5	0/5	ND	ND	ND	ND	ND
E. coli	0/5	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	ND	ND	ND	ND	ND
K. pneumoniae	0/5	0/5	3/5	2/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	ND	ND	ND	ND	ND
M. morganii	0/5	2/5	2/5	3/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	ND	ND	ND	ND	ND
Proteus spp.	0/5	1/5	1/5	0/5	0/5	0/5	0/5	1/5	2/5	2/5	0/5	ND	ND	ND	ND	ND
P. aeruginosa	0/5	0/5	1/5	1/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	ND	ND	ND	ND	ND
S. marcescens	1/5	2/5	0/5	0/5	1/5	0/5	0/5	1/5	1/5	5/5	0/5	ND	ND	ND	ND	ND
S. maltophilia	0/5	0/5	0/5	2/5	2/5	0/5	1/5	3/5	2/5	2/5	0/5	ND	ND	ND	ND	ND
total	1/55	6/55	10/55	12/55	8/55	5/55	2/55	8/55	12/55	11/55	1/55					

AMC, amoxicillin/clavulanate; TZP, piperacillin/tazobactam; CRO, ceftriaxone; CAZ, ceftazidime; FEP, cefepime; IPM, imipenem; MEM, meropenem; ATM, aztreonam; AMK, amikacin; SXT, trimethoprim/sulfamethoxazole; CIP, ciprofloxacin; MXF, moxifloxacin; RIF, rifampicin; VAN, vancomycin; TEC, teicoplanin; LZD, linezolid; ND, not done.

from human infections (one isolate per patient). Gram-positive isolates included six *Enterococcus faecalis* [five vancomycin-susceptible and one vancomycin-resistant (VanA type)], five *Enterococcus faecium* [four vancomycin-susceptible and one vancomycin-resistant (VanA type)], six *S. aureus* (two methicillin-susceptible *S. aureus*, three MRSA and one GISA) and five *S. pneumoniae* (three penicillin-susceptible and two penicillin-resistant). Gram-negative isolates were chosen at random to represent a spectrum of bacteria and resistance phenotypes encountered in clinical practice, e.g. β-lactam-resistant, quinolone-resistant and/or trimethoprim/sulfamethoxazole-resistant strains, and included five isolates of each of the following species: *Acinetobacter* spp., *Citrobacter freundii*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *E. coli*, *K. pneumoniae*, *M. morganii*, *Proteus* spp., *P. aeruginosa*, *S. marcescens* and *Stenotrophomonas maltophilia*.

Antimicrobial agents

The antibiotics tested in combination with tigecycline are shown in Table 1. Tigecycline was provided by Wyeth Research (Pearl River, NY, USA). All the other drugs were commercially available products.

MIC determination

MICs were determined using the broth microdilution method in Mueller–Hinton broth (MHB). *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 27736 and *P. aeruginosa* ATCC 27853 were used as quality control strains. Interpretative criteria for tigecycline MICs were defined on the basis of the United States Food and Drug Administration susceptibility breakpoints of $\leq$0.25 mg/L for streptococci and enterococci and $\leq$0.5 mg/L for *S. aureus*, and $\leq$2 mg/L for susceptibility, 4 mg/L for intermediate and $\geq$8 mg/L for resistance when testing Gram-negative organisms.[1,3]

Chequerboard studies

Antibiotic interactions were assessed by the chequerboard method in 96-well microtitre plates. The wells were inoculated with 10^5 cfu/mL, and the plates were incubated for 24 h at 35°C. Fractional inhibitory concentrations (FICs) were calculated as the MIC of drug A or B in combination/the MIC of drug A or B alone, and the FIC index (FICI) was obtained by adding the FIC values. FICIs of $\leq$0.5 were interpreted as synergistic, those of $>$0.5 but $\leq$4 were considered as indifferent, and those of $>$4 were interpreted as antagonistic.[8,9]

Time–kill assays

In selected cases, synergism and antagonism were also tested in time–kill experiments. Flasks containing MHB were inoculated with 10^6 cfu/mL of the test organism. For synergism, antibiotics were added to the flasks at concentrations equivalent to 0.25× the MIC for the specific isolate. For testing the agreement with the only case of antagonism observed by the chequerboard results (tigecycline in combination with imipenem, each drug was tested alone at the following concentrations): tigecycline 0.25× and 2× the MIC, imipenem 0.032× and 2× the MIC; and in combination at the following concentrations: tigecycline 0.25× the MIC plus imipenem 2× the MIC, and tigecycline 2× the MIC plus imipenem 0.032× the MIC, i.e. the highest drug combinations showing turbidity in microtitre plates.

Synergism, indifference and antagonism were defined as described previously.[8] Each time–kill experiment was repeated two times independently.

Results

Susceptibility results

Tigecycline was active (MIC 0.01–0.5 mg/L) against all of the 22 Gram-positive isolates tested regardless of their resistance pattern to other drugs. For the 55 Gram-negative test organisms, tigecycline was active (MIC $\leq$ 2 mg/L) against 46 (84%), borderline against 4 (7%; 3 *Proteus* spp. and 1 *M. morganii*), and ineffective (MIC $\geq$ 8 mg/L) against 5 (9%; all being *P. aeruginosa*) isolates.

FICIs—Gram-positive isolates

Over the 286 individual antibiotic and bacteria combinations with the Gram-positive isolates (Table 1), synergism (FICI $\leq$0.5) occurred in 21 of 286 (7%) cases and indifference (FICI $>$0.5 but $\leq$4) in 265 of 286 (93%) cases, irrespective of the drug resistance pattern to other drugs. No antagonism (FICI $>$4) was detected. High rates of synergism occurred against *Enterococcus* spp. (synergism in 7 of 11 isolates) when tigecycline was combined with rifampicin. The combination of tigecycline with amoxicillin/clavulanate was also synergistic against two (both MRSA) of the six *S. aureus* isolates.

FICIs—Gram-negative isolates

Over the 605 individual antibiotic and bacteria combinations with the Gram-negative isolates (Table 1), synergism occurred in 76 of 605 (13%) cases and indifference in 528 of 605 (87%) cases. Synergism was observed mainly with trimethoprim/sulfamethoxazole against *S. marcescens* (five of five isolates), *Proteus* spp. (two of five) and *S. maltophilia* (two of five); with cefepime and imipenem against *E. cloacae* (three of five); with ceftazidime against *M. morganii* (three of five); with ceftriaxone against *K. pneumoniae* (three of five); and with aztreonam against *S. maltophilia* (three of five isolates). Other synergisms occurred in various isolates with various antibiotic combinations, including with amikacin against a total of 12 of the 55 organisms. Antagonism occurred only in one case (0.2% of the tests) with imipenem against an *S. marcescens* isolate.

Time–kill assays

In selected cases (three Gram-positive isolates and nine Gram-negative isolates), synergism or antagonism was further tested in time–kill experiments [see Figures S1 and S2, available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/)]. For Gram-positive bacteria, time–kill experiments confirmed that tigecycline plus rifampicin against enterococci was more active than either drug alone. In addition, they revealed a bactericidal synergism between tigecycline and piperacillin/tazobactam against one penicillin-resistant pneumococcal isolate.

Time–kill experiments with Gram-negative isolates confirmed the bacteriostatic synergism between tigecycline and imipenem against *E. cloacae* 1085, tigecycline and ceftazidime against *M. morganii* 48, tigecycline and trimethoprim/sulfamethoxazole against *P. mirabilis* 119 and *S. marcescens* 220, tigecycline and amikacin against *S. maltophilia* 58 and *P. mirabilis* 35, and tigecycline and aztreonam against *S. maltophilia* 59. In addition, bactericidal synergism was observed between tigecycline and amikacin against *Proteus vulgaris* 60.

We also tested the only case of antagonism revealed by the chequerboard method against one isolate of *S. marcescens*. When 0.25$\times$ MIC of tigecycline was mixed with increasing concentrations of imipenem, the MIC of imipenem reproducibly increased by 4$\times$. However, this antagonism was limited to this sub-MIC concentration and did not occur at greater concentrations of tigecycline. Likewise, when 0.032$\times$ MIC of imipenem was added to increasing concentrations of tigecycline, the MIC of tigecycline consistently increased by 4$\times$. When we tested tigecycline in combination with imipenem by time–kill assays at the highest drug combinations showing turbidity in microplates, the antagonism was confirmed when tigecycline at 0.25$\times$ the MIC was combined with imipenem at 2$\times$ the MIC. However, antagonism was not detected when tigecycline at 2$\times$ the MIC was combined with imipenem at 0.032$\times$ the MIC.

The results of time–kill assays agreed reasonably well with the chequerboard method: for the 34 combinations examined with both methods, synergy results by chequerboard were confirmed by time–kill studies in 10/20 (50%) occasions, indifference in 11/13 (85%) and antagonism in 1/1 (100%).

Discussion

The present results indicate that the interaction of tigecycline with other drugs against Gram-positive and Gram-negative bacteria was essentially indifferent. Thus, tigecycline could be used safely with other antibacterial compounds, for instance, in empirical antibiotherapy requiring a very broad-spectrum antibiotic coverage or in intra-abdominal polymicrobial infection involving a possible tigecycline-resistant *Pseudomonas*. An antagonism was observed in only one single case (0.1% of cases), with tigecycline in combination with imipenem. This antagonism was limited to a very sub-MIC concentration of tigecycline (0.25$\times$ the MIC). A speculative explanation for this observation is that sub-MIC concentrations of either of the drugs could induce drug efflux of the partner compound. As the phenomenon occurred in a restricted window of sub-MIC concentrations, its potential clinical relevance is unclear.

The fact that drug interactions between tigecycline and other compounds were essentially indifferent supports previous results using the chequerboard method.[10] However, the present experiments also revealed a number of interesting synergisms. These synergisms were of either of two types, i.e. (i) bacteriostatic, as observable by chequerboard and time–kill assays, and/or (ii) bactericidal, as revealed only by time–kill assays. Two of the synergisms revealed by the chequerboard method tended to be both drug-class and organism dependent, including tigecycline and rifampicin against *Enterococcus* spp. and tigecycline and trimethoprim/sulfamethoxazole against *E. cloacae*, *Proteus* spp. and *S. maltophilia*. Both synergisms could be of clinical relevance, as the organisms they target belong to potentially problematic multiresistant species. The generalization of these synergisms to additional isolates of these species and their possible relevance in infection models might be worth testing. Other synergisms were

non-specifically spread over several drugs and organisms and might be more difficult to interpret. Eventually, time–kill assays disclosed a clear bactericidal synergism between tigecycline and β-lactams against one tested *S. pneumoniae* and tigecycline and amikacin against one tested *P. vulgaris*. Although these preliminary observations need to be investigated further, they might open new perspectives for the utilization of tigecycline with other antibacterials in specific types of infections.

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Transparency declarations

None to declare.

Supplementary data

Figures S1 and S2 are available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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