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POST-ANTIBIOTIC AND SYNERGIC EFFECTS OF FLUOROQUINOLONES AND CEFTAZIDIME IN COMBINATION AGAINST *PSEUDOMONAS* STRAINS*

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Infectious complications are still the leading cause of morbidity and mortality in seriously ill patients [1–3]. To combat the resistance of (mainly Gram-negative, non-fermentative) bacteria, e.g. *Pseudomonas aeruginosa,* to a wide spectrum of antibiotics, drug combination therapy has been widely adopted as standard clinical practice since the late 1990s. β-Lactam combinations are not optimal and the potential of nephrotoxicity and ototoxicity from aminoglycosides has caused clinicians to evaluate new possibilities, such as combinations of fluoroquinolones and β-lactams. We examine here the synergic and post-antibiotic effects (PAEs) of ciprofloxacin, ofloxacin and pefloxacin-ceftazidime combinations against 6 clinical *Pseudomonas* isolates.

The fluoroquinolone-ceftazidime combinations were not only synergic against *Comamonas (P.) testosteroni*, but had double the PAEs of the two drugs alone. The ciprofloxacin-ceftazidime combination had a longer PAE against *P. aeruginosa* isolate 1 than ciprofloxacin alone. The combinations, however, did not have longer PAEs than those of the single drugs against the other 5 *P. aeruginosa* isolates.

Keywords: Post-antibiotic effect (PAE) – synergism – antibiotic combinations – ciprofloxacin – ceftazidime – *Pseudomonas*

INTRODUCTION

Infectious complications are still the leading cause of morbidity and mortality in patients with neutropenia, cancer, endocarditis and even cystic fibrosis [3, 4, 9]. During the 1960s, there were only a few antimicrobial agents and the drugs were used in monotherapy. After the 1970s, extended-spectrum carboxypenicillins, aminoglycosides and later the third-generation cephalosporines were introduced, and combination therapy (mainly β-lactam antibiotics plus aminoglycosides) was extensively used in the treatment of seriously ill patients and nosocomial infections from the early 1980s. The changing spectrum of bacterial pathogens, their resistance and the availability of potent broad-spectrum antibiotics (carbapenems and newer fluorinated quinolones) with the ability to exert a bactericidal effect against a variety of organisms led again to the use of monotherapy in the 1990s. Following the emergence of

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bacterial resistance to a wide spectrum of antibiotics, the use of drug combination therapy has been widely adopted as standard clinical practice since the late 1990s. The double β-lactam combinations are not optimal, and the potential of nephrotoxicity and ototoxicity from aminoglycosides has caused some clinicians to evaluate new possibilities, such as combinations of fluoroquinolones and β-lactam agents.

Pseudomonas aeruginosa and other *Pseudomonas* species are opportunistic pathogens capable of causing serious infections. Only a few antimicrobial agents, such as ceftazidime, cefepime, quinolones and carbapenems, display potent antibacterial activity against these species, which are often multiresistant and can play important roles in the colonization or infection of patients with cystic fibrosis, cancer or neutropenia.

In previous papers, we described an automated method for measurement of the post-antibiotic effects (PAEs) of different antipseudomonal antibiotics [8], and a method for measuring the synergic effects of antibiotic combinations [7] against *Pseudomonas* isolates.

The aim of this study was to examine whether there is a connection between the synergic effects and PAEs of combinations of fluoroquinolones with ceftazidime against clinical *Pseudomonas* isolates [8].

MATERIALS AND METHODS

Six clinical isolates of *Pseudomonas* species were investigated. Three of them were described previously [4]; the 3 new isolates were resistant to trovafloxacin, ciprofloxacin, ofloxacin and pefloxacin.

Ofloxacin (Hoechst AG.), pefloxacin (Rhone Poulenc Santé/EGIS), ciprofloxacin (Bayer) and ceftazidime (Glaxo S.P.A.) were used. The minimum inhibitory concentrations (MICs) of the antibiotics were determined in Mueller-Hinton broth with the micro broth dilution method [1].

The combinations were examined by three different methods: disc diffusion, "checkerboard" titration and the time-killing method. The criterion of synergism in "checkerboard" titration was a fractional inhibitory concentration (FIC) index not higher than 0.5 [1, 5, 7]. The time-killing method was carried out with subcultures of the test bacteria made from fresh blood agar cultures in Mueller-Hinton broth and incubated overnight. The same inoculum was used in fresh Mueller-Hinton broth containing no antibiotic, one or other of the antibiotics in 1/2×MIC concentration, or the same amounts of antibiotics in combination, and the cultures were incubated for 24 h at 37 °C. The CFU/mL values of the cultures were determined at the beginning and after 2, 4, 6 and 24 h of incubation. Synergism was defined as a ≥100-fold increase in killing at 24 h (as measured by colony counts) for the combination in comparison with the more active of the single drugs [2, 5, 7]. The PAE was determined as previously described by Craig and Gudmundson [1]: the subcultures were incubated for 2 h with the antibiotics, and the drugs were then removed by washing. Some changes were made: (a) subcultures were kept in an ice-cold water-bath during wash-

ing; (b) 200 µl aliquots were placed into the wells of a microtiter plate for automatic measurement with an Anthos HT III (Anthos Labtec Instruments, Austria) microplate reader, and the OD was measured at 620 nm every 30 min., in parallel with colony counting; (c) neither the controls nor the cultures treated with antibiotics were diluted after washing [4]. The PAE was defined as the difference in time required for the cultures to reach a chosen point (A50) on the absorbance curve, where A50 was 50% of the maximum absorbance of the control culture (9): PAE = T – C [1].

RESULTS

The MICs of quinolones and ceftazidime are listed in Table 1. The isolates differed in sensitivity not only against fluoroquinolones, but also against ceftazidime. Table 2 shows the FIC indices of the tested combinations against the chosen microorganisms.

Table 1
Minimum inhibitory concentrations (MICs) of antipseudomonal antibiotics against six clinical isolates of *Pseudomonas* species

Isolates	MIC (mg/L) (resistance breakpoints)			
	CIP (≥4)	OFL (≥8)	PEFL (≥8)	CAZ (≥32)
C. (P.) testosteroni	1	4	1	128
P. aeruginosa isolate 1	0.125	4	2	2
P. aeruginosa isolate 2	1	16	16	8
P. aeruginosa isolate 3	16	32	32	64
P. aeruginosa isolate 4	32	64	128	32
P. aeruginosa isolate 5	16	16	32	16

Abbreviations: CIP: ciprofloxacin, OFL: ofloxacin, PEFL: pefloxacin, CAZ: ceftazidime, *According to NCCLS M100-S10 (M7); January, 2000.

Table 2
Fractional inhibitory concentration (FIC) indices of different antibiotic combinations for six *Pseudomonas* isolates, by "checkerboard" titration

FIC indices	Combination		
	Ciprofloxacin – ceftazimide	Ofloxacin – ceftazidime	Pefloxacin – ceftazidime
C. (P.) testosteroni	0.312	0.25	0.375
P. aeruginosa isolate 1	0.75	0.516	0.517
P. aeruginosa isolate 2	1.0	0.75	1.0
P. aeruginosa isolate 3	0.75	0.75	0.75
P. aeruginosa isolate 4	0.5	0.375	0.75
P. aeruginosa isolate 5	0.5	0.75	0.625

Table 3
Synergic effects of fluoroquinolones in combination with ceftazidime, using the "killing curve" method, and 1/2×MICs of the antibiotics

Combinations	Synergism observed with isolates					
	C. (P.) testosteroni	*P. aeruginosa* isolate 1	*P. aeruginosa* isolate 2	*P. aeruginosa* isolate 3	*P. aeruginosa* isolate 4	*P. aeruginosa* isolate 5
CIP + CAZ	++++	++++	++	++	++	++++
OFL + CAZ	++++	ND	ND	–	+	–
PEF + CAZ	++++	–	ND	+	+	–

Abbreviations: CIP – ciprofloxacin, OFL – ofloxacin, PEF – pefloxacin, CAZ – ceftazidime, ND – not determined.

Table 4
Post-antibiotic effects (PAEs) in hours of the combination of ciprofloxacin and ceftazidime against *Pseudomonas* isolates

Antibiotics or Combination	PAEs					
	C. (P.) testosteroni	*P. aeruginosa* isolate 1	*P. aeruginosa* isolate 2	*P. aeruginosa* isolate 3	*P. aeruginosa* isolate 4	*P. aeruginosa* isolate 5
CIP, 1/2×MIC	1.8	2.5	0.1	0.0	0.0	1.1
CAZ, 1/2×MIC	1.1	0.0	0.0	0.0	0.0	0.0
CIP + CAZ						
Each 1/2×MIC	3.8	4.1	0.5	0.0	0.0	1.1
OFL, 1/2×MIC	1.6	0.5	0.1	0.1	1.1	0.7
CAZ, 1/2×MIC	0.1	0.0	0.0	0.0	0.4	0.0
OFL + CAZ						
Each 1/2×MIC	1.6	0.6	ND	0.2	1.35	0.7
PEF, 1/2×MIC	1.8	10.1	1.8	1.0	0.0	0.9
CAZ, 1/2×MIC	1.3	0.5	0.0	0.0	0.0	0.0
PEF + CAZ						
Each 1/2×MIC	ND	ND	1.8	1.6	0.0	0.7

Abbreviations: CIP – ciprofloxacin, OFL – ofloxacin, PEF – pefloxacin, CAZ – ceftazidime, ND – not determined.

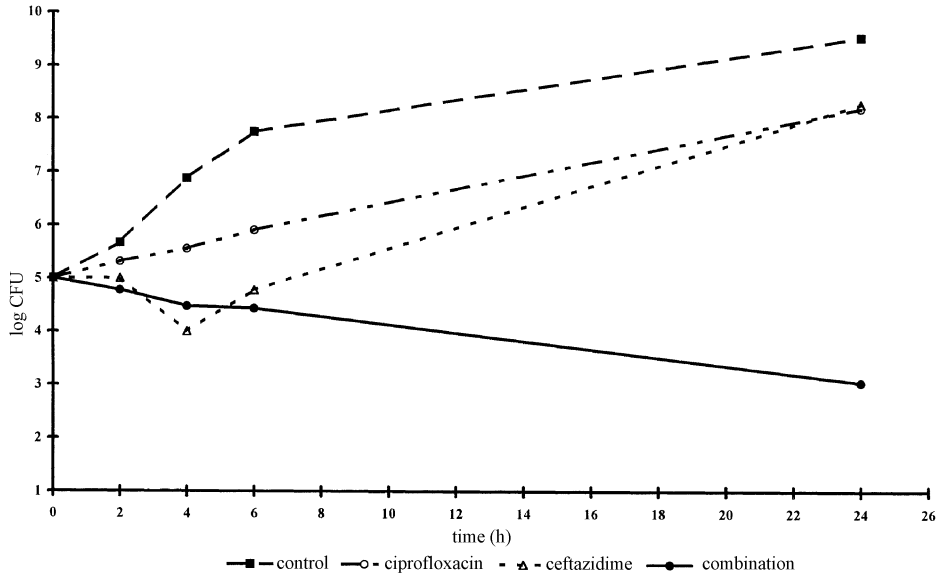

Fig. 1. Synergic effect of the combination of ciprofloxacin and ceftazidime against *C. (P.) testosteroni* as revealed by the "killing curve" method

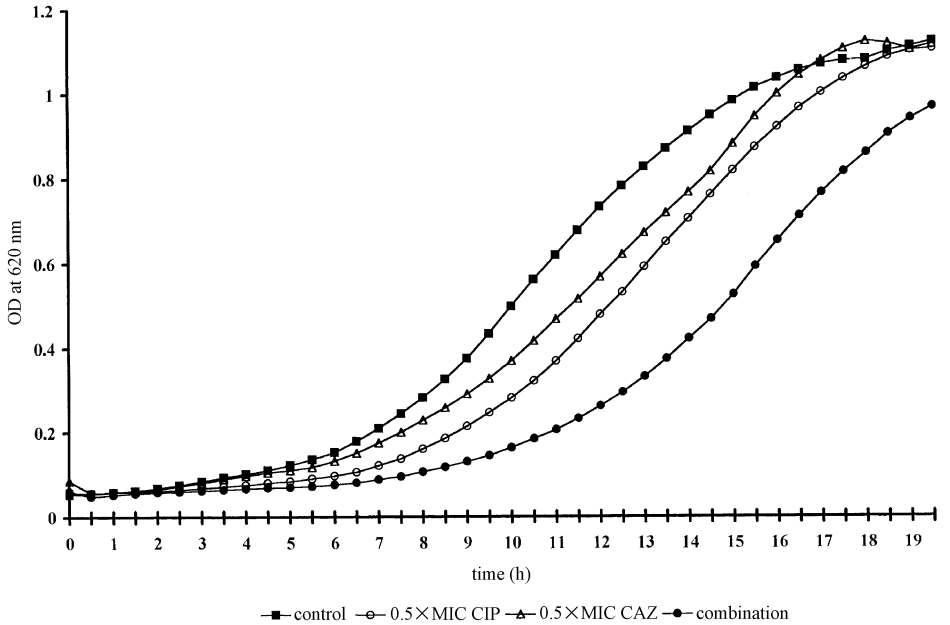

Fig. 2. The PAE of the combination of ciprofloxacin and ceftazidime against *C. (P.) testosteroni*

The ciprofloxacin-ceftazidime combination exhibited a synergic effect against 3 of the 6 isolates, while all combinations displayed a synergic effect against *Comamonas (Pseudomonas) testosteroni*. The synergic effects of the fluoroquinolone-ceftazidime combinations against the isolates, obtained by the "killing curve" method, are presented in Table 3. The ciprofloxacin plus ceftazidime combination showed synergy against all isolates.

The PAEs of ciprofloxacin, ofloxacin and pefloxacin were 0.1–5.6, 0.0–1.6 and 0.0–10.1 h, respectively, depending on the isolate tested (Table 4). The ciprofloxacin plus ceftazidime combination had longer PAEs against three of them; this combination gave the longest PAE. The ofloxacin plus ceftazidime combination had no PAE, while the pefloxacin plus ceftazidime combination exhibited a PAE against only one isolate.

Figures 1 and 2 depict the synergic effect and PAE of the ciprofloxacin-ceftazidime combination against *C. (P.) testosteroni*. The combinations not only had a synergic effect, but also showed a longer PAE against this strain.

DISCUSSION

Combined antibiotic therapy is mainly used in special units of hospitals. The combinations of aminoglycosides and cephalosporines are most commonly adopted. The aminoglycosides are nephrotoxic, so that replacement of these drugs would be desirable. The possible candidates include the fluoroquinolones.

The results of the first investigation of the ciprofloxacin plus ceftazidime combination against the rare clinical isolate of *C. (P.) testosteroni* was promising: the combination not only proved synergic, but it had approximately twice as long a PAE as that of either of the drugs alone. The combination of ciprofloxacin plus ceftazidime also had a 2.0 h PAE against *P. aeruginosa* isolate 1 as compared with ciprofloxacin alone. However, the combinations did not have longer PAEs than those of the single drugs against the other 5 *P. aeruginosa* isolates.

Although the PAE seems to be antibiotic, concentration and strain-dependent, and the combinations of fluoroquinolones with ceftazidime exhibit a synergic effect against approximately 30% of *P. aeruginosa* isolates, the PAEs of these antibiotic combinations were rare and also appeared to be strain-specific. The combinations did not have longer PAEs than those of the single fluoroquinolones against most of the *P. aeruginosa* isolates tested.

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