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Investigation of *In Vitro* Antagonistic and Synergistic Effects of Finafloxacin in Combination with Other Antibiotics

C. Y. GOH[1], S. B. NG[1], M. EVERETT[1] and A. VENTE[2]

[1]MerLion Pharmaceuticals, Singapore [2]MerLion Pharmaceuticals, Berlin, Germany

Contact information:

Andreas Vente
Merlion Pharmaceuticals GmbH
Robert-Rossle-Strasse 10
13125 Berlin, Germany
Phone : +49 30 9489 4050
Fax: +49 30 9489 4051
E-mail: **vente@merlionpharma.de**

Revised Abstract

Background:
Finafloxacin (FIN) is a novel member of the fluoroquinolone class of antibiotics. Specifically, FIN belongs to a new 8-cyano subclass that contains a novel base component which confers improved antibacterial activity under acidic conditions. Combination antibiotic therapy has been one of the options considered when dealing with multi-drug resistant bacteria infection. The aim of this study was to identify potential antagonistic or synergistic interactions between FIN and other commonly used antibiotics.

Methods:
Chequerboard experiments were performed at pH 5.8 and 7.2 under otherwise standard CLSI conditions using FIN in combination with meropenem (MER), ceftazidime (CTZ), amikacin (AMK) or colistin (COL) against *P. aeruginosa* ATCC 27853 and tigecycline (TIG), vancomycin (VAN), daptomycin (DAP) or linezolid (LZD), against MRSA ATCC 33591. Combinations were scored on the basis of the fractional inhibitory concentration index (FICI). FIN in combination with AMK was also tested against three fluoroquinolone-resistant *E.coli* UTI isolates and one susceptible strain (ATCC 25922). Time-kill studies were performed at synergistic drug combinations observed in the chequerboard experiment, and also at combinations of sub-MIC concentrations.

Results:
None of the drug combination exhibited antagonism. Most antibiotic combinations showed partial synergistic or additive effects (FICI: > 0.5 ≤1) under both acidic and neutral conditions, except FIN-MER and FIN-AMK which showed synergy (FICI: ≤ 0.5) at pH 7.2. Synergy was also observed for FIN-AMK against one UTI isolate at pH 5.8. Time-kill studies confirmed that the synergistic effects were bactericidal rather than bacteriostatic. Higher drug concentrations were required to attain synergistic bactericidal effect.

Introduction

Finafloxacin (FIN) is a novel member of the fluoroquinolone class of antibiotics. Specifically, FIN belongs to a new 8-cyano subclass. The agent contains a novel base component which confers improved antibacterial activity under acidic conditions[1], where the activity of many existing fluoroquinolones is impaired.

Combination antibiotic therapy has been one of the options considered when dealing with multi-drug resistant bacteria infection. However, there is a possibility of antagonism arising from the combination of certain antibiotics. Therefore, it is important to ensure that the use of FIN in combination therapy does not cause antagonistic effects.

The aim of this study was to identify potential antagonistic or synergistic interactions between FIN and other commonly used antibiotics.

[1]W. Stubbings *et. al*., 2011. In vitro spectrum of activity of finafloxacin, a novel, pH-activated fluoroquinolone, under standard and acidic conditions. Antimicrob. Agents Chemother. 55: 4394-4397.

[2]RL. White *et. al*., 1996. Comparison of three different in vitro methods of detecting synergy: time-kill, chequerboard, and E test. Antimicrob. Agents Chemother. 40: 1914-1918.

Results

In the chequerboard experiments most antibiotic combinations showed additive effect (FICI: > 0.5 ≤1) under both acidic and neutral conditions. When tested against *P. aeruginosa* ATCC 27853 and *E. coli* UTI clinical isolate FIN-AMK showed synergy (FICI: ≤ 0.5) at pH 7.2 and pH 5.8, respectively. Borderline synergy (FICI = 0.5) was observed for FIN-MER against *P. aeruginosa* at pH 7.2 for two out of three experiments.

In the time-kill studies no synergy was observed for either FIN-AMK or FIN-MER at the chequerboard synergistic drug concentrations. However, FIN-AMK showed enhanced bactericidal effects at early time points when it was used at a higher drug concentration of 0.5x MIC, as compared to the single most active agent. The enhanced effect was observed against both *P. aeruginosa* ATCC 27853 and *E. coli* UTI clinical isolate. Increasing the drug concentrations for FIN-MER to 0.5x MIC showed slight improvement in the bactericidal effect.

Table 1. Mean FICI of FIN-AB combination under both acidic and neutral conditions.

Antibiotic	Organism	AB MIC*	FIN MIC*	Mean FICI**	
		ph 5.8/7.2	pH 5.8/7.2	pH 5.8	pH 7.2
Meropenem	*P. aeruginosa* ATCC 27853	0.5/0.5	0.5/4	0.63	0.54
Amikacin	*P. aeruginosa* ATCC 27853	32/2	0.5/4	0.57	**0.50**
Colisitin (Pol E)	*P. aeruginosa* ATCC 27853	0.5/0.5	0.5/4	0.58	0.74
Ceftazidime	*P. aeruginosa* ATCC 27853	4/2	0.5/4	0.67	0.60
Daptomycin	*MRSA ATCC 33591*	1/0.5	0.06/0.13	0.59	0.63
Linezolid	*MRSA ATCC 33591*	1/1	0.06/0.13	1.00	0.63
Amikacin	UTI *E. coli 003/3*	32/4	16/64	0.53	0.83
Amikacin	UTI *E. coli 005/5*	32/8	4/16	0.54	0.71
Amikacin	UTI *E. coli 008/4*	16/2	16/128	**0.34**	0.55
Amikacin	*E. coli* ATCC 25922	16/4	0.0078/0.031	**0.40**	0.71

*MIC (mg/L) **Mean FICI (n = 3) of Best FIN-AB combination Synergy = 0.5 (bold)

Figure 1. Synergy time-kill of FIN-AMK against *E. coli* urinary tract infection (UTI) clinical isolate.

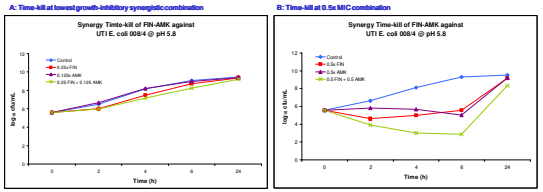
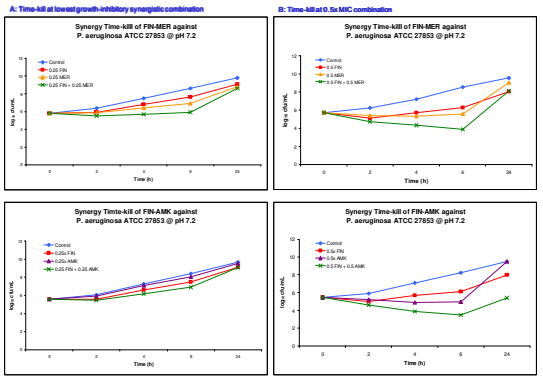


Figure 2. Synergy time-kill of FIN-MER & FIN-AMK against *P. aeruginosa* ATCC 27853.



Methods

Chequerboard synergy test
Chequerboard experiments were performed at pH 5.8 and 7.2 under otherwise standard CLSI conditions using FIN in combination with meropenem (MER), ceftazidime (CTZ), amikacin (AMK) or colistin (COL) against *P. aeruginosa* ATCC 27853 and tigecycline (TIG), vancomycin (VAN), daptomycin (DAP) or linezolid (LZD), against MRSA ATCC 33591. Combinations were scored on the basis of the fractional inhibitory concentration index (FICI). FIN in combination with AMK was also tested against three fluoroquinolone-resistant *E. coli* UTI isolates and one susceptible strain (ATCC 25922).

Time-kill studies
Kill kinetics of AMK, MER and FIN were determined by incubating an inoculum of ~5 x 10^5 cfu/mL of either *P. aeruginosa* or *E. coli* with the drug concentrations that showed synergy in the checkerboard experiments, and at 1 dilution below the MICs (0.5x MIC).

Synergy was defined as a ≥ 2 $\log_{10}$ decrease in colony count by the drug combination compared to the most active single agent. Indifference was defined as < 2 $\log_{10}$ increase or decrease in colony count; antagonism was defined as ≥ 2 $\log_{10}$ increase in colony count by the drug combination compared to the most active single agent[2].

Conclusions

- **No antagonism was observed when FIN was used in combination with the antibiotics tested**
- **FIN-AMK & FIN-MER showed synergistic bacteriostatic effects**
- **Both combinations at 0.5x MIC showed enhanced bactericidal effects at early time points, compared to the individual drugs at the same concentration**
- **Further studies at higher concentrations are warranted**